**The μ opiate receptor as a candidate gene for pain: Polymorphisms, variations in expression, nociception, and opiate responses**

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**ABSTRACT** There are differences between human individuals and between mouse strains in levels of μ opiate receptor (μOR) expression, responses to painful stimuli, and responses to opiate drugs. One of the best candidates for contributing to these differences is variation at the μOR gene locus. Support for this idea comes from analyses of the human and murine μOR genes. Assessments of individual differences in human μOR expression add further support. Studies with mice, including knockout-transgenic, quantitative trait locus, and strain-comparison studies, also strongly support the possibility that μOR gene alleles would be strong candidates for contributing to individual differences in human nociception and opiate drug responses. This paper reviews current analyses of the murine and human μOR genes, their important variants, and correlations between these variants and opiate influences on pain.

Opiates remain major weapons in pain therapy, but individual differences in the effectiveness of these drugs and in their side effects can be a major limitation for effective pain treatment for many patients. A number of lines of evidence now indicate convincingly that the morphine-preferring μ opiate receptor (μOR) is the major site for the analgesic action of most clinically important opiate drugs.

The powerful analgesic effects of morphine and related drugs focus attention on morphine-preferring μORs and their endogenous and exogenous agonists. A number of laboratories, including our own, have had success in cloning μOR cDNAs and genomic sequences from several species (1–4), thereby opening new avenues from which to approach this receptor’s neurobiology and its relationships with nociceptive responses. This work has laid substantial groundwork for genetic analyses, although it remains incomplete (see below).

Data from animal models provide powerful motivation to search out and understand possible genetic bases for individual differences in levels of human μOR gene expression. Recent data from transgenic mice provide important information about the role of μOR expression levels in mouse models of human pain (4, 5). The data indicate strongly that the μOR gene product is the principal route for opiate effects on nociception. Morphine is not analgesic without μORs. Prototypical δ and κ agonists can also function poorly without μORs (refs. 6 and 7; I.S. and G.R.U., unpublished observations).

Several studies of the mice that lack μOR provide evidence that μORs are important for baseline nociception (ref. 4; see also ref. 5). Nociceptive thresholds vary in gene dose-dependent fashions in such mice. Mice with no μORs have lower nociceptive thresholds than heterozygous knockouts that have 50% of wild-type receptor densities. These heterozygous mice, in turn, have lower nociceptive thresholds than wild-type mice with intact μORs.

Mouse-strain comparisons and studies in recombinant inbred mouse lines also provide powerful models for possible sources and consequences of genetic variation in humans. Strain-comparison studies have identified both reduced antinociceptive responses to morphine and lower levels of μOR expression in some mouse strains, although these are correlations that do not directly document causal relationships between differences in μOR expression levels and observed differences in morphine responses (8–10). Differences in morphine responses between DBA and C57 mice as well as the B×D recombinant inbred strains derived from these parental lines can be mapped by using quantitative trait locus approaches (11–14). Berrettini and coworkers have mapped a significant portion of the genetic variance in morphine preference to the vicinity of the μOR locus by using quantitative-trait-locus approaches (13, 14). Belknap et al. (15) have also found that markers near this chromosomal locus correlate with mouse analgesic responses to 16 mg/kg morphine in hot-plate test assays. This data set derives from a genomic marker somewhat distant from the mouse μOR locus, a single analgesic measure, and a single, relatively high morphine dose. However, the data do fit with those from morphine-preference studies. They also correlate with maximum bound determinations for [3H]naloxone binding densities in the brains of the same species, performed under binding conditions that should predominantly label μORs.

Recently, we have identified a murine μOR gene 5' flanking-region polymorphism that lies much closer to candidate μOR promoter/enhancer regions (I.S. and G.R.U., unpublished observations). This simple sequence repeat has striking correlations with both levels of μOR expression and the extent of morphine antinociception in the B×D recombinant inbred lines (see below). Preliminary analyses suggest that the allelic status at this marker correlates with the baseline nociceptive thresholds for hot-plate assays in mice of eight tested strains (see below). Replication of this finding and its extension to more strains and to opiate responsiveness in them could provide striking evidence that a nearby region has sequence variants that have functional consequences for the level of μOR expression and/or its regulation.

