2.3. Comparison with Participants in Treatment Settings: Recruitment Criteria and Facilities

The ASI-J was administered to 111 drug abusers in treatment settings to examine its reliability and validity [19]. These participants were recruited at three hospitals and two recovery facilities between January 2002 and September 2004. Of the 111 drug abusers, 55 were included in the present study using the following criteria: at least 18 years old, male Japanese with a history of methamphetamine abuse, diagnosed as a drug abuser or drug dependent based on DSM-IV criteria, and an inpatient or outpatient at a Japanese mental hospital or recovery center or a person who was recovering from stimulant abuse and working in a recovery center. Patients in the acute phase of psychosis were excluded.

The numbers of participants from each facility were 23 (six inpatients, 17 outpatients, 0 recovering) from Tokyo Metropolitan Matsuzawa Hospital in Tokyo, 10 (one inpatient, nine outpatients, 0 recovering) from the National Center of Neurology and Psychiatry Musashi Hospital in Kodaira, two (one inpatient, one outpatient, 0 recovering) from Fukko-kai Tarumi Hospital in Kobe, 17 (eight inpatients, three outpatients, six recovering) from Self Support Services in Tokyo, and three (three inpatients, 0 outpatient, 0 recovering) from GAIA in Naha. Inpatients were asked about their status during the 30 days prior to their admission to the substance treatment facility. Outpatients and recovering patients were simply asked about their status during the 30 days prior to the ASI interview.

2.4. Statistical Analysis

Significant differences between two ASI datasets were examined using the χ^2 test for categorical data and Mann-Whitney-Wilcoxon test for continuous data. We used the Mann-Whitney-Wilcoxon test because ASI data include many nonparametric variables and zero values [21]. The associations between continuous variables were analyzed by Spearman's rank correlation coefficient. All analyses were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of Participants

Table 1 shows the general characteristics of the two groups (*i.e.*, inmates and patients). No significant differences were found in age between the two groups. Inmates had significantly lower education levels than patients. The majority of inmates had been married and had held a full-time job during the past three years. Half of the inmates lived with a sexual partner. In contrast, less than half of the patients had ever married or had a full-time job. Only one-fifth of the patients had ever lived with a sexual partner. The inmates more frequently had a history of juvenile delinquency and had more criminal charges of illicit drug use that resulted in conviction than patients. Crimes other than illicit drug use were more common among inmates than patients. Inmates had less access to mental health service, were given less medication for psychological problems, and received less psychiatric or drug treatment than patients.

3.2. Severity Score and Related Items

As shown in Table 2, inmates had significantly higher composite scores (CSs) related to drug use and legal status than patients. In contrast, they had significantly lower CSs related to employment/support and psychiatric symptoms. The CSs for medical status, alcohol use, and family/social relationships did not differ between the two groups. Similar to the CSs, the interviewer severity ratings (ISRs) of the inmates were significantly higher for drug use and legal status and significantly lower for employment/support than patients. No significant differences were found for the other ISR variables between the two groups.

Table 1. Characteristics of participants in each group.

	Inmates (n = 52)	**Patients (n = 55)**	***p* values**
Age, *M* (±SD, *range*)	38.0 (±1.5, 25–75)	35.9 (±1.2, 22–60)	*n.s.*
Years of education, *M* (±SD)	9.8 (±1.7)	11.6 (±2.3)	$p < 0.001$***
Never married, *n* (%)	9 (17.3%)	36 (65.5%)	$p < 0.001$***
Full-time employment past 3 years, *n* (%)	40 (76.9%)	24 (43.6%)	$p < 0.001$***
Lived with sexual partner past 3 years, *n* (%)	30 (57.7%)	12 (21.8%)	$p < 0.001$***
Lived with parent(s) past 3 years, *n* (%)	26 (50.0%)	24 (43.6%)	*n.s.*
Charged with juvenile delinquency, *n* (%)	42 (80.8%)	35 (63.6%)	$p = 0.049$*
Charges resulting in conviction (illicit drugs), *M* (±SD)	2.67 (±1.7)	0.98 (±1.3)	$p < 0.001$***
Charges resulting in conviction (other than illicit drugs), *n* (%)	30 (57.7%)	16 (29.1%)	$p = 0.003$**
Chronic medical problems, *n* (%)	20 (38.5%)	19 (34.5%)	*n.s.*
Medication for any psychological emotional problem, *n* (%)	16 (30.8%)	40 (72.7%)	$p < 0.001$***
History of psychiatric treatment, *n* (%)	10 (19.2%)	26 (47.3%)	$p = 0.002$**
Any drug detoxification treatments, *n* (%)	3 (5.8%)	18 (32.7%)	$p < 0.001$***
Any drug abuse treatment programs, *n* (%)	7 (13.5%)	45 (81.8%)	$p < 0.001$***
Any alcohol treatment programs, *n* (%)	2 (3.8%)	7 (12.7%)	$p = 0.098$

n, number of participants; *M*, mean; SD, standard deviation; *n.s.*, not significant.
Note: Mann-Whitney Wilcoxon test was used for the statistical comparison for Age and Years of education. The χ^2 test was used for the other categories.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 2. ASI-J composite scores (CSs) and interviewer severity ratings (ISRs).

ASI-J area		**Inmates (n = 52) Mean (SD)**	**Patients (n = 55) Mean (SD)**	***p* values**
Medical status	(CS)	0.06 (0.02)	0.10 (0.03)	*n.s.*
	(ISR)	1.04 (0.25)	0.64 (0.22)	*n.s.*
Employment/support	(CS)	0.45 (0.04)	0.65 (0.04)	$p < 0.001$**
	(ISR)	2.85 (0.43)	4.60 (0.40)	$p = 0.002$**

Table 2. *Cont.*

Alcohol use	(CS)	0.18 (0.03)	0.11 (0.02)	*n.s.*
	(ISR)	2.13 (0.38)	1.16 (0.29)	$p = 0.086$
Drug use	(CS)	0.20 (0.03)	0.14 (0.02)	$p = 0.033$*
	(ISR)	6.56 (0.24)	4.51 (0.38)	$p < 0.001$***
Legal status	(CS)	0.47 (0.02)	0.03 (0.01)	$p < 0.001$***
	(ISR)	6.98 (0.13)	0.56 (0.21)	$p < 0.001$***
Family/social relationships	(CS)	0.26 (0.03)	0.21 (0.03)	*n.s.*
	(ISR)	4.19 (0.46)	2.95 (0.30)	$p = 0.055$
Psychiatric symptoms	(CS)	0.15 (0.03)	0.24 (0.03)	$p = 0.030$*
	(ISR)	2.44 (0.44)	3.05 (0.41)	*n.s.*

n.s., not significant.

