180 s without habituation to yield an ambulation score. Locomotor hyperactivity induced by morphine (10 mg/kg, i.p.) was expressed as the percentage increase in ambulation score compared with control mice (saline injection) and was calculated as follows: percentage increase = 100% × {[(ambulation score with morphine injection) − (ambulation score with saline injection)]/(ambulation score with saline injection)}. The average ambulation score in control mice was applied to the value of 'ambulation score with saline injection' for each mouse strain. In the hot-plate test, mice were placed on the hot-plate apparatus (Model MK-350B, Muromachi Kikai Co. Tokyo, Japan) that was maintained at 52 ± 0.2°C, and the latency to lick the hindpaws in response to the heat stimulus was measured with a 180 s cutoff time to minimize tissue damage. In the tail-flick test, the mice were loosely wrapped in a velvet towel and placed on the tail-flick apparatus (Model MK-330B, Muromachi Kikai Co. Tokyo, Japan). A light beam was focused on the tail approximately 1–3 cm from the base, and the latency to flick the tail vigorously in response to the heat stimulus was measured with a 15 s cutoff time to minimize tissue damage. Tail-flick latencies were measured three times per mouse with different light beam foci, and the average was considered the latency. The antinociceptive effect of morphine was expressed as a percentage of the maximal possible effect (%MPE) and was calculated as follows: %MPE = 100% × {[(latency with morphine injection) − (latency with saline injection)]/[(cut-off time) − (latency with saline injection)]}. The average latency in control mice was applied to the value of 'latency with saline injection' for each mouse strain.

Sequence analysis

Genomic DNAs were prepared from the tail or liver of each mouse strain and subjected to polymerase chain reaction (PCR) amplification of the *Oprm1* gene. The coding and noncoding region of the *Oprm1* gene was amplified by KOD dash DNA polymerase (Toyobo, Osaka, Japan) under the following conditions: 94°C for 5 min, 25 cycles at 94°C for 1 min, 60 or 65°C for 1 min, 74°C for 3 min, followed by 74°C for 7 min. These PCR products were subjected to PCR for sequencing by using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA). The PCR conditions were as follows: 96°C for 1 min, 25 or 30 cycles at 96°C for 10 s, 50 or 55°C for 5 s, followed by 60°C for 4 min. The PCR products for sequencing were purified using the QIAquick PCR Purification Kit and then sequenced with a PRISM 3100 genetic analyzer (Applied Biosystems). DNASTAR v.7.0 (DNAstar, Madison, Wisconsin, USA) was used to analyze and assemble nucleotide sequences. The nucleotide sequences in the 5′ flanking region and 5′ UTR (8.5 kbp), the coding region (1197 bp), and the 3′ UTR (10178 bp) of the *Oprm1* gene in nine strains were compared with those in the B6 strain (which was the only laboratory strain used in this study). The difference in total number of nucleotides per 100 nucleotides in the nine strains compared with the B6 strain was calculated and used as an index for nucleotide differences among the 10 inbred strains.

Statistical analyses

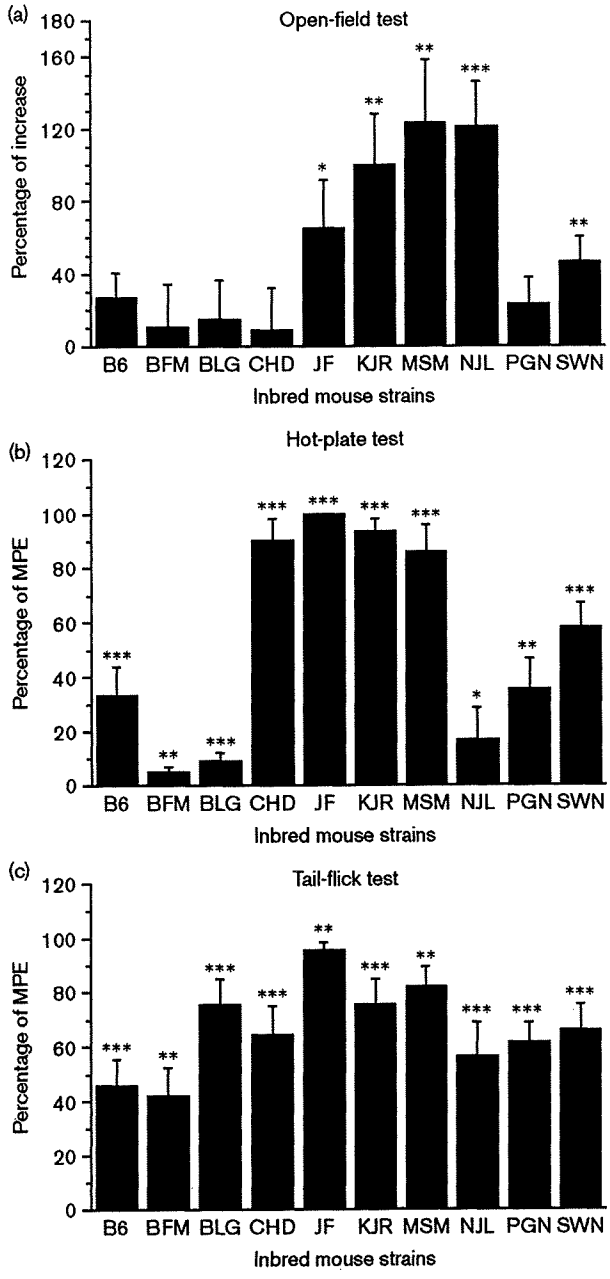
All statistical analyses were performed using StatView software (SAS Institute Inc., Cary, North Carolina, USA). The antinociceptive effect and locomotor hyperactivity induced by morphine were statistically evaluated using the Mann–Whitney unpaired nonparametric *U*-test for each mouse strain. We also examined differences among mouse strains in the percentage increase in ambulation score and the %MPE of antinociception induced by morphine using Kruskal–Wallis nonparametric analysis of variance (ANOVA) followed by the Games–Howell post-hoc test. Spearman's nonparametric rank correlations were calculated between repeat number and the behavioral data (percentage increase in ambulation score induced by morphine and %MPE of antinociception induced by morphine). In the haplotype analyses, repeat numbers of each STR were classified into long (L) and short (S) repeat length groups. Differences in the percentage increase in ambulation score and the %MPE of antinociception induced by morphine were analyzed among the haplotype groups consisting of the GA, T, and TA STRs using Kruskal–Wallis nonparametric ANOVA followed by the Games–Howell post-hoc test. $P < 0.05$ was considered statistically significant.

Results

In this study, we used 10 inbred mouse strains derived from fancy (JF), laboratory (B6), and wild mice (BFM, BLG, CHD, KJR, MSM, NJL, PGN, and SWN). The effects of morphine on locomotor activity were examined in these inbred strains in the open-field test (Fig. 1a). Locomotor activity was significantly higher in the morphine-treated group than in the saline-treated group in the JF ($P < 0.05$), KJR, MSM, SWN ($P < 0.01$), and NJL ($P < 0.001$) strains, but not in the B6, BFM, BLG, CHD, and PGN strains (Fig. 1a, Mann–Whitney *U*-test). Kruskal–Wallis nonparametric ANOVA revealed no significant differences in percentage increase in locomotor activity among the strains tested.

The antinociceptive effects of morphine were examined in the hot-plate and tail-flick tests (Fig. 1b and c, respectively). In the hot-plate test, the response latency was significantly longer in the morphine-treated group than in the saline-treated group in all of the strains tested [$P < 0.05$ (NJL), $P < 0.01$ (BFM and PGN), $P < 0.001$ (B6, BLG, CHD, JF, KJR, MSM, and SWN); Mann–Whitney *U*-test]. Kruskal–Wallis nonparametric ANOVA revealed significant differences in the %MPE of morphine-induced antinociception among the strains tested

