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Elucidation of mu-Opioid Gene Structure: How Genetics Can Help Predict Responses to Opioids

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Abstract

Opioid drugs are among the most commonly used and effective human analgesics. To date, the clinical benefits of opioid analgesics have not been fully realized due to substantial individual variations in the responses to opioids, insufficient drug dosing, and a high rate (up to 66%) of adverse events. As such, there is a substantial need to identify the genetic and molecular biological mechanisms that mediate individual responses to opioid therapy. Recent discoveries show that genetic variations in the μ -opioid receptor (*OPRM1*) gene locus play an essential role in inter-individual responses. The majority of genetic association studies have focused on the A118G polymorphism, which codes for a non-synonymous change in *OPRM1* exon 1. In addition to the A118G polymorphism, another functional SNP (rs563649), which is located within an alternatively-spliced *OPRM1* isoform (MOR-1K), has been identified. The MOR-1k isoform codes for 6TM *OPRM1* isoforms that display excitatory rather than the inhibitory cellular effects, which are characteristic of the canonical 7TM isoforms. Thus, stimulation of the 6TM isoforms may engage the molecular mechanisms mediating opioid-dependent hyperalgesia, tolerance and dependence. Future clinical and basic studies that seek to identify the functional genetic variants within *OPRM1* locus, and associated molecular mechanisms, will result in a better understanding of individual responses to opioid therapy and ultimately to the development new pharmacotherapeutics and diagnostic tools.

Keywords

analgesic response to opioids; mu-opioid receptor (MOR); opioid receptor gene (*OPRM1*) structure; genetic association study; 6 transmembrane (6TM) MOR

Opioid analgesics are the most widely used drugs to treat moderate to severe pain. Unfortunately, these agents produce significant individual side effects consisting of miosis,

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pruritus, sedation, nausea and vomiting, cognitive impairment, constipation, hypotension and life-threatening respiratory depression (Rowlingson and Murphy, 2000; Ready, 2000; Inturrisi, 2000; Goldstein, 2002). Even when the correct clinical protocol is followed for the use of opioids, an effective and safe dose of an opioid for an individual patient can be difficult to predict, which reflects the considerable inter-individual variability in the clinical responses to opioid analgesics. In fact, the minimal effective analgesic concentration (MEAC) for opioids, such as morphine, pethidine, alfentanil and sufentanil, vary among patients by factors of 5 to 10 (Glass, 2000; Camu and Vanlersberghe, 2002). As such, there is a substantial need to develop new biological markers that will provide valid and reliable predictions of individual responses to opioid therapy.

The μ -opioid Receptor as a Critical Contributor to Opioid Responses

Results from animal and human studies suggest that variations in opioid analgesia and side-effect profiles are influenced by multiple factors, including genetic variability. Among genetic factors, allelic variants in the gene locus coding for μ -opioid receptor (MOR) have been suggested to play a critical role in the variation in the responses to MOR agonists (Thompson et al, 2000; Duguay et al, 2004; Klepstad et al, 2004). MOR1 is the primary target of both endogenous and exogenous opioid analgesics, which mediates basal nociception as well as μ -opioid receptor agonists responses (Matthes et al, 1996; Sora et al, 1997; Uhl et al, 1999; Edwards et al, 2006). Both animal and human studies have indicated that reduced basal nociceptive sensitivity is associated with greater opioid analgesia (Mogil et al, 1999; Edwards et al, 2006). Identified genetic polymorphisms in the human *OPRM1* gene, which codes for MOR1, are the primary candidates for sources of clinically relevant variability in opiate sensitivity and baseline nociception (Mogil, 1999; Uhl et al, 1999; Han et al, 2004). Several polymorphisms have been found in the promoter, coding and intron regions of the gene that are associated with pharmacological and physiological effects mediated by MOR1 stimulation (for review see (Lotsch and Geisslinger, 2005)). However, one of the most commonly investigated MOR genetic variants involves a single nucleotide polymorphism (rs1799971) in exon 1 of *OPRM1*, in which an adenine to guanine substitution exists at position 118 (A118G or 118A→G). This missense mutation causes a substitution of asparagine for aspartic acid at amino acid position 40 in the N-terminal extracellular domain, which corresponds to the loss of a putative glycosylation site (Bond et al, 1998). The functional molecular consequences of this non-synonymous change has been widely studied, but has not been fully elucidated. Early studies reported that the 118G receptor displayed increased binding affinity and potency for endogenous ligands (Bond et al, 1998), though other studies failed to replicate this finding or found impaired signaling through the receptor (Befort et al, 2001; Beyer et al, 2004). Consistent with the latter findings, the majority of the human genetic association studies that have examined the responses to opioids show that carriers of the 118G variant allele demonstrate poor responses to opioids (Table 1), although the opposite or no effect have been reported as well (Lotsch and Geisslinger, 2005). Furthermore, in twin studies few functional non-synonymous *OPRM1* mutations have also been identified (Table 2). It is unclear how common these rare familial mutations are in the human population and it is not known if they aggregate within specific haplotypes. Since *OPRM1* gene locus is situated within two large haploblocks, with one long functional haplotype spanning the entire gene locus, the joint effect of several functional SNPs and interactions between them have been described (Shabalina et al, 2009). Collectively, the current literature provides evidence for the existence of other functional SNPs within the *OPRM1* gene locus in addition to A118G.

