Alternative Splicing of G Protein–Coupled Receptors: Relevance to Pain Management

Folabomi A. Oladosu, BS; William Maixner, PhD, DDS; and Andrea G. Nackley, PhD

From the Center for Pain Research and Innovation, University of North Carolina, Chapel Hill.

CME Activity

Target Audience: The target audience for *Mayo Clinic Proceedings* is primarily internal medicine physicians and other clinicians who wish to advance their current knowledge of clinical medicine and who wish to stay abreast of advances in medical research.

Statement of Need: General internists and primary care physicians must maintain an extensive knowledge base on a wide variety of topics covering all body systems as well as common and uncommon disorders. *Mayo Clinic Proceedings* aims to leverage the expertise of its authors to help physicians understand best practices in diagnosis and management of conditions encountered in the clinical setting.

Accreditation: Mayo Clinic College of Medicine is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

Credit Statement: Mayo Clinic College of Medicine designates this journal-based CME activity for a maximum of 1.0 *AMA PRA Category 1 Credit(s).*™ Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Learning Objectives: On completion of this article, you should be able to (1) explain the importance of G protein–coupled receptors to pain signaling and modulation, (2) explain the basic concepts of alternative splicing, and (3) describe how individual variability in alternative splicing of G protein–coupled receptors may contribute to variability in the nature of pain as well as responses to analgesic drugs.

Disclosures: As a provider accredited by ACCME, Mayo Clinic College of Medicine (Mayo School of Continuous Professional Development) must ensure balance, independence, objectivity, and scientific rigor in its educational activities. Course Director(s), Planning Committee members, Faculty, and all others who are in a position to control the content of this educational activity are required to disclose all relevant financial relationships with any commercial interest related to the subject matter of the educational activity. Safeguards against commercial bias have been put in place. Faculty also will disclose any off-label and/or investigational use of pharmaceuticals or instruments discussed in their presentation. Disclosure of this information will be published in course materials so that those participants in the activity may formulate their own judgments regarding the presentation.

In their editorial and administrative roles, William L. Lanier, Jr, MD, Terry L. Jopke, Kimberly D. Sankey, and Nicki M. Smith, MPA, have control of the content of this program but have no relevant financial relationship(s) with industry.

Dr Maixner is a co-founder and equity stock holder in Algynomics, Inc, a company providing research services in personalized pain medication and diagnostics, and a patent holder with UNC Proove Bioscience. In addition, he receives consulting fees from National Institutes of Health, APS, and Orthogen with research funding support from National Institutes of Health/National Institute of Dental and Craniofacial Research.

Dr Nackley receives research support from the following sources: National Institutes of Health/National Institute of Neurological Disorders and Stroke R01 NS072205 (Principal Investigator); National Institutes of Health/National Institute of Neurological Disorders and Stroke P01 NS045685 (Principal Investigator); National Institutes of Health/National Institute of Dental and Craniofacial Research U01 DE017018 (Investigator); National Vulvodynia Association (NVA) (Principal Investigator); and UNC NCTraCS 50KR81417 (Principal Investigator).

Method of Participation: In order to claim credit, participants must complete the following:

1. Read the activity.
2. Complete the online CME Test and Evaluation. Participants must achieve a score of 80% on the CME Test. One retake is allowed.

Visit www.mayoclinicproceedings.com, select CME, and then select CME articles to locate this article online to access the online process. On successful completion of the online test and evaluation, you can instantly download and print your certificate of credit.

Estimated Time: The estimated time to complete each article is approximately 1 hour.

Hardware/Software: PC or MAC with Internet access.

Date of Release: 8/01/2015

Expiration Date: 7/31/2017 (Credit can no longer be offered after it has passed the expiration date.)

Privacy Policy: http://www.mayoclinic.org/global/privacy.html

Questions? Contact dletcsupport@mayo.edu.

Abstract

Drugs that target G protein–coupled receptors (GPCRs) represent the primary treatment strategy for patients with acute and chronic pain; however, there is substantial individual variability in both the efficacy and adverse effects associated with these drugs. Variability in drug responses is due, in part, to individuals' diversity in alternative splicing of pain-relevant GPCRs. G protein–coupled receptor alternative splice variants often exhibit distinct tissue distribution patterns, drug-binding properties, and signaling characteristics that may impact disease pathology as well as the extent and direction of analgesic effects. We review the importance of GPCRs and their known splice variants to the management of pain.

 ■ Mayo Clin Proc. 2015;90(8):1135-1151

Pain is a multidimensional sensory and emotional experience that generally can be categorized into one of 4 types.[1] *Nociceptive pain* is an acute response to environmental stimuli that warns of potential or actual tissue damage. In the event of actual damage, inflammatory and/or neuropathic pain may occur. *Inflammatory pain* occurs in response to damage of tissues and infiltration of immune cells, while *neuropathic pain* occurs

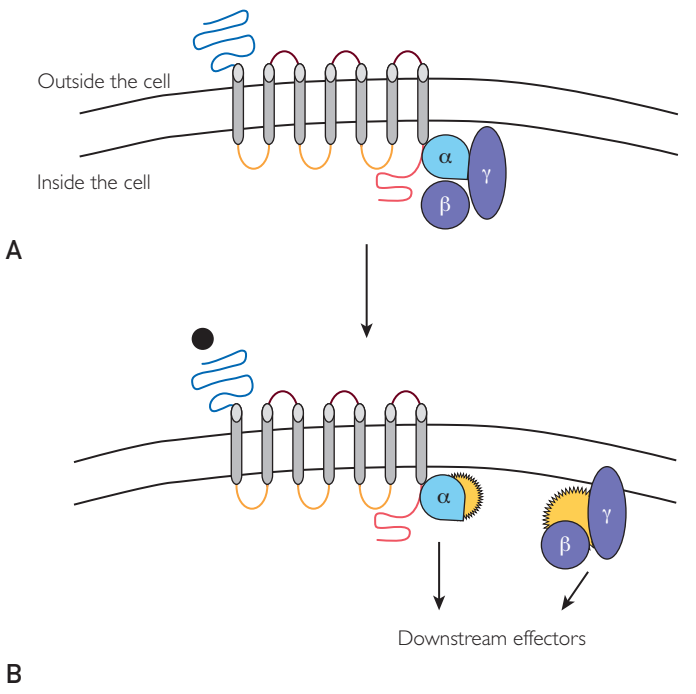

FIGURE 1. G protein—coupled receptor (GPCR) structure and function. A, G protein—coupled receptors are composed of 7-transmembrane domains (gray) interconnected by 3 intracellular (orange) and 3 extracellular (purple) loops. On the end of the first and last transmembranes are the N-terminus (blue) and C-terminus (red), respectively. As its name suggests, a GPCR is bound to a trimeric G protein composed of α and β/γ subunits. B, When a ligand (black circle) binds to a GPCR, the associated G protein separates into the α and β/γ subunits. These subunits then stimulate a variety of downstream effectors that produce changes in cellular activity (see Table 1).

in response to damage of nerves. Inflammatory and neuropathic pain typically serve to promote wound healing and repair; however, in many cases, the pain outlasts the stimulus and becomes chronic. Unlike inflammatory and neuropathic pain, *functional or idiopathic pain* is characterized by perpetual abnormalities in sensory processing that occur in the absence of direct inflammation or nerve damage.

Acute and chronic pain are primarily treated with pharmacological agents that promote analgesia. The principle target of a variety of analgesic drugs including opioids, cannabinergics, and antidepressants is G protein—coupled receptors (GPCRs). On activation, GPCRs initiate molecular changes resulting in excitation or inhibition of nerve, immune, and glial cells important for the onset and maintenance of pain. Although the critical role of GPCRs in pain biology and management is well established, reliably effective therapeutics with minimal adverse effects are lacking. Interindividual variability in response to a given analgesic is largely due to variation at the genetic level. Of particular interest are genetic variants in alternative splice regions that alter protein coding of the messenger RNA (mRNA), giving rise to proteins that differ in form and function (ie, alternative splice variants). This review highlights the importance of alternative splicing in the regulation of GPCRs involved in the transmission and modulation of pain.

TABLE 1. Common G Proteins and Their Intracellular Effects

G protein	Effectors	Overall impact
Gαs	Activates adenylate cyclase → ↑ cAMP	Cellular excitation (pronociceptive)
Gαq	Activates PLCβ → ↑ intracellular Ca^{++} levels	Cellular excitation (pronociceptive)
Gαi/o	Inhibits adenylate cyclase → ↓ cAMP	Cellular inhibition (antinociceptive)

Ca^{++} = calcium; cAMP = cyclic adenosine monophosphate; PLCβ = phospholipase C-β; → = results in; ↑ = increased.

GPCRS ARE RELEVANT FOR THE TREATMENT OF PAIN

The human genome encodes approximately 800 distinct GPCRs, 70% of which contribute to pain or pain-related phenotypes.[2] G protein—coupled receptors interact with a tremendous variety of signaling mediators, ranging from small molecules to large peptides and proteins. Although each receptor has the ability to induce a range of functional intracellular changes, all GPCRs possess a distinct and evolutionarily conserved architecture. Each canonical or classic receptor comprises 7 transmembrane (TM) proteins that span the cellular membrane. These TM proteins are interconnected by intracellular and extracellular loops (Figure 1). In addition, there are amino acid chains known as *N-terminus* and *C-terminus tails*, which are attached to the first and last TM, respectively. As alluded by its name, every GPCR is coupled to a G protein, which acts as a molecular switch to regulate cellular activity (Table 1).

The resulting structure created by the TM segments and loops provides interactive sites where ligands can bind. Ligands that bind to their receptor and initiate cell signaling are referred to as *agonists*. On binding, agonists

Mayo Clin Proc. ■ August 2015;90(8):1135-1151 ■ http://dx.doi.org/10.1016/j.mayocp.2015.06.010
www.mayoclinicproceedings.org

produce a conformational change of the GPCR and subsequent uncoupling of the associated G protein. Once uncoupled, the G protein separates into 2 subunits (the α and β/γ subunits), each of which initiates a chain of molecular reactions that affect cellular activity.[3] Depending on the type of G protein, the initiated downstream effects can promote cellular excitation or inhibition (Table 1). In general, agonists that activate pain-relevant GPCRs coupled to Gs typically produce pain, while those coupled to Gi typically inhibit pain.[2] Other ligands, known as *antagonists*, compete with agonists for the GPCR binding site and impede G protein uncoupling and downstream signaling events. Because of their ability to modulate cellular activity at each step of the pain pathway, GPCRs represent a popular pharmacological target for the management of clinical pain. In fact, over 60% of commonly prescribed analgesics work by binding to GPCRs.[3] Table 2 provides a summary of these GPCRs (opioid, cannabinoid [CB], adrenergic, and serotonergic receptors) along with their associated G protein, endogenous ligands, and analgesic compounds.

