17. Haraguchi A, Ogai Y, Senoo E, Saito S, Suzuki Y, Yoshino A, Ino A, Yamaga K, Hasegawa M, Murakami M, Murayama M, Ishikawa T, Higuchi S, Ikeda K.: Application of the addiction severity index Japanese version (ASI-J) to alcohol-dependent individuals. The Second USA-Japan Joint Workshop on Alcohol-Related Problems, 横浜 [2008/09/17].
18. 池田和隆, 高松幸雄, 山本秀子, 大谷保和, 原口彩子, 妹尾栄一: 薬物嗜好性抑制の基礎研究と臨床応用の可能性. 平成20年度アルコール・薬物依存関連学会合同学術総会, 横浜 [2008/09/15].
19. 福田謙一, 林田眞和, 井手聡一郎, 笠井慎也, 西澤大輔, 池田和隆: ヒト mu-オピオイド受容体遺伝子(*OPRM1*)の一塩基多型(SNPs)が、下顎骨切り術を受ける患者におけるフェンタニルの鎮痛効果に及ぼす影響. 第 29 回鎮痛薬・オピオイドペプチドシンポジウム, 富山 [2008/08/30].
20. 池田和隆: オピオイド感受性とミューオピオイド受容体遺伝子多型. 第29回鎮痛薬・オピオイドペプチドシンポジウム, 富山 [2008/08/30].
21. 井手聡一郎, 曽良一郎, 池田和隆: ATP 受容体サブタイプ $P2X_7$ 遺伝子多型と疼痛感受性との関連解析. 第 30 回日本疼痛学会, 福岡 [2008/07/20].
22. 福田謙一, 林田眞和, 池田和隆: 硬膜外鎮痛ができない症例に対する術後痛対策とそれに関連する遺伝子多型. 第33回日本外科系連合学会学術集会, 浦安 [2008/06/12].
23. 池田和隆, 西澤大輔, 笠井慎也: 鎮痛のファーマコゲノミクス. 第33回日本外科系連合学会学術集会, 浦安 [2008/06/12].

G. 知的財産権の出願・登録状況

1. 特許取得

1. 池田和隆, 笠井慎也, 林田眞和, 樋口進: POMC 遺伝子解析による薬物感受性の評価方法 [出願] 特許庁, PCT 出願 PCT-JP2008-058083 [2008/04/25]

2. 実用新案登録

なし

3. その他

特記すべきことなし

Drug and Alcohol Dependence 101 (2009) 20–26

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Application of the Relapse Risk Scale to alcohol-dependent individuals in Japan: Comparison with stimulant abusers☆

Yasukazu Ogai[a], Masahiro Yamashita[b], Keiko Endo[c], Ayako Haraguchi[a], Yoko Ishibashi[a], Tatsuya Kurokawa[d], Tatsuyuki Muratake[e], Ryoichi Suga[b], Toru Hori[f], Mitsuru Umeno[g], Nozomu Asukai[a], Eiichi Senoo[a], Kazutaka Ikeda[a,*]

[a] Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan
[b] Nakajo Daini Hospital, 2941 Nakajoki, Tokamachi, Japan
[c] Urabe Mental Health Clinic, 5-36-8 Oshima, Koto-ku, Tokyo, Japan
[d] Hirakawa Hospital, 1076 Miyama-cho, Hachioji, Japan
[e] Niigata University Graduate School of Medical and Dental Sciences, 1-754 Asahimachi, Niigata, Japan
[f] National Center of Neurology and Psychiatry, Musashi Hospital, 4-1-1 Ogawahigashi-cho, Kodaira, Japan
[g] Tokyo Metropolitan Matsuzawa Hospital, 2-1-1 Kamikitazawa, Setagaya-ku, Tokyo, Japan

ARTICLE INFO

Article history:
Received 26 March 2008
Received in revised form 5 September 2008
Accepted 17 October 2008
Available online 11 December 2008

Keywords:
Alcohol
Relapse risk
Craving
Scale
Japanese

ABSTRACT

Objective: To develop and validate the Alcohol Relapse Risk Scale (ARRS) for Japanese alcohol-dependent individuals and to compare the features of relapse risk for alcohol-dependent individuals with those for stimulant abusers.
Methods: The ARRS is a multidimensional self-rating scale consisting of 32 items based on the Stimulant Relapse Risk Scale (SRRS). Two hundred eighteen inpatients and outpatients with a history of alcohol dependence (181 males and 36 females) were recruited, provided informed consent, and were administered the ARRS. The Visual Analog Scale (VAS) for alcohol craving, current state of drinking, and data on relapse within 1 month after the rating were used for validation.
Results: Exploratory factor analysis highlighted five factors: stimulus-induced vulnerability (SV), emotionality problems (EP), compulsivity for alcohol (CA), lack of negative expectancy for alcohol (NE), and positive expectancy for alcohol (PE). Cronbach's α coefficient for each of the subscales ranged from .55 to .90 and was .90 for the total ARRS, indicating their adequate internal consistency. SV, EP, CA, PE, and total ARRS were significantly correlated with the VAS and current drinking state, supporting their concurrent validity. SV and total ARRS were significantly correlated with relapse, suggesting that the ARRS is useful for predicting relapse risk in alcohol-dependent individuals, similar to the SRRS for stimulant abusers. Compared with stimulant abusers, alcohol-dependent individuals tended to express their desires related to relapse more honestly on the scales.
Conclusions: The ARRS has multidimensional psychometric properties that are useful for assessing the various aspects of alcohol relapse risk.

1. Introduction

In 2003, approximately 800,000 adults out of the Japanese general population of 120 million presented with alcohol dependence, making this group one of the largest among the various mental disorders (Osaki et al., 2005). A serious problem with the treatment of alcohol-dependent individuals is the very low rate of complete abstinence (about 20%; Noda et al., 2001).

Some clinical studies have examined psychosocial factors related to relapse in individuals with alcohol dependence. Relapse-promoting factors include anxiety (Lucht et al., 2002), craving (Gordon et al., 2006), negative mood, childhood sexual abuse (Walitzer and Dearing, 2006), and psychological distress (Sander and Jux, 2006). Some researchers have placed emphasis on relapse-inhibiting factors such as self-efficacy, social support, coping (Brown et al., 1995), other-efficacy beliefs (Demmel et al., 2006), spirituality (Gordon et al., 2006), peer support group attendance, and continuing care program involvement (Miller et al., 1999).

☆ English and Japanese versions of the Alcohol Relapse Risk Scale (ARRS) can be found by accessing the online version of this paper at http://dx.doi.org by entering doi:10.1016/j.drugalcdep.2008.10.021.

* Corresponding author. Tel.: +81 3 3304 5701x508; fax: +81 3 3329 8035.
E-mail address: ikedak@prit.go.jp (K. Ikeda).

0376-8716/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.drugalcdep.2008.10.021

Zywiak et al. (2006) developed the "Relapse Questionnaire" and examined its inner multiple construction of relapse-onset factors of alcohol dependence with a follow-up study. This study revealed three factors: Negative Affect/Family Influences, Craving/Cues, and Social Pressure. These factors appear to cover most of the psychosocial factors related to alcohol relapse. This questionnaire, however, is intended for relapsed patients, not for the prediction of relapse risk. Additionally, difficulty with administering the questionnaire remains problematic because it is part of a research interview.

No multidimensional scale that measures relapse risk for alcohol dependence currently exists in Japan. To further advance the development of medicines and programs for the prevention of relapse, scales for the appropriate assessment of relapse risk are necessary. We previously developed a 48-item multidimensional scale for the measurement of relapse risk for Japanese patients with stimulant dependence (i.e., the Stimulant Relapse Risk Scale, SRRS; Ogai et al., 2007) based on the Marijuana Craving Questionnaire (Heishman et al., 2001). The reliability and validity of this scale were demonstrated by analyzing 100 inpatients and outpatients with a history of stimulant abuse in Japan. Exploratory factor analysis revealed five subscales: "anxiety and intention to use drug" (AI), "emotionality problems" (EP), "compulsivity for drug use" (CD), "positive expectancy and lack of control over drug" (PL), and "lack of negative expectancy for drug use" (NE). AI, PL, NE, and total SRRS scores were significantly and positively related to relapse within 3 and 6 months. Shaffer et al. (2004) proposed a common etiology for addiction to stimulants, alcohol, and other drugs. Relapse risk may be similar between stimulant abusers and alcohol-dependent individuals.

In the present study, we developed a multidimensional scale, the Alcohol Relapse Risk Scale (ARRS), based on the SRRS. Forty-eight items in the ARRS reflect a variety of relapse risk factors, such as intention, compulsivity, expectancy for alcohol, and emotional problems. We administered the ARRS to 218 inpatients and outpatients with a history of alcohol dependence in Japan and examined its inner structure, reliability, and validity. Moreover, certain relapse risk factors appear to be common between stimulants and alcohol; we therefore compared the relapse risk for alcohol-dependent individuals with that for stimulant abusers.

2. Methods

2.1. Participants

A total of 218 patients (29 inpatients, 182 outpatients, and 7 unknown patients) with a history of alcohol dependence participated in the study (Table 1). They were recruited for ongoing research studies at Nakajo Daini Hospital, Tokamachi (n = 68), National Center of Neurology and Psychiatry, Musashi Hospital, Kodaira (n = 63), Tokyo Metropolitan Matsuzawa Hospital, Tokyo (n = 34), Urabe Mental Health Clinic, Tokyo (n = 30), and Hirakawa Hospital, Hachioji (n = 20). All of these treatment facilities specialized in the treatment of alcohol dependence. The participants comprised 181 males and 36 females, ranging in age from 28 to 81 years (mean = 53.6, S.D. = 11.06).

Table 1
Characteristics of the ARRS participants.

Items	Values
Number of participants	218
Age ($M \pm$ S.D.)	53.6 ± 11.5
Gender (% female)	16.6
Treatment state (N)	
Inpatients	29
Outpatients	182
Unknown	7
Participants with follow-up (%)	56.9
Current drinking/not drinking (N)	55/163
Relapse/no relapse within 1 month (N)	31/93
VAS (current, 0–10; $M \pm$ S.D.)	2.07 ± 2.51
VAS (past 2 weeks, 0–10; $M \pm$ S.D.)	2.47 ± 3.20
CES-D (0–60; $M \pm$ S.D.)	16.82 ± 11.06
GHQ-12 (0–12; $M \pm$ S.D.)	2.32 ± 3.63

N: number of participants, M: mean, S.D.: standard deviation.

The five eligibility criteria were the following: at least 20 years of age, a history of alcohol dependence, diagnosed as alcohol-dependent on the basis of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV; American Psychiatric Association, 1994), an inpatient or outpatient at a Japanese mental hospital, and the ability to understand Japanese. The study was approved by the Institutional Review Board of each facility. After an explanation of the research by a psychiatrist or a psychologist, each participant provided informed written consent and completed the ARRS, the Visual Analog Scale (VAS) for alcohol craving, the Center for Epidemiological Studies Depression Scale Japanese version (CES-D) (Shima et al., 1985), the 12-item General Health Questionnaire Japanese version (GHQ-12) (Daibo and Nakagawa, 1985), and a questionnaire on alcohol experience and demographics. One hundred twenty-four participants also answered a follow-up questionnaire about their drinking state within 1 month after the rating.

2.2. Development of the Alcohol Relapse Risk Scale

The ARRS was developed on the basis of 48 preliminary items from the Stimulants Relapse Risk Scale (SRRS). For all items, "use the drug" was replaced by "drink alcohol." Two items related to illegal activities were replaced by behaviors related to alcohol drinking (i.e., "Even though I know I will be arrested, I would use the drug" was replaced with "Even though I know I will lose my family and/or job, I would drink alcohol"; "I want to obtain the drug even by working illegally" was replaced with "I want to drink alcohol even if it deteriorates my health").

Forty-three of the preliminary items comprised the five initial factors of alcohol relapse: (i) compulsivity (C), (ii) negative expectancy for alcohol (N), (iii) clear intention of alcohol drinking (I), (iv) positive expectancy for alcohol (P), and (v) emotional problems (E). The remaining five preliminary items were provided to measure the lack of insight into one's own mental condition (i.e., denial; e.g., "I am sure that I will not drink alcohol in the future").