OPRM1 Genomic and Isoform Structure

The MOR1 receptor is a member of G-protein-coupled receptors (GPCRs) family. It has an extracellular N-terminus and intracellular C-terminus, with seven membrane-spanning domains that comprise the binding pocket for exogenous drugs. MOR1 induces analgesia via pertussis toxin (PTX)-sensitive inhibitory G protein ($G_{i/o}$), which inhibits cAMP formation and Ca^{2+} conductance and activates K^{+} conductance, leading to hyper-polarization of cells thereby, exerting an inhibitory effect (North, 1986; Crain and Shen, 2000). The major form of *OPRM1*, also called MOR-1, is coded by exons 1, 2, 3 and 4, whereas exon 1 codes for first transmembrane domain and exon 2 and 3 code for the second through seventh transmembrane domains (Pasternak, 2004). There is growing evidence from rodent studies for an important role of alternatively-spliced forms of *OPRM1* in mediating opiate analgesia (Pasternak, 2004). The synergistic activities of these splice variants has been proposed to explain the complex pharmacology of μ -opioids (Pasternak, 2004). Though there is substantial discrepancy between the genomic organization of mouse *OPRM1* and the genomic organization of human *OPRM1*, (see Unigene databases, (Pasternak, 2004; Kvam et al, 2004; Pan; Doyle et al, 2007)), there are human homologues for each, or at least majority of the mouse *OPRM1* exons (Shabalina et al, 2009). There are two common splicing patterns of *OPRM1* that involve the C-terminus and N-terminus. C-terminus variants contain exons 1, 2 and 3 and code for all seven transmembrane domains, but differ structurally and functionally at the intracellular domain, a region important in signal transduction following receptor activation. There are also a number of variants that differ in their N-terminus, some of which encode for truncated receptors. All reported mouse N-terminus variants are initiated from exon 11. Exon 11 is located approximately 30 kb upstream of exon 1 and is under the control of a different upstream promoter, suggesting alternative regulation of transcription. Three of these variants are predicted to code for truncated receptors with only six transmembrane domains (6TM). Thus, the plausible biological role and functional significance of these truncated receptors represents an intriguing experimental and clinical question.

During the last few years, several human alternatively-spliced forms coding for 6TM receptor variants have been reported. First, a human MOR isoform MOR-3 has been cloned that is selectively activated by opiate alkaloids, but is insensitive to opioid peptides (Cadet et al, 2003). Unlike the mouse 6TM isoform, *MOR-3* mRNA is lacking exon 11 and seems to use a promoter upstream to exon 2. At the functional level, COS-1 cells transfected with the MOR-3 receptor exhibited a dose-dependent release of nitric oxide (NO) following treatment with morphine, but not with DAMGO. Importantly, naloxone blocks the effect of morphine on COS-1 cells transfected with MOR-3 (Cadet et al, 2003). Similar to the mouse 6TM receptor isoform, this isoform lacks an amino acid sequence of ~90 amino acids that constitute the extracellular N-terminal and the first transmembrane domain TMH1 and part of the first intracellular loop, but retains the ligand binding pocket that is distributed across conserved TMH2, TMH3, and TMH7 domains. Additionally, the MOR-3 isoform possesses unique intracellular C-terminal amino acid sequences that have been hypothesized to serve as coupling or docking domains required for constitutively expressed NO synthase (NOS) activation (Kream et al, 2007). Northern blot and RT-PCR results reveal the expression of this MOR-3 variant in human vascular tissue, mononuclear cells, polymorphonuclear cells, and human neuroblastoma cells (Cadet et al, 2003). Following the first reports of the human 6TM MOR isoform MOR-3, there has been an increase in the number of identified and characterized *OPRM1* isoforms that code for a 6TM receptor.