Opioid receptors are among the most well-known GPCRs that regulate the transmission and perception of pain. There are 4 opioid receptor subtypes: the μ-opioid receptor (MOR-1), the δ-opioid receptor, the κ-opioid receptor, and the nociceptin receptor. Of these subtypes, MOR-1 is the classic receptor responsible for analgesic responses to endogenous endorphins as well as exogenous drugs. On agonist binding to MOR-1, its associated Gαi protein is activated and produces cellular inhibition of pronociceptive neurons.[9] For this reason, opioids are used in the management of acute pain (such as that associated with surgery) as well as chronic pain disorders such as low back pain, extremity pain, and osteoarthritis.[10] Opioid antagonists, usually coadministered with opioid agonists to reduce the development of unwanted opioid effects, are also capable of producing analgesia independently of MOR-1.[11]

Cannabinoid receptors share similar signaling properties with MOR-1, making them attractive targets for clinical pain management. There are 2 CB receptor subtypes, CB_1 and CB_2, both of which couple to Gαi. Cannabinoid receptors play an important role in promoting analgesia in response to endocannabinoids such as 2-arachidonoylglycerol and anandamide. Commercially available CB agonists such as nabilone and tetrahydrocannabinol, which bind to both CB subtypes, are used to treat fibromyalgia and neuropathic pain.[12]

Adrenergic receptors (ARs), which mediate the physiologic responses to epinephrine and norepinephrine, represent another frequently targeted class of GPCRs. The adrenergic superfamily includes 3 subtypes respectively of α_1-ARs (α_{1A}-AR, α_{1B}-AR, α_{1D}-AR), α_2-ARs (α_{2A}-AR, α_{2B}-AR, α_{2C}-AR), and β-ARs (β_1-ARs, β_2-ARs, β_3-ARs). The α_2-AR couples to Gαi and promotes analgesia via cellular inhibition. Hence, α_2-AR agonists such as trazodone are used to promote analgesia. In contrast, α_1-AR, which is coupled to Gαq, facilitates cellular excitation of pronociceptive neurons, resulting in increased pain signaling. The β-ARs also facilitate pain signaling via Gαs signaling. To attenuate their excitatory contributions, α_1-AR and β-ARs are commonly used to treat a range of chronic pain disorders such as migraine, neuropathic pain, and fibromyalgia.

Finally, serotonin receptors, which mediate physiologic responses to the monoamine serotonin (or 5-hydroxytryptamine [5-HT]) play an important role in pain management.[8] The serotonin superfamily is quite large, including 7 general members: 5-HT_1 (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}), 5-HT_2 (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}), 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7. With the exception of the 5-HT_3 receptor, a ligand-gated ion channel, all 5-HT receptors are GPCRs. The effects of the 5-HT receptor family on pain are heavily dependent on the receptor subtype. Triptans target Gαi-coupled 5-HT_1 receptors, which promote analgesia via cellular inhibition, and normalize vascular changes associated with migraine.[13] Antidepressants promote chronic synaptic serotonin release that causes the down-regulation of Gαq-coupled 5-HT_2 receptors, thus attenuating their excitatory contributions to pain signaling. The 5-HT antagonists that target 5-HT_4 receptors in the central nervous system and the gastrointestinal (GI) tract are used in the treatment of migraine[14] and irritable bowel syndrome (IBS).[15] Meanwhile, the net effect of 5-HT_7 activation on pain is highly dependent on the location of the receptor. Activation of 5-HT_7 receptors on peripheral nerve terminals produces pain,[16,17] while activation in midbrain structures such as

TABLE 2. GPCRs Commonly Targeted for Clinical Pain Management

GPCR	G protein	Endogenous ligand	Prescribed analgesic			Known splice variant
			Reuptake Inhibitors	Agonist	Antagonist	
μ-Opioid receptor						
MOR-1	Gαi[4]	α-Endorphin β-Endorphin γ-Endorphin	NA	Alfentanil Buprenorphine Codeine Fentanyl Hydrocodone Hydromorphone Levorphanol Meperidine Methadone Morphine Oxycodone Oxymorphone Remifentanil Sufentanil Tapentadol Tramadol	Naloxone Naltrexone	Yes
Cannabinoid receptors						
CB_1	Gαi[5]	2-AG Anandamide	NA	Nabilone THC	Cannabidiol	Yes
CB_2	Gαi[5]	LPI NADA OAE		Nabilone THC	Cannabidiol	Yes
Adrenergic receptors						
α_1-AR	Gαq[6]	Epinephrine Norepinephrine	Amitriptyline (NET) Desipramine (NET)	NA	Amitriptyline Promethazine Nortriptyline Trazodone	Yes
α_2-AR	Gαi[6]		Desvenlafaxine (NET)	Clonidine	Trazodone	No
β_1-AR	Gαs[7]		Duloxetine (NET) Levorphanol (MAO) Meperidine (NET)	NA	Atenolol Nadolol Metoprolol Propranolol Timolol	No
β_2-AR	Gαs, Gαi[7]		Nortriptyline (NET) Tapentadol (NET)	NA	Nadolol Propranolol Timolol	No
β_3-AR	Gαs[7]		Venlafaxine (NET)		Nadolol Propranolol Timolol	Yes
Serotonin receptors						
5-HT_1	Gαi[8]	Serotonin	Amitriptyline (SERT) Desipramine (SERT) Desvenlafaxine (SERT) Duloxetine (SERT) Levorphanol (MAO)	Almotriptan Dihydroergotamine Eletriptan Frovatriptan Naratriptan Rizatriptan Sumatriptan Zolmitriptan	Trazodone	No

Continued on next page

Mayo Clin Proc. ■ August 2015;90(8):1135-1151 ■ http://dx.doi.org/10.1016/j.mayocp.2015.06.010
www.mayoclinicproceedings.org

TABLE 2. Continued

GPCR	G protein	Endogenous ligand	Prescribed analgesic: Reuptake Inhibitors	Prescribed analgesic: Agonist	Prescribed analgesic: Antagonist	Known splice variant
Serotonin receptors, continued						
5-HT_2	Gαq[8]		Nortriptyline (SERT) Trazodone (SERT)	Dihydroergotamine Methylergometrine	Amitriptyline Nortriptyline Promethazine Trazodone	Yes
5-HT_4	Gαs[8]		Venlafaxine (SERT)	Mosapride	NA	Yes
5-HT_6	Gαs[8]			NA	Amitriptyline Nortriptyline Trazodone	Yes
5-HT_7	Gαs[8]			NA	Amitriptyline Trazodone	Yes

2-AG = 2-arachidonoylglycerol; α_1-AR = α_1-adrenergic receptor; β_1-AR = β_1-adrenergic receptor; CB = cannabinoid; GPCR = G protein–coupled receptor; 5-HT = 5-hydroxytryptamine; LPI = lysophosphatidylinositol; MAO = monoamine oxidase; MOR-1 = μ-opioid receptor; NA = not applicable; NADA = *N*-arachidonoyl dopamine; NET = norepinephrine transporter; OAE = *O*-arachidonoyl ethanolamine (virodhamine); SERT = serotonin transporter; THC = tetrahydrocannabinol.

the periaqueductal gray alleviates pain associated with nerve injury.[18]

Although these conventional therapeutics are able to alleviate pain, their efficacy is limited to a subset of the population.[19] Additionally, their use is constrained by adverse effects, such as altered mental state, nausea, constipation, sedation, and life-threatening respiratory depression. Variability in patient response and adverse effect profiles is due, in part, to diversity in alternative splicing of GPCRs expressed in tissues that regulate pain processing. By expanding our understanding of GPCR alternative splice variants and their associated pharmacodynamic responses, we will be able to better predict patient-centered treatment outcomes.

ALTERNATIVE SPLICING ADDS TO THE DIVERSITY OF GPCR SIGNALING

Alternative splicing is an important mechanism of gene regulation, affecting approximately 90% of all genes within the human genome.[20] A single gene is able to generate exponential protein coding capabilities via alternative splicing. Before alternative splicing, a gene is first transcribed into precursor mRNA (pre-mRNA). The pre-mRNA sequence contains short-protein coding regions known as *exons*. Interspersed between the exons are longer non-coding regions known as *introns* (Figure 2). Before the sequence can be translated to produce protein, the introns and alternative exons within pre-mRNA are removed, or spliced, and the constitutive exons are brought together, resulting in the canonical mRNA transcript ready for protein synthesis. When alternative splicing occurs, however, the pre-mRNA is edited such that constitutive exons are removed from, or introns are retained in, the final mRNA transcript. The most common type of alternative splicing within the human genome is exon skipping,[21] in which constitutive exons are excluded from the final mRNA transcript.

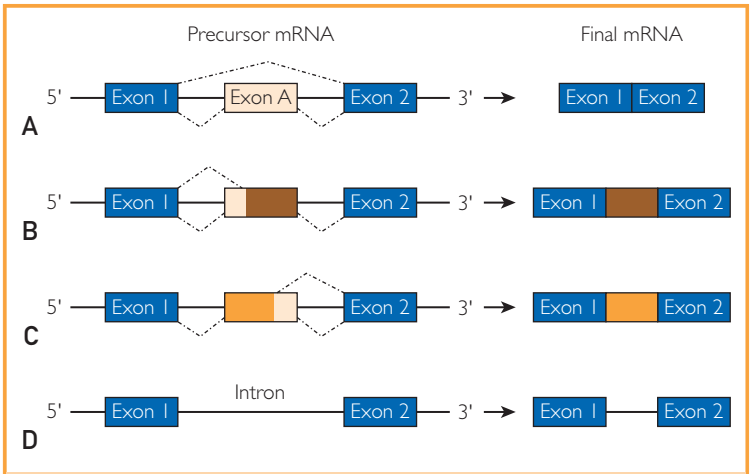

FIGURE 2. Different types of alternative splicing. The most common type of alternative splicing in animals is exon skipping (A), in which a constitutive exon is spliced from the final messenger RNA (mRNA) transcript. Alternative 3′ (B) and 5′ (C) splice sites provide additional junctions within an exon, resulting in partial splicing of the exonic mRNA sequence. D, Intron retention is a rare type of alternative splicing that occurs when an intron remains within the final mRNA transcript.