Fig. 1



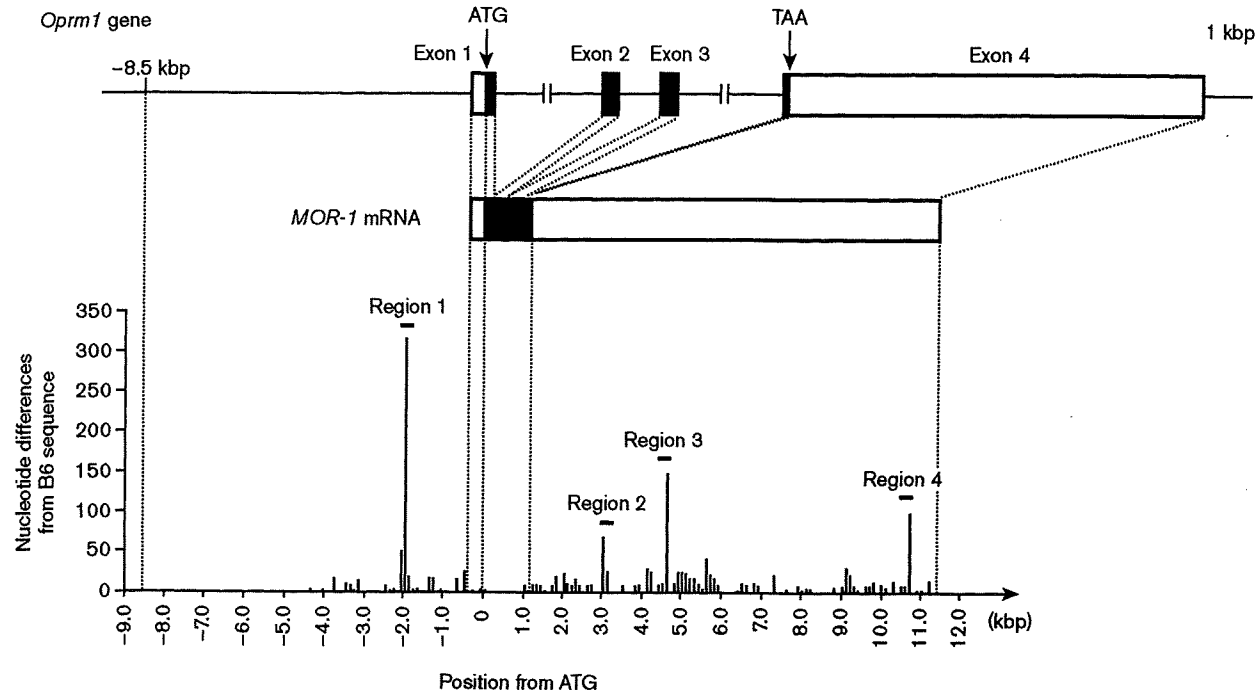
Variations in the effects of morphine in 10 inbred mouse strains, including wild-derived strains. Locomotor hyperactivity induced by morphine [10 mg/kg, intraperitoneally (i.p.)] was examined in the open-field test (a) in 10 inbred mouse strains derived from fancy (JF), laboratory (B6), and wild mice (BFM, BLG, CHD, KJR, MSM, NJL, PGN, and SWN). The effects of morphine are expressed as percentage increase in ambulation scores compared with control mice injected with saline. The antinociceptive effects induced by morphine (10 mg/kg, i.p.) were examined in the hot-plate (b) and tail-flick (c) tests. Antinociceptive effects are expressed as percentages of the maximal possible effects (%MPEs) of morphine. Each bar represents the mean ± SEM (n = 10–16). Asterisks indicate statistically significant differences between saline-treated and morphine-treated groups in each mouse strain (*P<0.05, **P<0.01, and ***P<0.001).

($P < 0.001$). The differences in morphine %MPE were statistically significant between mouse strains: B6-MSM, CHD-PGN, and JF-SWN ($P < 0.05$, Games–Howell post-hoc test); B6-CHD, B6-JF, B6-KJR, BFM-CHD, BFM-JF, BFM-KJR, BFM-MSM, BFM-SWN, BLG-CHD, BLG-JF, BLG-KJR, BLG-MSM, BLG-SWN, CHD-NJL, JF-NJL, JF-PGN, KJR-NJL, KJR-PGN, and MSM-NJL ($P < 0.001$). In the tail-flick test, response latencies were significantly longer in the morphine-treated group than in the saline-treated group in all of the strains tested [$P < 0.01$ (BFM, JF, and MSM), $P < 0.001$ (B6, BLG, CHD, KJR, NJL, PGN, and SWN); Mann–Whitney U-test]. Kruskal–Wallis nonparametric ANOVA revealed significant differences in the %MPE of morphine-induced antinociception among the strains tested ($P < 0.001$). The %MPE of morphine-induced antinociception was significantly different between B6 and JF, BFM and JF, and JF and PGN strains [$P < 0.05$ (JF-PGN), $P < 0.01$ (B6-JF and BFM-JF); Games–Howell post-hoc test]. These data indicated that the antinociceptive effects of morphine were highly variable among the 10 inbred strains derived from wild mice.

Among the mice used in this study, the B6, BFM, and PGN strains belong to the *Mus musculus domesticus* subspecies, and the others belong to the *Mus musculus musculus* subspecies [11]. The nucleotide sequences of the *Oprm1* gene were found to be similar within subspecies groups (Table 2). The percentage increase in locomotor activity was significantly different between the *domesticus* and *musculus* groups ($P = 0.0015$, Mann–Whitney U-test, data not shown). In the hot-plate and tail-flick tests, the %MPEs of morphine-induced antinociception also were significantly different between the *domesticus* and *musculus* groups ($P < 0.001$, Mann–Whitney U-test, data not shown). All of the *domesticus* strains exhibited low or intermediate morphine sensitivity, whereas most of the *musculus* strains showed high morphine sensitivity (Fig. 1), indicating that *Mus musculus musculus* strains are more sensitive to morphine than *Mus musculus domesticus* strains. Therefore, the Mishima battery of inbred mice, including both *domesticus* and *musculus* strains with wide variability in opioid sensitivity, may be useful for analyzing the genetic mechanisms underlying interstrain differences in opioid sensitivity and possibly various other behavioral and drug responses.

The mouse *Oprm1* gene consists of 14 exons that combine to yield 15 isoforms [19]. Among these isoforms, *MOR-1* is a major form that is encoded by exons 1–4 of the *Oprm1* gene (Fig. 2). We identified and compared the nucleotide sequences of the 5′ flanking region adjacent to *Oprm1* exon 1 and exons 1–4 by sequencing in the 10 strains. The total numbers of nucleotide differences among the 10 strains were 527 in the 5′ flanking region and 5′ UTR, nine in the coding region, and 992 in the 3′ UTR. The

Fig. 2



Nucleotide sequence variations in the *Oprm1* gene among the 10 inbred mouse strains. *MOR-1* mRNA was transcribed from four exons (exons 1–4) of the *Oprm1* gene. Nucleotide sequences were identified in the 5′ flanking region and 5′ untranslated region (UTR) [−8.5 kb to −1 from antithymocyte globulin (ATG)], coding region (1197 bp), and 3′ UTR (10178 bp) of the *Oprm1* gene in the 10 inbred mouse strains. The total numbers of nucleotide differences from the B6 sequence in nine inbred mouse strains (BFM, BLG, CHD, JF, KJR, MSM, NJL, PGN, and SWN) were counted in 100 bases. Four high-variability regions (regions 1–4) were identified in the 5′ flanking region and 3′ UTR of the *Oprm1* gene.

ratio of nucleotide differences per 100 nucleotides was 6.20 (527/8500) in the 5′ flanking region and 5′ UTR, 0.75 (9/1197) in the coding region, and 9.75 (992/10178) in the 3′ UTR, indicating the higher variability of nucleotide sequences in the 5′ flanking region and UTR of the *Oprm1* gene compared with the coding region. In the coding region of the *Oprm1* gene, nucleotides were different among the 10 strains at nucleotide positions 24 (G or A), 1071 (A or G), and 1179 (A or G) from the ATG translation initiation codon, which do not cause amino acid substitution (Table 1). In contrast, in four regions at the 5′ flanking region and 3′ UTR of the *Oprm1* gene (region 1–4), total nucleotide differences were greater than in the other regions (Fig. 2). In region 1 of the 5′ flanking region of the *Oprm1* gene (−6501 to −6600 bp from ATG in the B6 *Oprm1* gene sequence), 319 total nucleotides were different among the 10 strains and mainly resulted from GA STR variation (Fig. 2 and Table 2). The GA polymorphic STR was located from −6499 to −6572 bp from ATG in the B6 *Oprm1* gene and ranged between 14 and 36 repeats among the 10 strains (Table 2). Region 2 (1801–1900 bp from TAA in the B6 *Oprm1* gene sequence), region 3 (3401–3500 bp from TAA), and region 4 (9501–9600 bp from TAA) in the 3′ UTR of the *Oprm1* gene contained T, TA, and CA/CT STRs in the range of 20–39, 7–32, 9–17/26–43 repeats, respectively, that produced high variations in nucleotide differences in each region (68, 148, and 99 nucleotide differences in regions 2, 3, and 4, respectively; Fig. 2 and Table 2). In the PGN mouse strain, the nucleotide sequence of the *Oprm1* 3′ UTR was identical to the B6 strain (Table 2).