Expansion of the OPRM1 Gene Locus

In our recent work, we applied new comparative genomic approaches that generated evidence that the human *OPRM1* gene is orthologous to the mouse *OPRM1* gene and is thus much more complex than previously appreciated (Shabalina et al, 2009). We hypothesized that all 20 of the reported mouse exons, or a substantial number of these exons, have analogous exons within the human *OPRM1* gene locus. Applying a unique set of bioinformatic comparative analytical approaches to mouse and human genomic *OPRM1* gene, we identified 10 new potential exons, and two new putative promoters (Shabalina et al, 2009). Our results suggest that at least one new alternatively spliced form of the *OPRM1* gene, *MOR1K*, codes for a receptor variant that results in greater sensitivity to noxious stimuli and poorer responses to morphine (Shabalina et al, 2009). Importantly, the *MOR1K* isoform codes for a 6TM rather than 7TM G protein coupled receptor (GPCR). Although similar to the *MOR-3* variant reported by Cadet (Cadet et al, 2003), *MOR-1K* codes for a truncated 6TM MOR receptor with a different intracellular domain and tissue distribution. In contrast to MOR-3, MOR-1K is expressed in brain tissues, but not in vascular tissues or leukocytes. Unlike the mouse MOR-1K isoform, where transcription is initiated from an alternative promoter upstream to exon 11, the 5' start site of MOR-1K isoform has been mapped to a novel *OPRM1* promoter region that is located upstream of exon 13 (Shabalina et al, 2009). We also reported that agonists of the 6TM receptor produce dose-dependent excitatory rather than the classic inhibitory cellular effects observed for the major 7TM MOR1 isoform. Specifically, stimulation of the MOR-1K 6TM isoform with morphine produces a dose-dependent increase in intracellular cAMP and Ca⁺⁺, and increases the cellular release of nitric oxide (Gris et al, 2010). These observations provided the basis for the intriguing hypothesis that the "excitatory shift" in MOR signaling depends, at least in part, on the stimulation of the 6TM isoform, and thus contributes to opioid-induced hyperalgesia, tolerance and dependence (Gris et al, 2010).

We are also conducting a comprehensive bioinformatic study of the molecular structure of human exon 11. We demonstrated that the exon 11 region shows the strongest conservation between man and mouse genomes (Shabalina et al, 2009). Shortly after this bioinformatic prediction, the human exon 11 was cloned (Xu et al, 2009). Four new human isoforms *MOR-1G1*, *MOR-1G2*, *MOR-1I* and *MOR-1H* were identified and were shown to contain exon 11 spliced either to exon 2 or to exon 1 through a variable 3' splice site. These isoforms code for either the extended 7TM receptor isoforms or the 6TM receptor isoform that are identical to the MOR-1K isoform we cloned (Shabalina et al, 2009). Importantly, the cloned exon 11 corresponds to our predicted exon 11 and the predicted 3' splice site reported by Pan and coworkers is situated between two alternative 3' spliced sites of the cloned exon we identified. The 5' spliced site of the cloned exon 11 was not mapped by Pan and coworkers (Xu et al, 2009), but instead was predicted by using bioinformatic approaches (Fig. 6). The successful cloning of human exons 11 and 13 by Pan and coworkers (Xu et al, 2009) and by our (Shabalina et al, 2009) groups validates our approach to predicting the extended *OPRM1* structure (Shabalina et al, 2009).

Genetic Variability within OPRM1 Alternative Exons

We also recently proposed that SNPs located within the new functional elements of *OPRM1* gene affect the activity or regulate the relative amount of corresponding alternatively-spliced *OPRM1* forms that are contribute to human variability to opioid responses. Our association analysis between a set of SNPs densely situated within the newly identified functional elements and measures of human pain perception and analgesic responses to morphine identified four new potentially functional SNPs (unpublished data, Shabalina et al, 2009). Importantly, all significantly associated SNPs are situated within the new functional

elements of *OPRM1*. This suggests that alternative exons represent an important target for genetic variability and may be a significant source of variation in common clinical phenotypes, including responses to opioid drugs. Since modifications of the function of the main receptor form may have dramatic consequences on genetic fitness and may not reach significant frequency in the general population, genetic variations in alternative exons that are expressed at low levels or only under specific conditions may lead to more subtle phenotypic differences that underlie the observed variations in MOR1-dependent phenotypes. In addition to the substantial biological significance of these findings, these results are likely to be of substantial clinical importance, as conceptually new tactics can now be considered for the identification of new functional SNPs that define the analgesic efficacy and side effects of opioids commonly used in clinical practice.

Thus, we demonstrated that SNP rs563649, which is located within a structurally conserved internal ribosomal binding site (IRES) in the 5'UTR of the novel isoform MOR-1K, affects both mRNA levels and translation efficiency of these variants (Shabalina et al, 2009). The higher receptor expression is driven by the minor T allele and is associated with greater sensitivity to noxious stimuli and poorer responses to morphine (Shabalina et al, 2009), confirming the potential excitatory cellular effects of 6TM MOR isoform. We propose to use similar approaches to identify the molecular genomic mechanisms that underlie other functional polymorphisms within the *OPRM1* gene locus and MOR alternatively spliced isoforms.