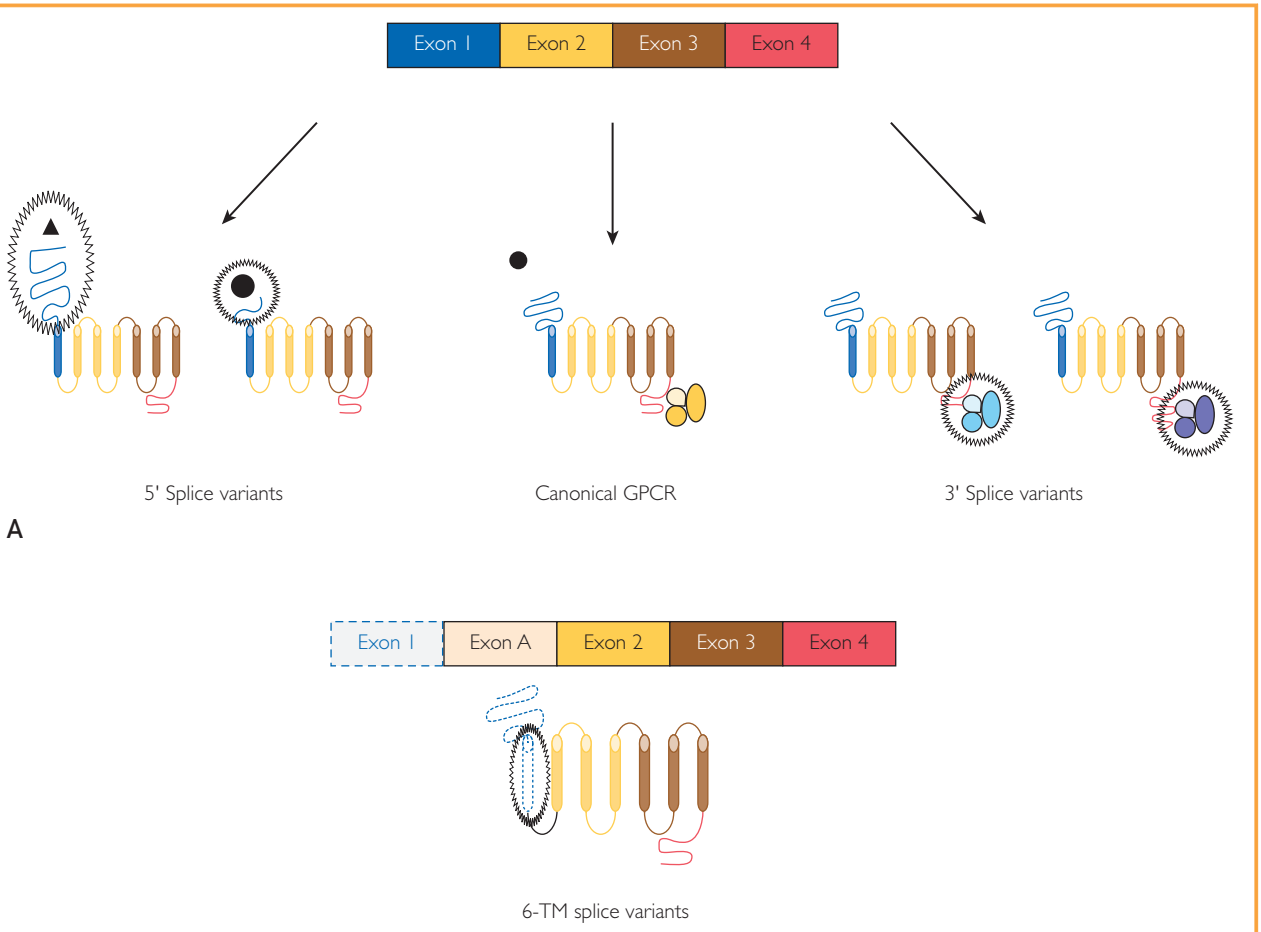

FIGURE 3. Structural variations in G protein–coupled receptors (GPCRs) as a result of alternative splicing. Exons within the messenger RNA transcript serve as coding regions for specific sections of protein. Alternative splicing events that change or remove exonic sequences can produce GPCR splice variants with corresponding changes in protein composition and/or structure. A, Splicing events that lead to alterations in exon 1 can yield GPCRs with truncated N-termini that affect ligand binding, while events that lead to alterations in exon 4 can yield GPCRs with truncated C-termini that affect G protein coupling and signaling. B, Splicing events can also lead to skipping of an exon that codes for a unit of the GPCR, such as a transmembrane, thus yielding a truncated GPCR lacking the encoded section, such as a 6-transmembrane (6-TM) splice variant.

Another common type of alternative splicing is splice site selection, in which the portion of an exon is spliced out because of the presence of a nucleotide sequence that facilitates splicing activity.[21] Intron retention is another type of alternative splicing in which an intron remains in the final mRNA transcript. Each type of alternative splicing will render an mRNA transcript and corresponding protein that is structurally different from the canonical protein produced from the standard template (Figure 3).

Accumulating evidence suggests that alternative splicing substantially adds to the functional diversity of the human genome and that variations in these processes produce pathologic states.[22] The presence of multiple GPCR splice variants allows for essential, precisely regulated differences in expression (eg, tissue-specific expression)[23] as well as in agonist binding,[24] agonist-induced internalization,[25] and intracellular signaling dynamics.[25,26] Some alternative splice variants even display functional characteristics opposite to the canonical form.[27-29] Allelic variants that alter the ratio of functionally distinct protein isoforms through alternative splicing may produce changes in the direction of pain-relevant

Mayo Clin Proc. ■ August 2015;90(8):1135-1151 ■ http://dx.doi.org/10.1016/j.mayocp.2015.06.010
www.mayoclinicproceedings.org

GPCR pharmacodynamics (eg, coupling to stimulatory vs inhibitory G protein effector systems), yet remain understudied. A PubMed search of the term *alternative splicing pain* yields only 87 relevant original research articles. Most are focused on ion channels such as voltage-gated calcium channels[30] and transient receptor potential channels,[31,32] with only 12 articles focusing on GPCRs. This is an important area of study because identification of GPCR splice variants differentially expressed in individuals with altered pain perception and/or analgesic responses will help elucidate novel targets for the development of individualized treatment strategies.

FUNCTIONAL GPCR ALTERNATIVE SPLICE VARIANTS

Examples of alternative splice variants of pain-relevant GPCRs that exhibit diversity in expression and signaling profiles include the aforementioned MOR-1, CB receptors, adrenergic receptors, and serotonin receptors. Of additional interest are nociceptin, prostaglandin, and neurokinin receptors, which are not targeted by common analgesics but are critical for the induction and modulation of pain. Accumulating evidence from in vitro, preclinical, and clinical studies suggests that alternative splicing of these and other GPCR transcripts adds additional layers of complexity to GPCR signaling and pharmacodynamic responses (Table 3).

Opioid Receptors

The pharmacological manipulation of MOR-1 is an essential component of clinical pain treatment. Although the signaling characteristics of MOR-1 are well established, we are just beginning to understand the complex nature of genetic variants that contribute to alternative splicing. At least 20 MOR-1 splice variants have been identified in mouse and human genomes,[25] suggesting an array of potentially functional consequences that may occur with opioid administration.

Preclinical studies within the past 15 years have begun to reveal the functional properties of specific MOR-1 splice variants. Xu et al[90] provide evidence that the gene expression of MOR-1 splice variants represent compensatory responses to long-term opioid administration that stabilize or diminish the development of tolerance. Other studies have found that the presentation of some unwanted effects are due to the activation of MOR-1 splice variants. For example, Liu et al[37] have documented that because of its distinct C-terminus, the splice variant MOR-1D dimerizes with the gastrin-releasing peptide receptor in the mouse spinal cord to produce opioid-induced itch. Another splice variant known as MOR-1K, a truncated receptor lacking the N-terminus and first TM, has been implicated in the paradoxical increase in pain sensitivity known as opioid-induced hyperalgesia (OIH). In contrast to MOR-1, which typically couples to Gαi, MOR-1K couples to Gαs to activate adenylate cyclase (AC) and increase intracellular calcium, thus engaging pronociceptive signaling events that likely drive OIH.[29] A subsequent preclinical study in mice revealed that genetic knockdown of *MOR-1K* hindered the development of OIH and unmasked opioid analgesia.[91]

Additional studies investigating the functional characteristics of MOR-1 splice variants provide evidence that a set of these receptors promote opioid analgesia by providing exclusive binding sites for different opioids. Transgenic mice lacking exon 11, an exon that provides an alternative promoter region for the MOR transcript, have substantial reductions in the analgesic efficacies of heroin, fentanyl, and the morphine metabolite morphine-6β-glucuronide,[24] suggesting that exon 11–containing variants play a critical role in opioid analgesia. Exon 11–containing splice variants also mediate the analgesic effects of iodobenzoylnaltrexamide (IBNtxA), a novel synthetic opioid that produces 10 times the analgesic efficacy of morphine without producing respiratory distress, dependence, tolerance, or GI distress in rodents.[36,39,92] MOR-1 splice variants also promote analgesia by enhancing canonical receptor function. Single-TM splice variants MOR-1R and MOR-1S structurally enhance MOR-1 function by stabilizing the canonical 7-TM (the GPCR as a whole, indicating the total number of transmembrane segments the receptor has) receptor at the cellular membrane.[41] Collectively, these studies highlight the importance of MOR-1 alternative splice variants in mediating opioid analgesia, as well as adverse effects such as tolerance, itch, and OIH.

Although few preclinical studies have examined the opioid receptor-like nociceptin receptor (ORL-1), it may also play an influential role in opioid analgesia. Majumdar et al[39] found that the exon 11 splice variant MOR-1G

dimerizes with ORL-1 to provide a binding site for novel opioid IBNtxA, suggesting that ORL-1 interacts with MOR-1 splice variants to provide specific opioid binding sites. The contribution of ORL-1 to splice variant signaling is further complicated by the existence of its own splice variants, ORL-1_{Long} and ORL-1_{Short}.[93] Thus far, ORL-1_{Short} has been implicated in the regulation of the canonical receptor, indicating a possible influence over ORL-1 function.

CB Receptors

Both the CB_1 and CB_2 receptors undergo alternative splicing to yield variants differing at their N-terminal region. The CB_{1A} variant is truncated by 61 amino acids, with the first 28 amino acids completely different from the canonical CB_1.[49] Although its tissue distribution largely overlaps with that of CB_1, CB_{1A} exhibits decreased agonist binding and activity, which might be due to a lack of 2 glycosylation sites typically important for signal transduction.[94] The CB_{1B} variant lacks the first 33 N-terminus amino acids, and although it overlaps with CB_1 in a number of tissues, its abundant expression in fetal brain suggests it may play an important role in development.[48] Similar to CB_{1A}, CB_{1B} exhibits decreased agonist binding and activity.

The CB_2 variants are generated through the use of alternate promoters located upstream of the major coding exon 3.[53] The gene CB_{2A} is initiated from the more distal promoter and includes exons 1a and 1b spliced to exon 3, while CB_{2B} is initiated from the more proximal promoter and includes exon 2 spliced to exon 3. The CB_{2A} variant is predominantly expressed in testes and at lower levels in spleen and brain. In contrast, the CB_{2B} variant is predominantly expressed in spleen with very low expression in brain and no expression in testes. These tissue-specific distribution patterns may indicate specialized roles for the different splice variants with respect to pain modulation, immune response, and spermatogenesis.

Adrenergic Receptors

Adrenergic receptors play a key role in pain processing as well as cognition and cardiovascular function. While α_2-ARs, β_1-ARs, and β_2-ARs are highly relevant to the modulation of pain by endogenous and exogenous agonists, the genes encoding these receptors are intronless and not subject to alternative splicing. Among the remaining adrenergic receptors, the α_{1a}-AR subtype has been studied the most extensively with respect to alternative splicing.