Data from murine studies thus document (i) that μORs may well be key both for normal nociception and for normal opiate drug responses, (ii) that changes in μOR densities of 50%, or even less, can produce differences in both nociceptive re-

Abbreviation: μOR, μ opiate receptor.

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sponses and in their modulation by opiates, and (iii) that allelic variants at the μOR locus are strong candidates for contributing to these differences in mice and attractive candidates for producing such effects in humans.

Humans differ in their individual responses to pain and to opiates. Recent studies of twins document that individual differences in several types of pain are likely to have substantial genetic determinants. Genetic components to susceptibility to migraine pain are documented in studies of thousands of twin pairs, although family studies document substantial genetic heterogeneity in this disorder (16–18). Studies of concordance for self-reported menstrual pain also identify substantial genetic components (19). Interestingly, the heritabilities documented in these human studies (~0.5) fit nicely with those identified in murine strain-comparison and quantitative trait locus studies (20, 21).

Humans also differ from one another in μOR densities. Binding studies to postmortem brain samples and in vivo positron-emission tomography radioligand analyses both suggest 30–50% or even larger ranges of individual human differences in μOR densities. For example, Pfeiffer et al. (22) reported that median μOR binding in human frontal cortex was 2.3 pmol/g (SD = 0.52). Frost and coworkers (23, 24) noted that a measure of thalamic μOR binding with [11C]carfentanil was 3.8 pmol/g (SD = 1.4). If individuals in the upper third of the population are characterized by these data, they should express >45% (22) or >74% (23, 24) more μORs than the individuals in the lower third of the population. Mouse studies document that genetic differences of this magnitude in μOR expression can influence both baseline nociception and morphine responses, as noted above. Elucidation of the genetic bases for these differences in receptor expression would thus represent a substantial advance in our understanding of individual differences in nociceptive behaviors and drug responses.

Levels of expression of many, if not most, human genes differ from individual to individual. Many of these differences are thought to be based on differences in the cis-acting DNA sequences that normally act to provide cell-type-specific, appropriately regulated gene expression (25). Many of these DNA promoter and enhancer sequences are typically found in the 5′ ends of genes and often serve as recognition sites for regulatory DNA binding proteins.

Searches for the functional polymorphisms that contribute to these individual differences in gene expression can involve several steps. Cloning appropriate genomic sequences and characterizing the site(s) for transcriptional initiation so that 5′ flanking and other potential regulatory regions can be determined with confidence represents an important initial step. Identifying polymorphisms in these regions provides a second series of challenges (26). Seeking relationships between these polymorphisms and differences in levels of gene expression is a third step. We can then ask whether the identified polymorphic sequences predict differences not only in levels of μOR expression but also in opiate responses.

The information currently available in GenBank describes ~2 kilobases (kb) of murine and 0.2 kb of human μOR genomic sequences 5′ to the μOR translational start site (Fig. 1). Studies of rapid amplification of cDNA 5′ ends have suggested to other workers that two nearby regions provide the sites at which primer extension products terminate, which are thus potential transcriptional initiation sites (~793 and ~268 bp from the translational start site; refs. 27 and 28). Sequences from each of these two regions can support some expression of reporter genes in heterologous cell-expression systems. These sequences can even have enhanced expression in the SHSY5Y cells that normally express μOR at modest levels.

None of the reported primer extension products have the modified bases characteristic of mRNA capping, however. None of these sequences provide the 5′ untranslated-region length characteristic of most long mRNAs with relatively short coding sequences (see below). These ~268-bp and ~793-bp sequences might thus serve as true promoter/enhancer regions. If so, then human polymorphisms in these regions should be sought out, as only a moderate number have been reported thus far.

Initial searches in our laboratory, as well as more extensive work by Goldman and coworkers (29) and by L. Yu (personal communication), have failed to identify common human μOR protein coding-sequence variants that dramatically change the receptor’s function, although a modest alteration in affinity for the opioid peptide β-endorphin has been noted by Yu and coworkers (30).

These data are in accord with studies that document no convincing individual differences in μOR affinities among humans. The data also fit with the substantial μOR coding sequence conservation among species (2, 4, 22). Such information suggests that genetic components may be unlikely to provide commonly encountered individual differences through functionally different μOR protein sequences. The information contrasts with the abundant data, noted above, documenting frequent individual differences in levels of μOR expression.

Studies by Ko et al. (27) and by Liang and Carr (28) indicate that searches for possible promoter-region sequences must include the sequences located between ~268 and ~793 bp 5′ to the translational initiation sites tentatively identified by these workers. However, recently, we have also developed interesting results from comparisons of murine and human 5′ flanking sequences. These data could also suggest other sites at which to seek potential promoter-region polymorphisms in humans. Scatterplot comparisons of these species’ μOR sequences clearly show the area of high cross-species conservation at the ~268/~793-bp region identified by Ko et al. (27) and by Liang and Carr (28). These analyses also find another highly conserved region that seems to extend from ~2,500 to ~4,500 bp 5′ to the translational start (Fig. 2). Conceivably,
each of these regions could represent unusually highly conserved promoter/enhancer sequences. Polymorphisms from these two conserved regions would thus be likely candidates for marking functional, level-of-expression allelic μOR variants. Alternatively, these highly conserved regions could also reflect additional exon sequences. Under this scenario, even more 5′ sequences would suggest more prominent candidates for contributing to μOR regulation.

We and others have also identified a number of interesting μOR gene polymorphic markers. We identified a human polymorphism: an MspI restriction fragment length polymorphism (2). We used PCR to amplify the DNA and have sequenced >1 kb of DNA containing the −268/−793-bp region from 12 unrelated human individuals (volunteers who gave informed consent for studies conducted for the Intramural Research Program of the National Institute on Drug Abuse in Baltimore; Z.W. and G.R.U., unpublished observations). Sequence comparisons identify repetitive sequences in these regions that were not polymorphic in initial screens. Two other types of sequence variation have been identified. More than 20 single nucleotide polymorphisms have been identified in these sequences (see ref. 31). We are working to confirm these sequences and will study their frequencies in larger samples to establish their utility for correlations with receptor-expression densities and opiate-drug responses. Altering 5′ untranslated mRNA sequences could readily explain different levels of μOR mRNA stability or even translational efficacy and could contribute to the expression of differing levels of this protein in different individuals or cell types.

We also have identified a polymorphic repetitive element in >8 kb of murine 5′ flanking sequence (I.S. and G.R.U., unpublished observations). This murine polymorphism lies 2 kb 5′ to the translational start site, close to sequences recently identified as candidate μOR promoter/enhancer elements (see below).

Because data from comparisons of more 5′ human and mouse μOR genomic sequences also suggest the presence of additional exon(s) and more 5′ sites for transcriptional initiation or highly conserved regulatory regions, searches in more 5′ genomic regions also make sense. Workers are currently undertaking approaches consisting of cloning additional 5′ genomic sequence, searching for simple sequence repeat and single nucleotide polymorphisms, characterizing the individual differences in these polymorphic sites, and applying these polymorphisms to seek correlations with μOR expression levels and noiceptive responses. Most genes’ promoters have much of their functional anatomy within several thousand base pairs of their transcriptional initiation sites. However, other genes have promoter regions that extend for >10 or even >20 kb. Analyses of further μOR genomic 5′ flanking sequences could make great contributions to understanding this gene and would be quite likely to identify many of its important regulatory elements.

Information about μOR gene polymorphisms that can predict the likelihood of high or low levels of μ expression in an individual could allow drug treatments to be individualized. These data could aid in selecting analgesic agents and in optimizing dose ranges. They could thus improve pain management for individuals with acute or long-term pain problems. These data could suggest new therapeutic specificities and efficacies to even this well established opiate drug class that remains a major weapon for amelioration of pain states.
Thus, the extensive work required to identify such markers should be worthwhile.