Note: The Mann-Whitney Wilcoxon test was used for the statistical comparison.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3. ASI-J items answered differently by inmates and patients.

ASI-J items	**Inmates ($n = 52$)**	**Patients ($n = 55$)**	***p* values**
Employment/support			
paid working days in the past 30 days, *M* (SD)	13.3 (11.95)	8.6 (11.80)	$p < 0.05$*
received money (Yen) for working in the 30 days, *M* (SD)	204,370 (248,437)	116,000 (226,830)	$p < 0.001$***
received money (Yen) for illegal activities in the 30 days, *M* (*SD*)	111,440 (426,470)	10,070 (67,285)	$p < 0.01$**
received financial support, *n* (%)	17 (65.4)	34 (18.2)	$p < 0.001$***
someone depends on you for the majority of life, *M* (SD)	0.92 (1.82)	0.37 (1.26)	$p < 0.05$*
Drug use			
days of methamphetamine use in the past 30 days, *M* (SD)	13.0 (12.28)	1.4 (5.72)	$p < 0.001$***
days of multidrug use in the past 30 days, *M* (SD)	3.6 (8.99)	1.1 (5.66)	$p < 0.05$*
money (Yen) spent on drugs in the past 30 days, *M* (SD)	72,400 (76,508)	10,560 (32,238)	$p < 0.001$***
Legal status			
days of illegal activities for profit in the past 30 days, *M* (SD)	2.8 (8.25)	0.4 (2.69)	$p < 0.05$*
experience of arrest for drug charges, *M* (SD)	2.67 (1.66)	0.98 (1.25)	$p < 0.001$***
experience of arrest for parole violations, *M* (SD)	0.44 (0.50)	0.05 (0.29)	$p < 0.001$***
experience of arrest for assault, *M* (SD)	0.48 (0.93)	0.04 (0.18)	$p < 0.001$***
Psychiatric symptoms			
experience of hallucinations in the past 30 days, *n* (%)	1 (1.9)	8 (14.5)	$p < 0.001$***
prescribed medication for psychological and emotional problems in the past 30 days; *n* (%)	10 (19.2)	32 (58.2)	$p < 0.001$***
Other status (no difference in CS)			
days of alcohol use in the past 30 days, *M* (%)	12.15 (13.01)	5.60 (10.01)	$p < 0.001$***
money (Yen) spent on alcohol in the past 30 days, *M* (SD)	34,120 (66,473)	14,180 (45,545)	$p < 0.001$***
experience of serious problems with sexual partner in the past 30 days, *n* (%)	17 (32.0)	8 (13.0)	$p < 0.001$***

Table 3. *Cont.*

experiences of serious problems with sexual partner in their lifetime, *n* (%)	41 (79.0)	30 (56.0)	$p < 0.001$***
days of serious problems with their family in the past 30 days, *M* (%)	4.37 (9.29)	0.98 (2.87)	$p < 0.001$***

n, number of participants; *M*, mean; SD, standard deviation

Note: The Mann-Whitney Wilcoxon test was used for the statistical comparison with continuous values. The χ^2 test was used for the other categories.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Table 3 shows a list of the ASI-J items that inmates and patients answered differently. With regard to employment/support status, inmates had more paid work and received more money for work in the past 30 days than patients. Inmates also received more money from illegal activities than patients. Fewer inmates received financial support compared with patients. Inmates had more people who depended on them for the majority of food, shelter, *etc.*, than patients.

With regard to drug use, inmates reported significantly more days of "methamphetamine use in the past 30 days" and "multidrug use in the past 30 days" than patients. Inmates spent more money on drugs in the past 30 days than patients. No significant differences were found between the two groups in the drug-use CS items with the exception of these three items.

With regard to legal status, inmates more frequently engaged in illegal activities for profit in the past 30 days than patients. Inmates were arrested more frequently for drug charges, parole violations, and assault than patients.

With regard to psychiatric symptoms, inmates experienced fewer hallucinations and were prescribed less medication for psychological and emotional problems in the past 30 days than patients.

With regard to medical status, alcohol drinking status, and family/social relationships, no overall significant differences in CS were found between the two groups. However, significant differences were found in some of the items. For example, inmates reported significantly more days of alcohol use and spent more money on alcohol in the past 30 days than patients. Inmates also more frequently experienced serious conflicts with their family in the past 30 days than patients. Similarly, more inmates experienced serious problems with their sexual partner in the past 30 days and during their lifetime than patients.

3.3. Substance Use Behavior

Table 4 shows the number and percentage of participants who reported substance use/abuse lasting more than 1 year in their lifetime and who abused methamphetamine by injection. Table 4 also shows the average duration (standard deviation) of substance use/abuse of participants in prison and treatment settings. No significant difference was observed in experience of methamphetamine and alcohol abuse in their lifetime between inmates and patients. Inmates had more frequently experienced inhalant abuse and had less frequently experienced cannabis and methylenedioxymethamphetamine (MDMA) abuse than patients. Additionally, inmates had a significantly shorter duration of methamphetamine and MDMA abuse and a significantly longer duration of alcohol use than patients. Most inmates abused

methamphetamine by injection (80.8%), whereas a minority of patients used the injection route (41.8%). No participants in either group reported cocaine or opiate abuse.

Table 4. Lifetime prevalence of substance use/abuse lasting more than 1 year.