The human $ADRA_{1A}$ gene locus comprises more than 8 exons and codes for 15 known splice variants.[95] The canonical receptor is generated through splicing exon 1 (coding for the N-terminus and TMs 1 to 6) together with exon 2 (coding for TM7 [the specific transmembrane of a GPCR that is coded by a specific exon] and the C-terminus). Four C-terminus splice variants ($\alpha_{1a\text{-}2}$, $\alpha_{1a\text{-}3}$, $\alpha_{1a\text{-}4}$, $\alpha_{1a\text{-}5}$) have been identified that are generated through the use of additional acceptor sites at varying locations within, and distal to, exon 2. The $\alpha_{1a\text{-}2}$, $\alpha_{1a\text{-}3}$, and $\alpha_{1a\text{-}4}$ variants exhibit ligand binding properties and tissue distribution profiles similar to α_{1a}-AR, although $\alpha_{1a\text{-}3}$ and $\alpha_{1a\text{-}4}$ are absent in the kidney.[54-57] In contrast to α_{1a}-AR that couples to Gαq, these variants couple to Gαi to inhibit AC activity.[7] This diversity in α_{1a}-AR signaling may contribute to differential responses to α_1-AR antagonists used in the treatment of pain.

In addition, eleven 6-TM variants ($\alpha_{1a\text{-}6}$ through $\alpha_{1a\text{-}16}$) have been identified that are generated through exon skipping. These variants lack TM7, and their C-terminal tails are located extracellularly.[57] The truncated 6-TM variants are expressed in similar tissues as α_{1a}-AR but are localized exclusively within the cell and unable to bind α_1-AR agonists or directly mediate signal transduction. The 6-TM variants do, however, impair α_{1a}-AR ligand binding and trafficking to the cell surface. Thus, α_{1a}-AR 6-TM variants likely play an important physiologic role by modifying the function and expression of their parent 7-TM receptors.

One α_{1b}-AR splice variant has also been identified in human brain.[58] The α_{1b}-AR protein is generated through splicing of exons 1 and 2. In contrast to the canonical receptor, the $\alpha_{1a\text{-}2}AR$ includes an immediately adjacent sequence following exon 1 in its coding sequence and excludes exon 2 that codes for TM7. Tseng-Crank et al[58] also identified low levels of a truncated $ADRA_{1D}$ transcript, but the result was inconclusive and naturally occurring α_{1d}-AR variants were not observed. More work is required to determine the potential functional role of α_{1b}-AR and α_{1d}-AR variants.

The β_3-AR is primarily known for its ability to regulate energy metabolism and thermogenesis,[58] although evidence for its ability to

Mayo Clin Proc. ■ August 2015;90(8):1135-1151 ■ http://dx.doi.org/10.1016/j.mayocp.2015.06.010
www.mayoclinicproceedings.org

TABLE 3. Signaling, Tissue Distribution, and Function of Known GPCR Splice Variants

Receptor variant	G protein	Tissue distribution	Functional characteristics
Opioid receptors			
MOR-1	Gαi[4]	Brain, spinal cord > adrenal gland > small intestine[33]	
C-term variants			
MOR-1A		Brain[34]	OP binding → analgesia[35]
MOR-1B		Brain[34]	OP binding → analgesia[35]
MOR-1C		Brain[34]; agonist-induced reduction[36]	OP binding → analgesia[35]
MOR-1D		Brain[34]	OP-induced itch[37]
MOR-1E	Gαi[37]	Brain[34]	OP binding → analgesia[35]
MOR-1F		Brain[34]	OP binding → analgesia[35]
MOR-1O		Brain[34]	Unknown
MOR-1P		Brain[34]	Unknown
MOR-1U		Brain[34]	Unknown
MOR-1V		Brain[34]	Unknown
MOR-1W		Brain[34]	Unknown
MOR-1X		Brain[34]	Unknown
MOR-1Y		Brain[34]	OP binding → analgesia[38]
N-term variants			
MOR-1G		Brain[34]	Novel opioid binding[39]
MOR-1H		Brain[34]	OP binding → analgesia[40]
MOR-1I		Brain[34]	OP binding → analgesia[40]
MOR-1J		Brain[34]	OP binding → analgesia[40]
MOR-1K		Brain[34]	Contributes to OIH
MOR-1L	Gαs[29]	Brain[34]	OP binding → analgesia[40]
MOR-1M		Brain[34]	Unknown
MOR-1N		Brain[34]	Unknown
Single TM variants			
MOR-1Q		Brain[34]	Unknown
MOR-1R		Brain[34]	Stabilization of MOR-1[41]
MOR-1S		Brain[34]	Stabilization of MOR-1[41]
MOR-1T		Brain[34]	Unknown
MOR-1Z		Brain[34]	Unknown
MOR-1SV1		Brain (human neuroblastoma cell line)[42]	Unknown
MOR-1SV2		Brain (human neuroblastoma cell line)[42]	Unknown
ORL-1	Gαi[43]	Brain, immune cells, GI tract[44]	
ORL-1$_{Short}$		Brain > testis > heart, kidneys, muscle, spleen, thymus[45]	↓ Agonist binding[46]
ORL$_{Long}$		Brain > testis > muscle, spleen[45]	Unknown
Cannabinoid receptors			
CB1	Gαi[5]	Brain, sc, DRG > pituitary > heart, lung, uterus, testis, spleen, tonsils[47]	
N-term variants			
CB1A		Similar distribution to CB1 + kidney[48,49]	↓ Agonist binding, ↓ GTPγS activity[48]
CB1B		Fetal brain > GI tract, uterus, muscle > adult brain[48]	↓ Agonist binding, ↓ GTPγS activity[48]
CB2	Gαi[5]	Immune cells/tissues > glia and macrophages in brain/sc[47,50-52]	
N-term variants			
CB2A		Testis > spleen, leukocytes > brain[53]	Unknown
CB2B		Spleen > leukocytes[53]	Unknown
Adrenergic receptors			
α_{1a}	Gαq[6]	Liver, heart, brain > prostate, kidney, bladder[6]	
C-term variants			Pharmacology similar to α_{1a}[7,54-56]
α_{1a-2}	Gαi[7]	Liver, heart > prostrate, kidney[54,55]	
α_{1a-3}	Gαi[7]	Liver > heart, prostrate (absent in kidney)[54,55]	

Continued on next page

TABLE 3. Continued

Receptor variant	G protein	Tissue distribution	Functional characteristics
Adrenergic receptors, continued			
$\alpha_{1a\text{-}4}$	Gαi[7]	Liver, heart > prostrate (absent in kidney)[54,55]	
$\alpha_{1a\text{-}5}$			
6-TM variants		Liver, heart, hippocampus, and prostate; expressed intracellularly[57]	Impair α_{1a} binding and cell surface expression[57]
$\alpha_{1a\text{-}6}$			
$\alpha_{1a\text{-}7}$			
$\alpha_{1a\text{-}8}$			
$\alpha_{1a\text{-}9}$			
$\alpha_{1a\text{-}10}$			
$\alpha_{1a\text{-}11}$			
$\alpha_{1a\text{-}12}$			
$\alpha_{1a\text{-}13}$			
$\alpha_{1a\text{-}14}$			
$\alpha_{1a\text{-}15}$			
$\alpha_{1a\text{-}16}$			
α_{1b}	Gαq[6]	Liver, heart, brain (including cortex)[6]	
6-TM variant			
$\alpha_{1b\text{-}2}$		Expressed in hippocampus but absent in cortex[58]	Unknown
β_3	Gαs, Gαi[59,60]	Fat, immune cells/tissues > GI tract, DRG[59,61]	
C-term variants			
β_{3a} (mouse)	Gαs[7,62]	Fat > ileum > brain[63]	Unknown
β_{3b} (mouse)	Gαs, Gαi[7,62]	Brain > fat, ileum[63]	Unknown
Serotonin receptors			
5-HT_{2A}	Gαq[8]	Cortex, hippocampus, brainstem, olfactory > basal ganglia, limbic[8]	
6-TM variant			Impaired 5-HT–induced Ca^{++} signaling[64]
5-$HT_{2A\text{-}tr}$		Hippocampus, caudate, corpus callosum, amygdala, substantia nigra[64]	
5-HT_{2C}	Gαq[8]	Choroid plexus, striatum, hippocampus, hypothalamus, olfactory, sc[8,65]	
6-TM variant			Impaired 5-HT ligand binding[65]
5-HT_{2CT}		Choroid plexus, striatum, hippocampus, hypothalamus, olfactory, sc[65]	
C-term variant			Impaired 5-HT ligand binding[66]
5-$HT_{2C\text{-}R\text{-}COOH\Delta}$		Sc, cortex, cerebellum, medulla, caudate, amygdala, corpus callosum[66]	
5-HT_4	Gαs[8]	Intestine > brain > pituitary > uterus, testis > spleen > heart, kidney, lung, sc[67]	
C-term variants			
5-HT_{4A}	Gαs[68]	Intestine, brain > pituitary > uterus, testis > heart > spleen, lung, sc[67]	↑ Constitutive AC activity, ↑ isomerization, ↓ agonist internalization[69,70]
5-HT_{4B}	Gαs, Gαi[68,71]	Intestine, brain > pituitary > uterus > heart, spleen, lung, sc[67]	↑ Constitutive AC activity[68]
5-HT_{4C}	Gαs[68]	Intestine > pituitary > brain > uterus, testis, heart, spleen, sc[67]	↑ Constitutive AC activity[68]
5-HT_{4D}	Gαs[68]	Ileum, colon, but absent in brain[72,73]	20-Fold ↑ in agonist-induced cAMP activity[74]
5-HT_{4E}	Gαs[75]	Brain > testis > sc > intestine, pituitary, heart, prostate, ileum, colon[73]	↑ Constitutive AC activity[75]
5-HT_{4F}	Gαs[76]	Brain, ileum, colon[73]	Unknown
5-HT_{4G}	Gαs[77]	Brain, heart, ileum, colon[73]	Unknown

Continued on next page

Mayo Clin Proc. ■ August 2015;90(8):1135-1151 ■ http://dx.doi.org/10.1016/j.mayocp.2015.06.010
www.mayoclinicproceedings.org