Next, correlations between each polymorphic STR and morphine effects (i.e. the percentage increase in locomotor activity and %MPEs in the hot-plate and

Table 1 Number of nucleotide differences and amino acid substitutions in the coding region of the *Oprm1* gene among 10 inbred mouse strains compared with the B6 strain

Strains	Nucleotide differences (positions)	Amino acid substitutions
B6	–	–
BFM/2	2 (G24A, A1179G)	0
BLG2	1 (A1071G)	0
CHD	1 (A1071G)	0
JF1	1 (A1071G)	0
KJR	1 (A1071G)	0
MSM	1 (A1071G)	0
NJL	1 (A1071G)	0
PGN2	0	0
SWN	1 (A1071G)	0

Table 2 Number of nucleotide differences and repeat numbers in the 5′ flanking region, 5′ UTR, and 3′ UTR of the *Oprm1* gene in 10 inbred mouse strains compared with the B6 mouse strain

	5′ flanking region and 5′ UTR		3′ UTR				
Strains	GA repeat (region 1)	Others	T repeat (region 2)	TA repeat (region 3)	CA repeat (region 4)	CT repeat (region 4)	Others
B6	– (36 repeat)	–	– (26 repeat)	– (8 repeat)	– (13 repeat)	– (33 repeat)	–
BFM/2	18 (28)	12	1 (27)	2 (7)	8 (17)	14 (26)	52
BLG2	45 (14)	20	13 (39)	52 (32)	7 (9)	5 (31)	94
CHD	38 (18)	27	6 (20)	2 (9)	6 (10)	21 (43)	82
JF1	41 (16)	17	13 (39)	6 (11)	2 (12)	1 (33)	99
KJR	45 (14)	18	2 (28)	28 (22)	2 (14)	15 (40)	94
MSM	41 (16)	17	13 (39)	8 (12)	0 (13)	0 (33)	96
NJL	41 (16)	40	6 (20)	2 (9)	2 (14)	15 (40)	86
PGN2	25 (23)	19	0 (26)	0 (8)	0 (13)	0 (33)	0
SWN	43 (15)	20	11 (37)	32 (24)	0 (13)	1 (33)	93

UTR, untranslated region.
Data are expressed as number of nucleotide differences (repeat numbers) from the nucleotide sequences of the B6 *Oprm1* gene.

tail-flick tests) were examined. A significant inverse correlation was found between GA repeat number and the %MPE of morphine-induced antinociception in the tail-flick test (Spearman's correlation coefficient: $r = -0.689$, $P = 0.027$; Fig. 3a). In contrast, the T and TA repeat number variations were proportionally correlated with the %MPE of morphine-induced antinociception in the tail-flick test (Spearman's correlation coefficient, respectively: $r = 0.735$, $P = 0.016$, Fig. 3b; $r = 0.738$, $P = 0.015$, Fig. 3c). The other STRs were not significantly correlated with any morphine effects (data not shown). Additionally, no STRs were significantly correlated with the percentage increase or %MPE in the hot-plate test (data not shown).

We also carried out statistical analyses for the association between the effects of morphine and the haplotypes consisting of the GA, T, and TA STRs, which were associated with the antinociceptive effects of morphine. Repeat numbers of each STR were classified into long (L) and short (S) repeat length groups for the haplotype association study. In the case of the GA STR, 10 mouse strains were divided into two groups whose GA STRs consisted of greater than or equal to 20 repeats (L group) or lesser than 20 repeats (S group). Similarly, the mouse strains were divided into L ($\geq$ 30 and 20 repeats) and S groups ($<$ 30 and 20 repeats) for the T and TA repeats, respectively. We examined significant differences among haplotype groups in the percentage increase in ambulation score and the %MPE of antinociception induced by morphine using Kruskal–Wallis nonparametric ANOVA. The %MPE in the tail-flick test was significantly different among the GA-T and T-TA STR haplotype groups ($P = 0.027$ and $P = 0.019$, respectively), whereas no significant difference was observed among the GA-TA STR haplotype groups ($P = 0.088$). The other effects of morphine, such as percentage increase in the open-field test and %MPE in the hot-plate test, were not significantly different among the haplotype groups (data not shown). In the GA-T haplotype, the %MPE in the tail-flick test was significantly higher in the L–S group than in the S–L groups ($P = 0.040$, Games–Howell post-hoc test; Fig. 4a). In the GA-TA haplotype, the L–S group was not significantly different from the other groups in the %MPE in the tail-flick test, but this group trended higher than the S–L group ($P = 0.083$, Games–Howell post-hoc test; Fig. 4b). A post-hoc test could not be performed in the T-TA haplotype because the S–L group had only one mouse strain (Fig. 4c).

Discussion

This study demonstrated interstrain differences in the effects of morphine in the Mishima battery of inbred mouse strains that included wild-derived strains. This interstrain difference in the antinociceptive effects of morphine varied widely in the mouse battery. We identified four highly variable regions containing five novel STR polymorphisms in the 5′ flanking region and 3′ UTR of the *Oprm1* gene. Among the five STRs, repeat length variations of the GA, T, and TA STRs were associated with interstrain differences in morphine-induced antinociception.

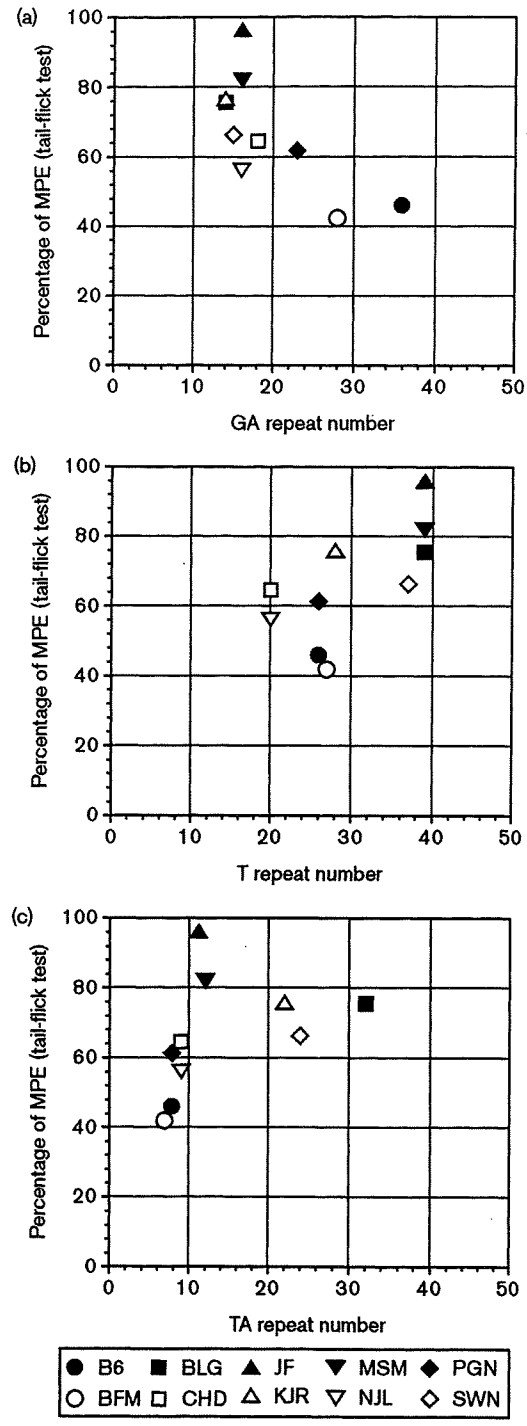
The antinociceptive effect of morphine in the hot-plate test was similar to that in the tail-flick test. The antinociceptive effects of morphine in some strains, such as BFM, BGL, and NJL, however, were different between the hot-plate and tail-flick tests. The hot-plate test measures the spinal–supraspinal response to thermal stimulation, whereas the tail-flick test measures the spinal reflex to noxious stimulation. Morphine induces antinociception through multiple pathways by suppressing the ascending nociceptive neurons within the spinal cord and brain and activating descending antinociceptive neurons from the brainstem. The differences in the antinociceptive effects of morphine between the hot-plate and tail-flick tests may be attributable to the different neural networks that are responsible for these actions. Furthermore, in this study, the antinociceptive effect of morphine in the hot-plate test varied more widely than in the tail-flick test, which is consistent with results from an earlier study by Mogil *et al.* [3]. This trend

also is observed in the nociceptive sensitivity among available inbred mouse strains [30]. The antinociceptive effect of morphine in the hot-plate test may be influenced by more genes, in addition to the *Oprm1* gene, than in the tail-flick test and was found to vary widely among the 10 mouse strains used in this study. Therefore, the *Oprm1* gene may contribute significantly to variations in the antinociceptive effects of morphine in the tail-flick test that resulted in the positive associations with some STR variations in the *Oprm1* gene.

Five STR polymorphisms were found in the 5′ flanking region and 3′ UTR of the mouse *Oprm1* gene in this study. In the 5′ flanking region of the mouse *Oprm1* gene, the distal and proximal promoters have been identified at 794 and 291–268 bp upstream from the start codon ATG of the mouse *Oprm1* gene, respectively [31–33]. Various *cis*-acting elements for transcription factors, such as AP-1, AP-2, CREB-binding protein, NF-κB, NRSF, NRSE, Oct-1, PU.1, Sox, Sp1, Sp3 isoform, and STAT6, have been identified at the distal and proximal promoter sites of the mouse *Oprm1* and human *OPRM1* genes [34]. The differences in the STRs may affect the interaction between these *cis*-acting elements and transcription factors that may influence morphine sensitivity.

Around the GA STR located at 1899–1973 bases upstream from ATG, *cis*-acting elements have not been reported earlier. By our analysis with the TRANSFAC v.7.0 transcription factor database (www.gene-regulation.com/cgi-*bin/pub/databases/transfac/search.cgi*) (BIOBASE Gmbh, Wolfenbüttel, Germany), putative *cis*-acting elements for transcription factors such as TAGATAAGAGAGA and AGAGAGATGAAAT for Oct-1 and ATAAGAGAG for Hoxa-3, however, exist around the GA repeat (data not shown). The GA repeat number was inversely related to the analgesic effects of morphine in the tail-flick test (Fig. 3). Transcription of *MOR-1* occurs through the transcription factors listed above in the absence of a TATA box and CAAT box [31,32,35–37]. Among *cis*-acting elements for these transcription factors, NRSE acts as a suppressor for *MOR-1* mRNA transcription at ATG (from −9 to 12 bp relative to ATG) [38,39]. The homologous sequence to the NRSE was not found near the GA STR, and therefore transcription suppressors are unlikely to be recruited to the long GA STR. The GA STRs at the 5′ flanking region of the genes were reported previously to silence their transcription by recruiting insulator proteins and transcription factors [40,41]. A possible explanation for the suppressive effect of the GA STR polymorphism is that the upstream and downstream sequences close to the GA STR polymorphism recruit some insulator proteins and transcription factors that cooperatively silence *MOR-1* transcription, and their transcriptional efficacy may be more suppressed depending on the length of the GA STR.