Other Important Genetic Contributors to Opioid Response

Marked individual variability in the pharmacokinetics of opioids as well as genetic variations in opioid-metabolizing enzymes, transporter and signal transduction elements have been reported (Somogyi et al, 2007). Several genes contribute to the pharmacodynamics and pharmacokinetics of opioids. For example, morphine, is particularly vulnerable to glucuronidation in the liver and brain by UDP-glucosyltransferase activity (mainly by UGT2B7) resulting in morphine-3-glucuronide (M3G) and M6G, which are the main metabolites of morphine (Lotsch et al, 2002). Following morphine administration, M3G levels are increased but it is biologically inactive. In concert, levels of M6G increase to a lesser extent but this substance is an agonist of MOR1, with an analgesic potency greater than morphine (Dahan et al, 2008). The transporter P-glycoprotein (PGP), which is encoded by the *ABCB1* gene, also plays an important role in the pharmacokinetics of morphine. It facilitates the uptake of morphine, but not M6G, into the brain (Thompson et al; Bostrom et al, 2005; Hamabe et al, 2006). The most consistent genetic contributors to morphine response reported in the literature so far include *ABCB1*, catechol-O-methyl transferase (COMT) and melanocortin-1 receptor (MC1R) (Somogyi et al, 2007; Diatchenko et al, 2007). Thus combined effects of genes affecting pharmacokinetics and pharmacodynamics of opioids should be considered in clinical association studies that examine opioid responses.

Search for Functional SNPs

A focused study of the *OPRM1* gene locus is a critical first step in advancing our understanding of genetic differences in the responses to opioids. MOR1 is the major binding target for most clinically used opioid therapeutics and is a potent source of the currently recognized genetic variability of responses to opioids; yet, there is a striking deficiency in our knowledge of the genomic and genotypic structure of *OPRM1*. Because of the inherent difficulties in studying the molecular genetics of *OPRM1*, such as the very low abundance of the mRNA, the unusual sequence structure that is not optimal for current molecular biological tools, and some degree of divergence between human and mouse transcriptomic

structure, this very important drug target lags behind the vast majority of other gene loci in terms of our understanding of its molecular and genetic structure.

Furthermore, it is crucially important to study the molecular mechanisms that underlie the observed associations mediated by specific SNPs in order to identify the *functional* allelic variants rather than variants that are merely associated genetic markers. This will permit a major advancement in our understanding of opioid receptor biology, pharmacodynamic responses to opioid administration, and will enable the development of reliable pharmacogenomic tests that predict the efficacy and side-effects of opioids.

Summary

MOR agonists are the most widely used analgesics, prescribed for both acute postoperative pain and chronic pain conditions; yet, there is substantial individual variation in drug responses of which we have very limited understanding and ability to control. Thus, the identification of new functional SNPs within alternative exons of *OPRM1* is of considerable importance to the field of medical genetics. Existing evidence suggests that recently studied *OPRM1* SNPs are not just another group of genetic variations that affect *OPRM1* function, but instead are very important contributors to the *OPRM1* genetic variability and receptor function. Further analysis of *OPRM1* gene locus for the presence of functional SNPs within new potential alternative exons and other functional elements is required. The intriguing hypothesis that the analgesic efficacy and/or side effect profile of morphine are associated with and predicted by SNPs identified in new alternative exons and other functional elements rather than constitutive exons (Shabalina et al,2009) is an area of future investigation. Further genotypic analysis of the *OPRM1* gene locus and receptor function hold great promise in elucidating a set of genetic markers that can be used to r predict the safety and efficacy of opioids on an individual basis.

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Reference List

1. Befort K, Filliol D, Decaillet FM, Gaveriaux-Ruff C, Hoehe MR, Kieffer BL. A single nucleotide polymorphic mutation in the human mu-opioid receptor severely impairs receptor signaling. *J Biol.Chem.* 2001; 276:3130–3137. [PubMed: 11067846]
2. Beyer A, Koch T, Schroder H, Schulz S, Holtt V. Effect of the A118G polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human mu-opioid receptor. *J Neurochem.* 2004; 89:553–560. [PubMed: 15086512]
3. Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc.Natl.Acad.Sci.U.S.A.* 1998; 95:9608–9613. [PubMed: 9689128]
4. Bostrom E, Simonsson US, Hammarlund-Udenaes M. Oxycodone pharmacokinetics and pharmacodynamics in the rat in the presence of the P-glycoprotein inhibitor PSC833. *J Pharm.Sci.* 2005; 94:1060–1066. [PubMed: 15799017]