TABLE 3. Continued

Receptor variant	G protein	Tissue distribution	Functional characteristics
Serotonin receptors, continued			
5-HT_{4I}	Gαs[78]	Brain, ileum, colon, heart[73]	↑ Constitutive AC activity[79]
5-HT_{4N}	Gαs[72]	Brain, heart, esophagus[73]	Antagonist GR113808 acts as partial agonist[76]
2nd EL loop variant			
5-HT_{4H}	Gαs[76]	GI tract[76]	
5-HT_6	Gαs[8]	Cortex, hippocampus, olfactory, striatum, amygdala, acumbens[8]	
6-TM variant			Impaired binding to 5-HT and LSD[80]
5-$HT_{6\text{-tr}}$		Cortex, hippocampus, cerebellum, thalamus, substantia nigra, caudate[80]	
5-HT_7	Gαs[8]	Brain, heart, GI tract, muscle, kidney, astrocytoma, glia[81,82]	
C-term variants			
5-HT_{7a}	Gαs[83]	Brain, heart, GI tract, spleen, lung, astrocytoma, glia[81-83]	Unknown
5-HT_{7b}	Gαs[84]	Brain, heart, GI tract, spleen, lung, astrocytoma, glia[81,82,84]	↑ Constitutive AC activity[84]
5-HT_{7d}	Gαs[84]	Heart, GI tract, ovary, testis, spleen, lung, astrocytoma[81]	Exhibit agonist-independent internalization[85]
Prostaglandin E receptors			
EP3	Gαi[86]	Kidney > uterus > stomach > brain, thymus, heart, spleen[86]	
C-term variants			
$EP3_{A/I}$	Gαi, $Gα_{12}$[86]	Unknown	↓ Constitutive AC activity[86]
$EP3_{B/II}$	Gαi, $Gα_{12}$[86]	Unknown	↓ AC activity[86]
$EP3_{C/III}$	Gαi, Gαs[86]	Unknown	↓ Or ↑ constitutive AC activity[86]
$EP3_D$	Unknown	Unknown	Unknown
$EP3_E$	Unknown	Unknown	Unknown
$EP3_F$	Unknown	Unknown	Unknown
Neurokinin receptor			
NK-1R	Gαq/11[87]	Brain, GI tract, lung, thyroid, immune cells[88]	
$NK\text{-}1R_{truncated}$	Unknown	Unknown	Impaired SP-induced calcium release[89]

AC = adenylate cyclase; Ca^{++} = calcium; cAMP = cyclic adenosine monophosphate; CB = cannabinoid; C-term = carboxyl terminus; DRG = dorsal root ganglion; EL = extracellular loop; EP3 = prostaglandin E receptor 3; GI = gastrointestinal; GPCR = G protein–coupled receptor; 5-HT = 5-hydroxytryptamine; LSD = lysergic acid diethylamide; MOR-1 = μ-opioid receptor; NK = neurokinin receptor; N-term = amino terminus; OIH = opioid-induced hyperalgesia; OP = opioid; ORL-1 = opioid receptor-like nociceptin receptor; sc = spinal cord; SP = substance P; TM = transmembrane; → = receptor concentration greater (in given tissue) than; ↓ = decreased; ↑ = increased.

promote functional and neuropathic pain is emerging.[62,63,96] The gene encoding $β_3$-AR undergoes alternative splicing within the coding region to yield 2 C-terminal splice variants differing with respect to tissue expression, G protein signaling profiles, and regulatory properties.[56,64,97] The $β_{3A}$-AR and $β_{3B}$-AR splice variants contain completely unique terminal chains that are 13 and 17 amino acids long, respectively. The $β_{3a}$-AR is primarily enriched in fat tissue and couples exclusively to Gαs, while the $β_{3b}$-AR is primarily enriched in brain and couples to both Gαs and Gαi. In addition, the $β_{3a}$-AR exhibits increased agonist-induced extracellular acidification, a measure of cyclic adenosine monophosphate–independent cellular activity. Their unique tissue distribution and signaling profiles, together with the known functional role of $β_3$-ARs, could indicate that $β_{3a}$-ARs play a greater role in lipolysis/thermogenesis and that $β_{3b}$-ARs in brain mediate pain. Although these studies were conducted in mice, it is important to note that the human $β_3$-AR contains a substantial number of genetic variants that are predicted to regulate alternative splicing.[65,66]

Serotonin Receptors

Serotonin receptors play a key role in pain processing as well as mood and GI function.[8]

Of the 5-HT_1 (A, B, D-F), 5-HT_2 (A-C), 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 GPCR family members, the 5-HT_{2A}, 5-HT_{2C}, 5-HT_4, 5-HT_6, and 5-HT_7 receptors are known to undergo alternative splicing.

The human 5-HT_2 receptor subtypes (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}) couple to Gαq proteins to promote the transient release of intracellular calcium. One truncated splice variant of *5-HT_{2A}* (*5-$HT_{2A\text{-}tr}$*) has been identified that utilizes alternate splice donor and acceptor sites to yield a 3-TM receptor with 57 unique amino acids in the C-terminal region.[64] The 5-$HT_{2A\text{-}tr}$ is coexpressed with 5-HT_{2A} in most brain tissues but is unable to couple to the calcium pathway. Two truncated splice variants of 5-HT_{2C} (5-HT_{2CT} and 5-$HT_{2C\text{-}R\text{-}COOH\Delta}$) have also been identified. Similar to 5-$HT_{2A\text{-}tr}$, the *5-HT_{2CT}* variant utilizes alternate splice donor and acceptor sites to yield a 3-TM receptor with 19 unique amino acids in the C-terminal region.[65] The *5-$HT_{2C\text{-}R\text{-}COOH\Delta}$* variant retains an extra 90 nucleotides from intron 5 in the TM4 splice site, resulting in a 3-TM receptor with a short C-terminus.[66] Compared with the canonical 5-HT_{2C} receptor, the truncated variants exhibit similar expression patterns but have impaired 5-HT ligand binding and G protein coupling.[65,66] Although the relative importance of these truncated 5-HT_2 splice variants in humans remains unknown, they are conserved in rats and mice,[66] in which their expression levels increase following nerve injury.[98]

The 5-HT_4 receptor couples preferentially to Gαs, and although widely expressed, the highest levels are found in the intestine.[78] Agonists targeting 5-HT_4 are beneficial in alleviating abdominal pain associated with IBS. Of all the 5-HT receptors, 5-HT_4 possesses the greatest diversity in alternative splicing. At least 10 splice variants have been identified that vary with respect to their tissue distribution and function. Nine C-terminus variants (5-HT_{4a}, 5-HT_{4b}, 5-HT_{4c}, 5-HT_{4d}, 5-HT_{4e}, 5-HT_{4f}, 5-HT_{4g}, 5-HT_{4i}, 5-HT_{4n}) have been identified that are identical up to amino acid Leu358, after which they vary in sequence and length.[73] Additionally, one variant (*5-HT_{4HB}*) has been identified that includes exon h coding for 14 additional amino acids in the second extracellular loop.[76] The 5-HT_{4a}, 5-HT_{4b}, 5-HT_{4c}, and 5-HT_{4e} variants are expressed in most tissues, with distribution patterns similar to the canonical form.[67,73] In contrast, the 5-HT_{4f} variant is found in the brain and GI tract but is absent in the heart and other tissues.[22] Meanwhile, the 5-HT_{4d} and 5-HT_{4h} variants are expressed exclusively in the GI tract.[73,76,78] Although all of the 5-HT_4 splice variants display typical ligand binding properties, some have notable functional differences. Both of the GI-specific 5-HT_{4d} and 5-HT_{4h} variants have a tendency to recognize 5-HT antagonists as partial agonists.[74,76] Furthermore, the 5-HT_{4d} variant exhibits a remarkable 20-fold increase in cyclic adenosine monophosphate formation following application of the 5-HT_4 agonist renzapride.[74] The 5-HT_{4b} variant is unique in its ability to couple to Gαi as well as Gαs proteins, suggesting its diverse signaling capabilities in the GI tract, brain, and other tissues.[71] In the absence of ligand binding, most C-terminus variants exhibit heightened constitutive AC activity.[68-70,72,74,75] The ability of GPCRs to increase basal AC activity has been reported previously and can result in physiologic functions of the receptor that are largely independent of endogenous ligands or exogenous drugs.[99] Collectively, these studies illustrate the high degree of tissue and signaling specificity for a number of 5-HT_4 splice variants that may be attractive targets for the development of new, more selective drugs for the treatment of IBS among other conditions.

The 5-HT_6 receptor is unique in that it is expressed almost exclusively in the central nervous system.[8] A 3-TM splice variant of HTR_6 (*5-$HT_{6\text{-}tr}$*) has been identified in brain that is generated through different splice donor and acceptor sites.[80] The corresponding receptor includes the TM1-3 and 10 unique amino acids in its C-terminus. In contrast to 5-HT_6, the expression of 5-$HT_{6\text{-}tr}$ is limited to substantia nigra and caudate. The 5-$HT_{6\text{-}tr}$ receptor is able to translocate to the membrane, yet is unable to bind serotonin. This splice variant may have a yet-to-be-determined function or be indicative of abnormalities due to pathologic state.

The 5-HT_7 receptor is expressed on primary afferent nociceptors, as well as in pain-relevant brain regions where it couples to Gαs to mediate the transmission and modulation of pain. Three splice variants of 5-HT_7 (5-HT_{7a}, 5-HT_{7b}, 5-HT_{7d}) have been identified that are all generated through alternative splicing of the second intron

Mayo Clin Proc. ■ August 2015;90(8):1135-1151 ■ http://dx.doi.org/10.1016/j.mayocp.2015.06.010
www.mayoclinicproceedings.org

located near the C-terminal coding region. The 5-HT_{7a} and 5-HT_{7b} variants have tissue expression profiles and functional characteristics similar to the canonical receptor, although 5-HT_{7b} has been found to exhibit considerably higher constitutive AC activity when expressed in stable cell lines.[100] The 5-HT_{7d} variant is predominantly expressed in smooth muscle tissues such as the heart and GI tract[84] and displays unique functional characteristics. Compared with the canonical 5-HT_7 receptor and the 5-HT_{7a} and 5-HT_{7b} variants, the 5-HT_{7d} variant displays agonist-independent internalization (even in the presence of antagonist) and associated reductions in agonist-induced AC activity.[85] It has been suggested that differences in the functional characteristics of 5-HT_7 variants are due to specific features of their carboxyl tails, leading to differential interactions with protein partners that mediate their activity, trafficking, and/or internalization.[85,101]

Prostaglandin E Receptor 3

Prostaglandins, such as prostaglandin E2, are a product of cyclooxygenase that facilitates pain transmission through binding to the prostaglandin E receptor 3 (EP3 receptor). Activation of the Gαi-coupled EP3 receptor has been reported to produce analgesia[102] but also to promote human immunodeficiency virus–induced inflammation[103] and sensitization of trigeminal nociceptors.[104] These contradictory effects may be due to the presence of EP3 splice variants. To date, 6 C-terminus splice variants (EP_{3A} through EP_{3F}) have been identified. Of these, the EP_{3C} receptor exhibits the most unique signaling characteristics because it is able to couple to Gαs as well as Gαi.[86] The dual coupling of the EP_{3C} variant to different G proteins may explain the ability of EP3 ligands to produce both analgesia and hyperalgesia.