Fig. 3

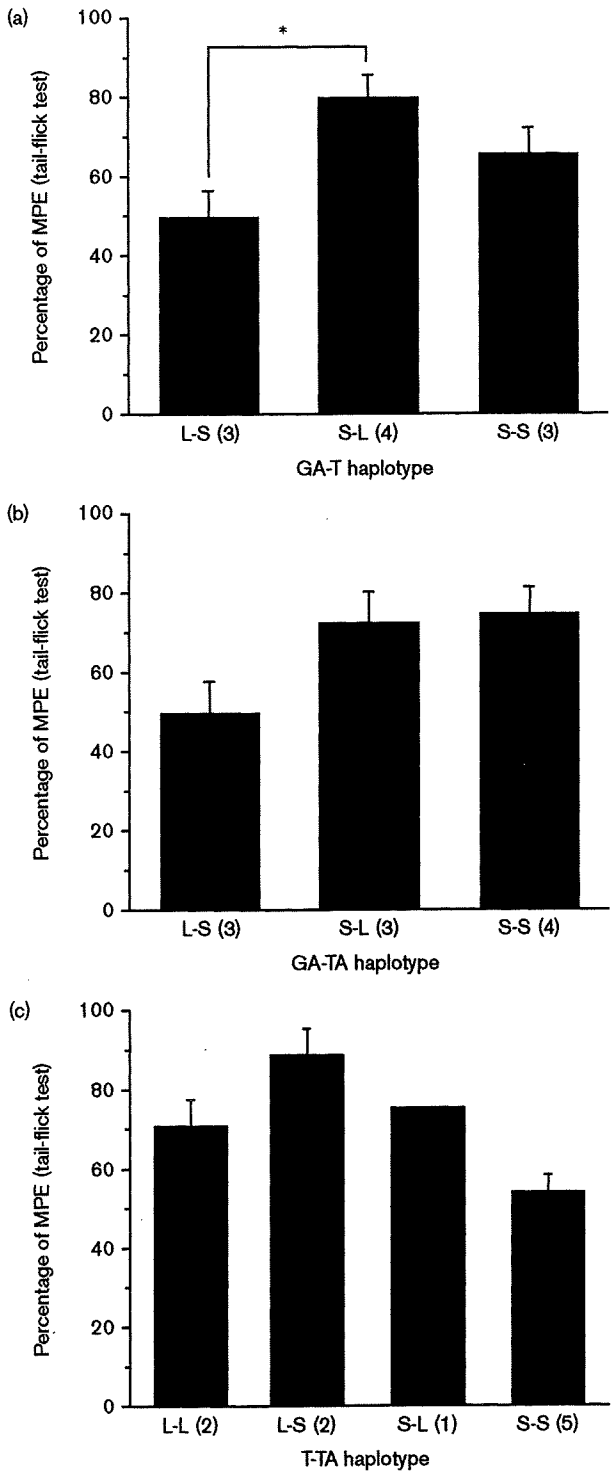


Association of the GA, T, or TA repeat diversities in the *Oprm1* gene with the antinociceptive effects of morphine. The GA repeat length at region 1 (a), the T repeat length at region 2 (b), and the TA repeat length at region 3 (c) of the *Oprm1* gene varied from 18 to 45, 20 to 39, and 7 to 32 in the 10 inbred mouse strains, respectively. The lengths of the GA, T, and TA repeats were inversely and proportionally related with percentage of the maximal possible effect (%MPE) of morphine-induced antinociception examined in the tail-flick test ($r = -0.689$, 0.735, and 0.738, respectively; $P < 0.05$).

In the 3′ UTR of the mouse *Oprm1* gene, T, TA, and CA/CT repeats were located 1850–1875, 3433–3448, 9497–9523/9524–9592 bases from the TAA translational stop codon of *MOR-1* in the B6 strain, respectively (Fig. 2). The gene structure of the human *OPRM1* gene is similar to that of the mouse *Oprm1* gene [34,42], including a long 3′ UTR of over 10 kbp [43,44]. In the 3′ UTR, approximately 2 kb regions at both ends of the 3′ UTR were similar between mouse *Oprm1* and human *OPRM1* genes [43]. Regions corresponding to the novel STR polymorphism regions identified in the mouse *Oprm1* gene in this study have not been found in the human *OPRM1* gene, at least not in a Japanese population. The human *OPRM1* gene, however, contains other STRs such as T and CA repeats in their 3′ UTR at positions 7069–7099 and 11132–11168 bases upstream from TAA, respectively, although these STR polymorphisms have not been examined. In the other regions, such as intron 2 and 4, the human *OPRM1* gene also contains some STRs, and the possibility of an association was reported between substance dependence and alleles at the CA STR locus in *OPRM1* intron 2 [45,46]. Noncoding regions in genes, such as introns and UTRs, often contain repeat sequences that frequently have wide genetic diversity in their lengths within a population and between populations. The STRs in the human *OPRM1* gene may be associated with morphine sensitivity. During gene expression in eukaryotes, 3′ UTRs are known to contribute to subcellular localization, degradation/stability, and translation of mRNA. A major class of *cis*-acting elements that regulate mRNA stability include AU-rich elements (AREs), often found in the 3′ UTR of short-lived mRNA [47,48]. In the *Oprm1* gene and *OPRM1* gene, several ARE motifs have been found at 4–5 and 8–9 kb, and 4–5 and 11–12 kb downstream from TAA in their 3′ UTR, respectively [49]. AREs mainly recruit mRNA degradation proteins, but mRNA stabilizing proteins, such as HuR and pp32, also have been reported to bind and to be recruited to AREs [50,51]. These STRs in the 3′-UTR, especially the T and TA repeat STRs, may contribute to mRNA stability by affecting the binding of mRNA degradation or stabilizing proteins that may consequently lead to the high morphine sensitivity observed in mouse strains with long T and TA STRs.

In addition to the *Oprm1*, other genes such as *Abcb1a* (ABC transporter gene), *Arrb2* (β-arrestin gene), *Cacna1e* (R-type calcium channel gene), *Hrh1, 2* (histamine

Fig. 4



Comparison of the antinociceptive effects of morphine among the short tandem repeat (STR) haplotype groups. The mouse strains were divided into long (L) and short (S) groups by repeat number of the GA, T, and TA STRs. The percentages of the maximal possible effects (%MPEs) of morphine in the tail-flick test were compared among the GA-T (a), GA-TA (b), and T-TA (c) STR haplotype groups. Regarding the GA-T STR haplotype, %MPE in the tail-flick test was higher in the L–S group than in the S–L group. The number of mouse strains in each haplotype group is indicated in parentheses. The asterisk indicates a statistically significant difference between haplotype groups (*$P<0.05$).

receptor gene), and *Kcnj3*, *6*, *9* (inwardly rectifying potassium channel gene) have been shown using gene-knockout mice to be involved in opioid sensitivity [52]. Additionally, human gene association studies revealed some genetic variations in *ABCB1*, *ARRB2*, *COMT* (methyl transferase gene), and *CYP2D6* (P450 subtype gene) associated with opioid sensitivity [24,52]. In the Mishima battery of inbred mouse strains, possible variations in these genes also may be associated with opioid sensitivity.

In conclusion, we found regions with high genetic diversity in the 5′ flanking region and 3′ UTR of the mouse *Oprm1* gene using the Mishima mouse battery that included wild-derived strains and identified three STRs associated with morphine-induced antinociception. This mouse battery may be useful for elucidating the molecular mechanisms involving STRs in the *Oprm1* noncoding region underlying interindividual differences in opioid sensitivity.

Acknowledgements

The authors are grateful to Dr Y. Ogai, Ms J. Hasegawa, and Ms E. Kamegaya (Tokyo Institute of Psychiatry) for their suggestion of statistical analysis and skilled technical assistance. The authors also thank all members in the animal facility at the National Institute of Genetics for maintaining and providing wild-derived inbred mice. This study was supported by the Japanese Ministry of Health, Labour and Welfare (H17-pharmaco-001), the NIG Cooperative Research Program (2005A39, 2006A41, 2007B9), and The Naito Foundation.