Substance	Inmates (n = 52)	Patients (n = 55)	p values
Methamphetamine abuse, n (%)	50 (96.2)	52 (94.5)	*n.s.*
Duration (years), M (SD)	4.8 (0.63)	8.2 (0.91)	$p = 0.001$**
Drug use by injection, n (%)	42 (80.8)	23 (41.8)	$p < 0.001$***
Alcohol use, n (%)	40 (76.9)	41 (74.5)	*n.s.*
Duration (years), M (SD)	17.6 (1.39)	15.3 (1.48)	$p = 0.0116$*
Cannabis abuse, n (%)	6 (11.5)	23 (41.8)	$p < 0.001$***
Duration (years), M (SD)	6.5 (3.55)	7.0 (1.28)	*n.s.*
Methylenedioxymethamphetamine abuse, n (%)	0 (0)	6 (10.9)	$p = 0.014$*
Duration (years), M (SD)	0 (0)	2.3 (0.56)	$p = 0.015$*
Inhalant abuse, n (%)	32 (61.5)	18 (32.7)	$p = 0.003$**
Duration (years), M (SD)	4.1 (0.56)	3.6 (0.65)	*n.s.*

n, number of participants; M, mean; SD, standard deviation; *n.s.*, not significant

Note: The Mann-Whitney Wilcoxon test was used for the statistical comparison with continuous values. The χ^2 test was used for the other categories.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

3.4. Psychiatric Symptoms

The number and percentage of participants who reported psychiatric symptoms in their lifetime are shown in Table 5. The inmates experienced less major depression, anxiety and tension, and hallucinations in their lifetime than patients. Additionally, no significant associations were found between the duration of methamphetamine abuse and these psychiatric symptoms using Spearman's rank correlation coefficient analysis in both groups (rs = −0.006–0.186, *n.s.*). Moreover, inmates received less prescribed medication in their lifetime than patients. Although these psychiatric symptoms were not common among inmates, the lifetime prevalence of suicidal behavior and trouble controlling violence was not significantly lower than in patients.

Table 5. Lifetime prevalence of psychiatric symptoms.

Symptoms	Inmates (n = 52) n (%)	Patients (n = 55) n (%)	p values
Serious depression (a)	9 (17.3)	24 (43.6)	$p = 0.003$**
Serious anxiety or tension (a)	14 (26.9)	25 (45.5)	$p = 0.047$*
Hallucinations (a)	3 (5.8)	22 (40.0)	$p < 0.001$***

Table 5. *Cont.*

Trouble understanding, concentrating, or remembering (a)	18 (34.6)	18 (32.7)	*n.s.*
Trouble controlling violent behavior (b)	20 (38.5)	28 (50.9)	*n.s.*
Serious suicidal thoughts (b)	22 (42.3)	29 (52.7)	*n.s.*
Suicide attempts (b)	13 (25)	18 (32.7)	*n.s.*
Prescribed medication (b)	16 (30.8)	40 (72.7)	$p < 0.001$***

n, number of participants; *n.s.*, not significant

Note: According to the ASI counting rule, (a) counts only long-lasting symptoms lasting more than three months, and (b) counts symptoms that do not last long. The χ^2 test was used for statistical comparisons.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

3.5. Variables Related to Drug Abuse Severity

Similarities and differences were observed between the two groups in correlations between the drug use CS and some of the other variables. In both groups, the psychiatric status CS (inmates: $r = 0.410$, $p < 0.05$; patients: $r = 0.283$, $p < 0.05$) and the number of days that participants experienced psychological and emotional problems in the past 30 days (inmates: $r = 0.374$, $p < 0.05$; patients: $r = 0.274$, $p < 0.05$) were significantly and positively correlated with the drug use CS.

For inmates, the legal status CS ($r = 0.328$, $p < 0.05$), days of alcohol problems in the past 30 days ($r = 0.309$, $p < 0.05$), and subjective feelings of trouble with alcohol problems in the past 30 days ($r = 0.283$, $p < 0.05$) were significantly and positively correlated with the drug use CS. Additionally, the number of close friends ($r = -0.276$, $p < 0.05$) and serious problems with their children in the past 30 days ($r = -0.366$, $p < 0.05$) were significantly and negatively correlated with the drug use CS.

For patients, the employment/support status CS ($r = 0.281$, $p < 0.05$), treatment for any psychological and emotional problems in a hospital ($r = 0.352$, $p < 0.05$), and serious depression in the past 30 days ($r = 0.383$, $p < 0.01$) were significantly and positively correlated with the drug use CS.

4. Discussion

4.1. Characteristics of Methamphetamine Abusers

In the present study, significant differences were found in the backgrounds and characteristics of Stimulant Control Law inmates and participants from treatments settings. The CSs of inmates were higher for drug use and legal status and lower for employment/support and psychiatric symptoms than patients. A relatively high CS for drug status in the inmates would be attributable to their use of illegal drugs in the 30 days before arrest. Interestingly, inmates abused more frequently and spent more money on both methamphetamine and alcohol than patients. Significant correlations were observed between some items related to alcohol problems and the drug use CS in inmates. These results are consistent with the results of Russell *et al.* [22], indicating that a history of alcohol use is one of the risk factors

for methamphetamine use. For Japanese inmates, methamphetamine abuse may have some association with problematic alcohol drinking.

The inmates tended to have a better employment status than patients. Most of the inmates had regular jobs, received wages, and did not receive financial support. That is, they lived a relatively financially independent life compared with patients, although many of them received a substantial amount of money illegally. Additionally, the inmates tended not to experience psychotic symptoms, such as hallucinations, and not to access medical services for their addictive problems. Many patients received treatment for the distress associated with psychotic symptoms rather than for the drug abuse itself [23]. These results indicate that inmates abused drugs within a range before their arrest, but their psychiatric symptoms were apparently not as severe as those among patients.

Problems appeared to exist in the inmates' environments while they were young. Many of them had less education and a history of juvenile delinquency. These results are consistent with the results of Miura *et al.* [7] in which the number of admissions to a juvenile detention home significantly predicted methamphetamine use during adolescence. Moreover, a history of inhalant abuse was found in more than half of the inmates. Inhalant abuse, such as paint thinner abuse, was found to be a significant problem leading to methamphetamine abuse among young Japanese [2]. Our results, combined with these previous results, suggest that a troubled childhood may lead to illegal drug use in adulthood.