Each item was rated on a three-point Likert-type scale with a score ranging from 1 to 3 based on the participant's level of agreement with each statement. The anchors were "Strongly Disagree and Disagree," "Neither Agree nor Disagree," and "Strongly Agree and Agree." A three-point scale was used to reflect patient feedback, indicating the difficulty in answering a five-point scale. The following written instruction was given: "Please describe your state during the past week. For each statement below, please circle one answer that best describes you."

2.3. Measurements of concurrent validity

To evaluate the concurrent validity of the ARRS, the VAS was administered to the participants to measure their subjective desires for alcohol. The VAS was composed of two questions: "Please rate your current state of craving" and "Please rate your strongest craving for alcohol in the past 2 weeks." Participants answered each question by placing a vertical mark on a 100-mm horizontal line, labeled "not at all" on the left and "extremely" on the right end. The current state of drinking (i.e., drinking or not drinking) was also asked.

Participants also answered the CES-D and GHQ-12 that measured their emotional problems. These scales were used to examine the concurrent validity of the factor "emotionality problems" in the ARRS.

2.4. Measurements of predictive validity

To evaluate relapse risk, relapse within 1 month after the ARRS rating was investigated. Relapse was operationally defined as "consumption of any alcohol after the ARRS rating" and was determined from the patients' self-reports and/or their psychiatrists. Of 124 participants whose information was available, 16 were drinking at baseline and follow-up, 15 were abstinent at baseline but drinking at follow-up, 8 were drinking at baseline but not at follow-up, and 85 were abstinent throughout. Thirty-one participants who were drinking at follow-up were considered relapsed patients.

2.5. Questionnaire on alcohol experience and demographic factors

The participants were also asked to complete a short questionnaire to determine their age and gender, the day the questionnaire was completed, and the principal type of alcohol they were drinking (or had consumed). The questions also asked the date on which the patient had last consumed alcohol, the period of abstinence, the number of years they had consumed alcohol, other problems apart from alcohol, perceived stress, perceived social support, and the availability of social support for their problems.

Table 2
Promax rotated factor pattern for the 43-item ARRS.

	Factor				
	1	2	3	4	5
Factor 1: Stimulus-induced vulnerability (SV)					
(C 35) If alcohol is placed in front of me, I would drink it.	**.855**	−.240	.083	.078	.023
(I 9) It would be difficult for me to refuse if someone placed alcohol in front of me.	**.849**	−.063	−.155	−.007	.056
(C 24) If someone held alcohol under my nose, I would not be able to refuse it.	**.838**	.047	−.017	−.066	−.018
(I 27) I would drink alcohol if my friends offered it to me on a street.	**.823**	.059	−.043	−.096	−.037
(I 42) I might drink alcohol at a party or a gathering.	**.661**	.084	−.074	−.051	.142
(C 29) I would drink alcohol if I am alone.	**.580**	−.118	.187	.156	.083
(I 46) I will drink alcohol in near future.	**.536**	.095	−.079	−.051	.185
(C 47) I want to drink alcohol even if it deteriorates my health.	**.522**	−.038	.226	.067	.004
(C 32) If my friend gave me alcohol, I would drink it even in the hospital.	**.479**	−.056	.272	.027	−.133
Factor 2: Emotionality problems (EP)					
(E 22) I feel lonely.	−.046	**.823**	−.114	−.019	−.015
(E 15) I am not motivated to do anything.	.112	**.733**	.119	−.290	−.236
(E 28) I am anxious about my future.	.225	**.565**	−.175	.319	−.031
(E 33) I cannot control my feeling.	.149	**.554**	−.029	.020	−.054
(E 7) I am annoyed by words from others.	−.150	**.486**	.112	−.049	.052
(E 10) I am irritated.	−.297	**.476**	.354	.009	.198
(E 34) I have significant job-related problems.	.107	**.442**	.060	.293	−.165
(E 25) I feel bored.	.149	**.404**	.079	−.236	.102
Factor 3: Compulsivity for alcohol (CA)					
(C 44) I want alcohol even if I have to steal.	.098	.029	**.702**	−.036	−.137
(C 13) I would do almost anything in order to drink alcohol.	−.109	.044	**.534**	.047	.128
(C 40) I would do anything to get money for alcohol.	.132	−.013	**.502**	.001	.047
Factor 4: Lack of negative expectancy for alcohol (NE)					
(N 30) If I drink alcohol, I think it would badly influence my job (reverse-coded).	.103	.047	−.079	**.798**	−.143
(N 23) I would not be able to control myself if I drink alcohol (reverse-coded).	−.141	.118	.145	**.541**	−.146
(N 12) If I drink a small amount of alcohol, I would not be able to stop drinking (reverse-coded).	−.001	−.129	.282	**.464**	.198
(N 39) I would feel restless if I drank alcohol (reverse-coded).	−.213	.013	.103	**.441**	.156
Factor 5: Positive expectancy for alcohol (PE)					
(P 43) If I drink alcohol, I will feel everything is going well.	.169	−.197	.039	−.025	**.780**
(P 45) If I drink alcohol, I will feel invigorated.	.181	.085	.016	−.005	**.541**
(P 26) Alcohol would save me from feeling lonely.	.186	.252	−.003	.011	**.410**
Ambiguous items					
(I 38) If I had a large sum of money, I would want to buy alcohol.	**.564**	−.089	.443	**−.107**	−.001
Other items					
(I 11) I am dying to drink alcohol.	.370	.094	.320	−.201	.103
(C 48) Even though I know I will lose my family or job, I would drink alcohol.	.341	.169	.158	.168	−.044
(E 3) The feeling I used to have while drinking alcohol sometimes comes back.	.297	.124	.092	.123	.103
(E 5) I feel a constant need to put something in my mouth.	.266	.219	−.140	−.040	.068
(E 36) I feel tired due to impatience.	−.092	.395	.227	.224	.111
(E 31) I occasionally have nightmares.	−.034	.328	.107	.008	.200
(N 20) I am afraid of withdrawal due to alcohol dependence (reverse-coded).	−.058	.326	.050	.124	.015
(E 8) I am anxious about relapse.	.163	.161	.312	−.040	−.037
(E 14) I feel easier than before (reverse-coded).	.085	−.124	−.332	.391	.123
(E 1) I want to find a job or need to improve my work environment.	.256	.158	−.025	.357	
(N 2) I need to make the most of my friend's (and AA's) support (reverse-coded).	.001	.000	−.175	.296	.089
(N 18) Thinking about my family, I can no longer drink alcohol (reverse-coded).	−.216	−.075	.055	.257	−.020
(P 16) I recall the relief from feeling blue from the time I was drinking alcohol.	−.027	.282	−.076	.102	.379
(I 4) There are times I want to drink alcohol.	.305	.119	−.130	−.063	.328
(P 41) If I drink alcohol, I would be less nervous.	.229	.300	.003	.028	.311

E: emotionality problems; C: compulsivity; I: clear intention of alcohol use; P: positive expectancy for alcohol; N: negative expectancy for alcohol in terms of the initial five concepts. Numbers followed by single parentheses indicate the order in the ARRS. Values higher than 0.4 are in bold.

2.6. Statistical analysis

Raw scores for the negatively worded items (items 2, 6, 12, 14, 17, 18, 19, 20, 21, 23, 30, 37) were reversed to make these items positively correlated with other items. The inner structure of the 43-item ARRS without the items that assessed insight into the patient's mental condition was examined by exploratory factor analysis using a principal factor method with promax rotation to detect simple structure. Exploratory factor analysis was used instead of confirmatory factor analysis because the inner structure of the ARRS was expected to be different from that of the SRRS. The items that assessed insight into mental condition were excluded from factor analysis of the relapse risk structure because they were added to the questionnaire to distinguish patients who are "in denial." Factors were extracted on the basis of their eigenvalues (>1) and the scree plot. Only the items loading higher than 0.4 were retained in the analyses, and all items cross-loading at higher than 0.4 were removed. The reliability of the extracted factor scales was checked by calculating Cronbach's α value. Concurrent and predictive validity of the subscales and inter-subscale correlations were analyzed by calculating Pearson's product-moment correlation coefficient. With regard to predictive validity, logistic regression analysis was also used to examine whether the ARRS subscales as independent variables predict relapse as a dependent variable. All subscales of the ARRS were analyzed at the same time. For analysis of current state of drinking and relapse, "drinking" and "relapse" were coded as 1, and "not drinking" and "no relapse" were coded as 0.

Additionally, the function of the five items that assessed insight into mental condition was examined. Relationships among insight into mental condition, relapse, and the period of abstinence were analyzed by Fisher's exact test. Median split (i.e., median of the five items' average scores = 2.0; period of abstinence = 150) was used to divide the variables into two groups. Data on relapse and the period of abstinence for 66 participants were used for the analysis.

All analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 12.0 for Windows.

Table 3
Cronbach's α for each subscale of the ARRS and correlation of the ARRS against current drinking, VAS, CES-D, GHQ-12, and relapse.

	Cronbach's α	Correlation					
		Current drinking state	VAS (current craving)	VAS (craving in the past 2 weeks)	CES-D	GHQ-12	Relapse (1 month)
ARRS subscale [number of items]							
Stimulus-induced vulnerability (SV) [9]	.897	.497**	.472**	.604**	.306**	.495**	.268**
Emotionality problems (EP) [8]	.794	.197**	.255**	.386**	.537**	.578**	.131
Compulsivity for alcohol (CA) [3]	.730	.196**	.212**	.350**	.319**	.219	.004
Lack of negative expectancy for alcohol (NE) [4]	.785	.165*	.011	−.003	−.158*	−.316**	.169
Positive expectancy for alcohol (PE) [3]	.545	.341**	.272**	.480**	.328**	.435**	.178
Total ARRS [27]	.864	.410**	.357**	.541**	.445**	.394**	.215*

Note: Reliability was calculated according to Cronbach's alpha. Concurrent validity was calculated according to correlation of ARRS against current drinking, VAS, CES-D, and GHQ-12. Predictive validity was calculated according to correlation of ARRS against relapse.
* $p<.05$.
** $p<.01$.

3. Results

3.1. Factor analysis

Exploratory factor analysis of the ARRS scores for 218 participants revealed five factors with eigenvalues of 9.38, 4.60, 2.55, 2.33, and 1.96. These factors accounted for 47.54% of the overall variance (26.60%, 8.10%, 4.84%, 4.40%, and 3.62% for factors 1–5, respectively). Cronbach's α values for factors 1, 2, 3, 4, 5, and all items were .89, .79, .73, .78, .54, and .86, respectively. The factors were subsequently rotated using the promax method. Of the original 43 items, 27 were retained and 16 were discarded. The factor structure after the promax rotation is shown in Table 2. Cronbach's α values for each subscale and the total ARRS (all 27 extracted items) are shown in Table 3.

The first factor had significant loadings for nine items, including all seven items that reflect stimulus-induced vulnerability (e.g., "If alcohol is placed in front of me, I would drink it" and "It would be difficult for me to refuse if someone placed alcohol in front of me"). The two remaining items reflected intention and desire to drink alcohol (e.g., "I will drink alcohol in the near future" and "I want to drink alcohol even if it deteriorates my health"). The first factor, therefore, was labeled "stimulus-induced vulnerability" (SV).

Eight items loaded exclusively on the second factor. All of these items reflected emotional problems related to alcohol consumption (e.g., "I feel lonely" and "I am not motivated to do anything"). This factor, therefore, was labeled "emotionality problems" (EP).

The third factor had significant loadings for three items, all of which reflecting compulsivity for alcohol (e.g., "I want alcohol even if I have to steal" and "I would do almost anything to drink alcohol"). This factor, therefore, was labeled "compulsivity for alcohol" (CA).

The fourth factor comprised four items that had originally been classified as negative expectancy for alcohol drinking (e.g., "If I drink alcohol, it would badly influence my job" and "I would not be able to control myself if I drink alcohol"). This factor, therefore, was labeled "lack of negative expectancy for alcohol drinking" (NE).

The fifth factor comprised three items that reflected positive expectancy about alcohol drinking (e.g., "If I drink alcohol, I will feel everything is going well" and "If I drink alcohol, I will feel invigorated"). The fifth factor, therefore, was labeled "positive expectancy for alcohol" (PE). Although the internal consistency of this factor was insufficient, its items were retained in the scale because positive expectancy is a significant factor of relapse risk in stimulant abusers (Ogai et al., 2007).