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Naunyn-Schmiedeberg's Arch Pharmacol (2008) 377:269–281
DOI 10.1007/s00210-007-0205-3

SPECIAL ARTICLE

Candidate gene polymorphisms predicting individual sensitivity to opioids

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Received: 10 August 2007 / Accepted: 18 October 2007 / Published online: 13 November 2007

Abstract Significant interindividual differences in opioid sensitivity can hamper effective pain treatment and increase the risk for substance abuse. Elucidation of the genetic mechanisms involved in the interindividual differences in opioid sensitivity would help establish personalized pain management. Studies using gene knockout mice have revealed that genes encoding some metabolic enzymes, opioid transporters, and opioid system signal transduction mediators may be candidate genes to predict appropriate kinds and doses of opioids for individuals. Recently, various databases on knockout mice, pharmacogenetics, and gene polymorphisms have been rapidly consolidated. Such information should aid in developing and improving the methods of predicting interindividual differences in opioid sensitivity. In the near future, it will be possible to predict the appropriate kinds and doses of opioids for individuals by analyzing genetic variations contributing to opioid sensitivity.

Keywords Opioid sensitivity · Interindividual differences · mu opioid receptor · Genetic polymorphisms

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Introduction

Pain is an essential physiological mechanism by which animals and humans prevent themselves from developing and/or aggravating tissue injury. It is clear, however, that excessive pain is harmful to a living body because it can evoke significant adverse reactions and severely deteriorate quality of life. Therefore, it is crucial to adequately control such severe pain. Opioid drugs, including morphine and fentanyl, are widely used as analgesics to control moderate to severe pain. Audits of cancer pain report that in 80% of patients, satisfactory pain relief can be achieved by the use of opioids and other analgesics based on the World Health Organization (WHO) guidelines (Jadad and Browman 1995). Unfortunately, however, opioids also can produce multiple adverse effects, such as physiological dependence, tolerance, respiratory depression, nausea, vomiting, and constipation (Ikeda et al. 2005; Schug and Gandham 2006). Thus, the balance between analgesic efficacy and adverse side effects needs to be considered when opioid dosing is established (McQuay 1999). In addition, considerable interindividual variations exist in the analgesic efficacy and side effect profiles of opioids, which often hamper effective pain management with opioid drugs (Kalso and Vainio 1990; McQuay 1999; Klepstad et al. 2003; Fig. 1). For example, the minimal effective analgesic concentration (MEAC) of fentanyl required for satisfactory analgesia varies from 0.2 to 2.0 ng/ml among patients (Glass et al. 2000). Such variability of opioid sensitivity has been attributed to environmental, psychological, and genetic factors. Although environmental and psychological factors may alter pain response and opioid sensitivity through cognitive and emotional process such as fear and anxiety, many genetic factors, including genomic variations and gene copy number, affect opioid pharmacokinetics and

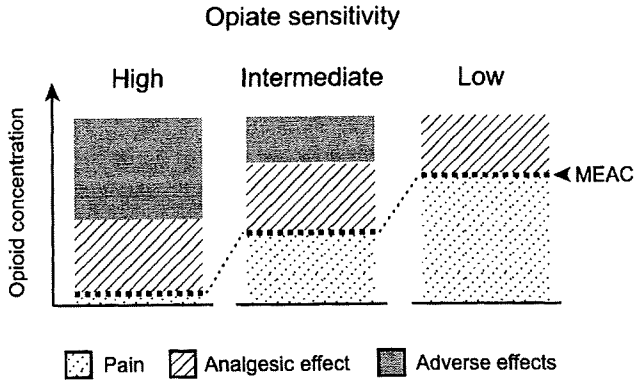

Fig. 1 Model for inter-individual differences in opioid sensitivity. Minimal effective analgesic concentration (*MEAC*; indicated by *bold dotted lines*) is five to tenfold different among individuals (Glass et al. 2000), and this is a purported cause of wide variations in clinical response to opioids among individuals. The *dotted*, *striped*, and *gray areas* indicate ranges of the plasma opioid concentrations that produce no analgesia, analgesia, and severe adverse side effects, including deep sedation and respiratory depression, respectively. Opioids, at plasma concentrations that produce analgesia in subjects with "intermediate" opiate sensitivity, may fail to produce analgesia in those with "low" opioid sensitivity and may produce severe adverse side effects in those with "high" opioid sensitivity

pharmacodynamics. Thus, the prediction of therapeutic efficacy of opioids in individuals based on information on such variability in opioid pharmacology is a prerequisite for establishing adequate personalized pain management with opioids. For this reason, exploring genetic factors that affect opioid pharmacology has significant importance.

In this brief review, we will focus on and summarize candidate genes that may affect the pharmacokinetics and pharmacodynamics of various opioids.

Candidate genes for predicting opioid efficacy in individual patients

Opioids exert their activities by interacting with their receptors in the central and peripheral nervous systems as well as in non-neuronal sites such as intestine epithelial cells and immuno cells and are metabolized and eliminated from the body. The analgesic action of opioids is dependent on the metabolic enzymes, transporters, and molecules involved in the opioid signal transduction pathways. There are numerous enzymes which metabolize lipid-soluble opioids into more water-soluble metabolites to facilitate elimination. Evidence in animals and humans indicates that some drug-metabolizing enzymes and transporters, including cytochrome P450 (CYP), uridine 5′-diphosphate (UDP)-glucuronosyltransferases (UGT), and adenosine triphosphate (ATP)-binding cassette (ABC) transporters, are involved in opioid metabolism, the flux of opioids into and out of sites of opioid action, and interindividual differences in opioid concentrations in the body and brain. Opioids exert their activities via three types of opioid receptors, designated μ (mu), κ (kappa), and δ (delta; Mansour et al. 1988; Kieffer 1995). Among these three opioid receptors, morphine and fentanyl particularly interact with the μ opioid receptor (MOP). MOP couples to $G_{i/o}$ proteins and regulates adenylate cyclase, inwardly rectifying potassium channels, voltage-dependent calcium channels, and other mediators that trigger subsequent signal transduction pathways.

Cytochrome P450

CYPs and associated monooxygenases are the principal family of drug-metabolizing enzymes. More than 72 CYP isoforms that have distinct yet overlapping substrate specificities have been identified in humans. Morphine is *N*-demethylated by CYP2C8 or CYP3A4 (Projean et al. 2003; Table 1). Codeine is converted to norcodeine by CYP3A4 and *O*-demethylated by CYP2D6 to form morphine which has been shown to account for most, if not all, of codeine's analgesic activity (Caraco et al. 1996; Poulsen et al. 1996; Gasche et al. 2004). CYP2D6 is under polymorphic genetic control, which leads to a wide variety of metabolic activities (reviewed in Zanger et al. 2004). Individuals are normally classified as either poor metabolizer (PM) or extensive metabolizer (EM) depending on CYP2D6 enzyme activity. In PMs, codeine is converted to very little morphine (1.84 nmol mg^{-1} h^{-1}), whereas EMs produce significantly greater concentrations of morphine (11.80±3.47 nmol mg^{-1} h^{-1}; Dayer et al. 1988). Similarly, EMs excrete more morphine via urine (6.5% of dose) than PMs (1.1%) after intake of codeine (Hedenmalm et al. 1997). Pain threshold increases in EMs after codeine intake ($p<0.05$), whereas there are no significant changes in pain threshold in PMs (Sindrup et al. 1990). Approximately 5–10% of Caucasian populations in Europe and North America lack the functional action of the CYP2D6 enzyme due to inactive mutations in both alleles of the *CYP2D6* gene, and they are PMs of debrisoquine and numerous other drugs (Sachse et al. 1997; Eckhardt et al. 1998). Furthermore, CYP2D6 activity is highly variable in EMs and distributed as much as 10,000-fold among individuals (Bertilsson et al. 1991). CYP2D6 also catalyzes the conversion of dihydrocodeine, hydrocodone, oxycodone, and tramadol to dihydromorphine, hydromorphone, oxymorphone, and tramadol metabolite M1, respectively (Otton et al. 1993; Kirkwood et al. 1997; Subrahmanyam et al. 2001; Lalovic et al. 2004). In EMs, dihydrocodeine is metabolized to dihydromorphine (0.192±0.075 ml min^{-1} g^{-1}) more than tenfold more than in PMs (0.015 ml min^{-1} g^{-1}; Kirkwood et al. 1997). The metabolic clearance of 10 mg hydrocodone to hydromorphone was eight times faster in EMs (28±10.3 ml h^{-1} kg^{-1}) than in PMs (3.4±

Table 1 CYP isotypes catalyzing various opioids

	CYP isotypes							
	1A2	2B6	2C8	2C19	2D6	3A4	3A5	References
Morphine	–	–	N	–	–	N	–	Projean et al. 2003
Fentanyl	–	–	–	–	–	N	N	Labroo et al. 1997; Klees et al. 2005
Methadone	N	N(S)	N(R,S)	N(R)	–	N(R,S)	–	Foster et al. 1999; Wang and DeVane 2003 Gerber et al. 2004; Ferrari et al. 2004
Codeine	–	–	–	–	O	N	–	Gasche et al. 2004
Dihydrocodeine	–	–	–	–	O	N	–	Fromm et al. 1995; Kirkwood et al. 1997
Hydrocodone	–	–	–	–	O	N	–	Otton et al. 1993; Hutchinson et al. 2004
Oxycodone	–	–	–	–	O	N	N	Lalovic et al. 2004
Buprenorphine	–	–	–	–	–	N	–	Kobayashi et al. 1998
Tramadol	–	N	–	–	O	N	–	Subrahmanyam et al. 2001