Methamphetamine-induced psychosis is reported frequently in Japanese patients diagnosed with methamphetamine dependence [6]. Additionally, Wada and Fukui [24] reported that five years of methamphetamine use is considered a turning point in terms of the occurrence of psychotic symptoms, suggesting that a shorter duration of methamphetamine abuse in inmates may be related to less frequent psychosis. However, no significant association was found between the duration of methamphetamine abuse and lifetime experience of psychosis in the inmates and patients in the present study. Interestingly, inmates reported fewer psychotic symptoms, such as hallucinations, than patients, although no significant differences were found in age between the two groups. This result may reflect the fact that inmates with acute psychosis were excluded from the study. Nonetheless, the present results suggest that inmates and patients may have different backgrounds contributing to vulnerability to methamphetamine-induced psychosis. Further studies with more inmate and patient samples using multivariate statistical analysis will be needed to reveal the factors leading to vulnerability to methamphetamine-induced psychosis in methamphetamine dependence/abuse.

With regard to route of methamphetamine administration, intravenous injection was used by most of the inmates, whereas a minority of patients used this route. These results are consistent with the results of Matsumoto *et al.* [6] in which methamphetamine-injecting subjects had more extensive criminal records than smoking subjects. Matsumoto *et al.* investigated outpatients and reported significant differences in life circumstances between injecting and smoking abusers, and injecting abusers appeared to have greater antisocial tendencies than smoking abusers. Moreover, Matsumoto *et al.* reported that although methamphetamine abusers often claimed that smoking is safer than injection, no significant differences were found in the overall occurrence of psychotic symptoms between injection and smoking. Our results consistently showed that the injection route, which was used by most of the inmates, might not be a factor causing critical psychotic problems.

The inmates were less frequently admitted to treatment facilities, possibly because of their less frequent episodes of psychosis. The inmates also expressed their psychological problems not as depression or anxiety, but rather as uncontrollable violence, suicide attempts, and interpersonal relationship problems with their families and sexual partners. These results suggest that inmates expressed their psychological problems outwardly rather than viewed their psychological problems as an inner conflict. For inmates, the correct perceptions of their problems may be especially important for treatment [25]. Treatment programs should be implemented not only for patients but also for inmates.

4.2. Strengths and Limitations of the Study

The present study provides basic statistical information about Japanese methamphetamine abusers in correctional settings measured by the ASI compared with abusers in treatment settings. Although Wada *et al.* [3] reported that most Japanese methamphetamine abusers belonged to correctional settings rather than treatment settings, characteristics of methamphetamine abusers in prisons have not been as well studied as those of abusers in hospital settings. The results of this study indicate many differences in the quantity and quality of methamphetamine abuse between inmates and patients. Additionally, the present study showed that the ASI could be an effective tool not only for patients but also for inmates to grasp the severity of their problems in multiple areas. The accumulation of data on incarcerated methamphetamine abusers in Japan might extend the use of the ASI as an interview tool.

One possible limitation of the present study was the sampling procedure. The participants were not recruited randomly but were limited to inmates who gave informed consent and whose doctors recognized their ability to be interviewed. Therefore, the data of this study were not obtained from methamphetamine abusers in prison as a whole, but rather only from cooperative inmates with a relatively low severity of methamphetamine dependence. Additionally, Shizuoka prison especially treated offenders who were imprisoned for the first time, and offenders mainly came from Tokyo or Shizuoka prefectures. Consequently, the results of this study reflect only a portion of the methamphetamine abuse prisoner population. Inmates and patients were also recruited at different time-points, although the situation regarding methamphetamine use in Japan minimally changed from 2002 to 2007. Moreover, the large number of statistical tests performed in the present study might make some results significant by chance. Another limitation was the relatively low sample size. A subsequent study with more subjects from other correctional facilities and multivariate statistical analysis will be necessary to confirm our conclusions.

In the future, the wide use of the ASI for methamphetamine abusers in correctional facilities will enable systematic collection of basic information from these subjects which may aid in more effective intervention. The ASI may be useful for selecting adequate treatment and re-education programs for inmates. Additionally, utilization of the ASI as a common tool among facilities that treat methamphetamine abusers, such as hospitals (or other treatment facilities), legal facilities (e.g., prisons or probation offices), and research institutes, may contribute to more effective treatment and research.

5. Conclusions

These findings suggest that Japanese methamphetamine abusers in correctional settings have many characteristics and environmental backgrounds that are different from abusers in medical settings. Methamphetamine abusers in correctional settings may need to have their specific problems assessed, including trouble with mental health and access to support facilities.

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SYSTEMATIC MAPPING OF PAIN-RELATED QTL USING CONSOMIC MOUSE STRAINS : ADVANTAGE OF USING WILD-DERIVED STRAINS

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ABSTRACT

Pain sensitivity has proved to be extremely variable among human individuals. One of the most important factors for such variations in pain-related phenomena is genetic diversity. A variety of mouse strains are reportedly suitable animal models for investigating the genetic basis of large individual differences in pain sensitivity. Laboratory strains have been reported to exhibit different behavioral traits due to variations in their genetic background. However, they show low genetic polymorphism because the original colony bred to produce the strains comprises a relatively low number of mice belonging to the subspecies *Mus musculus domesticus*. The low heterogeneity of laboratory strains makes their behavioral phenotype less variable. Therefore, the use of inbred strains derived from different mouse subspecies for pain-related phenotype studies is a great advantage. Several research groups have been involved in the long-term process of establishing a variety of wild-derived inbred strains from wild mice captured all over the world after at least 20 generations of brother-sister mating. The genetic diversity of wild-derived strains is advantageous for the analysis of phenotypic differences among strains. We previously identified a marked variety in pain and morphine sensitivity in a series of wild-derived inbred strains. In particular, we found that the MSM/Ms (MSM) strain established from the Japanese wild mouse, *Mus musculus molossinus*, one of the

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subspecies of the musculus subspecies group, exhibits significant differences in pain-related phenotypes compared to C57BL/6 (B6). In order to study genetic factors associated with these differences, we used a panel of consomic strains, established by replacing each B6 chromosome by that of MSM on a B6 background. Our study identified multiple chromosomes related to reduced pain sensitivity in both hot-plate and tail-flick tests. Further mapping using subconsomic strains carrying a shorter segment of the chromosome allowed the successful characterization of a locus related with reduced pain sensitivity using the hot-plate test. Thus, this review reports on the usefulness of consomic strains for genetic analyses of pain-related phenotypes in mice.

INTRODUCTION

Pain is an important stimulus for animals to recognize tissue injury, environmental danger and disease, but surplus pain degrades quality of life. Although many analgesics have been developed to reduce human pain, dependence, tolerance, and sensitivity to them vary significantly among individuals, and no ideal drugs have yet been developed. One of the most important factors causing individual differences in pain-related phenomena is genetic diversity (Lariviere et al., 2002). Laboratory strains have been reported to show different behavioral traits due to differences in genetic background. In this regard, selecting the best genetic strain for objective behavioral study is recommended (Crawley et al., 1997). However, laboratory mice have been domesticated during the process of establishing laboratory strains, causing them to lose their characteristic behavior due to domestication. In addition to the obedient phenotype, most laboratory strains show low genetic polymorphism since the original colony of the laboratory strains comprises a relatively small number of mice mostly belonging to *M. m. domesticus* subspecies (Bonhomme and Guénet, 1996; Ferris et al., 1982; Yonekawa et al., 1980). Low heterogeneity in laboratory strains makes the behavioral phenotype less variable among them. Therefore, behavioral tests may show non significant values as a consequence of their low genetic variability. On the other hand, results using inbred strains derived from different mouse subspecies are valuable for behavioral and brain research in the field of neurological science.

WILD MICE AND WILD STRAINS

The *Mus musculus* species is divided into four major subspecies groups: domesticus, musculus, castaneus, and bactrianus (Fig. 1) (Bonhomme and Guénet, 1996; Moriwaki et al., 1994; Silver, 1995). These four subspecies groups are genetically different according to their diversity of biochemical markers and mtDNA patterns. Further taxonomic subdivisions have been applied using their geographical distribution and morphological characteristics, although these local subspecies have been classed as heterogeneous with the major subspecies groups according to their genetic profiles. Furthermore, genetic admixture between these major subspecies groups have been observed. Japanese mice, *M. m. molossinus*, show that genetic admixture has occurred between the musculus and castaneus groups (Yonekawa et al., 1988). Some research groups including us have been involved in establishing a variety of inbred strains from wild mice captured in different areas of the world (Bonhomme and Guénet,

1996; Gregorová and Forejt, 2000; Moriwaki et al., 1994). The descendants of these original mice were established as wild-derived inbred strains (wild strains) after at least 20 generations of brother-sister matings. These genetically defined wild strains have proven to be useful for a variety of genetic and behavioral studies due to the high frequency of genetic polymorphism among the strains (Koide et al., 2000). A panel of wild-derived strains including a reference laboratory strain, B6, is now known as the Mishima battery (Table 1) (Furuse et al., 2002). One of these wild derived inbred strains, MSM, was originally captured in Mishima city, Japan, and classified as *M.m. molossinus* (Abe et al., 2004; Ogasawara et al., 2005; Moriwaki et al., 1994). In the last part of this review, we focus on a comparison of MSM with B6.

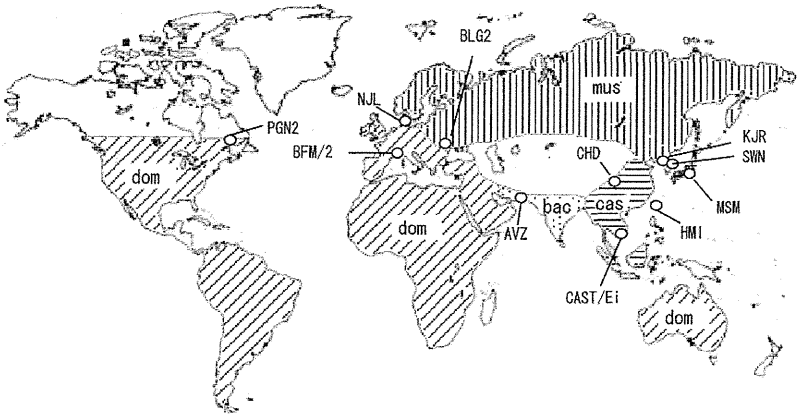

Figure 1. Geographical distribution of subspecies groups for wild mice and origin of wild-derived strains. Dom, domesticus subspecies group; mus, musculus subspecies groups; cas, castaneus subspecies group. Each subspecies group is categorized based on the genetic profile of wild mice which were systemized further in a taxonomic way. Wild-derived strains and their origin are indicated.

Table 1. A list of the Mishima battery of mouse strains

Origin	Strain	Subspecies group	Subspecies	Place of collection
Laboratory	C57BL/6J	domesticus		
Laboratory	DBA/1J	domesticus		
Wild mice	PGN2/Ms	domesticus	*M.m.domesticus*	Canada
Wild mice	BFM/2Ms	domesticus	*M.m.brevirostris*	France
Wild mice	HMI/Ms	castaneus	*M.m.castaneus*	Taiwan
Wild mice	CAST/Ei	castaneus	*M.m.castaneus*	Thailand
Wild mice	NJL/Ms	musculus	*M.m.musculus*	Denmark
Wild mice	BLG2/Ms	musculus	*M.m.musculus*	Bulgaria
Wild mice	SWN/Ms	musculus	*M.m.yamasinai*	Korea
Wild mice	KJR/Ms	musculus	*M.m.yamasinai*	Korea
Wild mice	MSM/Ms	musculus	*M.m.molossinus*	Japan
Fancy mice	JF1/Ms*	musculus	*M.m.molossinus*	Denmark*

* JF1 was found in Denmark, but characterized as a Japanese fancy mouse by a genetic study (Koide et al., 1998).

GENETIC PROFILES OF WILD AND LABORATORY STRAINS

Several studies characterizing genomic sequence polymorphisms have been carried out in order to analyze the genetic profiles of these wild strains clearly. Koide and colleagues (Koide et al., 2000) analyzed polymorphism frequency in microsatellite markers among wild strains by SSLP analysis (Fig. 2). These results showed that polymorphism frequencies among wild strains are much higher than among laboratory strains. The frequency of simple sequence length polymorphism (SSLP) for two laboratory strains, B6 and DBA/2, was 49% but increased to 83.7% when B6 was compared with MSM. This result indicates that higher diversity can be expected by adding these wild strains to the mouse resource pool of laboratory strains.

	MSM	JF1	KJR	SWN	HMI	CAST	BLG2	NJL	BFM/2	B6	DBA/1
MSM		45.2	69.2	73.1	87.5	88.5	81.7	72.1	92.3	83.7	86.5
JF1			64.4	60.6	85.6	88.5	78.8	73.1	92.3	82.7	80.8
KJR				67.3	82.7	92.3	83.7	77.9	91.3	89.4	89.4
SWN					84.6	87.5	75	76	91.3	87.5	91.3
HMI						69.2	64.6	87.5	89.4	87.5	88.5
CAST							87.5	88.5	86.5	88.5	86.5
BLG2								77.9	85.6	86.5	93.3
NJL									87.5	84.6	91.3
BFM/2										83.7	80.8
B6											49
DBA/1											

Figure 2. A matrix diagram showing degrees of polymorphism based on SSLP typing for 104 different microsatellite markers distributed in the entire genome. The value indicates the frequency of polymorphism between two strains. Two laboratory strains are indicated by grey shadowing. This figure is modified from Koide et al. (2000).

Triplet-repeat sequences are frequently observed in the coding regions. Two trinucleotide repeats, CAG and CAA, coding for poly-glutamines have been studied by many research groups since repeat length contributes to differences in protein function or diseases. We systematically searched for genes carrying a CAG repeats in the public databases and found 62 loci carrying CAG/CAA trinucleotide repeat. We then analyzed variations of the repeat length in the 62 loci among 16 inbred mouse strains by PCR amplification following sequence analysis. We found that the maximum repeat number was 37 and 51.6% of the loci maintained their length among strains. Higher polymorphism frequency was observed when the repeat number was over 10. Phylogenetic relationships among the 16 inbred mouse strains were analyzed using the CAG/CAA repeat numbers in the coding regions. The branching patterns from reconstructed Neighbor-joining (NJ) trees showed that 14 inbred strains, excluding ZBN and AVZ, were clustered into 3 groups: domesticus, castaneus and musculus

(Fig. 3). Laboratory strains B6 and DBA/2 were also shown to co-cluster into the domesticus subspecies group, as previously mentioned.

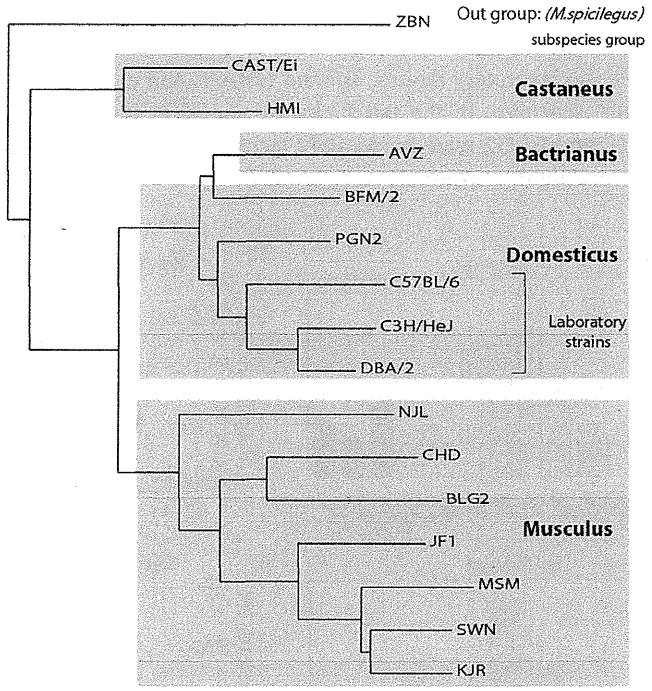

Figure 3. Phylogenetic tree for wild-derived strains. A Neighbor-joining tree was constructed based on CAG/CAA repeat polymorphism for 31 repeat loci of 16 inbred mouse strains. Three subspecies groups, domesticus, castaneus, and musculus, were clearly different in terms of their genetic profile. The AVZ strain is categorized as a bactrianus subspecies group, but this group is not well differentiated. This figure is modified from Ogasawara et al. (2005).

In addition to these repetitive sequences, 21 nuclear genes were characterized in the polymorphism of these wild-derived strains (Liu et al., 2008). A neighbor-joining tree constructed from the sequence polymorphism data showed clustering of wild-derived strains into three subspecies groups, and coclustering of a laboratory strain, C57BL/10, into the domesticus subspecies group.

These data indicate that three subspecies groups, domesticus, castaneus, and musculus, are genetically different from each other. Wild-derived strains classified into different subspecies are genetically different and very useful for locating genetic polymorphisms in the desired gene/genes.

PAIN AND CAPSAICIN SENSITIVITY IN WILD STRAINS

In order to study the strain differences in pain sensitivity, Koide and colleagues conducted tail-flick (Fig. 4B) and hot-plate tests at 52°C (Fig. 4A) on a Mishima battery (Koide et al., 2000). In this study, only females were used. It is thought that the hot plate

response at 52°C mediates the central response to moderate heat stimuli, but the tail flick response mediates the spinal reflex to high temperatures. As a result of these tests, significant strain-effects were observed in both hot-plate (licking) and tail-flick tests. The results clearly showed that diversity in terms of pain sensitivity exists among strains. In the hot-plate test, three strains, JF1, KJR and SWN, proved to be insensitive to heat, and MSM was moderately insensitive, while the laboratory strains, B6 and DBA/1, and castaneus strains, CAST/Ei and HMI, were highly sensitive. In the tail-flick test, the KJR strain proved to be insensitive, MSM, JF1, SWN, and BLG2 were moderately insensitive, but CAST/Ei, HMI, NJL, BFM/2, B6 and DBA/1 were highly sensitive.

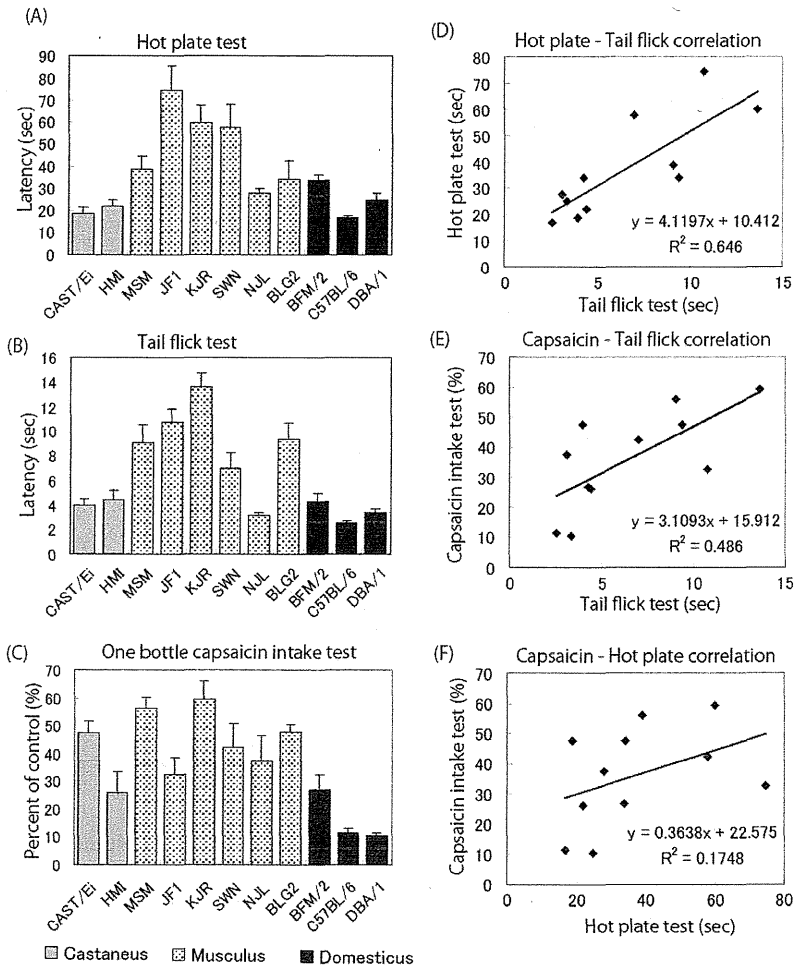

Figure 4. Strain difference in pain sensitivities and correlation between different pain tests. Strain comparison of sensitivities in (A) hot-plate, (B) tail-flick, and (C) capsaicin intake tests. Correlation analyses of different pain sensitivities. (D) Correlation between hot-plate and tail-flick sensitivities, (E) correlation between capsaicin sensitivity and tail-flick sensitivity, (F) correlation between capsaicin sensitivity and hot-plate sensitivity. Data for hot-plate and tail-flick tests were taken from a previous paper (Koide et al. 2000). Data from capsaicin sensitivity were taken from our previous paper (Furuse et al., 2002).

In order to determine the relationship between hot-plate and tail-flick tests, we conducted Pearson's correlation analysis of data from the two pain sensitivity tests (Fig. 4D). A comparison between both tests showed a high correlation (R^2=0.646), suggesting the existence of a partially overlapping underlying mechanism of strain difference for pain sensitivity.

These results also raised the possibility that there could be strain differences for capsaicin perception among these strains, since perception for both hot taste and a moderate level of heat is mediated by the same sensory receptor, *Trpv1* (*vanilloid receptor 1*, a nonselective cation channel in the membrane of primary sensory nerve endings) (Caterina et al., 1999; Caterina et al., 2000; Caterina et al., 1997). Capsaicin is the chemical component of hot chili peppers which causes its hot taste and stimulates the physiological pain system. Since response to both moderate heat (> 43°C) and capsaicin are mediated by *Trpv1*, a similar sensitivity to the hot-plate test at moderate temperature (52°C) and capsaicin intake test was expected. Thus, Koide and colleagues (Furuse et al. 2002) carried out 1-bottle capsaicin intake tests (Fig. 5) on a Mishima battery to measure hot-taste sensitivity (capsaicin intake is expressed as a percentage of baseline water intake). Results at 15 μM of capsaicin are shown in Figure 4C. When fluid intake was compared among strains, the KJR and MSM strains consumed 15 μM of capsaicin solution at 60% of the control water intake, which was significantly higher than for all other strains (a post hoc analysis, $P < 0.03$). BFM/2, BLG2, CAST/Ei, HMI, JF1, NJL and SWN strains consumed 15 μM capsaicin at 26 – 47% of the controls. Two strains, B6 and DBA/1, consumed 15 μM capsaicin solutions at 7 - 11% of the controls, which was significantly lower than for all other strains (a post hoc analysis, $P < 0.01$). The KJR and MSM strains were less sensitive to capsaicin, but two laboratory strains, B6 and DBA/1, were highly sensitive.

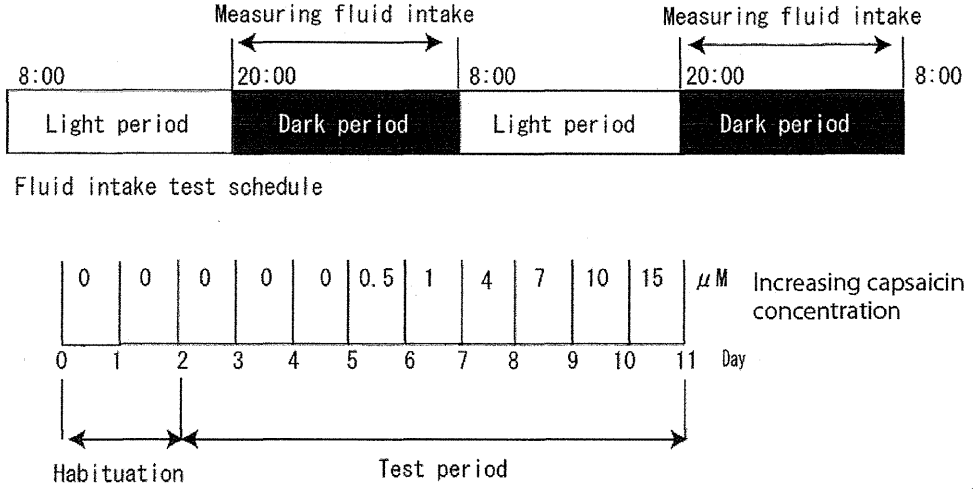

Figure 5. Method for the 12-h 1-bottle test. Test schedule for measuring capsaicin sensitivity.

To determine the relationship between capsaicin sensitivity and other pain sensitivity tests, we conducted Pearson's correlation analysis on data from the combination with the capsaicin sensitivity test and either the hot-plate test or tail-flick test. Neither combinations of capsaicin - hot plate test nor capsaicin - tail flick test showed a high correlation (R^2=0.486

and 0.175, respectively) suggesting an independent basis for the strain difference in sensitivity to capsaicin and heat sensation (Fig. 4E,F).

Concerning the functional basis for pain sensitivity, it is possible that further variations in the central pathways may be associated with the strain differences in pain sensitivity. Pain perception can be modified by two types of endogenous analgesic systems: opioid-dependent and opioid-independent (Mogil, 1999). Therefore, investigation of the relationship between pain sensitivities and opioid-dependent pathways is important to understand the basic mechanisms for different pain sensitivities in these mouse strains.

DIFFERENCES IN OPIOID PATHWAYS

Opioid drugs are among the most used analgesics in human history, since some of them are available as natural compounds extracted from the juice of *Papaver somniferum*, such as morphine and codeine. Both natural and synthetic (including fentanyl and methadone) opioids have been widely used in clinical practice to alleviate many types of pain from general surgery to terminal cancer. However, it is known that there is a large individual diversity in opioid sensitivities in human populations. The existence of large inter-individual differences in the response to opioid analgesics complicates appropriate pain treatment in clinical practice. This individual diversity in opioid sensitivity is thought to be caused by complex interactions of psychological, environmental, and genetic factors. For this reason, understanding the genetic basis of an individual difference in morphine sensitivity would be useful information for developing better analgesic drugs and an appropriate administration method for morphine-related drugs.

Ikeda and colleagues have investigated morphine effects in the Mishima battery (Shigeta et al., 2008). The morphine effect on spontaneous activity was examined in these strains with the open-field test (Fig. 6A). Half of the strains, JF1, KJR, MSM, SWN, and NJL, showed significantly increased open-field ambulation when morphine was administered.

The antinociceptive effects of morphine were examined using hot-plate and tail-flick tests (Fig. 6B and 6C, respectively; Shigeta et al., 2008). In the hot-plate test, response latency was significantly longer when mice were treated with morphine than with saline in all of the strains tested. For the tail-flick test, the response latency was significantly longer in the morphine-treated group than in the saline-treated group for all of the strains tested. Next, we tried to measure the antinociceptive effect of morphine by scoring it as a percentage of the maximal possible effect (%MPE), which was calculated as follows: %MPE = {[(latency at morphine injection) – (latency at saline injection)] / [(cut-off time) – (latency at saline injection)]} x 100%.

When we calculated the %MPE of morphine antinociception, diversity of morphine sensitivity was observed among strains (Fig.7). Particularly, we found that the CHD, KJR, JF1 and MSM strains showed a pronounced response to morphine for multiple tests in each strain. These data indicated that morphine effects were highly variable among mouse strains.

We have shown that there is a wide diversity of sensitivities in pain-related tests and the antinociceptive effect of morphine. In order to investigate the functional relationship between pain sensitivity and morphine sensitivity, we conducted Pearson's correlation analysis. The results showed a moderate correlation of nociceptive response and the morphine effect in the

hot-plate test (Fig. 8A), and a high correlation between nociceptive response and the morphine effect in the tail-flick test (Fig. 8B).

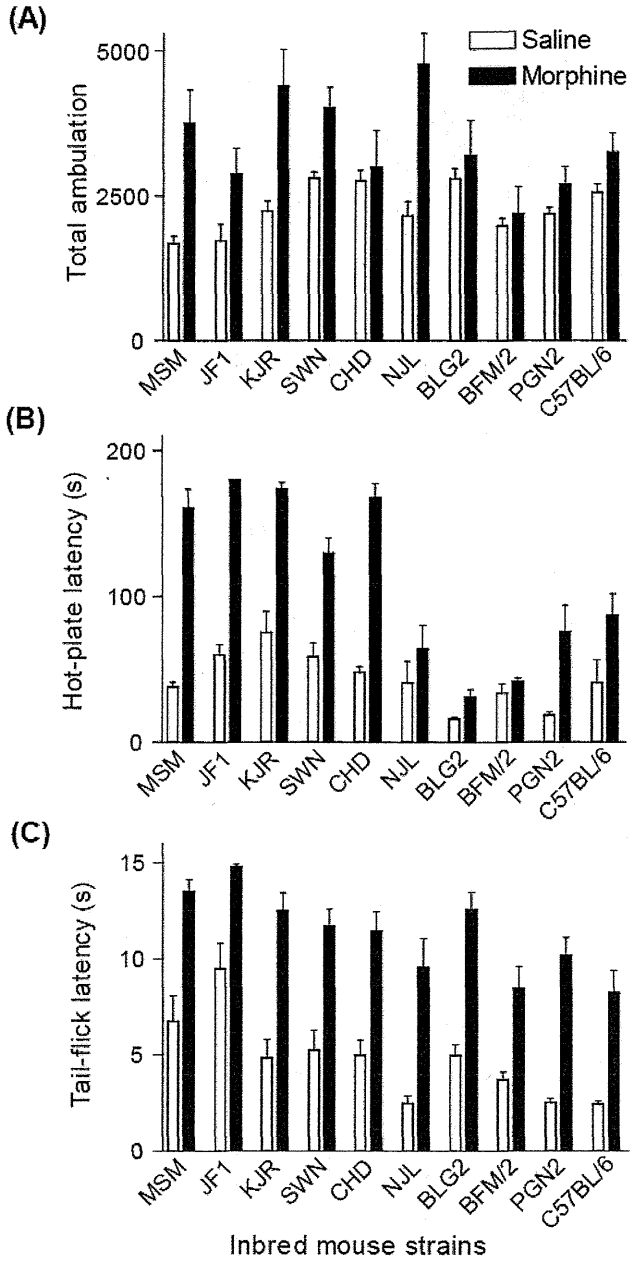

Figure 6. Comparison of strain differences in morphine sensitivity in 10 inbred mouse strains. Effects of morphine were compared after administration of saline or morphine (10 mg/kg) intraperitoneally. Effect on spontaneous activity in open-field test (A), hot-plate sensitivity (B), and tail-flick sensitivity (C) were examined. Each bar represents the mean±SEM. Data on tests for open-field, hot-plate, and tail-flick were taken from a previous paper (Shigeta et al., 2008).