Additionally, we analyzed the function of the five items for assessing insight into mental condition. Cronbach's α values for these items were .68. The association between insight into mental condition and relapse was not significant regardless of the duration of abstinence.

3.2. Basic statistics of the ARRS and inter-subscale correlations

Table 4 presents means, standard deviations, and inter-correlations of the five ARRS factor scales (subscales). No significant correlations were found between "stimulus-induced vulnerability" (Factor 1) and "lack of negative expectancy for drug use" (Factor 4). The other subscales exhibited low to moderate positive inter-correlations.

3.3. Concurrent validity of the ARRS

Correlation coefficients between the ARRS scores (i.e., subscale scores and total score for the 27 items) and the variables that were measured to examine the concurrent validity were calculated (Table 3). The current state of drinking was significantly and positively correlated with the scores for stimulus-induced vulnerability, emotionality problems, compulsivity for alcohol, positive

Table 4
Mean and S.D. of the ARRS and inter-subscale correlations.

	Mean (S.D.)	SV	EP	CA	NE	PE
ARRS subscale (range: 1–3)						
Stimulus-induced vulnerability (SV)	1.53 (0.53)	–	.446**	.404**	−.076	.560**
Emotionality problems (EP)	1.80 (0.53)		–	.362**	−.318**	.507**
Compulsivity for alcohol (CA)	1.20 (0.38)			–	−.276**	.375**
Lack of negative expectancy for alcohol (NE)	1.94 (0.59)				–	−.250**
Positive expectancy for alcohol (PE)	1.54 (0.61)					–
Total ARRS (range: 1–3)	1.62 (0.34)					

S.D.: standard deviation.
** $p<.01$.

Table 5
Logistic regression analysis of each ARRS subscale as independent variable and relapse within 1 month as dependent variable.

	Coefficient *B* (SE)	Wald statistic (d.f.)	*p*-Value	Odds ratio	95% Confidence interval
ARRS subscale					
Stimulus-induced vulnerability (SV)	.994 (.565)	3.095 (1)	.079	2.703	.893–8.186
Emotionality problems (EP)	.392 (.549)	.508 (1)	.476	1.479	.504–4.342
Compulsivity for alcohol (CA)	−.351 (.691)	.258 (1)	.612	.704	.182–2.729
Lack of negative expectancy for alcohol (NE)	1.008 (.461)	4.771 (1)	.029	2.740	1.109–6.769
Positive expectancy for alcohol (PE)	.574 (.499)	1.323 (1)	.250	1.77	5.668–4.721

Note: Predictive validity was also calculated according to odds ratio of ARRS against relapse. 118 data was used for analysis.

expectancy for alcohol drinking, and total ARRS. The current state of drinking was significantly and negatively correlated with lack of negative expectancy. The two VAS scores for alcohol craving ("current craving" and "craving in the past two weeks") were also significantly and positively correlated with the scores for stimulus-induced vulnerability, emotionality problems, compulsivity for alcohol, positive expectancy, and total ARRS. Additionally, the CES-D and GHQ-12 scores were significantly and positively correlated with the scores for all subscales, with the exception of compulsivity for alcohol in the GHQ-12 and total ARRS.

3.4. Predictive validity of the ARRS

Table 3 also presents correlations between the ARRS scores and relapse within 1 month. Relapse was significantly and positively correlated with stimulus-induced vulnerability and total ARRS. Craving in the past 2 weeks (measured by VAS; $r = 0.317$) and the period of abstinence ($r = -0.252$) were significantly correlated with relapse. A significant and positive relationship was found between lack of negative expectancy in the ARRS and participants' compliance at follow-up ($r = 0.199$; 127 participants approved, 75 refused, and 20 were not asked). Logistic regression analysis (Table 5) revealed that lack of negative expectancy significantly and positively predicted relapse (odds ratio = 2.740, $p < .05$), and stimulus-induced vulnerability showed a tendency toward positively predicting relapse (odds ratio = 2.703, $p = .079$).

3.5. Gender differences and differences between inpatients and outpatients

The relationship between ARRS scores and current state of drinking and the relationship between ARRS scores and relapse were differentiated by treatment state (i.e., inpatient *vs.* outpatient) and gender (i.e., male *vs.* female) (Table 6). Among outpatients, a significant positive correlation was observed between lack of negative expectancy and current state of drinking. For inpatients, in contrast, the current state of drinking was significantly and negatively correlated with lack of negative expectancy.

For males, stimulus-induced vulnerability, positive expectancy, and total ARRS were significantly and positively correlated with relapse. For females, lack of negative expectancy was significantly and positively correlated with relapse.

4. Discussion

The present study developed the ARRS to assess relapse risk for Japanese alcohol-dependent individuals and statistically examined its inner structure, reliability, and validity. Five factors were found, and their internal consistency, concurrent validity, and predictive validity were revealed. Notably, part of the ARRS was related to relapse, implying its potential application for relapse prediction. Our findings demonstrated that the ARRS has multidimensional psychometric properties. Thus, the ARRS may be useful for assessing various aspects of relapse risk in alcohol-dependent individuals, similar to the SRRS for stimulant abusers.

Some similarities were found in the multidimensional structures of the ARRS and the SRRS; emotionality problems (Factor 2), compulsivity for alcohol (Factor 3), lack of negative expectancy (Factor 4), and positive expectancy (Factor 5) were similar to "emotionality problems," "compulsivity for drug," "lack of negative expectancy," and "positive expectancy" of the SRRS subscales, respectively. Factor 2 revealed negative emotional states (e.g., anxiety and negative mood) that have been shown previously to be related to relapse in alcohol-dependent individuals (Lucht et al., 2002; Walitzer and Dearing, 2006). Factor 3 was considered to reflect craving based on "obsessive compulsive theory" (Anton, 2000). Factor 4 and Factor 5 were considered to reflect craving based on "expectancy theory" (Jones et al., 2001). Positive expectancy for substance increases the risk of relapse, whereas negative expectancy for substance (understanding the harmful effects of the substance) decreases the risk. The above four factors are risk factors that may trigger relapse in alcohol-dependent individuals, as well as in stimulant abusers.

Differences were also found between the ARRS and the SRRS. "Stimulus-induced vulnerability" (Factor 1) in the ARRS and "anxiety and intention to drug use" in the SRRS, both of which relating to relapse, have differences in content. This may reflect the fact that alcohol-dependent individuals often encounter environmental stimuli related to alcohol because it is not illegal and is commonly

Table 6
Correlation of the ARRS against relapse by treatment state and gender.

	Correlation with current state of drinking				Correlation with relapse	
	Inpatient ($N = 29$)	Outpatient ($N = 182$)	Male ($N = 183$)	Female ($N = 35$)	Male ($N = 97$)	Female ($N = 27$)
ARRS subscale						
Stimulus-induced vulnerability (SV)	.615**	.447**	.543**	.201	.344**	.075
Emotionality problems (EP)	.656**	.082	.312**	−.378*	.185	−.005
Compulsivity for alcohol (CA)	.565**	.094	.229**	.032	.037	−.087
Lack of negative expectancy for alcohol (NE)	−.399**	.289**	.156*	.267	.076	.503**
Positive expectancy for alcohol (PE)	.664**	.262**	.435**	−.157	.280**	−.149
Total ARRS	.690**	.341**	.512**	−.031	.357**	−.046

* $p < .05$.
** $p < .01$.

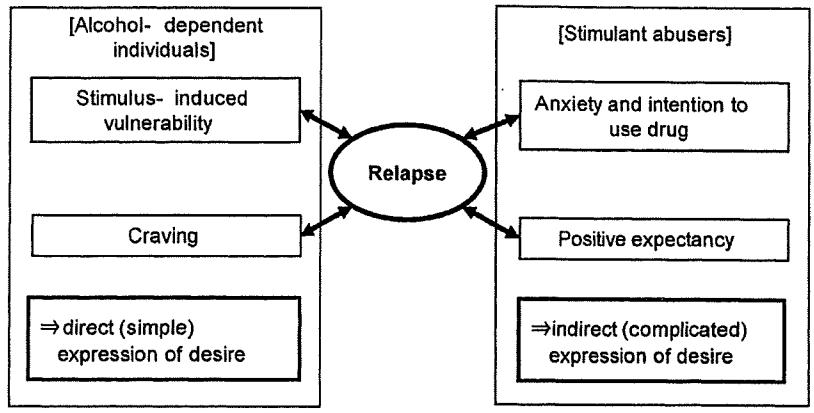

Fig. 1. Differences in the factors related to relapse between alcohol-dependent individuals and stimulant abusers. In alcohol-dependent individuals, stimulus-induced vulnerability in the ARRS and craving measured by the VAS were significantly related to relapse within 1 month. Anxiety, intention, and positive expectancy in the SRRS in stimulant abusers were significantly related to relapse within 3 months. These results indicate that alcohol-dependent individuals express signs of relapse more directly, such as craving for alcohol, and appear to be easily influenced by the environment. Stimulant abusers, in contrast, express signs of relapse indirectly, such as anxiety and expectancy, which are inner feelings.

consumed. In fact, social pressure is one of the relapse risks for alcohol-dependent individuals (Zywiak et al., 2006).

With regard to concurrent validity, the scores for total ARRS and all subscales except lack of negative expectancy were significantly correlated with the current state of drinking and the VAS scores. Specifically, the coefficients for stimulus-induced vulnerability and positive expectancy for alcohol were more than 0.4. These results suggest that stimulus-induced vulnerability and positive expectancy are important factors that govern the severity of alcohol dependence and craving related to the subjective desire for alcohol, although the difference in time frames of the ARRS (past 1 week) and the VAS (the current and past 2 weeks) must be considered.

With regard to predictive validity, the scores for stimulus-induced vulnerability and total ARRS were significantly correlated with relapse within 1 month, suggesting that these scores predict relapse risk. This was supported by the nearly significant prediction shown in the logistic regression analysis. The correlation between the scores for stimulus-induced vulnerability and relapse supports the hypothesis of a prior study of more than 900 individuals in which relapse was found to be triggered by social pressure such as temptation from alcohol-drinking friends (Zywiak et al., 2006). Particularly in Japanese collectivistic culture, refusing an offer to drink alcohol at a party is difficult. According to Hendershot et al. (2005), alcohol use is influenced by cultural background. Thus, the influence of culture on stimulus-induced vulnerability (e.g. "It would be difficult for me to refuse if someone placed alcohol in front of me") may be a more prevalent risk factor in Japan.

Interestingly, the logistic regression analysis showed that lack of negative expectancy significantly predicted relapse. This result suggested that lack of understanding of negative effects of alcohol drinking was an important factor leading to relapse. This result was also consistent with a report in which relapsed alcohol-dependent individuals and their families reported that "reduced cognitive vigilance" was the most common relapse sign (Malhotra et al., 1999). The differences between correlation analysis (Table 3) and logistic regression analysis (Table 5) relating Factor 4 with relapse indicate that some indirect effects of Factor 4 on relapse via other factors (e.g., positive expectancy) counterbalanced the direct effect of Factor 4 in the correlation score.

Although preliminary, stimulus-induced vulnerability and lack of negative expectancy in the ARRS and craving measured by the VAS among alcohol-dependent individuals were significantly related to relapse within 1 month. In stimulant abusers, in contrast, anxiety, intention, positive expectancy, and lack of negative expectancy were significantly related to relapse within 3 months. These results indicate that alcohol-dependent individuals express signs of relapse more directly, such as craving for alcohol, and appear to be easily influenced by the environment. In contrast, stimulant abusers express signs of relapse indirectly through inner feelings such as anxiety and expectancy (Fig. 1).

Other subscales (e.g., emotionality problems, compulsivity for alcohol, and positive expectancy) were not related to relapse. However, the significant correlations of these subscales with stimulus-induced vulnerability, lack of negative expectancy, and craving measured by the VAS suggest that these factors may have an indirect effect on relapse. Additionally, internal consistency of positive expectancy was low.

The relationships among the ARRS, current state of drinking, and relapse were influenced by treatment state and gender. Among inpatients, higher negative expectancy was associated with the risk of current drinking. By contrast, lower negative expectancy in outpatients was associated with the risk of current drinking. These results may reflect the fact that inpatients check into hospitals because they are more aware of the risk of drinking than outpatients. With regard to gender, higher vulnerability and positive expectancy were related to higher risk of relapse in males. By contrast, lower negative expectancy in females was related to high risk of relapse. These results were consistent with a report showing that alcohol-dependent males had a more positive affect during the week before relapse than females (McKay et al., 1996). The above results suggest the necessity of gender-specific intervention.