N, *N*-demethylation or *N*-dealkylation; *O*, *O*-demethylation; – not catalyzed or not determined

2.4 ml h^{-1} kg^{-1}). Furthermore, pretreatment with quinidine, a selective CYP2D6 inhibitor, in the EMs reduced their clearance to levels similar to those in PMs (5.0±3.6 ml h^{-1} kg^{-1}), and the maximal plasma concentration for hydromorphone was five times higher in EMs than in PMs or in EMs pretreated with quinidine (Otton et al. 1993). Concentrations of *O*-demethyltramadol differ between PMs [median (one third quartile), 0 ng h^{-1} ml^{-1}; range, 0–11.4 ng h^{-1} ml^{-1}] and EMs [median (one third quartile), 66.5 ng h^{-1} ml^{-1}; range, 17.1–118.4 ng h^{-1} ml^{-1}) after tramadol administration for postoperative analgesia (Stamer et al. 2007). As presented above, for all opioids with the 4,5-epoxymorphinan structure (i.e., codeine, dihydrocodeine, hydrocodone, and oxycodone), *O*-demethylation by the CYP2D6 isotype results in the formation of more potent agonists than substrates, and it is clear that genetic variations, including PM/EM allelic polymorphisms in the *CYP2D6* gene, strongly influence interindividual differences in opioid efficacy. In addition to CYP2D6, CYP2B6, 2C19, 3A4, and 3A5 isoforms also are involved in opioid metabolism (Table 1). Thus, genetic polymorphisms in these *CYP* genes also would cause interindividual variations in the plasma levels of opioids and their efficacies. In addition, administration of therapeutic drugs for other diseases may inhibit CYP activity, alter opioid blood levels, and result in interindividual differences in opioid efficacy.

UDP-glucuronosyltransferases

UDP-glucuronosyltransferases (UGTs) catalyze glucuronidation, which is an additional reaction of the glycosyl group from a nucleotide sugar to numerous compounds (Fujimoto and Way 1957; Yoshimura et al. 1969). Twenty-eight UGT isoforms have been identified in humans. The UGT2B7 isoform is expressed in the human brain, liver, and kidney, and has been shown to catalyze glucuronidation of almost all opioids in vitro (reviewed in King et al. 2000). Furthermore, it has been reported that 60–80% of a given dose of morphine is excreted via urine as glucuronidated metabolites (Yeh 1975; Mitchell et al. 1991). Morphine possesses hydroxyl groups at the 3- and 6- positions in the molecule and is glucuronidated by UGT2B7 at the 3- and 6-hydroxyl positions to form morphine-3-glucuronide (M-3-G) and morphine-6-glucuronide (M-6-G), respectively (Oguri et al. 1970; Yeh et al. 1977). The major metabolite M-3-G has been shown to lack any analgesic properties and is implicated as an antagonist at opioid peptide receptors (Smith et al. 1990; Lipkowski et al. 1994). However, M-3-G does not bind to any opioid receptors (Löser et al. 1996), suggesting that M-3-G may interact with other receptors and cause behavioral manifestations of antagonism to opioid agonist effects. The minor metabolite M-6-G is 50-fold more potent as an analgesic than morphine (Shimomura et al. 1971; Abbott and Palmour 1988; Osborne et al. 1988), although the contribution of M-6-G to analgesia is extremely variable when morphine is administered (range, 0.1–66%; Murthy et al. 2002). These results implicate UGT2B7 in morphine's analgesic efficacy. The C802T single nucleotide polymorphism (SNP) (rs7439366 in dbSNP database provided by the National Center for Biotechnology Information, USA) is a well-studied polymorphism in the human *UGT2B7* gene which results in a histidine-to-tyrosine substitution at amino acid position 268 (His268Tyr). M-3-G and M-6-G concentrations are lower in 802C/C individuals (M-3-G median, 152 ng/ml; range, 30–434 ng/ml; M-6-G median, 18 ng/ml; range, 0–66 ng/ml) than in 802C/T and 802T/T individuals (802C/T median, 242 ng/ml; range, 33–1381 ng/ml; 802T/T median, 43 ng/ml; range, 0–193 ng/ml; Sawyer et al. 2003). However, some studies have demonstrated that the His268Tyr polymorphism cannot account for the considerable variations

in the efficiency of opioid glucuronidation by UGT2B7 (Bhasker et al. 2000; Holthe et al. 2002, 2003).

ATP-binding cassette transporters

The ABC transporter superfamily contains membrane proteins that transport a variety of substrates across extra- and intracellular membranes. P-glycoprotein, the encoded product of the human multidrug resistance (MDR1) gene (*ABCB1*), is extensively distributed in brain endothelia and kidney epithelia and is an essential component of the blood–brain barrier. MDR1 has been demonstrated to be involved in morphine's cellular membrane permeability in vitro (Callaghan and Riordan 1993). Studies with *Mdr1a* gene knockout mice have revealed that MDR1 transports morphine into the bloodstream in brain capillary endothelial cells (Schinkel et al. 1995). In addition to morphine levels, MDR1 regulates the levels of fentanyl and methadone in the brain (Thompson et al. 2000), although it cannot transport other opioids such as M-3-G, M-6-G, and oxycodone (Wandel et al. 2002). Another study using *Mdr1a* knockout mice has shown that MDR1 is not involved in the transport of M-6-G across the blood–brain barrier (Bourasset et al. 2003). Another ABC transporter, multidrug resistance protein (MRP3; ABCC3), transports M-3-G and M-6-G in the basolateral membrane of polarized cells in vitro (Zelcer et al. 2005). *Mrp3* knockout mice exhibited increased levels of M-3-G in the liver and bile and a 50-fold reduction in plasma levels of M-3-G, suggesting that MRP3 excretes morphine-glucuronide from the liver into the bloodstream (Zelcer et al. 2005). Genetic variations in the genes encoding ABC transporters are associated with a wide variety of human disorders with Mendelian genetic and complex inheritance (Dean et al. 2001). The C3435T variation in exon 26 of the human *ABCB1* gene (rs1045642) is associated with the expression level and function of MDR1, although it is a synonymous SNP (Ile1145Ile) (Hoffmeyer et al. 2000). 3435C/C individuals have significantly lower duodenal MDR1 expression and the highest plasma levels of digoxin, a substrate of MDR1. The C3435T SNP is associated with the miotic effects of the MDR-1 substrate loperamide when MDR-1 inhibitor quinidine is coadministered (Skarke et al. 2003b). However, there is no association between the C3435T SNP and plasma levels and miotic or respiratory depressive effects of loperamide (Pauli-Magnus et al. 2003; Skarke et al. 2003b). There are no significant differences in the allele and genotype frequencies of the individual SNPs, including the C3435T SNP and haplotypes, between opioid-dependent and -non-dependent subjects (Coller et al. 2006). Furthermore, the *ABCB1* haplotype based on only the G2677T(A) (Ser893Ala(Thr), rs2032582) and also C3435T SNPs is not associated with the miotic effects of levomethadone in healthy subjects (Lötsch et al. 2006). In contrast, the *ABCB1* haplotype constructed from five SNPs [A61G (Asn21Asp, rs9282564), G1199A (Asn400Ser, rs2229109), C1236T (Gly412Gly, rs1128503), G2677T (A), and C3435T] influences daily methadone dose requirements (Coller et al. 2006). Thus, the haplotype of these genetic variations in the *ABCB1* gene is expected to affect the absorption and concentration of numerous other substrates of MDR1, such as morphine.

μ Opioid receptors

Much pharmacological data using genetically modified mice have shown that the MOP is a preferred target of morphine, and it appears to play a crucial role in mediating major clinical effects of morphine, including analgesia, tolerance, and dependence (Uhl et al. 1999; Kieffer and Gavériaux-Ruff 2002). For example, morphine-induced analgesia is abolished in MOP knockout mice (Matthes et al. 1996; Sora et al. 1997; Loh et al. 1998). Furthermore, heterozygous MOP knockout mice possess approximately one half of the amount of MOP protein than wild-type mice and exhibit intermediate efficacy of morphine between homozygous MOP knockout mice and wild-type mice (Sora et al. 1997; Loh et al. 1998), suggesting that MOP levels influence opioid efficacy in a gene dosage-dependent manner. CXB7/ByJ (CXBK) mice are a useful model for altered opioid efficacy (Bailey 1971). The CXBK mouse strain exhibits a marked decrease in morphine-induced analgesia compared to their progenitor mice (Ikeda et al. 1999; Table 2). In CXBK mice, the expression level of MOP mRNA is also reduced to 60% of that in the progenitor mice due to an insertional mutation in the *Oprm1* 3′ untranslated region (UTR; Ikeda et al. 2001; Han et al. 2006; Kasai et al. 2006). Similarly to these animals, genetic variations in the human MOP gene (*OPRM1*) altering the expression of MOP (e.g., SNPs in the promoter region or 3′ UTR) may affect opioid efficacy. Thus far, more than 100 genetic polymorphisms have been identified in the *OPRM1* gene, and four linkage disequilibrium (LD) blocks have been found in the *OPRM1* gene by haplotype analysis in Japanese subjects (Ide et al. 2006). Polymorphisms in the *OPRM1* gene may be promising candidates associated with opioid sensitivity.

The A118G polymorphism (rs1799971) is a tag SNP of the first LD block containing exon 1 and causes an asparagine-to-aspartic acid substitution at amino acid position 40 (Asn40Asp) in the extracellular N-terminal domain of the MOP. The Asn^{40} amino acid is a putative *N*-linked glycosylation site (Asn-*X*-Cys/Ser/Thr, where *X* is any amino acid) in the *OPRM1* gene. The binding affinity

Table 2 Gene knockout and mutant mice showing altered opioid effects

Knockout genes (coding proteins) or mutant mice	Analgesia (opioids)	Other phenotypes in opioid effects	Reference
Opioid peptides and receptors			
Penk1 (Enkephalin)		Abolished morphine tolerance	Nitsche et al. 2002
Oprm1 (μ Opioid receptor)	Abolished (morphine)		Matthes et al. 1996; Sora et al. 1997; Loh et al. 1998
CXB7/ByJ (CXBK) mice	Reduced (morphine)		Ikeda et al. 1999
Oprk1 (κ Opioid receptor)	Abolished (U50,488H)		Simonin et al. 1998
Oprd1 (δ Opioid receptor)	Abolished (DPDPE)	Abolished morphine tolerance	Zhu et al. 1999
Receptors, channels and transporters			
Adora1 (Adenosine A1 receptor)	Maintained/Reduced (morphine)		Johansson et al. 2001; Wu et al. 2005
Adra1b (Adrenergic receptor α1B)		Reduced hyperactivity induced by morphine	Drouin et al. 2002
Adra2a (Adrenergic receptor α2A)	Increased (morphine, tramadol)		Özdoğan et al. 2006
Adrb2 (Adrenergic receptor β2)		Reduced morphine dependence and tolerance	Liang et al. 2007
Cacna1e (R-type calcium channel)	Increased (morphine)	Reduced morphine tolerance	Yokoyama et al. 2004
Cckbr (Cholecystokinin B receptor)	Reduced (morphine)	Enhanced hyperactivity induced by morphine	Pommier et al. 2002
Chrm1 (Muscarinic acetylcholine receptor 1)	Increased (morphine)	Reduced rewarding effect of morphine	Carrigan and Dykstra 2007
Chrm5 (Muscarinic acetylcholine receptor 5)		Reduced rewarding effect of morphine	Basile et al. 2002; Yamada et al. 2003
Cnr1 (Cannabinoid receptor 1)	Maintained (morphine)	Reduced rewarding effect of morphine	Ledent et al. 1999; Martin et al. 2000; Cossu et al. 2001
Drd2 (Dopamine receptor 2)	Increased (morphine, M-6-G, U50,488H, naloxone benzoylhydrazone)		King et al. (2001)
		Reduced rewarding effect of morphine	Elmer et al. 2005
Drd3 (Dopamine receptor 3)		Enhanced rewarding effect and hyperactivity induced by morphine	Narita et al. 2003
Grin2a (NMDA receptor 2A)		Reduced morphine tolerance	Miyamoto et al. 2004
Gria1 (AMPA1 receptor)		Reduced morphine tolerance	Vekovischeva et al. 2001
Hrh1 (Histamine H1 receptor)	Increased (morphine)		Mobarakeh et al. 2002
Hrh2 (Histamine H2 receptor)	Increased (morphine)		Mobarakeh et al. 2006
Kcna1 (Voltage-gated potassium channel)	Reduced (morphine)		Clark and Tempel 1998
Kcnj3 (Inwardly-rectifying potassium channel 1)	Reduced (morphine)		Marker et al. 2004
Kcnj6 (Inwardly-rectifying potassium channel 2)	Reduced (morphine)		Marker et al. 2002, 2004; Mitrovic et al. 2003
Weaver mutant mice	Reduced (morphine)		Ikeda et al. 2000
Kcnj9 (Inwardly-rectifying potassium channel 3)	Reduced (morphine)	Reduced morphine and fentanyl tolerance	Marker et al. 2002; Terman et al. 2004
Mc1r (e/e) spontaneous mutant mice	Increased (M-6-G, pentazocine)		Mogil et al. 2003, 2007
Oprl1 (Nociceptin receptor)	Abolished (naloxone benzoylhydrazone)		Noda et al. 1998
Prlhr (Prolactin-releasing peptide receptor)	Increased (morphine)	Reduced morphine tolerance	Laurent et al. 2005

Table 2 (continued)

Knockout genes (coding proteins) or mutant mice	Analgesia (opioids)	Other phenotypes in opioid effects	Reference
Slc6a2 (Norepinephrine transporter)	Increased (morphine)		Bohn et al. 2000a
Slc6a3 (Dopamine transporter)		Enhanced rewarding effect of morphine	Spielewoy et al. 2000
		Reduced hyperactivity induced by morphine	
Tacr1 (Tachykinin receptor 1)		Reduced rewarding effect of morphine	Ripley et al. 2002
Neurotransmitters and mediators			
Pnoc (Orphanin FQ/nociceptin)		Hyperdependence and hypertolerance to morphine	Kest et al. 2001; Chung et al. 2006
Dopamine deficient mice	Reduced (morphine)	Maintained rewarding effect of morphine	Hnasko et al. 2005
Dbh (Dopamine β-hydroxylase)	Reduced (morphine)	Reduced rewarding effect of morphine	Jasmin et al. 2002; Olson et al. 2006
Ntf5 (Neurotrophin 5)		Reduced morphine tolerance	Smith et al. 2003
Tac1 (Tachykinin)		Abolished rewarding effect of morphine	Murtra et al. 2000
Intracellular signal transduction molecules			
Adcy5 (Adenylate cyclase 5)	Reduced (morphine)	Reduced morphine dependence, reward and tolerance	Kim et al. 2006
Adcy8 (Adenylate cyclase 8)		Reduced morphine tolerance	Li et al. 2006
Alox12 (Arachidonate 12-lipooxygenase)	Increased (morphine)		Walters et al. 2003
Arrb2 (β2 Arrestin)	Increased (morphine)	Reduced morphine tolerance	Bohn et al. 1999, 2000b, 2002
		Enhanced rewarding effect of morphine	Bohn et al. 2003
Camk4 (Calmodulin-dependent protein kinase IV)		Reduced morphine tolerance	Ko et al. 2006
Cdk5 (Cyclin-dependent kinase 5)		Reduced rewarding effect of morphine	Narita et al. 2005
Gnaz (Gz protein α subunit)		Hypertolerance to morphine	Hendry et al. 2000; Leck et al. 2004
Gnb5 (Guanine nucleotide binding protein β5)		Reduced morphine tolerance	Sánchez-Blázquez et al. 2003
Grasp (GRP1-associatted scaffold protein)	Reduced (morphine)	Reduced rewarding effect of morphine	Ogawa et al. 2007
Plcb1 (Phospholipase Cβ1)	Reduced (morphine)	Reduced morphine tolerance	Liu et al. 2006
Plcb3 (Phospholipase Cβ3)	Increased (morphine)		Xie et al. 1999
Prkce (Protein kinase Cε)		Enhanced rewarding effect of morphine	Newton et al. 2007
Rgs9 (Regulator of G-protein signaling 9)	Increased (morphine)	Enhanced rewarding effect of morphine	Zachariou et al. 2003
		Reduced morphine tolerance	Sánchez-Blázquez et al. 2003
Others			
Creb1 (cAMP response element binding protein 1)		Reduced or enhanced rewarding effect of morphine	Walters et al. 2005
Hmox2 (Heme oxygenase 2)		Abolished morphine tolerance	Liang et al. 2003
Il6 (Interleukin 6)	Reduced (morphine)		Bianchi et al. 1999
Lmx1b (LIM homeobox transcription factor 1β)	Reduced (morphine, U50,488H, DPDPE)		Zhao et al. 2007
Nrcam (Neuron-glia-CAM-related cell adhesion molecule)	Reduced reward effect of morphine		Ishiguro et al. 2006
Plat (Tissue plasminogen activator)		Reduced rewarding effect and hyperactivity induced by morphine	Nagai et al. 2004; Yan et al. 2007

and potency of the endogenous opioid peptide β-endorphin to the OPRM1-Asp^{40} variant receptor are threefold higher than that to the OPRM1-Asn^{40} wild-type receptor in AV-12 cells and *Xenopus* oocytes in vitro (Bond et al. 1998). The expression of *OPRM1* mRNA from the 118A allele is more abundant than that from the 118G allele by measuring allelic imbalance of mRNA expression (Zhang et al. 2005). However, these findings have not been replicated in other studies (Befort et al. 2001; Beyer et al. 2004). The agonist-binding and functional coupling between OPRM1-Asn^{40} wild-type and OPRM1-Asp^{40} variant receptors are not different in transiently expressed COS cells (Befort et al. 2001). Agonist-induced internalization, desensitization, and resensitization have been shown to be similar between wild-type and variant receptors expressed in HEK293 cells (Beyer et al. 2004). Therefore, the functional significance of the A118G polymorphism remains unclear.