Neurokinin-1 Receptor

Neurokin-1 receptors (NK-1Rs) are targets for the endogenous propain ligand substance P. Their activation results in Gαq-mediated increases in intracellular calcium levels and production of proinflammatory cytokines.[89] Alternative splicing of the NK-1R yields a truncated variant ($NK\text{-}1R_{truncated}$) that lacks the C-terminus and has functional properties that differ from the canonical receptor. Unlike NK-1R, activation of the $NK\text{-}1R_{truncated}$ variant does not result in increased levels of calcium or nuclear activity of factor κB. Instead, activation of $NK\text{-}1R_{truncated}$ results in decreased phosphorylation of protein kinase C and levels of interleukin 8. A recent clinical study reported the utility of an NK-1R antagonist in the treatment of chronic pain conditions and anxiety.[105] Results from functional studies of the $NK\text{-}1R_{truncated}$ variant suggest that splice variant–specific agonists may also be useful for pain management.

CLINICAL RELEVANCE OF FUNCTIONAL GENE REGULATORY EVENTS

Given the extensive list of alternative GPCR splice variants and their known impact on signaling and pharmacodynamics, it is expected that these variants have important clinical implications for pain management. Major strides in both preclinical and clinical research are still needed before we can reliably predict a patient's treatment response on the basis of their splice variant expression profile. Such strides have been made, however, in the study of another type of gene variation, single-nucleotide polymorphisms (SNPs). Like alternative splicing, SNPs within key pain-related genes can result in changes that subsequently affect the encoded protein. For example, SNPs in the gene encoding catechol-O-methyltransferase (COMT) (an enzyme that metabolizes catecholamines) are indicative of abnormalities in COMT function and predictive of chronic pain risk and treatment response. Human genetic association studies have revealed that the rs4680 SNP, alone or in combination with other nearby SNPs, is predictive of temporomandibular disorder and fibromyalgia onset.[106,107] Subsequent molecular studies found that these SNPs alter the thermostability and/or structure of the *COMT* transcript,[108] explaining why patients with functional pain disorders[109-111] and exacerbated postoperative pain[112-115] exhibit decreased levels of COMT alongside increased levels of catecholamines. Preclinical studies further revealed that elevated levels of epinephrine/norepinephrine resulting from low COMT activity lead to increased pain through activation of β-ARs.[116,117] Coming full circle, results from a randomized controlled trial documented that the β-AR antagonist propranolol provides considerable pain relief for patients who have SNPs associated with decreased levels of

COMT.[118] Together, these findings highlight the impact of gene regulation on pain as well as the utility of genetic and protein biomarkers in identifying a subgroup of patients who will benefit from specific therapies.

In a similar fashion, we believe that measurement of alternative GPCR splice variants can be used as a diagnostic tool to provide personalized pain treatment. This procedure is already being done in patients with cancer. In vitro studies examining the role of NK-1R alternative splicing in breast cancer cells revealed that overexpression of the NK-1R$_{truncated}$ variant promotes tumorigenesis.[119,120] A complementary clinical study further documented that individuals with overexpression of the *NK-1R$_{truncated}$* variant were at increased risk for colitis-associated carcinoma, whereas expression levels of the canonical NK1R remained consistent between cases and controls.[121]

Just as the study of alternative splicing is beginning to inform diagnosis and management of patients with cancer, the study of alternative splicing in pain-relevant GPCRs has great potential to advance the current state of clinical care for patients with chronic pain. Additionally, this line of inquiry may lead to the advent of new pain therapies such as IBNtxA, a novel opioid analgesic specifically targeting 6-TM μ-opioid splice variants.[122]

CONCLUSION

G protein–coupled receptors play a major role in modulating the activity of a chorus of cells involved in the transmission, modulation, and perception of pain. For this reason, GPCRs are the primary target of many pharmacological interventions used in the management of acute and chronic pain. Nonetheless, the use of these medications is limited because of variability in analgesic efficacy and adverse effect profiles. These limitations are partly attributed to genetic differences that influence alternative splicing of pain-relevant GPCRs. The functional importance and implications of the diversity of GPCRs in contributing to the pathophysiology of clinical pain is just beginning to emerge. More research, especially in the clinical arena, is necessary to further investigate the functions of specific GPCR splice variants, as well as the dynamic interactions between multiple variants of the same canonical receptor, within the context of pain. This line of inquiry will evolve our understanding of pain mechanisms and inform the design of new and clinically useful drugs that target specific alternative splice variants altered in a subset of patients.

Abbreviations and Acronyms: **AC** = adenylate cyclase; **AR** = adrenergic receptor; **CB** = cannabinoid; **COMT** = catechol-O-methyltransferase; **EP3** = prostaglandin E receptor 3; **GI** = gastrointestinal; **GPCR** = G protein–coupled receptor; **5-HT** = 5-hydroxytryptamine; **IBNtxA** = iodobenzoylnaltrexamide; **IBS** = irritable bowel syndrome; **mRNA** = messenger RNA; **MOR-1** = μ-opioid receptor; **NK-1R** = neurokinin 1 receptor; **OIH** = opioid-induced hyperalgesia; **ORL-1** = opioid receptor-like nociceptin receptor; **pre-mRNA** = precursor mRNA; **SNP** = single-nucleotide polymorphism; **TM** = transmembrane

Grant Support: Dr Nackley receives research support from the following sources: National Institutes of Health/National Institute of Neurological Disorders and Stroke R01 NS072205 (Principal Investigator); National Institutes of Health/National Institute of Neurological Disorders and Stroke P01 NS045685 (Principal Investigator); National Institutes of Health/National Institute of Dental and Craniofacial Research U01 DE017018 (Investigator); National Vulvodynia Association (NVA) (Principal Investigator); and UNC NCTraCS 50KR81417 (Principal Investigator).

Potential Competing Interests: Dr Maixner is a co-founder and equity stock holder in Algynomics, Inc, a company providing research services in personalized pain medication and diagnostics, and a patent holder with UNC Proove Bioscience. In addition, he receives consulting fees from National Institutes of Health, APS, and Orthogen with research funding support from National Institutes of Health/National Institute of Dental and Craniofacial Research.

Correspondence: Address to Andrea G. Nackley, PhD, Center for Pain Research and Innovation, University of North Carolina, Room 5506, Koury Oral Health Sciences Bldg, 385 S Columbia St, Chapel Hill, NC 27599-7455 (anackley@unc.edu). Individual reprints of this article and a bound reprint of the entire Symposium on Pain Medicine will be available for purchase from our website www.mayoclinicproceedings.org.

The Symposium on Pain Medicine will continue in an upcoming issue.

REFERENCES

1. Woolf CJ. American College of Physicians, American Physiological Society. Pain: moving from symptom control toward mechanism-specific pharmacologic management. *Ann Intern Med*. 2004;140(6):441-451.
2. Stone LS, Molliver DC. In search of analgesia: emerging roles of GPCRs in pain. *Mol Interv*. 2009;9(5):234-251.
3. Lundstrom K. An overview on GPCRs and drug discovery: structure-based drug design and structural biology on GPCRs. *Methods Mol Biol*. 2009;552:51-66.
4. North RA. Opioid receptor types and membrane ion channels. *Trends Neurosci*. 1986;9:114-117.
5. Matsuda LA. Molecular aspects of cannabinoid receptors. *Crit Rev Neurobiol*. 1997;11(2-3):143-166.

6. Michelotti GA, Price DT, Schwinn DA. α_1-Adrenergic receptor regulation: basic science and clinical implications. *Pharmacol Ther*. 2000;88(3):281-309.
7. Summers RJ, Broxton N, Hutchinson DS, Evans BA. The Janus faces of adrenoceptors: factors controlling the coupling of adrenoceptors to multiple signal transduction pathways. *Clin Exp Pharmacol Physiol*. 2004;31(11):822-827.
8. Hannon J, Hoyer D. Molecular biology of 5-HT receptors. *Behav Brain Res*. 2008;195(1):198-213.
9. Connor M, Christie MJ. Opioid receptor signalling mechanisms. *Clin Exp Pharmacol Physiol*. 1999;26(7):493-499.
10. Boudreau D, Von Korff M, Rutter CM, et al. Trends in long-term opioid therapy for chronic non-cancer pain. *Pharmacoepidem Drug Saf*. 2009;18(12):1166-1175.
11. Hutchinson MR, Zhang Y, Brown K, et al. Non-stereoselective reversal of neuropathic pain by naloxone and naltrexone: involvement of toll-like receptor 4 (TLR4). *Eur J Neurosci*. 2008;28(1):20-29.
12. Lynch ME, Campbell F. Cannabinoids for treatment of chronic non-cancer pain; a systematic review of randomized trials. *Brit J Clin Pharmacol*. 2011;72(5):735-744.
13. Johnston MM, Rapoport AM. Triptans for the management of migraine. *Drugs*. 2010;70(12):1505-1518.
14. Terrón JA. Is the 5-HT$_7$ receptor involved in the pathogenesis and prophylactic treatment of migraine? *Eur J Pharmacol*. 2002;439(1-3):1-11.
15. Kim JJ, Khan WI. 5-HT7 receptor signaling: improved therapeutic strategy in gut disorders. *Front Behav Neurosci*. 2014;8:396.
16. Meuser T, Pietruck C, Gabriel A, Xie G-X, Lim K-J, Pierce Palmer P. 5-HT7 receptors are involved in mediating 5-HT-induced activation of rat primary afferent neurons. *Life Sci*. 2002;71(19):2279-2289.
17. Rocha-González HI, Meneses A, Carlton SM, Granados-Soto V. Pronociceptive role of peripheral and spinal 5-HT$_7$ receptors in the formalin test. *Pain*. 2005;117(1-2):182-192.
18. Li S-F, Zhang Y-Y, Li Y-Y, Wen S, Xiao Z. Antihyperalgesic effect of 5-HT$_7$ receptor activation on the midbrain periaqueductal gray in a rat model of neuropathic pain. *Pharmacol Biochem Behav*. 2014;127:49-55.
19. American Pain Society. *Principles of Analgesic Use in the Treatment of Acute Pain and Cancer Pain*. 4th ed. Glenview, IL: American Pain Society; 1999.
20. Wang ET, Sandberg R, Luo S, et al. Alternative isoform regulation in human tissue transcriptomes. *Nature*. 2008;456(7221):470-476.
21. Keren H, Lev-Maor G, Ast G. Alternative splicing and evolution: diversification, exon definition and function. *Nat Rev Genet*. 2010;11(5):345-355.
22. Baralle D, Baralle M. Splicing in action: assessing disease causing sequence changes. *J Med Genet*. 2005;42(10):737-748.
23. Kilpatrick GJ, Dautzenberg FM, Martin GR, Eglen RM. 7TM receptors: the splicing on the cake. *Trends Pharmacol Sci*. 1999;20(7):294-301.
24. Pan Y-X, Xu J, Xu M, Rossi GC, Matulonis JE, Pasternak GW. Involvement of exon 11-associated variants of the mu opioid receptor MOR-1 in heroin, but not morphine, actions. *Proc Natl Acad Sci U S A*. 2009;106(12):4917-4922.
25. Pasternak GW, Pan Y-X. Mu opioids and their receptors: evolution of a concept. *Pharmacol Rev*. 2013;65(4):1257-1317.
26. Sato M, Hutchinson DS, Bengtsson T, et al. Functional domains of the mouse β_3-adrenoceptor associated with differential G protein coupling. *J Pharmacol Exp Ther*. 2005;315(3):1354-1361.
27. Boise LH, González-García M, Postema CE, et al. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell*. 1993;74(4):597-608.
28. Cascino I, Fiucci G, Papoff G, Ruberti G. Three functional soluble forms of the human apoptosis-inducing Fas molecule are produced by alternative splicing. *J Immunol*. 1995;154(6):2706-2713.
29. Gris P, Gauthier J, Cheng P, et al. A novel alternatively spliced isoform of the mu-opioid receptor: functional antagonism. *Mol Pain*. 2010;6(1):33.
30. Lipscombe D, Andrade A, Allen SE. Alternative splicing: functional diversity among voltage-gated calcium channels and behavioral consequences. *Biochim Biophys Acta*. 2013;1828(7):1522-1529.
31. Frühwald J, Camacho Londoño J, Dembla S, et al. Alternative splicing of a protein domain indispensable for function of transient receptor potential melastatin 3 (TRPM3) ion channels. *J Biol Chem*. 2012;287(44):36663-36672.
32. Zhou Y, Suzuki Y, Uchida K, Tominaga M. Identification of a splice variant of mouse TRPA1 that regulates TRPA1 activity. *Nat Commun*. 2013;4:2399.
33. Peng J, Sarkar S, Chang SL. Opioid receptor expression in human brain and peripheral tissues using absolute quantitative real-time RT-PCR. *Drug Alcohol Depend*. 2012;124(3):223-228.
34. Xu J, Lu Z, Xu M, et al. Differential expressions of the alternatively spliced variant mRNAs of the μ opioid receptor gene, OPRM1, in brain regions of four inbred mouse strains. *PLoS One*. 2014;9(10):e111267.
35. Pasternak GW. Insights into mu opioid pharmacology: the role of mu opioid receptor subtypes. *Life Sci*. 2001;68(19-20):2213-2219.
36. Wieskopf JS, Pan Y-X, Marcovitz J, et al. Broad-spectrum analgesic efficacy of IBNtxA is mediated by exon 11-associated splice variants of the mu-opioid receptor gene. *Pain*. 2014;155(10):2063-2070.
37. Liu X-Y, Liu Z-C, Sun Y-G, et al. Unidirectional cross-activation of GRPR by MOR1D uncouples itch and analgesia induced by opioids. *Cell*. 2011;147(2):447-458.
38. Pan Y-X. Diversity and complexity of the mu opioid receptor gene: alternative pre-mRNA splicing and promoters. *DNA Cell Biol*. 2005;24(11):736-750.
39. Majumdar S, Grinnell S, Le Rouzic V, et al. Truncated G protein-coupled mu opioid receptor MOR-1 splice variants are targets for highly potent opioid analgesics lacking side effects. *Proc Natl Acad Sci U S A*. 2011;108(49):19778-19783.
40. Pan YX, Xu J, Mahurter L, Bolan E, Xu M, Pasternak GW. Generation of the mu opioid receptor (MOR-1) protein by three new splice variants of the *Oprm* gene. *Proc Natl Acad Sci U S A*. 2001;98(24):14084-14089.
41. Xu J, Xu M, Brown T, et al. Stabilization of the μ-opioid receptor by truncated single transmembrane splice variants through a chaperone-like action. *J Biol Chem*. 2013;288(29):21211-21227.
42. Choi HS, Kim CS, Hwang CK, et al. The opioid ligand binding of human μ-opioid receptor is modulated by novel splice variants of the receptor. *Biochem Biophys Res Commun*. 2006;343(4):1132-1140.
43. Hawes BE, Graziano MP, Lambert DG. Cellular actions of nociceptin: transduction mechanisms. *Peptides*. 2000;21(7):961-967.
44. Currò D, Yoo JH, Anderson M, Song I, Del Valle J, Owyang C. Molecular cloning of the orphanin FQ receptor gene and differential tissue expression of splice variants in rat. *Gene*. 2001;266(1-2):139-145.
45. Arjomand J, Evans CJ. Differential splicing of transcripts encoding the orphanin FQ/nociceptin precursor. *J Neurochem*. 2001;77(3):720-729.
46. Xie G, Ito E, Maruyama K, et al. An alternatively spliced transcript of the rat nociceptin receptor ORL1 gene encodes a truncated receptor. *Brain Res Mol Brain Res*. 2000;77(1):1-9.
47. Galiègue S, Mary S, Marchand J, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem*. 1995;232(1):54-61.
48. Ryberg E, Vu HK, Larsson N, et al. Identification and characterisation of a novel splice variant of the human CB1 receptor. *FEBS Lett*. 2005;579(1):259-264.
49. Shire D, Carillon C, Kaghad M, et al. An amino-terminal variant of the central cannabinoid receptor resulting from

alternative splicing [published correction appears in *J Biol Chem*. 1996;271(52):33706]. *J Biol Chem*. 1995;270(8):3726-3731.

50. Cosenza-Nashat MA, Bauman A, Zhao ML, Morgello S, Suh HS, Lee SC. Cannabinoid receptor expression in HIV encephalitis and HIV-associated neuropathologic comorbidities. *Neuropathol Appl Neurobiol*. 2011;37(5):464-483.
51. Benito C, Núñez E, Tolón RM, et al. Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci*. 2003;23(35):11136-11141.
52. Yiangou Y, Facer P, Durrenberger P, et al. COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurol*. 2006;6:12.
53. Liu QR, Pan CH, Hishimoto A, et al. Species differences in cannabinoid receptor 2 (CNR2 gene): identification of novel human and rodent CB2 isoforms, differential tissue expression and regulation by cannabinoid receptor ligands. *Genes Brain Behav*. 2009;8(5):519-530.
54. Chang DJ, Chang TK, Yamanishi SS, et al. Molecular cloning, genomic characterization and expression of novel human α_{1A}-adrenoceptor isoforms. *FEBS Lett*. 1998;422(2):279-283.
55. Price RR, Morris DP, Biswas G, Smith MP, Schwinn DA. Acute agonist-mediated desensitization of the human α_{1a}-adrenergic receptor is primarily independent of carboxyl terminus regulation: implications for regulation of α_{1a}AR splice variants. *J Biol Chem*. 2002;277(11):9570-9579.
56. Daniels DV, Gever JR, Jasper JR, et al. Human cloned α_{1A}-adrenoceptor isoforms display α_{1L}-adrenoceptor pharmacology in functional studies. *Eur J Pharmacol*. 1999;370(3):337-343.
57. Cogé F, Guenin SP, Renouard-Try A, et al. Truncated isoforms inhibit [^{3}H]prazosin binding and cellular trafficking of native human α_{1A}-adrenoceptors. *Biochem J*. 1999;343(pt 1):231-239.
58. Tseng-Crank J, Kost T, Goetz A, et al. The α_{1C}-adrenoceptor in human prostate: cloning, functional expression, and localization to specific prostatic cell types. *Br J Pharmacol*. 1995;115(8):1475-1485.
59. Strosberg AD. Structure and function of the β_3-adrenergic receptor. *Annu Rev Pharmacol Toxicol*. 1997;37:421-450.
60. Soeder KJ, Snedden SK, Cao W, et al. The β_3-adrenergic receptor activates mitogen-activated protein kinase in adipocytes through a G_i-dependent mechanism. *J Biol Chem*. 1999;274(17):12017-12022.
61. Kanno T, Yaguchi T, Nishizaki T. Noradrenaline stimulates ATP release from DRG neurons by targeting β_3 adrenoceptors as a factor of neuropathic pain. *J Cell Physiol*. 2010;224(2):345-351.
62. Sato M, Hutchinson DS, Evans BA, Summers RJ. Functional domains of the mouse beta(3)-adrenoceptor associated with differential G-protein coupling. *Biochem Soc Trans*. 2007;35(pt 5):1035-1037.
63. Evans BA, Papaioannou M, Hamilton S, Summers RJ. Alternative splicing generates two isoforms of the β_3-adrenoceptor which are differentially expressed in mouse tissues. *Br J Pharmacol*. 1999;127(6):1525-1531.
64. Guest PC, Salim K, Skynner HA, George SE, Bresnick JN, McAllister G. Identification and characterization of a truncated variant of the 5-hydroxytryptamine$_{2A}$ receptor produced by alternative splicing. *Brain Res*. 2000;876(1-2):238-244.
65. Canton H, Emeson RB, Barker EL, et al. Identification, molecular cloning, and distribution of a short variant of the 5-hydroxytryptamine2C receptor produced by alternative splicing. *Mol Pharmacol*. 1996;50(4):799-807.
66. Wang Q, O'Brien PJ, Chen CX, Cho DS, Murray JM, Nishikura K. Altered G protein-coupling functions of RNA editing isoform and splicing variant serotonin$_{2C}$ receptors. *J Neurochem*. 2000;74(3):1290-1300.
67. Medhurst AD, Lezoualc'h F, Fischmeister R, Middlemiss DN, Sanger GJ. Quantitative mRNA analysis of five C-terminal splice variants of the human 5-HT$_4$ receptor in the central nervous system by TaqMan real time RT-PCR. *Brain Res Mol Brain Res*. 2001;90(2):125-134.
68. Blondel O, Gastineau M, Dahmoune Y, Langlois M, Fischmeister R. Cloning, expression, and pharmacology of four human 5-hydroxytryptamine$_4$ receptor isoforms produced by alternative splicing in the carboxyl terminus. *J Neurochem*. 1998;70(6):2252-2261.
69. Claeysen S, Sebben M, Becamel C, Bockaert J, Dumuis A. Novel brain-specific 5-HT$_4$ receptor splice variants show marked constitutive activity: role of the C-terminal intracellular domain. *Mol Pharmacol*. 1999;55(5):910-920.
70. Pindon A, Van Hecke G, Josson K, et al. Internalization of human 5-HT4a and 5-HT4b receptors is splice variant dependent. *Bioscience Rep*. 2004;24(3):215-223.
71. Pindon A, van Hecke G, van Gompel P, Lesage AS, Leysen JE, Jurzak M. Differences in signal transduction of two 5-HT$_4$ receptor splice variants: compound specificity and dual coupling with Gαs- and Gαi/o-proteins. *Mol Pharmacol*. 2002;61(1):85-96.
72. Vilaró MT, Cortés R, Mengod G. Serotonin 5-HT$_4$ receptors and their mRNAs in rat and guinea pig brain: distribution and effects of neurotoxic lesions. *J Comp Neurol*. 2005;484(4):418-439.
73. Coupar IM, Desmond PV, Irving HR. Human 5-HT$_4$ and 5-HT$_7$ receptor splice variants: are they important? *Curr Neuropharmacol*. 2007;5(4):224-231.
74. Mialet J, Berque-Bestel I, Sicsic S, Langlois M, Fischmeister R, Lezoualc'h F. Pharmacological characterization of the human 5-HT$_{4(d)}$ receptor splice variant stably expressed in Chinese hamster ovary cells. *Br J Pharmacol*. 2000;131(4):827-835.
75. Claeysen S, Faye P, Sebben M, Taviaux S, Bockaert J, Dumuis A. 5-HT$_4$ receptors: cloning and expression of new splice variants. *Ann NY Acad Sci*. 1998;861:49-56.
76. Bender E, Pindon A, van Oers I, et al. Structure of the human serotonin 5-HT$_4$ receptor gene and cloning of a novel 5-HT$_4$ splice variant. *J Neurochem*. 2000;74(2):478-489.
77. Irving HR, Tochon-Danguy N, Chinkwo KA, et al. Investigations into the binding affinities of different human 5-HT$_4$ receptor splice variants. *Pharmacology*. 2010;85(4):224-233.
78. Brattelid T, Kvingedal AM, Krobert KA, et al. Cloning, pharmacological characterisation and tissue distribution of a novel 5-HT$_4$ receptor splice variant, 5-HT$_{4(i)}$. *Naunyn Schmiedebergs Arch Pharmacol*. 2004;369(6):616-628.
79. Vilaró MT, Doménech T, Palacios JM, Mengod G. Cloning and characterization of a novel human 5-HT$_4$ receptor variant that lacks the alternatively spliced carboxy terminal exon: RT-PCR distribution in human brain and periphery of multiple 5-HT$_4$ receptor variants. *Neuropharmacology*. 2002;42(1):60-73.
80. Olsen MA, Nawoschik SP, Schurman BR, et al. Identification of a human 5-HT$_6$ receptor variant produced by alternative splicing. *Brain Res Mol Brain Res*. 1999;64(2):255-263.
81. Mahé C, Bernhard M, Bobirnac I, et al. Functional expression of the serotonin 5-HT$_7$ receptor in human glioblastoma cell lines. *Br J Pharmacol*. 2004;143(3):404-410.
82. Mahé C, Loetscher E, Dev KK, Bobirnac I, Otten U, Schoeffter P. Serotonin 5-HT$_7$ receptors coupled to induction of interleukin-6 in human microglial MC-3 cells. *Neuropharmacology*. 2005;49(1):40-47.
83. Jasper JR, Kosaka A, To ZP, Chang DJ, Eglen RM. Cloning, expression and pharmacology of a truncated splice variant of the human 5-HT$_7$ receptor (h5-HT$_{7(b)}$). *Br J Pharmacol*. 1997;122(1):126-132.
84. Krobert K, Bach T, Syversveen T, Kvingedal A, Levy FO. The cloned human 5-HT7 receptor splice variants: a comparative characterization of their pharmacology, function and distribution. *Naunyn Schmiedebergs Arch Pharmacol*. 2001;363(6):620-632.
85. Guthrie CR, Murray AT, Franklin AA, Hamblin MW. Differential agonist-mediated internalization of the human

5-hydroxytryptamine 7 receptor isoforms. *J Pharmacol Exp Ther*. 2005;313(3):1003-1010.
86. Sugimoto Y, Narumiya S. Prostaglandin E receptors. *J Biol Chem*. 2007;282(16):11613-11617.
87. Lai JP, Ho WZ, Kilpatrick LE, et al. Full-length and truncated neurokinin-1 receptor expression and function during monocyte/macrophage differentiation. *Proc Natl Acad Sci U S A*. 2006;103(20):7771-7776.
88. Muñoz M, Coveñas R. Involvement of substance P and the NK-1 receptor in human pathology. *Amino Acids*. 2014;46(7):1727-1750.
89. Lai JP, Lai S, Tuluc F, et al. Differences in the length of the carboxyl terminus mediate functional properties of neurokinin-1 receptor. *Proc Natl Acad Sci U S A*. 2008;105(34):12605-12610.
90. Xu J, Faskowitz AJ, Rossi GC, et al. Stabilization of morphine tolerance with long-term dosing: association with selective upregulation of mu-opioid receptor splice variant mRNAs. *Proc Natl Acad Sci U S A*. 2015;112(1):279-284.
91. Oladosu F, O'Buckley S, Nackley AG. Elucidating the role of MOR-1K in opioid-induced hyperalgesia via siRNA gene knockdown. International Association for the Study of Pain Conference, October 6-11, 2014, Buenos Aires, Argentina. *International Association for the Study of Pain*. 2014.
92. Grinnell SG, Majumdar S, Narayan A, et al. Pharmacologic characterization in the rat of a potent analgesic lacking respiratory depression, IBNtxA. *J Pharmacol Exp Ther*. 2014;350(3):710-718.
93. Xie G-X, Meuser T, Pietruck C, Sharma M, Palmer PP. Presence of opioid receptor-like (ORL1) receptor mRNA splice variants in peripheral sensory and sympathetic neuronal ganglia. *Life Sci*. 1999;64(22):2029-2037.
94. Kaushal S, Ridge KD, Khorana HG. Structure and function in rhodopsin: the role of asparagine-linked glycosylation. *Proc Natl Acad Sci U S A*. 1994;91(9):4024-4028.
95. Hawrylyshyn KA, Michelotti GA, Cogé F, Guénin SP, Schwinn DA. Update on human alpha1-adrenoceptor subtype signaling and genomic organization. *Trends Pharmacol Sci*. 2004;25(9):449-455.
96. Silver K, Walston J, Yang Y, et al. Molecular scanning of the beta-3-adrenergic receptor gene in Pima Indians and Caucasians. *Diabetes Metab Res Rev*. 1999;15(3):175-180.
97. van Spronsen A, Nahmias C, Krief S, Briend-Sutren M-M, Strosberg AD, Emorine LJ. The promoter and intron/exon structure of the human and mouse β3-adrenergic-receptor genes. *Eur J Biochem*. 1993;213(3):1117-1124.
98. Nakae A, Nakai K, Tanaka T, Hosokawa K, Mashimo T. Serotonin 2C receptor alternative splicing in a spinal cord injury model. *Neurosci Lett*. 2013;532:49-54.
99. Milligan G. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. *Mol Pharmacol*. 2003;64(6):1271-1276.
100. Krobert KA, Levy FO. The human 5-HT_7 serotonin receptor splice variants: constitutive activity and inverse agonist effects. *Br J Pharmacol*. 2002;135(6):1563-1571.
101. Gellynck E, Heyninck K, Andressen KW, et al. The serotonin 5-HT_7 receptors: two decades of research. *Exp Brain Res*. 2013;230(4):555-568.
102. Natura G, Bär K-J, Eitner A, et al. Neuronal prostaglandin E2 receptor subtype EP3 mediates antinociception during inflammation. *Proc Natl Acad Sci U S A*. 2013;110(33):13648-13653.
103. Minami T, Matsumura S, Mabuchi T, et al. Functional evidence for interaction between prostaglandin EP3 and κ-opioid receptor pathways in tactile pain induced by human immunodeficiency virus type-1 (HIV-1) glycoprotein gp120. *Neuropharmacology*. 2003;45(1):96-105.
104. Patwardhan AM, Vela J, Farugia J, Vela K, Hargreaves KM. Trigeminal nociceptors express prostaglandin receptors. *J Dent Res*. 2008;87(3):262-266.
105. Tillisch K, Labus J, Nam B, et al. Neurokinin-1-receptor antagonism decreases anxiety and emotional arousal circuit response to noxious visceral distension in women with irritable bowel syndrome: a pilot study. *Aliment Pharmacol Ther*. 2012;35(3):360-367.
106. Diatchenko L, Nackley AG, Slade GD, Fillingim RB, Maixner W. Idiopathic pain disorders—pathways of vulnerability. *Pain*. 2006;123(3):226-230.
107. Diatchenko L, Slade GD, Nackley AG, et al. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum Mol Genet*. 2005;14(1):135-143.
108. Nackley AG, Shabalina SA, Tchivileva IE, et al. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science*. 2006;314(5807):1930-1933.
109. Cheung KMC. The relationship between disc degeneration, low back pain, and human pain genetics [editorial]. *Spine J*. 2010;10(11):958-960.
110. Orrey DC, Bortsov AV, Hoskins JM, et al. Catechol-O-methyltransferase genotype predicts pain severity in hospitalized burn patients. *J Burn Care Res*. 2012;33(4):518-523.
111. Smith SB, Reenilä I, Männistö PT, et al. Epistasis between polymorphisms in COMT, ESR1, and GCH1 influences COMT enzyme activity and pain. *Pain*. 2014;155(11):2390-2399.
112. Kolesnikov Y, Gabovits B, Levin A, Voiko E, Veske A. Combined catechol-O-methyltransferase and μ-opioid receptor gene polymorphisms affect morphine postoperative analgesia and central side effects. *Anesth Analg*. 2011;112(2):448-453.
113. Tan E-C, Lim ECP, Ocampo CE, Allen JC, Sng B-L, Sia AT. Common variants of catechol-O-methyltransferase influence patient-controlled analgesia usage and postoperative pain in patients undergoing total hysterectomy [published online ahead of print May 12, 2015]. *Pharmacogenomics J*. http://dx.doi.org/10.1038/tpj.2015.33.
114. Kambur O, Kaunisto MA, Tikkanen E, Leal SM, Ripatti S, Kalso EA. Effect of catechol-O-methyltransferase-gene (COMT) variants on experimental and acute postoperative pain in 1,000 women undergoing surgery for breast cancer. *Anesthesiology*. 2013;119(6):1422-1433.
115. Sadhasivam S, Chidambaran V, Olbrecht VA, et al. Genetics of pain perception, COMT and postoperative pain management in children. *Pharmacogenomics*. 2014;15(3):277-284.
116. Nackley AG, Tan KS, Fecho K, Flood P, Diatchenko L, Maixner W. Catechol-O-methyltransferase inhibition increases pain sensitivity through activation of both β2- and β3-adrenergic receptors. *Pain*. 2007;128(3):199-208.
117. Hartung JE, Ciszek BP, Nackley AG. β2- and β3-adrenergic receptors drive COMT-dependent pain by increasing production of nitric oxide and cytokines. *Pain*. 2014;155(7):1346-1355.
118. Tchivileva IE, Lim PF, Smith SB, et al. Effect of catechol-O-methyltransferase polymorphism on response to propranolol therapy in chronic musculoskeletal pain: a randomized, double-blind, placebo-controlled, crossover pilot study. *Pharmacogenet Genomics*. 2010;20(4):239-248.
119. Zhou Y, Zuo D, Wang M, et al. Effect of truncated neurokinin-1 receptor expression changes on the interaction between human breast cancer and bone marrow-derived mesenchymal stem cells. *Genes Cells*. 2014;19(9):676-691.
120. Zhou Y, Zhao L, Xiong T, et al. Roles of full-length and truncated neurokinin-1 receptors on tumor progression and distant metastasis in human breast cancer. *Breast Cancer Res Treat*. 2013;140(1):49-61.
121. