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Accelerated CommunicationCHROMOSOMAL LOCALIZATION OF OPIOID PEPTIDE  
AND RECEPTOR GENES IN THE MOUSE

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**Abstract:** Opiate receptors are the primary targets for the drugs of abuse morphine and heroin. In this study, we completed the localization on mouse chromosomes of the genes encoding mu (*Oprm*) and kappa (*Oprk*) receptors, as well as the genes for the opioid propeptides proenkephalin (*Penk*) and prodynorphin (*Pdyn*). The genetic mapping was performed using a panel of DNA samples from an interspecific cross [C3H/HeJ-*gld* and (C3H/HeJ-*gld* x *Mus spretus*)F<sub>1</sub>] that has been characterized for more than 800 markers throughout the genome. The genes are localized on mouse Chr 1 (*Oprk*, 10 cM from the centromere), Chr 2 (*Pdyn*, 75 cM from the centromere), Chr 4 (*Penk*, 1 cM from the centromere) and Chr 10 (*Oprm*, 10 cM from the centromere). Interestingly, the gene for the mu receptor is located in the same region as a Quantitative Trait Locus for high morphine consumption, thus raising the possibility of its direct role in drug abuse mechanisms.

**Key Words:** opioid peptides, opioid receptors, chromosomal localization, genes, drug addiction

### Introduction

Addiction is a complex phenomenon which combines sensitization, dependence and tolerance to an abuse drug. Apart from the idiosyncratic pharmacological consequences of the drug administration, there is a marked individual propensity for drug craving. Even though these distinct behaviors are the result of individual environmental histories, family and twin studies in human, and recombined inbred analysis in mouse, strongly suggest that these individual differences could be partially attributed to genetic diversity (for review, see ref. 1).

Morphine and morphine derivatives represent a homogeneous class of drugs of abuse. They mimic the effects of endogenous peptides subdivided into three families

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according to their respective protein precursors, pro-enkephalin, pro-dynorphin and pro-opiomelanocortin. Three classes of opiate receptors called mu ( $\mu$ ), delta ( $\delta$ ) and kappa ( $\kappa$ ) receptors, have been characterized, and subdivided as  $\mu$ 1,  $\mu$ 2,  $\delta$ 1,  $\delta$ 2, and  $\kappa$ 1,  $\kappa$ 2,  $\kappa$ 3 according to their pharmacological characteristics. Allelic differences of these opioid propeptides and receptors may potentially be responsible for the divergent behaviors observed upon the intake of morphine or morphine derivatives. In order to explore this possibility, we took advantage of the cloning of opioid propeptides and receptors to complete the chromosomal mapping of the genes encoding proenkephalin, prodynorphin and the  $\mu$  and  $\kappa$  type of opiate receptors in the mouse. Precise localization of these genes should allow to investigate if they are colocalized with previously reported loci known to be involved in susceptibility to abuse drugs.

### **Material and Methods**

**Generation of probes:** The prodynorphin probe (*Pdyn*) was a 1.7 kb fragment of dynorphin genomic DNA (2). Probes including those for proenkephalin (*Penk*; 3),  $\mu$  opiate receptor (*Oprm*, kind gift of Dr. L. Yu, Indiana University Medical Center) and  $\kappa$  opiate receptor (*Oprk*, kind gift of Dr. G.I. Bell, Univ. of Chicago) genes corresponded to the entire coding region. Other probes included pF-C for collagen  $\alpha$ 1 type III (*Col3a1*; kind gift of Dr. Maria Mudryj), pXMm128 for collagen  $\alpha$ 1 type IX (*Col9a1*., 4), a 400 bp *EcoRI-HindIII* rho1 cDNA for the GABA receptor rho1 gene (*Gabrr*, 5), pMS-1 for the *Mos* protooncogene (*Mos*, 6), pMC1 clone for the *myb* protooncogene (*Myb*, 7), pSAM10b clone for collagen  $\alpha$ 1 type X (*Col10a1*, 8), pIL1-1301 clone for interleukin 1a (*Il1a*, 9), and pTOP1-3' for topoisomerase 1 (*Top1*, 10).