5. Cadet P, Mantione KJ, Stefano GB. Molecular identification and functional expression of mu 3, a novel alternatively spliced variant of the human mu opiate receptor gene. *J.Immunol.* 2003; 170:5118–5123. [PubMed: 12734358]
6. Campa D, Gioia A, Tomei A, Poli P, Barale R. Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief. *Clin.Pharmacol.Ther.* 2008; 83:559–566. [PubMed: 17898703]
7. Camu F, Vanlersberghe C. Pharmacology of systemic analgesics. *Best.Pract.Res.Clin.Anaesthesiol.* 2002; 16:475–488. [PubMed: 12516886]
8. Chou WY, Wang CH, Liu PH, Liu CC, Tseng CC, Jawan B. Human opioid receptor A118G polymorphism affects intravenous patient-controlled analgesia morphine consumption after total abdominal hysterectomy. *Anesthesiology.* 2006; 105:334–337. [PubMed: 16871067]
9. Chou WY, Yang LC, Lu HF, Ko JY, Wang CH, Lin SH, Lee TH, Concejero A, Hsu CJ. Association of mu-opioid receptor gene polymorphism (A118G) with variations in morphine consumption for analgesia after total knee arthroplasty. *Acta Anaesthesiol.Scand.* 2006; 50:787–792. [PubMed: 16879459]
10. Crain SM, Shen KF. Antagonists of excitatory opioid receptor functions enhance morphine's analgesic potency and attenuate opioid tolerance/dependence liability. *Pain.* 2000; 84:121–131. [PubMed: 10666516]
11. Dahan A, van Dorp E, Smith T, Yassen A. Morphine-6-glucuronide (M6G) for postoperative pain relief. *Eur.J Pain.* 2008; 12:403–411. [PubMed: 17869146]
12. Diatchenko L, Nackley AG, Tchivileva IE, Shabalina SA, Maixner W. Genetic architecture of human pain perception. *Trends Genet.* 2007; 23:605–613. [PubMed: 18023497]
13. Doyle GA, Rebecca SX, Lin SS, Press DM, Grice DE, Buono RJ, Ferraro TN, Berrettini WH. Identification of three mouse mu-opioid receptor (MOR) gene (Oprm1) splice variants containing a newly identified alternatively spliced exon. *Gene.* 2007; 388:135–147. [PubMed: 17156941]
14. Duguay Y, Baar C, Skorpen F, Guillemette C. A novel functional polymorphism in the uridine diphosphate-glucuronosyltransferase 2B7 promoter with significant impact on promoter activity. *Clin.Pharmacol.Ther.* 2004; 75:223–233. [PubMed: 15001974]
15. Edwards RR, Haythornthwaite JA, Tella P, Max MB, Raja S. Basal heat pain thresholds predict opioid analgesia in patients with postherpetic neuralgia. *Anesthesiology.* 2006; 104:1243–1248. [PubMed: 16732096]
16. Fortin JP, Ci L, Schroeder J, Goldstein C, Montefusco MC, Peter I, Reis SE, Huggins GS, Beinborn M, Kopin AS. The mu-opioid receptor variant N190K is unresponsive to peptide agonists yet can be rescued by small-molecule drugs. *Mol.Pharmacol.* 2010; 78:837–845. [PubMed: 20702761]
17. Fukuda K, Hayashida M, Ikeda K, Koukita Y, Ichinohe T, Kaneko Y. Diversity of opioid requirements for postoperative pain control following oral surgery--is it affected by polymorphism of the mu-opioid receptor? *Anesth.Prog.* 2010; 57:145–149. [PubMed: 21174568]
18. Ginosar Y, Davidson EM, Meroz Y, Blotnick S, Shacham M, Caraco Y. Mu-opioid receptor (A118G) single-nucleotide polymorphism affects alfentanil requirements for extracorporeal shock wave lithotripsy: a pharmacokinetic-pharmacodynamic study. *Br.J Anaesth.* 2009; 103:420–427. [PubMed: 19605407]
19. Glass, PSA. Intravenous Drug Delivery Systems. In: Miller, RD., editor. *Anesthesia.* Livingston, Churchill: 2000. p. 377–411.
20. Goldstein FJ. Adjuncts to opioid therapy. *J.Am.Osteopath.Assoc.* 2002; 102:S15–S21. [PubMed: 12356036]
21. Gris P, Gauthier J, Cheng P, Gibson DG, Gris D, Laur O, Pierson J, Wentworth S, Nackley AG, Maixner W, Diatchenko L. A novel alternatively spliced isoform of the mu-opioid receptor: functional antagonism. *Mol.Pain.* 2010; 6:33. [PubMed: 20525224]
22. Hamabe W, Maeda T, Fukazawa Y, Kumamoto K, Shang LQ, Yamamoto A, Yamamoto C, Tokuyama S, Kishioka S. P-glycoprotein ATPase activating effect of opioid analgesics and their P-glycoprotein-dependent antinociception in mice. *Pharmacol.Biochem.Behav.* 2006; 85:629–636. [PubMed: 17134744]