One possible limitation to the present study was the sampling procedure. The participants were not recruited randomly but were limited only to inpatients or outpatients who gave informed consent and whose doctors recognized their ability to answer the questionnaire. Therefore, the data of this study were not obtained from alcohol-dependent individuals as a whole, including dropout patients and non-patients, but rather only from cooperative patients with a relatively low severity of alcohol dependence. Additionally, the relatively low availability of relapse data (124/218) may have influenced the assessment of the ARRS's predictive validity. Conducting follow-up surveys for dropout cases and recruiting participants from other facilities and programs, including Alcoholics Anonymous, are necessary to gain a better understanding of relapse risk in alcohol-dependent individuals. Another limitation of the present study was the relatively low sample size. A sample of 218 participants was rather small to sufficiently support the factor analysis of 43 items. In the present

study, the number of participants per item was 5.07. Because of the wide range of sample size recommended for factor analysis of five participants per item (Gorsuch, 1983) to ten participants per item (Everitt, 1975), further study of the ARRS with larger samples will reveal the detailed features of the ARRS factor structure.

The low to moderate correlation between relapse within 1 month and the ARRS subscales may indicate a limitation in the clinical utility of this scale. However, in the present study, the ARRS and the VAS similarly predicted relapse within 1 month. One month reflects the highest level of the hazard function and is the most clinically relevant. The ARRS will likely predict greater levels of variance than the VAS with longer follow-up periods because alcohol craving wanes as the period of abstinence increases (Tavares et al., 2005). Furthermore, the ARRS is anticipated to complement the use of the VAS because the ARRS assesses a wider range of constructs.

Although the results for the items assessing insight into mental condition were not significant, these items should remain to distinguish patients who are "in denial." The expressions of some items (e.g., "I would be fine without alcohol") posed difficulty in distinguishing dishonest from honest responses because recovering honest patients answer "agree" to these items. Changing the expressions of these items in the future is necessary.

The present results suggest that the ARRS is an effective tool with which psychiatrists, psychologists, social workers, and alcohol-dependent individuals themselves could assess the level of relapse risk, similar to the SRRS for stimulant abusers, although the predictive validity of the instrument is preliminary. The ARRS and SRRS may also contribute to the assessment of craving-inhibitory effects of pharmacotherapies and treatment programs. To improve the usefulness of these scales, further studies of at least the following are necessary: (i) cross-validity using other alcohol-dependent individuals with confirmatory factor analysis, (ii) modification of the ARRS for a better prediction of relapse, and (iii) utilization of the SRRS and ARRS as communication tools among facilities that treat stimulant abusers and alcohol-dependent individuals, such as hospitals (or other treatment facilities), legal facilities (e.g., prisons or probation offices), and research institutes.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgements

We are grateful to the volunteers for their participation in this study. We are also grateful to Drs. Hideko Yamamoto, Yukio Takamatsu, and Ms. Fumiyuki Chin for fruitful discussion.

Role of funding source: This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (government department) (17025054), Ministry of Health, Labor and Welfare of Japan (government department) (H19-Iyaku-023, 18A-3 for Nervous and Mental Disorders, H17-Pharmaco-001, H16-Iyaku-029), and the Japan Society for the Promotion of Science (quasi-governmental organization) (17730421, 17591238).

These organizations had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Contributors: Authors Yasukazu Ogai, Nozomu Asukai, Eiichi Senoo, and Kazutaka Ikeda designed the study and wrote the protocol. Author Yasukazu Ogai, Masahiro Yamashita, Keiko Endo, Ayako Haraguchi, Yoko Ishibashi, Tatsuya Kurokawa, Tatsuyuki Muratake, Ryoichi Suga, Toru Hori, and Mitsuru Umeno managed the literature searches and summaries of previous related work. Author Yasukazu Ogai undertook the statistical analysis, and author Yasukazu Ogai wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the on-line version, at doi:10.1016/j.drugalcdep.2008.10.021.

References

American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Press, Washington, DC.
Anton, R.F., 2000. Obsessive-compulsive aspects of craving: development of the Obsessive Compulsive Drinking Scale. Addiction 95 (Suppl. 2), 211–217.
Brown, S.A., Vik, P.W., Patterson, T.L., Grant, I., Schuckit, M.A., 1995. Stress, vulnerability and adult alcohol relapse. J. Stud. Alcohol 56, 538–545.
Daibo, I., Nakagawa, Y., 1985. Japanese version of GHQ (General Health Questionnaire) by Goldberg. Nihon Bunka Kagakusha, Tokyo.
Demmel, R., Nicolai, J., Jenko, D.M., 2006. Self-efficacy and alcohol relapse: concurrent validity of confidence measures, self-other discrepancies, and prediction of treatment outcome. J. Stud. Alcohol 67, 637–641.
Everitt, B.S., 1975. Multivariate analysis: the need for data, and other problems. Br. J. Psychiatry 126, 237–240.
Gordon, S.M., Sterling, R., Siatkowski, C., Raively, K., Weinstein, S., Hill, P.C., 2006. Inpatient desire to drink as a predictor of relapse to alcohol use following treatment. Am. J. Addict. 15, 242–245.
Gorsuch, R.L., 1983. Factor Analysis, 2nd ed. Erlbaum, Hillsdale, NJ.
Heishman, S.J., Singleton, E.G., Liguori, A., 2001. Marijuana Craving Questionnaire: development and initial validation of a self-report instrument. Addiction 96, 1023–1034.
Hendershot, C.S., MacPherson, L., Myers, M.G., Carr, L.G., Wall, T.L., 2005. Psychosocial, cultural and genetic influences on alcohol use in Asian American youth. J. Stud. Alcohol 65, 185–195.
Jones, B.T., Corbin, W., Fromme, K., 2001. A review of expectancy theory and alcohol consumption. Addiction 96, 57–72.
Lucht, M., Jahn, U., Barnow, S., Freyberger, H.J., 2002. The use of a symptom checklist (SCL-90-R) as an easy method to estimate the relapse risk after alcoholism detoxification. Eur. Addict. Res. 8, 190–194.
Malhotra, S., Malhotra, S., Basu, D., 1999. A comparison of the beliefs of Indian alcohol-dependent patients and their close family members on their reasons for relapse. Addiction 94, 709–713.
McKay, J.R., Rutherford, M.J., Cacciola, J., Kabasakalian-McKay, R., Alterman, A.I., 1996. Gender differences in the relapse experiences of cocaine patients. J. Nerv. Ment. Dis. 184, 616–622.
Miller, N.S., Ninonuevo, F., Hoffmann, N.G., Astrachan, B.M., 1999. Prediction of treatment outcomes: lifetime depression versus the continuum of care. Am. J. Addict. 8, 243–253.
Noda, T., Imamichi, H., Kawata, A., Hirano, K., Ando, T., Iwata, Y., Yoneda, H., 2001. Long-term outcome in 306 males with alcoholism. Psychiatry Clin. Neurosci. 55, 579–586.
Ogai, Y., Haraguchi, A., Kondo, A., Ishibashi, Y., Umeno, M., Kikumoto, H., Hori, T., Komiyama, T., Kato, R., Aso, K., Asukai, N., Senoo, E., Ikeda, K., 2007. Development and validation of the Stimulant Relapse Risk Scale for drug abusers in Japan. Drug Alcohol Depend. 88, 174–181.
Osaki, Y., Matsushita, S., Shirasaka, T., Hiro, H., Higuchi, S., 2005. Nationwide survey of alcohol drinking and alcoholism among Japanese adults. Nihon Arukoru Yakubutsu Igakkai Zasshi 40, 455–470.
Sander, W., Jux, M., 2006. Psychological distress in alcohol-dependent patients: evaluating inpatient treatment with the symptom checklist (SCL-90-R). Eur. Addict. Res. 12, 61–66.
Shaffer, H.J., LaPlante, D.A., LaBrie, R.A., Kidman, R.C., Donato, A.N., Stanton, M.V., 2004. Toward a syndrome model of addiction: multiple expressions, common etiology. Harv. Rev. Psychiatry 12, 367–374.
Shima, S., Shikano, T., Kitamura, T., Asai, M., 1985. New self-rating scales for depression. Seishin Igaku 27, 717–723.
Tavares, H., Zilberman, M.L., Hodgins, D.C., el-Guebaly, N., 2005. Comparison of craving between pathological gamblers and alcoholics. Alcohol. Clin. Exp. Res. 29, 1427–1431.
Walitzer, K.S., Dearing, R.L., 2006. Gender differences in alcohol and substance use relapse. Clin. Psychol. Rev. 26, 128–148.
Zywiak, W.H., Stout, R.L., Longabaugh, R., Dyck, I., Connors, G.J., Maisto, S.A., 2006. Relapse-onset factors in project MATCH: the Relapse Questionnaire. J. Subst. Abuse Treat. 31, 341–345.

Analgesic requirements after major abdominal surgery are associated with *OPRM1* gene polymorphism genotype and haplotype

Aims: The association between SNPs of the human *OPRM1* gene encoding the μ-opioid receptor and postoperative analgesic requirements in surgical patients remains controversial. Here, we evaluate whether any of the five tag SNPs (A118G, IVS2+G691C, IVS3+G5953A, IVS3+A8449G and TAA+A2109G) representing the four linkage disequilibrium blocks of the *OPRM1* gene influences postoperative analgesic requirements. **Materials & methods:** We studied 138 adult Japanese patients who underwent major open abdominal surgery under combined general and epidural anesthesia and received continuous postoperative epidural analgesia with opioids. **Results:** The 118G homozygous (GG) patients required 24-h postoperative analgesics more than 118A homozygous (AA) and heterozygous (AG) patients. Tag SNP haplotypes also were associated with 24-h postoperative analgesic requirements. **Conclusions:** These results suggest that *OPRM1* gene tag SNP genotypes and haplotypes can primarily contribute to prediction of postoperative analgesic requirements in individual patients undergoing major open abdominal surgery.

KEYWORDS: μ-opioid receptor, *OPRM1* gene, analgesic, association study, haplotype analysis, pain, SNP

Masakazu Hayashida, Makoto Nagashima, Yasuo Satoh, Ryoji Katoh, Megumi Tagami, Soichiro Ide, Shinya Kasai, Daisuke Nishizawa, Yasukazu Ogai, Junko Hasegawa, Hiroshi Komatsu, Ichiro Sora, Kenichi Fukuda, Hisashi Koga, Kazuo Hanaoka & Kazutaka Ikeda*

*Author for correspondence: Division of Psychobiology, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156–8585, Japan
Tel: +81 833 304 5701
Fax: +81 833 329 8035
ikedak@prit.go.jp

Opioid analgesics, such as morphine and fentanyl, are widely used for the treatment of moderate to severe pain. However, the analgesic efficacy of opioid analgesics varies widely among individuals [1,2]. Several studies in mice that lack the *Oprm1* gene encoding the mouse μ-opioid receptor (MOP) have now provided evidence that the MOP is the opioid receptor subtype that is essential for the analgesic effects of most clinically efficacious opioid drugs. For example, homozygous and heterozygous MOP knockout mice that display 0 and 50% of the levels of MOPs in wild-type mice display complete abolition and partial reductions of the analgesic effect of morphine, respectively [3–5]. Buprenorphine, a nonselective opioid receptor partial agonist, also has no analgesic effect in homozygous MOP knockout mice [6]. These observations are especially interesting because the distributions of δ-opioid receptors (DOPs) and κ-opioid receptors (KOPs) are not apparently altered in MOP knockout mice [3,4,7]. Furthermore, CXBK mice that express approximately half of the MOP levels of C57BL/6 and BALB/c progenitor strains show reduced analgesic effects of morphine [8,9]. These previous reports indicate that analgesia produced by most commonly used opioid analgesics depends crucially on MOP expression. Individual variation in MOP expression has also been recognized in humans [10]. Because at least one SNP in the human *OPRM1* gene contributes to the individual variation in MOP expression [10], some SNPs in the human *OPRM1* gene may result in individual differences in the clinical effects of opioids.

More than 250 SNPs and four substantial linkage disequilibrium (LD) blocks have been identified in the human *OPRM1* gene [2,11,12] (Figure 1). The A118G SNP in exon 1 represents the first LD block. Because the A118G SNP leads to an amino acid substitution in the human *OPRM1* gene that changes the putative *N*-glycosylation site [13], this SNP has been the most extensively studied in the context of the clinical effects of opioid analgesics. A number of studies have shown that the 118G allele is a risk factor for substance dependence, including opioid dependence [14–18]. Several recent studies investigating the association between the A118G SNP and morphine requirements in cancer patients and postoperative patients have suggested that this SNP has a significant association with morphine requirements [19–21], although other studies have not found such an association [22,23]. As the IVS2+G691C SNP in intron 2 representing the second LD block also has been shown to be a risk factor for substance dependence [12], the possibility cannot be excluded that SNPs on LD blocks other than the first LD block (e.g., IVS2+G691C) may influence the clinical effects of opioids. However, to date no study has systematically investigated

10.2217/14622416.9.11.1605 © 2008 Future Medicine Ltd **Pharmacogenomics** (2008) 9(11), 1605–1616 ISSN 1462-2416

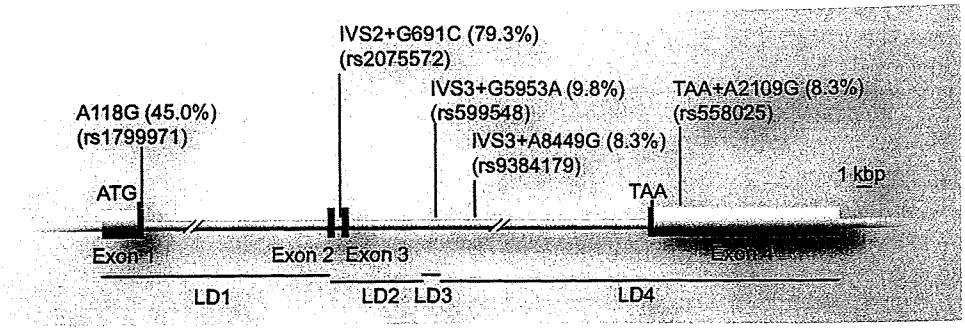

Figure 1. Schematic illustration of the *OPRM1* gene structure and polymorphisms. Values in parentheses are minor allele frequencies.
LD: Linkage disequilibrium.

possible associations between representative tag SNPs of the four LD blocks and the clinical effects of opioids.

Therefore, we examined, in the present retrograde study, whether any of the five tag *OPRM1* SNPs that represent four LD blocks covering all of the exons of the *OPRM1* gene could affect postoperative opioid or analgesic requirements in Japanese patients who had undergone major open abdominal surgery under combined general and epidural analgesia.

Methods

Subjects

The study protocol was approved by each Institutional Review Board at the Institute of Medical Science, The University of Tokyo (Tokyo, Japan), Toho University Sakura Hospital (Chiba, Japan) and the Tokyo Institute of Psychiatry (Tokyo, Japan). Enrolled in the study were 138 patients (American Society of Anesthesiologists Physical Status I or II, age 28–80 years, 80 males and 58 females) who were selected as subjects according to the following multistep process.

In the first step, anesthesiologists at the Research Hospital of the Institute of Medical Science and Toho University Sakura Hospital listed ex-patient or patient candidates who previously had undergone major open-abdominal surgery, including gastrectomy, colectomy, hepatectomy and pancreaticoduodenectomy for cancerous lesions, under combined general and epidural anesthesia and had received continuous epidural analgesia with fentanyl or morphine postoperatively during the period from January 2002 to December 2004 at either institution. In the second step, anesthesiologists at their respective institutions sent letters to these candidates explaining the outline of the study and reply cards on which the candidates could indicate their interest in participating in the study. In the third step, only candidates with interest in participating in the study sent the reply cards to a researcher (Kazutaka Ikeda [KI]) at the Tokyo Institute of Psychiatry. In the fourth step, the researcher (KI) sent these candidates explanatory letters, written informed consent forms, cotton swabs and test tubes to collect oral mucosa samples. In the fifth step, the candidates who agreed to participate in the study provided written informed consent forms and either whole-blood or oral mucosa samples for DNA analysis. Those subjects who chose to present oral mucosa samples wiped their own buccal mucosa with cotton swabs by themselves six times on each side, enclosed the cotton swabs in the test tubes, and sent the specimen and signed informed consent forms by mail to the researcher (KI). Those subjects who chose to provide whole-blood samples visited their respective hospitals to see an anesthesiologist and provided 10 ml of venous blood and signed the informed consent forms. The blood samples were transported to Mitsubishi Chemical Medience Corporation (Tokyo, Japan) on the day of blood sampling. Genomic DNA was purified from the blood and transported to the Tokyo Institute of Psychiatry. The study subjects also were asked to rate the pain intensity they had at rest during the first 24-h postoperative period using a five-point verbal numerical rating scale (NRS; 0: no pain; 1: mild pain; 2: moderate pain; 3: severe pain; 4: most severe pain imaginable).

Postoperative pain management

Postoperative pain was managed primarily with continuous epidural analgesia with fentanyl or morphine. Fentanyl or morphine was diluted with 0.25% bupivacaine in a total volume of 100 ml and infused through the catheter placed in the lower thoracic or upper lumbar epidural space at a constant rate of 2 ml h^{-1} using a syringe-type infuser (ISJ12-B2060, Coopdech Syringector®,

Daiken-iki, Osaka, Japan) [24]. The dose of fentanyl or morphine was determined at the discretion of anesthetists who were in charge of anesthesia. Whenever the patient complained of significant postoperative pain despite the epidural analgesia, appropriate doses of opioids, including buprenorphine, pentazocine and pethidine, were systemically administered at the discretion of surgeons. In some patients, appropriate doses of nonsteroidal anti-inflammatory drugs (NSAIDs), including diclofenac and flurbiprofen, were systemically administered for analgesia. The nurses in the postanesthetic care unit and the ward recovery room recorded the residual volume of the analgesic solution in the Syringector® infuser every 2 h, from which the dose of fentanyl or morphine epidurally administered during the first 24 h after surgery was determined. The anesthesiologists examined the hospital records and collected clinical data of the study subjects, including age, gender, body weight, postoperative diagnosis, surgical procedure, site and duration of surgery, dose of fentanyl or morphine epidurally administered during the first 24-h postoperative period, and dose of rescue analgesics administered during the same period.

Dose conversion of opioids & NSAIDs

To allow for intersubject comparisons of opioid doses required during the first 24-h postoperative period, doses of opioid analgesics used during this period were converted to the dose of the systemic fentanyl equivalent. Epidural fentanyl is equivalent to systemic fentanyl [25,26]. Systemic fentanyl is 100-times more potent than systemic morphine [27]. The dose of epidural morphine required for pain relief after abdominal surgery is approximately a fifth that of systemic morphine [28]. Systemic pentazocine is a third as potent as systemic morphine, and systemic buprenorphine is 30-times more potent than systemic morphine [29]. Systemic pethidine 120 mg is equipotent with systemic morphine 10 mg [30]. Therefore, epidural fentanyl 100 µg, epidural morphine 2 mg, systemic morphine 10 mg, systemic buprenorphine 333 µg, systemic pentazocine 30 mg, and systemic pethidine 120 mg were converted to the equivalent systemic fentanyl dose of 100 µg. Opioid requirements in the first 24-h postoperative period were determined as the sum of systemic fentanyl equivalent doses of all opioids used during the first 24-h postoperative period.

Similarly, to allow for intersubject comparisons of total analgesic doses required during the first 24-h postoperative period, doses of NSAIDs were also converted to the equivalent dose of systemic fentanyl. Systemic flurbiprofen, diclofenac and indomethacin are equivalent [31,32]. The analgesic effect of systemic diclofenac 1 mg kg^{-1} is comparable to that of systemic fentanyl 1 µg kg^{-1} [33,34]. Therefore, systemic flurbiprofen 1 mg kg^{-1} and systemic diclofenac 1 mg kg^{-1} were converted to the systemic fentanyl equivalent dose of 1 µg kg^{-1}. Analgesic requirements in the first 24 h postoperative period were determined as the sum of systemic fentanyl equivalent doses of all opioids and NSAIDs used for analgesia during the first 24-h after surgery.

Genotyping

DNA was extracted from the oral mucosa or whole-blood samples by the conventional phenol-chloroform method or by using a QIAamp DNA mini Kit (Qiagen CA, USA) according to the manufacturer's instructions. Purified genomic DNA was stored at 4°C until used. Based on a previous report, genotyping was performed for the five representative SNPs, designated as tag SNPs (selected by using HaploBlockFinder v.0.7 [35,101], in the human *OPRM1* gene, including A118G in exon 1 as the tag SNP of the first LD block, IVS2+G691C in intron 2 as the tag SNP of the second LD block, IVS3+G5953A in intron 3 as the tag SNP of the third LD block, IVS3+A8449G in intron 3 as the tag SNP of the fourth LD block, and TAA+A2109G in the 3′ untranslated region as the representative tag SNP of the absolute LD block [2,12,36].

For genotyping the five selected SNPs in the *OPRM1* gene, direct sequencing was adopted. To perform direct sequencing, primers were designed to cover each polymorphic site within the *OPRM1* gene on the basis of the reference genomic contig sequence in the National Center of Biotechnology Information database (Genbank Accession number NT-025741). All of the primers used for genotyping are shown in Table 1. PCR was performed with forward and reverse primers for each region in Table 1 in a final volume of 10 µl containing 1 × KOD Dash buffer, 0.2 mM dNTPs, 0.2 µM concentration of each primer, 0.25 U KOD Dash polymerase (Toyobo Co., Ltd., Tokyo, Japan), and 5–50 ng extracted genomic DNA as the template. The PCR program was the following: 94°C for 5 min followed by 30 cycles of 94°C for 50 s, 55–70°C for 30 s, and 72°C for 1–2 min, with a final extension at 72°C for 8 min. Following the cycle sequencing reaction with a BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied

Table 1. Primers used for genotyping *OPRM1* SNPs.

Target SNP	Region	Sequence (5′>3′)	Forward/reverse	Primer No.
A118G	Exon 1	CTC CCT TCC AGC CTC CGA ATC C	Forward	P1F
		CTC TTT CAT CCT CCC GCC CAA CA	Reverse	P2R
		ACT TGT CCC ACT TAG ATG GCA	Forward	P3F
		ACT TGT CCC ACT TAG ATG GCG	Forward	P4F
		CAA TCA CTG TCC GTG GTC TCC	Reverse	P5R
		GGA GAA TGT CAG ATG CTC AGC	Forward	P6F
IVS2+G691C	Intron 2	CTG GAG CCG CCT AGA GAC TTT	Forward	P7F
		GGA GTC CAG CAG ACG ATG AAC	Reverse	P8R
		TAA AAT TAT CAA GTG GCT GAC TAC	Forward	P9F
		CAA GAT GAA GAC TGC CAC CAA	Forward	P10F
		AGT ACC CTG CCC TTC CAG AG	Forward	P11F
		AGA GAG CAT GCG GAC ACT C	Reverse	P12R
IVS3+G5953A	Intron 3	ATT AAT ATG GCA AGT CCA GTC C	Forward	P13F
		CAA ACA GCC AGG AAG AGG TAG AAT	Forward	P14F
		GAC CAT GCT GAG AAG AGT GAA	Reverse	P15R
		AAT AAG ATA TCC CAT CCC AGA C	Reverse	P16R
		CTG TAG AAC TGA AAG AAT AGC C	Reverse	P17R
IVS3+A8449G	Intron 3	GGG AGG CTA GAA ACA AGA TTC	Forward	P18F
		CGT CTA TGA CTT CTA CTC TAC TGC	Forward	P19F
		GCC TAT ATT TAT TTG GTA TCT GAT	Reverse	P20R
TAA+A2109G	3′UTR	ATG GGC TAG GAT GGT TTC	Forward	P21F
		CTT TGC AGA GGT GTT TTC	Reverse	P22R
		TTT ATC AGA AAC CTT AGC CCA TCC	Reverse	P23R
		ATT GCT TTT GCT CAT CAG GC	Forward	P24F

UTR: Untranslated region.

Biosystems [ABI], CA, USA) according to the manufacturer's instructions and purification of the PCR products, DNA sequences of the fragments were determined using the automated sequencer ABI PRISM® 3100 Genetic Analyzer (ABI). The genotyping results for the A118G, IVS2+G691C and IVS3+G5953A SNPs were confirmed by recently developed modified membrane protein explorer (MPEX) and Sequence-Specific Primer Cycle Elongation-Fluorescence Correlation Spectroscopy (SSPCE-FCS) methods described in detail previously [36]. Furthermore, allele-specific PCR was performed to confirm incongruous results for the A118G SNP. To perform allele-specific PCR, two forward primers (whose 3′ ends were specified for detecting the A or G allele at the A118G polymorphic site) and a reverse primer were used. The sequences of the forward primers specific for the A and G alleles were 5′-ACTTGTCCCACTTAGATGGCA-3′ and 5′-ACTTGTCCCACTTAGATGGCG-3′, respectively, and the P2R primer was used as the reverse primer (Table 1). PCR was performed in a final volume of 10 µl containing 1 × GoTaq® Green Master Mix (Promega, WI, USA), 0.4 µM concentration of each primer and 5–50 ng extracted genomic DNA as the template. The PCR program was the following: 96°C for 5 min followed by 30 cycles of 96°C for 30 s, 62°C and 64°C for 30 s for the forward primer specific for the A and G alleles, respectively, and 72°C for 2 min, with a final extension at 72°C for 10 min. Following this, the DNA fragments amplified with both primer pairs were analyzed by electrophoresis using 1–2%

agarose gel and ethidium bromide staining for visualization under ultraviolet illumination to detect allele-specific PCR products.

Statistical analyses

Statistical analysis was performed with SPSS v.12.0 for Windows (SPSS Inc., IL, USA). Because of the relatively small sample size, univariate analysis was performed in the first step using linear regression analysis or analysis of variance (ANOVA) to detect possible associations between any one of the clinical or genomic parameters that may affect the analgesic efficacy of opioids (e.g., age, gender and genotype of the five SNPs) and 24-h postoperative opioid or analgesic requirements. Parameters showing significant associations ($p < 0.05$) with 24-h postoperative opioid or analgesic requirements were retained for the subsequent multivariate analysis of covariance (ANCOVA), followed by *post hoc* testing with Fisher's probability of least significant difference test. $p < 0.05$ was considered statistically significant. Data are presented as mean ± standard deviation (range). To adjust multiple tests for SNPs that were in linkage disequilibrium with each other, we used the SNP spectral decompostion (SNPSpD) Web interface program [37,38,102]. The adjusted significant threshold was $p = 0.0127$. Power analyses were performed using G*Power version 3.0.5 [39].

Haplotype analyses were performed using the HPlus software package v.2.5 (Fred Hutchinson Cancer Research Center, WA, USA) that employs expectation–maximization with a modified progressive ligation computational algorithm to infer haplotypes [40]. To adjust multiple tests in haplotype analyses, we corrected the p-values using Benjamini–Hochberg multiple test correction [41] with R software version 2.5.1 [103]. Haplotype data are presented as mean ± standard error.

Results

Of 285 candidates to whom letters were mailed explaining the outline of the study, 138 (79 males and 59 females) aged 28–80 years gave written informed consent and participated in the study. A total of 67, 57, 10 and 4 patients underwent gastrectomy for stomach cancer, colectomy for colon cancer, hepatectomy for primary or metastatic liver cancer, and pancreaticoduodenectomy for pancreas or bile duct cancer, respectively. Demographic, anesthetic and surgical data of these study subjects are shown in Table 2.

A total of 78 and 60 patients received continuous postoperative epidural analgesia with fentanyl and morphine, respectively. Doses of fentanyl and morphine epidurally infused during the first 24-h postoperative period were 386 ± 149 µg (range: 120–900 µg) and 6.04 ± 1.60 mg (range: 2–10 mg), respectively. For rescue analgesics, 40 patients received systemic opioids, two patients received both systemic opioids and NSAIDs, and 20 patients received NSAIDs during the first 24-h postoperative period. Doses of epidural opioids, including fentanyl and morphine, and rescue analgesics, including pentazocine, buprenorphine, pethidine, diclofenac and flurbiprofen, administered during the first 24-h postoperative period are shown in Table 3. Postoperative opioid requirements and postoperative analgesic requirements during the first 24-h postoperative period, expressed as equivalent systemic fentanyl doses, were 386 ± 143 µg (range, 120–900 µg) and 393 ± 146 µg (range, 120–950 µg), respectively.

The distributions of the A118G, IVS2+G691C, IVS3+G5953A, IVS3+A8449G, and TAA+A2109G SNP genotypes are shown in Table 4. No observed genotype frequencies were significantly different from Hardy–Weinberg equilibrium.

Univariate analysis revealed that age and the A118G SNP had a significant association ($p < 0.05$) with 24-h postoperative opioid

Table 2. Patient demographic and surgical data.

Patient demographic	Surgical data
Age (years)	63.1 ± 9.8 (28–80)
Male/female	79/59
Body weight (kg)	55.4 ± 10.4 (30–80)
Sites of surgery	Stomach (n = 67); colon (n = 57)
	Liver (n = 10); pancreas and duodenum (n = 4)
Duration of surgery (min)	223 ± 98 (40–619)
NRS pain score (retrospect)	1.59 ± 1.26 (0–4)

Data are expressed as number or mean ± SD (range).
NRS: Numerical rating scale.

Table 3. Doses of analgesics used during the first 24 h after surgery.

Analgesic	Number	Mean ± SD	Range
Primarily pain management (opioids)			
Opioids			
Epidural fentanyl (μg)	n = 78	388 ± 152	(120–900)
Epidural morphine (mg)	n = 60	6.04 ± 1.6	(2–10)
Rescue analgesics (opioids)			
Opioids			
Pentazocine (mg)	n = 26; alone n = 24, with buprenorphine n = 1, with diclofenac n = 1	30.6 ± 27.3	(15–135)
Buprenorphine (μg)	n = 16; alone n = 14, with pentazocine n = 1, with diclofenac n = 1	450 ± 171	(200–800)
Petidine (mg)	n = 1	35	
NSAIDs			
Diclofenac (mg)	n = 15; alone n = 12, with pentazocine n = 1, with buprenorphine n = 1, with flurbiprofen n = 1	35 ± 22.8	(25–100)
Flurbiprofen (mg)	n = 8; alone n = 7, with diclofenac n = 1	62.5 ± 23.1	(50–100)

Data are expressed as mean ± SD (range).
NSAIDs: Nonsteroidal anti-inflammatory drugs, SD: Standard deviation.

requirements (p = 0.0008 and p = 0.0341, respectively), but other factors, including IVS2+G691C, IVS3+G5953A, IVS3+A8449G, TAA+A2109G, body weight, gender, duration of surgery, site of surgery and NRS pain score, did not have significant associations with 24-h postoperative opioid requirements (p > 0.05). Multivariate analysis employing the two dependent variables (age and the A118G SNP) revealed that both had significant associations with 24-h postoperative opioid requirements (age × A118G SNP genotype, p = 0.0231; age, p < 0.0001; A118G SNP genotype, p = 0.0085). When multiple testing corrections were applied, age and the A118G SNP also had significant associations with 24-h postoperative opioid requirements. Younger age was associated with greater 24-h postoperative opioid requirements (r = -0.280, p = 0.0009). Opioid requirements in the 24-h postoperative period were significantly greater in patients homozygous for the 118G allele (GG) than in patients homozygous for the 118A allele (AA; p = 0.0245, *post hoc* test) and in patients heterozygous for the 118A allele (AG; p = 0.0080, *post hoc* test).

Using a similar statistical analysis, age and the A118G SNP had significant associations with 24-h postoperative analgesic requirements (age × A118G SNP genotype, p = 0.0382; age, p < 0.0001; A118G SNP genotype, p = 0.0136). When multiple testing corrections were applied, age also had a significant association, and the A118G SNP tended toward an association with 24-h postoperative analgesic requirements. Younger age was associated with greater 24-h postoperative analgesic requirements (r = -0.312, p = 0.0002; Figure 2). Analgesic requirements in the 24-h postoperative period were significantly greater in patients homozygous for the 118G allele (GG) than in patients homozygous for the 118A allele (AA; p = 0.0167, *post hoc* test) and in patients heterozygous for the 118A allele (AG; p = 0.0059, *post hoc* test; Figure 3). Both association analyses with 24-h postoperative analgesic and opioid requirements showed similar results.

By contrast, none of the five SNPs, gender, age, body weight, duration of surgery, or site of surgery had a significant association with NRS pain scores (p > 0.05). Therefore, the A118G SNP genotype had a significant association with 24-h postoperative opioid and analgesic requirements, but not with NRS pain scores, indicating that 118G homozygous patients required significantly more opioids or analgesics compared with 118A homozygous or heterozygous patients to obtain a similar degree of pain relief.

Table 4. Distribution of the 5 tag SNP genotypes examined in the *OPRM1* gene.

SNP	Genotype
A118G	AA: 41 (29.7%)/AG: 70 (50.7%)/GG: 27 (19.6%)
IVS2+G691C	GG: 6 (4.3%)/GC: 45 (32.6%)/CC: 87 (63.0%)
IVS3+G5953A	GG: 112 (81.2%)/GA: 25 (18.1%)/AA: 1 (0.7%)
IVS3+A8449G	AA: 116 (84.1%)/AG: 21 (15.2%)/GG: 1 (0.7%)
TAA+A2109G	AA: 116 (84.1%)/AG: 21 (15.2%)/GG: 1 (0.7%)

Data are expressed as number (%) of subjects.

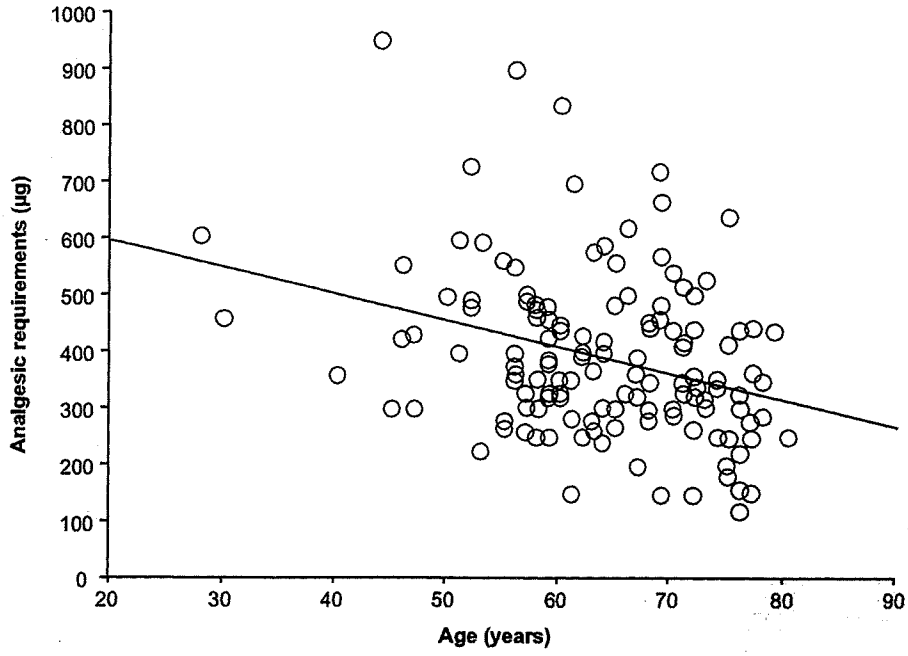

Figure 2. Association between age and 24-h postoperative analgesic requirements. Scatterplot of patient age versus 24 h postoperative analgesic requirements. Each data point represents one individual.

Statistical power analyses for the ANCOVA revealed that the expected power (1 minus Type II error probability) was 71% for Cohen's conventional 'medium' effect size of 0.25 when the sample size was 129.

We further analyzed the haplotype-based associations of the five *OPRM1* gene tag SNPs with 24-h postoperative opioid and analgesic requirements (Table 5). Logistic regression analyses revealed that age and *OPRM1* gene haplotype were significantly associated with 24 h postoperative opioid requirements (age × *OPRM1* gene haplotype, $p < 0.0001$; age, $p < 0.0016$; *OPRM1* gene haplotype, $p < 0.0001$) and analgesic requirements (age × *OPRM1* gene haplotype, $p < 0.0001$; age, $p < 0.0023$; *OPRM1* gene haplotype, $p < 0.0001$). In the seven estimated haplotypes, the G allele in the A118G SNP existed only in haplotype No. 1 (GCGAA), which was the most common haplotype (44.6 ± 2.9%). Both opioid and analgesic 24-h postoperative requirements were less in the other haplotypes when compared with the No. 1 GCGAA haplotype. Haplotypes No. 3 (AGAAA) and No. 5 (AGGAA) showed significantly less opioid and analgesic requirements than haplotype No. 1. These significant differences also were found in 24-h postoperative analgesic requirements after performing multiple testing corrections, with the exception of haplotype No. 3. Haplotype No. 4 (AGGGG) also tended to show less opioid and analgesic requirements than haplotype No. 1, although the differences did not reach statistical significance ($p < 0.1$ and $p < 0.1$, respectively; Table 5). Haplotypes No. 3 (AGAAA), No. 4 (AGGGG) and No. 5 (AGGAA), which all contain the A allele at the A118G SNP and the G allele at the IVS2+G691C SNP, showed significantly (or a trend toward significantly) less opioid and analgesic requirements than haplotype No. 1, a haplotype that contains a different allele at these positions.

Discussion

Our study used a group of patients who underwent major open abdominal surgery. Pain experienced by patients after such surgery can be severe and involve both somatic and visceral components [12,36]. The present data demonstrated that 24-h postoperative analgesic requirements varied according to the A118G SNP genotype in the *OPRM1* gene, the tag SNP representing the first LD block of the *OPRM1* gene [12,36]. 118G homozygous (GG) patients required significantly more analgesics compared with 118A homozygous (AA) and heterozygous (AG) patients to achieve a similar degree of pain relief, evaluated by NRS pain scores. Furthermore, our present report on haplotype-based association analyses in the *OPRM1* gene also showed a

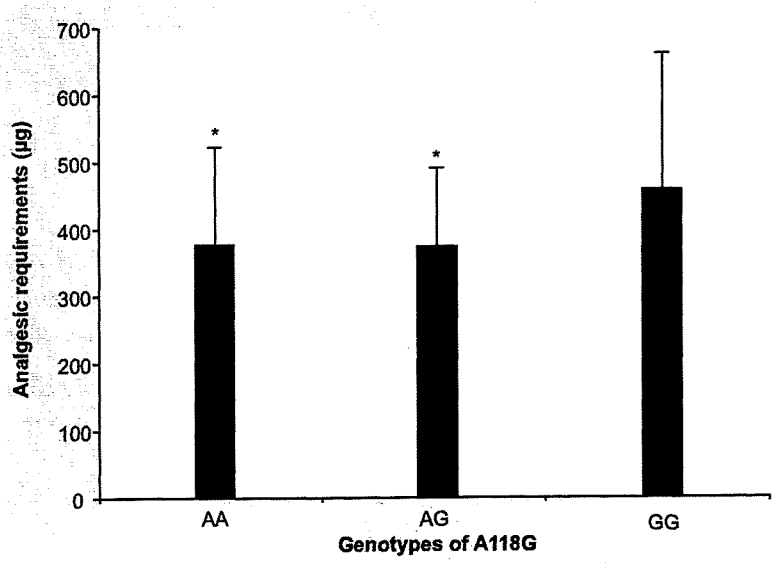

Figure 3. Association between A118G SNP and 24-h postoperative analgesic requirements. Averages of 24-h postoperative analgesic requirements in patients with the AA genotype (n = 41), AG genotype (n = 70) and GG genotype (n = 27). Data are expressed as mean ± standard deviation, *$p < 0.05$ versus patients with the GG genotype.

significant association with postoperative analgesic requirements. The haplotype containing the G allele at the A118G SNP showed higher analgesic requirements during the 24-h postoperative period than the other haplotypes. This report shows that haplotypes of *OPRM1* gene polymorphisms are more significantly associated with analgesic requirements in individual patients than the A118G SNP alone.

For genetic-association studies with pain or analgesic sensitivity, several points need to be carefully considered. The first consideration is pain modality. The present results reflect genetic differences in postoperative analgesic requirements after major open abdominal surgery and suggest that opioid effects are clinically relevant to the A118G SNP and are reduced in 118G homozygous patients. Our finding that 118G homozygous patients required more analgesic doses for pain relief is consistent with a previous study in patients with cancer pain [21]. Cancer pain varies according to various types and stages of the disease. In contrast, the present study used a model of acute postoperative pain, a more standardized pain model compared with cancer pain [2].

The second consideration is the analgesics employed and their administration routes. Absorption, metabolism and excretion all vary among analgesics and their administration routes. Although we used various analgesics, including relatively small amounts of nonopioid drugs as rescue analgesics, most of the analgesics used in the present retrograde study were drugs mainly acting on MOP [6]. All subjects in the present study received epidural morphine or fentanyl. To our knowledge, all genetic-association studies of opioid sensitivity have used patients who received systemic opioid administration, with the exception of one study by Landau *et al.* who reported that the 304G variant at the A304G SNP in the *OPRM1* gene significantly reduced intrathecal fentanyl requirements [42]. The various analgesics used and the various routes of administration may be important limitations of the present study.

The third consideration is subject demography. Although gender-related differences in pain sensitivity have been reported [43], analgesic requirements were not associated with gender, but rather associated with age in our present analyses. Ethnic differences may also play a role. Further analyses considering these three points are necessary to confirm the present findings.

Several recent studies have investigated the association between the A118G *OPRM1* SNP and postoperative morphine requirements in surgical patients, but have yielded inconsistent results [19,20,22,23]. Chou *et al.* found that out of 120 Taiwanese patients who underwent total knee arthroplasty, 118G homozygous (GG) patients received significantly more morphine doses than 118A homozygous (AA) and heterozygous (AG) patients during the first 48 h after surgery [19]. The same group of investigators also found in 80 Taiwanese patients after total abdominal hysterectomy that 118G homozygous (GG) patients required significantly more morphine doses than 118A homozygous (AA) patients during the first 24-h postoperative period, and 24-h postoperative morphine doses in heterozygous (AG) patients were intermediate between those in 118G and 118A homozygotes [20]. The results of the present study are generally consistent with these two previous studies. In contrast, Coulbault *et al.* failed to find a significant association between the A118G SNP and 24-h postoperative morphine requirements in 74 French patients after colorectal surgery [22]. Similarly, Janicki *et al.* failed to find a significant association between the A118G SNP and postoperative morphine requirements in 101 subjects in a postanesthetic care unit (95 Caucasian–Americans, two African–Americans, and four Hispanic–Americans) who underwent laparoscopic procedures [23]. The results of our study are clearly inconsistent with the studies by Coulbault *et al.* and Janicki *et al.*

Table 5. Association between *OPRM1* gene tag SNP haplotype and 24-h postoperative opioid and analgesic requirements.

	Estimated haplotypes	Frequency (SE)	Opioid requirements			Analgesic requirements		
			Coefficient (SE)	*Z-score*	*p-value*	*Coefficient (SE)*	*Z-score*	*p-value*
1	GCGAA	0.446 (0.029)						
2	ACGAA	0.330 (0.028)	-220.3 (133.6)	-1.65	0.099	-207.9 (139.1)	-1.49	0.135
3	AGAAA	0.094 (0.017)	-459.3 (180.6)	-2.54	0.011	-397.0 (185.7)	-2.14	0.033
4	AGGGG	0.069 (0.016)	-290.9 (176.3)	-1.65	0.099	-315.1 (179.4)	-1.76	0.079
5	AGGAA	0.029 (0.010)	-653.8 (205.6)	-3.18	0.001	-689.6 (209.3)	-3.29	0.001
6	ACGGA	0.015 (0.007)	-301.5 (351.9)	-0.86	0.392	-318.3 (370.6)	-0.86	0.390
7	AGGAG	0.015 (0.008)	-646.1 (850.1)	-0.76	0.447	-678.9 (872.8)	-0.78	0.437

Haplotype-based associations were analyzed by comparing haplotypes with the most common haplotype No. 1 (GCGAA).
SE: Standard error.

The discrepancies between the aforementioned studies and the present study may be explained by ethnic differences in the frequency of the A118G variant. The frequency of the 118G allele ranges from 5 to 15% in Caucasian–American and African–American populations [2]. In the studies by Coulbault *et al.* and Janicki *et al.*, the 118G allelic frequencies were 12 and 16%, respectively, and their studies included only two and one 118G homozygous patients, respectively [19,20,22,23]. The limited number of 118G homozygous patients in these previous studies should have precluded reliable statistical analysis regarding the effect of the A118G SNP on postoperative morphine requirements. However, the 118G allelic frequency in Asian populations ranges from 35 (Chinese) to 47% (Indian) [44], and the 118G allelic frequency in a Taiwanese population in the two studies by Chou *et al.* were 34 and 25%, respectively [19,20,22,23]. The greater number of 118G homozygous patients included in these studies (n = 18 and n = 13, respectively) may have allowed more reliable statistical analysis of the influence of the A118G SNP on postoperative morphine requirements. In the present study, in a Japanese population, the genotype frequency of the A118G SNP was 29.7% (41/138) for AA, 50.7% (70/138) for AG and 19.6% (27/138) for GG, with much higher G allelic frequency (44.9%) compared with previous studies. Clearly, such genotype distributions in the Japanese population enabled us to more reliably analyze the influence of the A118G SNP. The results from the present study, together with the results from previous studies [19–21], strongly suggest that opioid analgesics were less effective in subjects with the G allele of the A118G *OPRM1* SNP than in subjects with the A allele. Therefore, subjects with the G allele required more opioid or analgesic doses than subjects with the A allele to achieve similar degrees of pain relief.

The A118G substitution leads to an amino acid substitution of asparagine at position 40 with aspartate (Asn40Asp), the putative *N*-glycosylation site in the human MOP protein. This 40Asp resulted in a threefold increase in β-endorphin binding affinity *in vitro* compared with wild-type (40Asn) receptors [13]. Others have reported that this substitution caused differences in levels of binding capacity, forskolin-induced cyclic adenosine monophosphate (cAMP) accumulation and agonist-induced cAMP accumulation induced by several opioid agonists in *in vitro* assays [45]. Although some contradictory data have been reported [46], such alterations in ligand binding affinity may result in alterations in the analgesic efficacy of opioids [19]. Recently, Zhang *et al.* showed that in human autopsy brain tissues, *OPRM1* mRNA expression from the 118G allele was 1.5–2.5-fold less abundant than that from the 118A allele [10]. They also showed that transfection into Chinese hamster ovary cells of a cDNA representing the coding region of *OPRM1* carrying adenosine, guanosine, cytidine and thymidine in position 118 resulted in 1.5-fold lower mRNA levels only for *OPRM1-118G*, and more than tenfold lower *OPRM1* protein levels [10]. Such dramatic effects of an *OPRM1-118G* variant on both mRNA and protein yield may clearly explain why subjects with the G allele required more opioids than the other subjects for similar degrees of pain relief after major open abdominal surgery. Furthermore, these assumptions appear to be consistent with observations in mice that the

24 Hayashida M, Imamura S: Current status of portable disposable infusers. *Masui* 55, 1118–1127 (2006).

25 Loper KA, Ready LB, Downey M *et al.*: Epidural and intravenous fentanyl infusions are clinically equivalent after knee surgery. *Anesth. Analg.* 70, 72–75 (1990).

26 Guinard JP, Carpenter RL, Chassot PG: Epidural and intravenous fentanyl produce equivalent effects during major surgery. *Anesthesiology* 82, 377–382 (1995).

27 Pathak KS, Brown RH, Nash Jr CL, Cascorbi HF: Continuous opioid infusion for scoliosis fusion surgery. *Anesth. Analg.* 62, 841–845 (1983).

28 Klinck JR, Lindop MJ: Epidural morphine in the elderly: a controlled trial after upper abdominal surgery. *Anaesthesia* 37, 907–912 (1982).

29 Hoskin PJ, Hanks GW: Opioid agonist-antagonist drugs in acute and chronic pain states. *Drugs* 41, 326–344 (1991).

30 Bahar M, Rosen M, Vickers MD: Self-administered nalbuphine, morphine and pethidine: comparison, by intravenous route, following cholecystectomy. *Anaesthesia* 40, 529–532 (1985).

31 Buchanan WW, Kassam YB: European experience with flurbiprofen: a new analgesic/anti-inflammatory agent. *Am. J. Med.* 80, 145–152 (1986).

32 Sunshine S, Olson NZ: Nonnarcotic analgesics. In: *Textbook of Pain.* Wall PD, Melzack R (Eds), Churchill Livingstone, Edinburgh, UK, 923–942 (1994).

33 Basar H, Yilmaz E, Ozcan S *et al.*: Four analgesic techniques for shockwave lithotripsy: eutectic mixture local anesthetic is a good alternative. *J. Endourol.* 17, 3–6 (2003).

34 Ozcan S, Yilmaz E, Buyukkocak U, Basar H, Apan A: Comparison of three analgesics for extracorporeal shock wave lithotripsy. *Scand. J. Urol. Nephrol.* 36, 281–285 (2002).

35 Zhang K, Jin L: HaploBlockFinder: haplotype block analyses. *Bioinformatics* 19, 1300–1301 (2003).

36 Imai K, Ogai Y, Nishizawa D, Kasai S, Ikeda K, Koga H: A novel SNP detection technique utilizing a multiple primer extension (MPEX) on a phospholipid polymer-coated surface. *Mol. Biosyst.* 3, 547–553 (2007).

37 Li J, Ji L: Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity* 95, 221–227 (2005).

38 Nyholt DR: A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am. J. Hum. Genet.* 74, 765–769 (2004).

39 Faul F, Erdfelder E, Lang AG, Buchner A: G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 39, 175–191 (2007).

40 Zhao LP, Li SS, Khalid N: A method for the assessment of disease associations with single-nucleotide polymorphism haplotypes and environmental variables in case-control studies. *Am. J. Hum. Genet.* 72, 1231–1250 (2003).

41 Benjamini Y, Hochberg Y: Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Statist. Soc. Ser. B* 57, 289–300 (1995).

42 Landau R, Kern C, Columb MO, Smiley RM, Blouin JL: Genetic variability of the μ-opioid receptor influences intrathecal fentanyl analgesia requirements in laboring women. *Pain* 139(1), 5–14 (2008).

43 Fillingim RB: Sex, gender, and pain: women and men really are different. *Curr. Rev. Pain* 4, 24–30 (2000).

44 Tan EC, Tan CH, Karupathivan U, Yap EP: μ opioid receptor gene polymorphisms and heroin dependence in Asian populations. *Neuroreport* 14, 569–572 (2003).

45 Kroslak T, LaForge KS, Gianotti RJ, Ho A, Nielsen DA, Kreek MJ: The single nucleotide polymorphism A118G alters functional properties of the human μ opioid receptor. *J. Neurochem.* 103, 77–87 (2007).

46 Beyer A, Koch T, Schroder H, Schulz S, Hollt V: Effect of the A118G polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human μ-opioid receptor. *J. Neurochem.* 89, 553–560 (2004).

Websites

101 HaploBlockFinder http://cgi.uc.edu/cgi-bin/kzhang/haploBlockFinder.cgi

102 SNPSpD Web interface program http://genepi.qimr.edu.au/general/daleN/SNPSpD/

103 The R Foundation for Statistical Computing www.r-project.org/

Association of morphine-induced antinociception with variations in the 5′ flanking and 3′ untranslated regions of the μ opioid receptor gene in 10 inbred mouse strains

Yoshihiro Shigeta[a,b,*], Shinya Kasai[a,*], Wenhua Han[a], Harumi Hata[a], Akinori Nishi[a,c], Yukio Takamatsu[a], Yoko Hagino[a], Hideko Yamamoto[a], Tsuyoshi Koide[c], Toshihiko Shiroishi[d], Kiyoto Kasai[b], Koichi Tsunashima[b], Nobumasa Kato[b] and Kazutaka Ikeda[a]

Objective **Genetic factors are hypothesized to be involved in interindividual differences in opioid sensitivity. Inbred mouse strains that are genetically different and isogenic within each strain are useful for elucidating the genetic mechanisms underlying the interindividual differences in opioid-induced analgesia.**

Methods **We examined the effects of morphine in 10 inbred mouse strains, including wild-derived strains that have a wide range of genetic diversity, including BLG2, CHD, KJR, MSM, NJL, PGN2, and SWN. We also performed full sequencing of the 5′ flanking region and exons of the mouse μ opioid receptor gene *Oprm1* and analyzed the association between genotypes and phenotypes in these mice.**

Results **The effects of morphine on locomotor activation and antinociception varied among the inbred strains. The nucleotide differences that cause amino acid substitutions were not found in the *Oprm1* gene in the inbred strains analyzed in this study. In the 5′ flanking region and 3′ untranslated region of the *Oprm1* gene, four highly variable regions containing novel short tandem repeat polymorphisms (GA, T, TA, and CA/CT) were identified. The GA, T, and TA repeat numbers were significantly associated with morphine-induced antinociception.**

Conclusion **These results suggest that the short tandem repeats in the 5′ flanking and 3′ untranslated regions of the μ opioid receptor gene are involved in interstrain differences in opioid sensitivity in mice. Wild-derived inbred mouse strains with different numbers of these repeats may be useful models for examining interindividual differences in opioid sensitivity. *Pharmacogenetics and Genomics* 18:927–936 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Pharmacogenetics and Genomics 2008, 18:927–936

Keywords: μ opioid receptor gene, antinociception, inbred mice, interindividual differences, morphine

[a]Division of Psychobiology, Tokyo Institute of Psychiatry, [b]Department of Neuropsychiatry, Graduate School of Medicine, The University of Tokyo, Tokyo, [c]Mouse Genomics Resource Laboratory and [d]Mammalian Genetics Laboratory, National Institute of Genetics, Mishima, Japan

Present address: Harumi Hata, Department of Public Health, Showa University School of Medicine, Shinagawa-ku, Koichi Tsunashima, Department of Psychiatry, Juntendo University School of Medicine, Bunkyo-ku, Nobumasa Kato, Department of Psychiatry, Showa University School of Medicine, Setagaya-ku, Tokyo, Akinori Nishi, Tsukuba Research Institute, Banyu Pharmaceutical Company, Ltd., Tsukuba, Ibaraki, Japan.

Correspondence to Kazutaka Ikeda, Division of Psychobiology, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan
Tel: +81 3 3304 5701 x508; fax: +81 3 3329 8035;
e-mail: ikedak@prit.go.jp

*Yoshihiro Shigeta and Shinya Kasai equally contributed to this study.

Received 25 February 2008 Accepted 17 June 2008

Introduction

Opioid drugs have been widely used to alleviate a variety of pain, such as pain following general surgery and pain experienced in terminal cancer. Large interindividual differences, however, have been observed in opioid sensitivity, which hamper appropriate pain treatment [1]. Interindividual differences in opioid sensitivity are attributable to multiple and interacting psychological, environmental, and genetic factors.

Environmental and genetic factors mainly cause the variability within and between inbred animals, respectively, because inbred animals are isogenic within each strain, barring the very rare occurrence of a new mutation [2]. Therefore, the use of inbred animals has been an effective strategy for investigating the heritability of traits associated with interindividual differences in opioid sensitivity. To date, various inbred and recombinant inbred mouse strains have been established, and interstrain differences among laboratory mice, such as BALB/c, C3H/HeJ, and C57BL/6, have been reported in opioid sensitivity, including antinociception [3–6], reward [7], antinociceptive tolerance [8], and physical dependence [9]. A Mishima battery of inbred mouse strains,

1744-6872 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins

DOI: 10.1097/FPC.0b013e32830d0b9e

including wild mice captured in various countries around the world, has shown high variations in thermal stimuli sensitivity, capsaicin intake, locomotor activity, and open-field behaviors, suggesting that this mouse battery may be useful for analyzing interstrain differences [10–13].

Opioids, including morphine and fentanyl, exert their activities by interacting with three types of opioid receptors, designated μ, κ, and δ [14]. Many pharmacological studies using genetically modified mouse strains have demonstrated that the μ opioid receptor (MOP) is a preferred target of morphine and that it seems to play a mandatory role in mediating the major clinical effects of morphine, including antinociception, tolerance, and dependence [15]. For example, homozygous MOP knockout mice are completely insensitive to morphine [16–18]. Furthermore, heterozygous MOP knockout mice possessing approximately one-half the amount of MOP protein in wild-type mice show a reduced but significant sensitivity to morphine, indicating that MOP expression levels influence the sensitivity to morphine in a manner dependent on the gene copy number [17,18].

The mouse MOP gene *Oprm1* spans over 120 kilobases (kb) and consists of multiple exons [19]. Numerous isoforms of *Oprm1* have been found. Among these isoforms, *MOR-1* is the most abundant transcript, containing a long 3′ untranslated region (UTR) of over 10 kb [19–21]. In the human *OPRM1* gene, more than 250 gene polymorphisms have been identified, and a few of them have been studied extensively considering their association with opioid efficacy and dependence [22–24]. Polymorphisms such as single nucleotide polymorphisms and short tandem repeats (STRs) in the mouse *Oprm1* gene may contribute to interstrain differences in morphine sensitivity. Thus, we hypothesized that inbred mouse strains with these polymorphisms may be useful tools for investigating the genetic mechanisms underlying human interindividual differences in opioid sensitivity.

In this study, we identified novel STR polymorphisms in the 5′ flanking and UTR regions of the mouse *Oprm1* gene in the Mishima mouse battery that includes wild-derived inbred mouse strains and analyzed the correlation between these STR polymorphisms and the effects of morphine.

Materials and methods

Animals

Ten inbred strains of mice were used in this study. Seven strains, BLG2 (BLG), CHD, KJR, MSM, NJL, PGN2 (PGN), and SWN were originally established as inbred strains from wild mice after 20 generations of full-sibling mating at the National Institute of Genetics (Mishima, Japan). The JF1 (JF) strain also was established from fancy mice at the National Institute of Genetics. As the coat color *s* gene possessed by the JF strain is known to relate to auditory disability, we used a spontaneous revertant at this allele with a black coat color, JF-s^+. The BFM/2 (BFM) strain was established at the National Institute of Genetics from the original stock of BFM mice that was kept at F12 in 1980 [25]. The C57BL/6 (B6) strain was obtained from Jackson Laboratories (Bar Harbor, Maine, USA). These inbred strains were derived from many countries throughout the world. *Mus musculus* is genetically divided into four subspecies: *bactrianus*, *castaneus*, *domesticus*, and *musculus* [26]. The B6, BFM, and PGN strains belong to the *Mus musculus domesticus* subspecies, and the others belong to the *Mus musculus musculus* subspecies [11]. All strains were maintained at the National Institute of Genetics in a temperature-controlled room at 23 ± 2°C under a 12 h light/12 h dark cycle (lights on 08:00–20:00 h) and had ad-libitum access to a standard laboratory diet and water. Throughout this study, 8–12-week-old males of each strain were used. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the National Institute of Genetics.

Behavioral tests

The effects of morphine on locomotor activity were examined in the open-field test. The antinociceptive effects of morphine also were examined using the tail-flick and hot-plate tests, which were slightly modified from the original methods developed by D'Amour and Smith [27] and Woolfe and MacDonald [28], respectively, as described earlier [29]. All behavioral tests were performed from 09:00 to 18:00 h. Morphine hydrochloride solution (10 mg/ml; Shionogi & Co. Ltd, Osaka, Japan) was diluted to 1 mg/ml with sterile saline on each experimental day. Morphine was administered intraperitoneally (i.p.) at a dose of 10 mg/kg. Saline was injected i.p. into control mice from each strain to control for ambulation scores and latencies. The number of mice used for morphine and saline injection, respectively, was the following: B6 ($n = 16$ and 14), BFM ($n = 11$ and 10), BLG ($n = 10$ and 10), CHD ($n = 11$ and 10), JF ($n = 11$ and 11), KJR ($n = 13$ and 13), MSM ($n = 12$ and 10), NJL ($n = 12$ and 11), PGN ($n = 10$ and 10), and SWN ($n = 11$ and 11). The open-field test was performed 10 min after morphine or saline injection (10 ml/kg), followed by the hot-plate and tail-flick tests.

In the open-field test, mice were monitored continuously using the infrared sensor system SCANET MV-20 plus (MELQUEST Co. Ltd, Toyama, Japan) during the light period to measure general locomotor activity. Each mouse was gently lifted by its tail with tweezers and placed in the center of the open-field arena that consisted of a transparent acrylic cage (W × D × H: 460 × 460 × 303 mm) with two infrared sensors that registered horizontal and vertical movements. Locomotor activity was recorded for