**Chromosome localization:** C3H/HeJ-*gld* and *Mus spretus* (Spain) mice and [(C3H/HeJ-*gld*  $\times$  *Mus spretus*)F<sub>1</sub>  $\times$  C3H/HeJ-*gld*] interspecific backcross mice were bred and maintained as previously described (11). *Mus spretus* was chosen as the second parent in this cross because of the relative ease of detection of informative restriction fragment length variants (RFLV) in comparison with crosses using conventional inbred laboratory strains. DNA isolated from mouse organs by standard techniques was digested with restriction endonucleases and 10  $\mu$ g samples were electrophoresed in 0.9% agarose gels. DNA was transferred to Nytran membranes (Schleicher & Schuell, Inc., Keene, NH, USA), hybridized at 65°C and washed under stringent conditions, all as previously described (12). Gene linkage was determined by segregation analysis (13). Gene order was determined by analyzing all haplotypes and minimizing crossover frequency between all genes that were determined to be within a linkage group. This method resulted in determination of the most likely gene order (14).

### **Results**

In order to determine the chromosomal location of the genes encoding proenkephalin, prodynorphin, and the opiate receptors  $\mu$  and  $\kappa$ , we analyzed a panel of DNA samples from an interspecific cross that has been characterized for over 800 genetic markers throughout the genome. The genetic markers included in this map span between 50 and 80 centi-Morgans (cM) on each mouse autosome and the X chromosome (15-16). Initially, DNA from the two parental mice [C3H/HeJ-*gld* and (C3H/HeJ-*gld*  $\times$  *Mus spretus*)F<sub>1</sub>] were digested with various restriction endonucleases and hybridized with the *Oprk*, *Oprm*, *Penk* and *Pdyn* cDNA probes to determine RFLVs for haplotype analyses. Informative RFLVs are indicated in Table 1.

Comparison of the haplotype distribution of the *Oprk*, *Oprm*, *Penk* and *Pdyn* RFLVs indicated that each segregated independently and mapped to four different mouse chromosomes (Figure 1). In 108 of the 114 meiotic events examined, the *Oprk* locus cosegregated with *Col9a1* gene on proximal mouse Chr 1. In 109 of the 114 meiotic events, the *Oprm* locus cosegregated with the *Myb* gene on proximal mouse Chr 10. In 111 of 114 meiotic events, the *Pdyn* locus cosegregated with the *Il1a* gene on mouse Chr 2. Finally, in 113 of 114 meiotic events, the *Penk* locus segregated with the *Mos* gene on proximal mouse Chr 4.

**TABLE 1**  
Restriction Fragment Length Variants.

Locus	Restriction enzyme	C3H/HeJ- <i>gld/gld</i>	<i>Mus spretus</i>
<i>Oprk</i>	<i>EcoRI</i>	3.2	5.0
<i>Oprm</i>	<i>BamHI</i>	5.8	5.2
<i>Pdyn</i>	<i>StuI</i>	6.0	5.0
<i>Penk</i>	<i>MspI</i>	4.3	3.0

Representation of the informative restriction fragment length variants used in this study for the genetic mapping of opiate-related genes in interspecific backcross mice. Size of restriction fragments is indicated in kilobase, as a function of mouse strain.

The haplotype distribution among the other genes localized to these mouse chromosomes is shown in Figures 1 (A-D). The best gene order (14)  $\pm$  the standard deviation (13) indicated the gene order : (centromere) *Oprk*- 5.3  $\pm$  2.1 cM - *Col9a1* - 6.1  $\pm$  2.3 cM - *Col3a1* ; (centromere) *Oprm* - 4.4  $\pm$  1.9 cM - *Myb* - 6.1  $\pm$  2.3 cM - *Col10a1*; (centromere) *Il1a* - 2.6  $\pm$  1.5 cM - *Pdyn* - 14.0  $\pm$  3.3 cM - *Top1* ; (centromere) *Mos* - 0.9  $\pm$  0.9 cM - *Penk* - 15.8  $\pm$  3.4 cM - *Gabrr*.

As indicated in table 2, these data give the positions of the genes with respect to composite maps of mouse chromosomes. These positions are : *Oprk*, mouse Chr 1,  $\sim$ 10 cM from centromere; *Oprm*, mouse Chr 10,  $\sim$ 9 cM from the centromere; *Pdyn*, mouse Chr 2,  $\sim$ 75 cM from the centromere; *Penk*, mouse Chr 4,  $\sim$ 1 cM from the centromere.

### Discussion

Opiate receptors are the endogenous targets for morphine and morphine derivatives, and the endogenous peptides enkephalins, dynorphins and  $\beta$ -endorphin are natural competitors at these sites. Therefore, any modification of these proteins is likely to result in a variation of the response to drug abuse. In this respect, Quantitative Trait Loci (QTL) mapping on chromosomes appears to be a powerful approach to unravel regions in the mouse genome potentially involved in such complex behaviors as drug addiction, and even more specifically high morphine consumption (17-18). We now report the genetic mapping in the mouse of the genes encoding for opiate-related proteins, as summarized in Table 2. During the completion of our work, Kozak et al. (19) using a different interspecific backcross, reported the same localization of opiate receptors  $\mu$  and  $\kappa$  in mouse at the one we now report. These localization are discussed in relation to established mutations in the mouse, or locus potentially involved in drug abuse behavior.

**TABLE 2**  
**Localization of the genes encoding opioid receptors and propeptides on mouse and human chromosomes.**

Proteine	Locus	Mouse	Human
δ receptor	Oprd	4 <sup>a</sup>	(1p)
κ receptor	Oprk	1	8q11.2 <sup>c</sup>
μ receptor	Oprm	10	6q24-25 <sup>d</sup>
Prodynorphin	Pdyn	2	20pter-p12 <sup>e</sup>
Proenkephalin	Penk	4	8q11.23-q12 <sup>e</sup>
Proopiomelanocortin	Pomc1	12 <sup>b</sup>	2p23 <sup>f</sup>
	Pomc2	19 <sup>b</sup>	

Values for the mouse are the chromosome number (see text for details). For human, the values represent the chromosome number, and the loci when determined. Value in brackets is not an actual determination, but a probable localization derived from the mouse mapping.

a: Ref. 30. b: Ref. 31. c: Ref. 20. d: Ref. 27. e: Ref. 24. f: Ref. 32-33.

*Oprk* is located more proximally on mouse chromosome 1 than any gene for which the human homologous position has been defined, and very recently the human κ receptor gene was found to be located at 8q11.2. Interestingly, there is in mice a "neuro-endocrine" mutation known as tumbler (*tb*) which has been reported at proximal mouse Chr 1. With this recessive mutation, homozygotes walk in a crab-like fashion, they may fall over, or jump when trying to go forward. They can swim but cannot hold on to a rope. Most homozygotes survive and can breed (21). No obvious lesions were reported in the central nervous system, but the endocrine and lymphoid tissues show some abnormalities. The cells of the anterior pituitary have a diminished amount of cytoplasm (22), and growth hormone cells show sign of degeneration by 90 days of age (23). To study if the κ receptor gene is directly responsible for this mutation would probably deserve further investigation.

The gene coding for prodynorphin is located on the mouse Chr 2. Its position would suggest that in human *Pdyn* is located in the pericentric region of Chr 20 since genes mapped distal to *Il1a* on mouse Chr 2 are part of a large conserved linkage group with this region of human Chr 20 (23). This is actually the case, as Litt et al (24) mapped human *Pdyn* gene at 20pter-p12. No particular mutations, or QTL mapping, has been reported in this area of mouse chromosome 2.

Position of *Penk* on proximal mouse chromosome 4 is in agreement with the localization of human *Penk* at 8q11.23-q12 (24). However, there is here a discrepancy with a report from Shapiro et al. (25) who mapped this gene on mouse Chr 9. It should be emphasized that this previous study in the mouse detected both polymorphic (segregating) and nonpolymorphic (nonsegregating) bands using a mouse *Penk* probe different from the one used in the present study. Giving the single band hybridizing to the *Penk* probe in our study and the mapping results highly consistent with the human gene localization, it is probable that Shapiro et al. mapped a *Penk* related sequence rather than the structural gene for this propeptide.

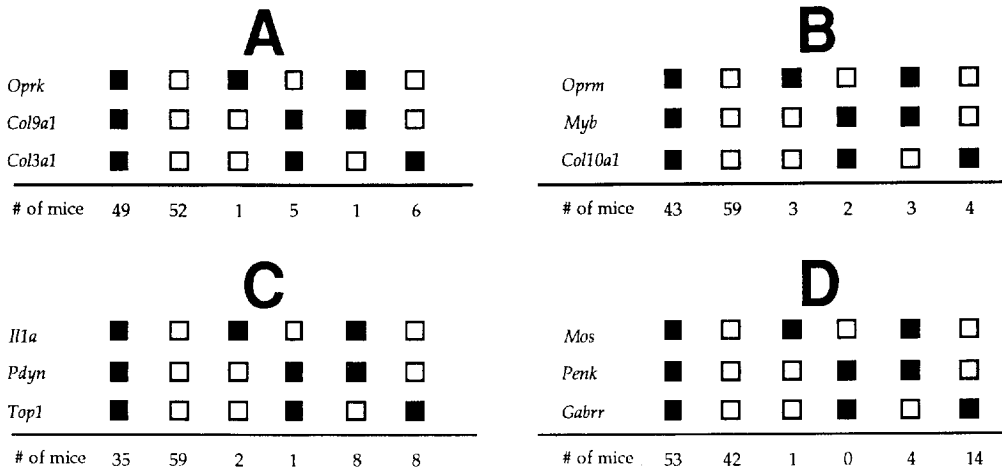


Fig. 1

Segregation of *Oprk* (A), *Oprm* (B), *Pdyn* (C) and *Penk* (D) on mouse chromosomes in [(C3H/HeJ-*gld* and (C3H/HeJ-*gld* x *Mus spretus*)F<sub>1</sub>] interspecific backcross mice. Filled boxes represent the homozygous C3H pattern and open boxes the F<sub>1</sub> pattern. The RFLV used to define *Oprk*, *Oprm*, *Pdyn* and *Penk* are defined in Table 1. The mapping of the other loci included in this figure have been previously published (5, 34, 10, 4), and are also included in composite maps of chromosome 1, 10, 2, and 4 (35, 36, 23, 37).

The only reported mutation in mice arising in this chromosomal area is waddler (*wd*), a recessive mutation. Homozygotes *Wd/Wd* can be identified at 14 days of age, their hindquarters sway from side to side in a smooth arc, and the mice often fall over on their hips, with no trembling or paralysis occurring. These animals are often smaller than their normal littermates, but viability is good, and many waddlers are fertile (26). Unfortunately, this line is now probably extinct, which will render any further testing rather speculative.

The gene for *Oprm* maps to a position on mouse chromosome 10 within a large conserved linkage group with the long arm of human chromosome 6. This would place human *Oprm* to chromosome 6 between bands q24 and q27, confirming a previous mapping at 6q24-25 (27). One major neuromuscular mutation, dystrophina muscularis (*dy*), lies nearby the *Oprm* locus. This recessive mutation is characterized in homozygotes by progressive weakness and paralysis beginning at about 3<sup>1/2</sup> weeks of age. Death occurs before 6 months of age, and the mice are usually sterile (28). This mutation is associated primarily with a defect in Schwann cell myelination (29). It is therefore unlikely that a defect in *Oprm* gene, i.e. in the  $\mu$  opiate receptor, would be responsible for the *dy* mutation.

More interesting is the location of *Oprm* in a region of mouse chromosome 10 where two QTL related to morphine effects have been mapped. One locus was reported to correlate with morphine-induced hypothermia (17), close to *Mpmv-5* region of chromosome 10. Much more excitingly, *Oprm* is located in the same region of proximal chromosome 10 as a recently reported QTL for high morphine consumption

(18). The maximum LOD score for this QTL mapped was near the D10Mit28 marker. Since the consensus position for D10Mit28 is 3 cM from the centromere, and that our data would place *Oprm* at 9 cM from the centromere, *Oprm* would appear to map within the highest LOD intervals for this QTL. In addition, only 1 crossover in 38 meiotic events ( $2.6 \text{ cM} \pm 1.5 \text{ cM}$ ) was observed between D10Mit28 and *Oprm* (data not shown), further indicating that *Oprm* is a very strong candidate gene for this QTL. Inbetween the two parental mouse strains, C57Bl/6 shows a very high consumption of morphine (~2-300 mg/day) as compared to DBA/2 (~10-20 mg/day). Therefore, to further verify this hypothesis, we are currently cloning the  $\mu$  opiate receptor in the two parental strains C57Bl/6 and DBA/2 to check for any differences in sequence or function.

In conclusion, we reported the mapping on mouse chromosomes of opioid-related genes encoding the  $\mu$  and  $\kappa$  opiate receptors, and the propeptides proenkephalin and prodynorphin, at respectively Chr10:9 (number, distance from the centromere in cM), Chr1:10, Chr4:1 and Chr2:75. These localizations should be informative for the assignment of mutations or QTL related genes to opiate function, and for a better understanding of the molecular changes which can possibly underlie drug addiction. In this respect, colocalization of the gene for the  $\mu$  opiate receptor and a quantitative trait locus for morphine consumption represents a very promising avenue for future investigations.

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