23. Han W, Ide S, Sora I, Yamamoto H, Ikeda K. A possible genetic mechanism underlying individual and interstrain differences in opioid actions: focus on the mu opioid receptor gene. *Ann.N.Y.Acad.Sci.* 2004; 1025:370–375. [PubMed: 15542738]
24. Hayashida M, Nagashima M, Satoh Y, Katoh R, Tagami M, Ide S, Kasai S, Nishizawa D, Ogai Y, Hasegawa J, Komatsu H, Sora I, Fukuda K, Koga H, Hanaoka K, Ikeda K. Analgesic requirements after major abdominal surgery are associated with OPRM1 gene polymorphism genotype and haplotype. *Pharmacogenomics.* 2008; 9:1605–1616. [PubMed: 19018716]
25. Inturrisi CE. Clinical pharmacology of opioids for pain. *Clin.J.Pain.* 2002; 18:S3–S13. [PubMed: 12479250]
26. Klepstad P, Rakvag TT, Kaasa S, Holthe M, Dale O, Borchgrevink PC, Baar C, Vikan T, Krokan HE, Skorpen F. The 118 A > G polymorphism in the human micro-opioid receptor gene may increase morphine requirements in patients with pain caused by malignant disease. *Acta Anaesthesiol.Scand.* 2004; 48:1232–1239. [PubMed: 15504181]
27. Koch T, Krosiak T, Averbeck M, Mayer P, Schroder H, Raulf E, Holtt V. Allelic variation S268P of the human mu-opioid receptor affects both desensitization and G protein coupling. *Mol.Pharmacol.* 2000; 58:328–334. [PubMed: 10908300]
28. Kream RM, Sheehan M, Cadet P, Mantione KJ, Zhu W, Casares F, Stefano GB. Persistence of evolutionary memory: primordial six-transmembrane helical domain mu opiate receptors selectively linked to endogenous morphine signaling. *Med.Sci.Monit.* 2007; 13:SC5–SC6. [PubMed: 18049446]
29. Kvam TM, Baar C, Rakvag TT, Kaasa S, Krokan HE, Skorpen F. Genetic analysis of the murine mu opioid receptor: increased complexity of Oprm gene splicing. *J.Mol.Med.* 2004; 82:250–255. [PubMed: 14991152]
30. Lotsch J, Geisslinger G. Are mu-opioid receptor polymorphisms important for clinical opioid therapy? *Trends Mol.Med.* 2005; 11:82–89. [PubMed: 15694871]
31. Lotsch J, Skarke C, Grosch S, Darimont J, Schmidt H, Geisslinger G. The polymorphism A118G of the human mu-opioid receptor gene decreases the pupil constrictory effect of morphine-6-glucuronide but not that of morphine. *Pharmacogenetics.* 2002; 12:3–9. [PubMed: 11773859]
32. Lotsch J, Stockmann A, Kobal G, Brune K, Waibel R, Schmidt N, Geisslinger G. Pharmacokinetics of morphine and its glucuronides after intravenous infusion of morphine and morphine-6-glucuronide in healthy volunteers. *Clin.Pharmacol.Ther.* 1996; 60:316–325. [PubMed: 8841154]
33. Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, Tzavara E, Hanoune J, Roques BP, Kieffer BL. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature.* 1996; 383:819–823. [PubMed: 8893006]
34. Mogil JS. The genetic mediation of individual differences in sensitivity to pain and its inhibition. *Proc.Natl.Acad.Sci.* 1999; 96:7744–7751. 1999. [PubMed: 10393892]
35. Mogil JS, Wilson SG, Bon K, Lee SE, Chung K, Raber P, Pieper JO, Hain HS, Belknap JK, Hubert L, Elmer GI, Chung JM, Devor M. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. *Pain.* 1999; 80:67–82. [PubMed: 10204719]
36. North RA. Membrane conductances and opioid receptor subtypes. *NIDA Res.Monogr.* 1986; 71:81–88. [PubMed: 2432429]
37. Oertel BG, Preibisch C, Wallenhorst T, Hummel T, Geisslinger G, Lanfermann H, Lotsch J. Differential opioid action on sensory and affective cerebral pain processing. *Clin.Pharmacol.Ther.* 2008; 83:577–588. [PubMed: 18030306]
38. Oertel BG, Schmidt R, Schneider A, Geisslinger G, Lotsch J. The mu-opioid receptor gene polymorphism 118A>G depletes alfentanil-induced analgesia and protects against respiratory depression in homozygous carriers. *Pharmacogenet.Genomics.* 2006; 16:625–636. [PubMed: 16906017]
39. Pan YX. Diversity and complexity of the mu opioid receptor gene: alternative pre-mRNA splicing and promoters. *DNA Cell Biol.* 2005; 24:736–750. [PubMed: 16274294]
40. Pasternak GW. Multiple opiate receptors: deja vu all over again. *Neuropharmacology.* 2004; 47 Suppl 1:312–323. [PubMed: 15464147]

41. Ravindranathan A, Joslyn G, Robertson M, Schuckit MA, Whistler JL, White RL. Functional characterization of human variants of the mu-opioid receptor gene. *Proc.Natl.Acad.Sci.U.S.A.* 2009; 106:10811–10816. [PubMed: 19528663]
42. Ready, LB. Acute Perioperative Pain. In: Miller, RD., editor. *Anesthesia*. Livingstone, Churchill: 2000. p. 2323-2350.
43. Reyes-Gibby CC, Shete S, Rakvag T, Bhat SV, Skorpen F, Bruera E, Kaasa S, Klepstad P. Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. *Pain*. 2007; 130:25–30. [PubMed: 17156920]
44. Rowlingson, JC.; Murphy, TM. Chronic Pain. In: Miller, RD., editor. *Anesthesia*. Livingston, Churchill: 2000. p. 7752-7755.
45. Shabalina SA, Zaykin DV, Gris P, Ogurtsov AY, Gauthier J, Shibata K, Tchivileva IE, Belfer I, Mishra B, Kiselycznyk C, Wallace MR, Staud R, Spiridonov NA, Max MB, Goldman D, Fillingim RB, Maixner W, Diatchenko L. Expansion of the Human mu-Opioid Receptor Gene Architecture: Novel Functional Variants. *Hum Mol.Genet.* 2009; 18:1037–1051. [PubMed: 19103668]
46. Sia AT, Lim Y, Lim EC, Goh RW, Law HY, Landau R, Teo YY, Tan EC. A118G single nucleotide polymorphism of human mu-opioid receptor gene influences pain perception and patient-controlled intravenous morphine consumption after intrathecal morphine for postcesarean analgesia. *Anesthesiology*. 2008; 109:520–526. [PubMed: 18719451]
47. Skarke C, Darimont J, Schmidt H, Geisslinger G, Lotsch J. Analgesic effects of morphine and morphine-6-glucuronide in a transcutaneous electrical pain model in healthy volunteers. *Clin.Pharmacol.Ther.* 2003; 73:107–121. [PubMed: 12545149]
48. Somogyi AA, Barratt DT, Collier JK. Pharmacogenetics of opioids. *Clin.Pharmacol.Ther.* 2007; 81:429–444. [PubMed: 17339873]
49. Sora I, Takahashi N, Funada M, Ujike H, Revay RS, Donovan DM, Miner LL, Uhl GR. Opiate receptor knockout mice define mu receptor roles in endogenous nociceptive responses and morphine-induced analgesia. *Proc.Natl.Acad.Sci.U.S.A.* 1997; 94:1544–1549. [PubMed: 9037090]
50. Tan EC, Lim EC, Teo YY, Lim Y, Law HY, Sia AT. Ethnicity and OPRM variant independently predict pain perception and patient-controlled analgesia usage for post-operative pain. *Mol.Pain*. 2009; 5:32. [PubMed: 19545447]
51. Thompson SJ, Koszdin K, Bernards CM. Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. *Anesthesiology*. 2000; 92:1392–1399. [PubMed: 10781286]
52. Tsai FF, Fan SZ, Yang YM, Chien KL, Su YN, Chen LK. Human opioid mu-receptor A118G polymorphism may protect against central pruritus by epidural morphine for post-cesarean analgesia. *Acta Anaesthesiol.Scand.* 2010; 54:1265–1269. [PubMed: 21039348]
53. Uhl GR, Sora I, Wang Z. The mu opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. *Proc.Natl.Acad.Sci.U.S.A.* 1999; 96:7752–7755. [PubMed: 10393893]
54. Xu J, Xu M, Hurd YL, Pasternak GW, Pan YX. Isolation and characterization of new exon 11-associated N-terminal splice variants of the human mu opioid receptor gene. *J Neurochem.* 2009; 108:962–972. [PubMed: 19077058]
55. Zhang W, Chang YZ, Kan QC, Zhang LR, Lu H, Chu QJ, Wang ZY, Li ZS, Zhang J. Association of human micro-opioid receptor gene polymorphism A118G with fentanyl analgesia consumption in Chinese gynaecological patients. *Anaesthesia*. 2010; 65:130–135. [PubMed: 20003118]
56. Zhang W, Yuan JJ, Kan QC, Zhang LR, Chang YZ, Wang ZY. Study of the OPRM1 A118G genetic polymorphism associated with postoperative nausea and vomiting induced by fentanyl intravenous analgesia. *Minerva Anesthesiol.* 2011; 77:33–39. [PubMed: 21150856]

Table 1

Carriers of MOR1 118G variant allele show poor responses for opioids.

	Freq. of Variant	Findings	Study Location	REF
N40D	11.1%	The G-allele reduced the potency of morphine-6-glucuronide, as measured by change in pupil diameter.	Germany	(Lotsch et al,2002)
	N/A	The G-allele reduced the potency of morphine and morphine-6-glucuronide, as measured by change in pupil diameter. G-allele carriers also experienced less nausea and vomiting after morphine-6-glucuronide exposure. The G-allele had no effect on the analgesic effect on morphine.	Germany	(Skarke et al,2003)]
	10.4%	Patients homozygous for the G-allele required more morphine to control pain associated with cancer their pain than did heterozygous or homozygous wild-type individuals.	Norway	(Klepstad et al,2004)
	24.5%	Patients homozygous for the G-allele self-administered the more morphine after knee arthroplasty and made more requests for drug infusion than did A-allele carriers. Genotype did not affect reported pain.	Taiwan	(Chou et al,2006)
	34.4%	Patients homozygous for the G-allele self-administered more fentanyl following total hysterectomy than patients homozygous for the A-allele during the first 24-hours after surgery.	Taiwan	(Chou et al,2006)
	N/A	Patients homozygous for the G-allele required higher serum alfentanil concentrations to achieve analgesia and showed lower incidence of respiratory depression than A-allele carriers.	Germany	(Oertel et al,2008)
	11.1%	G-allele carriers required more morphine to control pain associated with cancer than patients homozygous for the A-allele. There were significant joint effects of the A118G polymorphism and the COMT Val/Met polymorphism.	United States	(Reyes-Gibby et al,2007)
	N/A	Patients homozygous for the G-allele were less sensitive to the analgesic effects of morphine to control pain associated with cancer than A-allele carriers.	Italy	(Campa et al,2008)
	34.0%	G-allele carriers self-administered more intrathecal morphine following Cesarean section and reported more pain than patients homozygous for the A-allele. G-allele carriers experienced less nausea after morphine exposure.	Singapore	(Sia et al,2008)
	44.9%	Patients homozygous for the G-allele required more opioid analgesics following abdominal surgery than A-allele carriers to reach the same level of pain relief during the first 24-hours after surgery.	Japan	(Hayashida et al,2008)
	12.0%	G-allele homozygotes showed blunted ability of alfentanil to suppress activation of primary and secondary somatosensory cortices and insular cortex following carbon dioxide bursts applied to the nasal mucosa.	Germany	(Oertel et al,2006)]
	15.2%	G-allele carriers self-administered more alfentanil following extracorporeal shock wave lithotripsy and achieved a higher blood concentration of the drug. G-allele carriers reported more pain than patients homozygous for the A-allele.	Israel	Ginosar(Ginosar et al,2009)
	33.9 – 49.0%	Patients homozygous for the G-allele self-administered more morphine following Cesarian section and had higher pain scores than A-allele carriers. The G-allele homozygotes experienced less nausea and vomiting after morphine exposure than A-allele homozygotes.	Singapore	(Tan et al,2009)
	31.3%	G-allele carriers required more fentanyl to control pain following myomectomy or hysterectomy than patients homozygous for the A-allele during the first 24-hours after surgery. G-allele carriers were also more sensitive to electrical stimulation.	China	(Zhang et al,2010)
	32.6%	Patients homozygous for the G-allele exhibited decreased incidence of pruritis after epidural morphine administration following Cesarian section.	Taiwan	(Tsai et al,2010)
	46.3%	G-allele carriers exhibited shorter latency to pain perception following fentanyl administration after oral surgery when compared	Japan	(Fukuda et al,2010)

Freq. of Variant	Findings	Study Location	REF
	to patients homozygous for the A-allele. The G-allele was also associated with decreased analgesic efficacy of fentanyl when compared to the A-allele.		
32.4%	Patients homozygous for the G-allele required more fentanyl for pain relief following myomectomy or hysterectomy. Genotype did not mediate the incidence of nausea or vomiting.	China	(Zhang et al,2011)

Table 2Rare Functional Non-Synonymous *OPRM1* Mutations Identified in Twin Studies.

Polymorphism	Location	Consequence	Ref
N40D	N-terminus	See Table 1.	
S42T	N-terminus	Decreased morphine potency <i>in vitro</i>	(Ravindranathan et al,2009)
L85I	1 st Transmembrane domain	Increased internalization following morphine exposure <i>in vitro</i>	(Ravindranathan et al,2009)
S147C	3 rd Transmembrane domain	Increased morphine potency <i>in vitro</i>	(Ravindranathan et al,2009)
N190K	2 nd Intracellular loop	Naltrexone, naloxone, and buprenorphine produced agonist-like activity.	(Fortin et al,2010)
C192F	4 th Transmembrane domain	Decreased morphine potency <i>in vitro</i>	(Ravindranathan et al,2009)
R260H	3 rd Intracellular loop	Altered agonist-independent signaling <i>in vitro</i> , which may play a role in opioid tolerance.	(Fortin et al,2010)
R265H	3 rd Intracellular loop	Altered agonist-independent signaling <i>in vitro</i> , which may play a role in opioid tolerance.	[(Fortin et al,2010)
S268P	3 rd Intracellular loop	Reduced potency and efficacy of morphine <i>in vitro</i> and loss CaMKII receptor-mediated desensitization.	(Fortin et al), (Befort et al,2001), (Koch et al, 2000)