The A118G polymorphism is the most extensively studied polymorphism for clinical associations with opioid response and substance abuse. Approximately one half of the association studies have reported a positive correlation between 118G allele and opioid efficacy or abuse in various ethnic groups. The 118G allele has been shown to decrease the efficacy of morphine in both healthy subjects (Skarke et al. 2003a) and cancer patients (Klepstad et al. 2004; Chou et al. 2006). The OPRM1-Asp^{40} variant also reduces the potency of M-6-G (Lötsch et al. 2002; Skarke et al. 2003a). Furthermore, the A118G SNP has been reported to be a risk factor for substance abuse, such as alcohol and heroin dependence (Szeto et al. 2001; Schinka et al. 2002; Tan et al. 2003; Bart et al. 2004, 2005). In contrast, many controversial studies have shown no correlation between the A118G SNP and opioid efficacy, heroin, or alcohol dependence (Sander et al. 1998; Gscheidel et al. 2000; Franke et al. 2001; Ross et al. 2005; Xuei et al. 2007). In addition, meta-analyses with more than 8,000 subjects from 28 distinct samples and 1,208 subjects from 473 Han Chinese families also have shown no significant association between the A118G SNP and substance abuse (Arias et al. 2006; Glatt et al. 2007). Association studies in complex polygenetic disorders between genetic variations and clinical symptoms often show inconsistencies in the contributions of multiple factors in different populations. Additional studies are needed to determine whether these findings reflect the role for the *OPRM1* gene in opioid efficacy and abuse. Nevertheless, many results showing the positive association between the A118G SNP in the *OPRM1* gene and opioid response suggest that the A118G SNP has potential to predict adequate opioid dosages in individualized pain treatment. Several studies have demonstrated associations of other polymorphisms in the *OPRM1* gene with individual vulnerability to substance abuse.

The intervening sequence 2 (IVS2) + G691C SNP, which is the tag SNP of the second LD block, is associated with methamphetamine dependence/psychosis (Ide et al. 2006). Other studies have reported a positive association of the haplotypes from SNPs in the *OPRM1* 5′ regulatory region and polymorphisms such as C1031G in intron 2 and IVS2 + G31A with vulnerability to substance abuse (Hoehe et al. 2000; Szeto et al. 2001; Shi et al. 2002). These findings suggest that polymorphisms in the introns and UTRs of the *OPRM1* gene also might have roles in altering the transcription, stability, and translation of *OPRM1* mRNA, and functions of the MOP. Further studies are required to confirm the effects of polymorphisms on the expression and function of the MOP.

Molecules in opioid signaling pathways

Studies using gene knockout mice have suggested that a number of receptors, channels, transporters, neurotransmitters, and signal transduction molecules are implicated in the analgesic and adverse effects of opioids, especially morphine (Table 2). G-protein-activated inwardly rectifying K^+ (GIRK) channels are the proximal effectors activated by released β/γ subunits of the $G_{i/o}$ protein and play a crucial role in intracellular opioid signaling, whereas *N*-, *P/Q*-, and *R*-type voltage-dependent calcium channels are inhibited by β/γ subunits. In mammals, four subtypes of GIRK channels, GIRK1-4, have been identified (Kubo et al. 1993; Lesage et al. 1995; Wickman et al. 1997). Girk2 gene (*Kcnj6*) knockout and Girk3 gene (*Kcnj9*) knockout mice display hyperalgesia and reduced analgesic efficacy of morphine (Marker et al. 2002). *Weaver* mutant mice, which harbor a point mutation in the Girk2 pore domain, also exhibit decreased morphine-induced analgesia (Ikeda et al. 2000). These data suggest that Girk2 and Girk3 subunits are responsible for morphine-induced analgesia. *R*-type voltage-dependent calcium channel (Cav2.3) gene (*Cacna1e*) knockout mice exhibit altered analgesia and tolerance induced by morphine (Yokoyama et al. 2004).

Other receptors, transporters, and intracellular signal transduction molecules have been shown to be involved in the analgesic and rewarding effects of morphine (Table 2). Mice with gene knockout of the α2A adrenergic receptor, muscarinic acetylcholine receptor 1, dopamine receptor 2, histamine receptors H1 and H2, prolactin-releasing peptide receptor, norepinephrine transporter, arachidonate 12-lipooxygenase, β2arrestin, and phospholipase Cβ3, in addition to melanocortin-1 receptor spontaneous mutant mice, show enhanced analgesia induced by opioids. In contrast, adenosine A1 receptor, cholecystokinin B receptor, nociceptin receptor, dopa-

mine, dopamine β-hydroxylase, adenylate cyclase 5, tamalin, phospholipase Cβ1, interleukin 6, and Lmx1β deficient mice exhibit reduced opioid-induced analgesia. Lack of either transmitters or their receptors for dopamine, norepinephrine, and tachykinin especially affect the opioid response, suggesting that dopamine, norepinephrine, and tachykinin systems are regulators of the opioid signaling pathway. These results from mouse studies strongly indicate that both analgesic and some adverse effects of opioids such as dependence and analgesic tolerance are complex polygenetic symptoms.

Regarding the β2arrestin gene (*ARRB2*), there are significant differences between cancer patients needing and not needing opioid rotation in both genotype and allele frequencies for the T8622C (T840C in the coding sequence, Ser280Ser, rs1045280) SNP (Ross et al. 2005). Cancer patients who require switching to alternative opioids are more likely to carry the common T allele at this SNP. Catechol-*O*-methyl transferase (COMT) metabolizes dopamine, epinephrine, and norepinephrine to methoxytyramine, metanephrine, and normetanephrine, respectively. The A472G (Val158Met, rs4680) SNP is the most common SNP in the *COMT* gene. The regional μ opioid system response to pain is diminished in healthy volunteers with the Met/Met genotype compared to those with the Val/Met genotype (Zubieta et al. 2003). Caucasian cancer patients with the Val/Val genotype require more morphine (155± 160 mg/h) compared to Val/Met (117±100 mg/h) and Met/Met genotypes (95±99 mg/h; Rakvåg et al. 2005). Similarly, carriers of the Val/Val and Val/Met genotype require 63 and 23%, respectively, higher doses of morphine compared to carriers of the Met/Met genotype (Reyes-Gibby et al. 2007).

Future studies are necessary to identify the genetic variations in human genes implicated in opioid responses by gene-knockout mouse studies and to analyze the associations between these polymorphisms and interindividual differences in opioid efficacy.

Genome-wide association analyses to discover candidate genes

The International HapMap project began in 2002 and developed a haplotype map of the human genome to elucidate common patterns of human genetic variations. The complete dataset of phase I-containing SNP data for Ibadan (in Nigeria), Asian (Japanese in Tokyo and Han Chinese in Beijing), and northern and western European populations have been published (International HapMap Consortium 2005). The achievements of the HapMap project have been utilized to make genome-wide SNP genotyping arrays to assess the contribution of genetic varieties, particularly SNPs, to clinical traits. SNPs are known to exist per few hundred nucleotide bases in humans. In addition to SNPs, a number of genetic variations, including loss of heterozygosity (LOH) and copy number of genes, exist in human genomes. Therefore, it is difficult to analyze all genetic variations individually. The recent availability of high-density SNP genotyping and expression arrays enables researchers to undertake genome-wide association studies between various genetic variations, including more than 300,000 SNPs, LOH, copy number of genes, and some clinical traits such as disease phenotypes. These genotyping and expression arrays have strong potential to reveal the genes and their novel functions that have yet to be analyzed in opioid sensitivity.

Bioinformatics of knockout mice, genes, and genetic polymorphisms

A large number of gene knockout mice have been investigated for nociceptive sensitivity (Mogil and Grisel 1998; Mogil and Max 2006). Numerous genetic polymorphisms have been reported to be involved in the effects of opioids (Belfer et al. 2004). Bioinformatics and computational biology have been substantially developed, and various public databases have been constructed (e.g., Mouse Genome Informatics database, http://www.informatics.jax.org; Pharmacogenetics and Pharmacogenomics Knowledge Base, http://www.pharmgkb.org; SNP database, http://www.ncbi.nlm.nih.gov/SNP). These databases are useful for searching candidate genes and genomic variations for interindividual differences in opioid efficacy.

Conclusion

Until now, numerous gene-altered rodents have been established, and studies using these rodents have revealed that metabolic enzymes, receptors, transporters, and opioid signal mediators are involved in opioid sensitivity. Due to the development of genotyping technologies and the accumulation of information about genomic variations by human genome projects (e.g., HapMap), genomic polymorphisms associated with the function of these molecules and opioid sensitivity are rapidly increasing. Like many other polygenetic disorders, genetic factors such as SNPs have a major impact on interindividual differences in opioid sensitivity, although at present, results of clinical association studies are still limited and inconsistent for clinical applications. Further gene analyses with gene-altered rodents and human subjects will lead to methods of estimating the appropriate kinds and doses of opioids for individuals.

Acknowledgments We are grateful to Dr. Y. Ogai, Dr. D. Nishizawa, and Ms. J. Hasegawa (Division of Psychobiology, Tokyo Institute of Psychiatry) for valuable discussion and suggestions.

This study was supported by the Japanese Ministry of Health, Labour and Welfare (H17-pharmaco-001) and the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

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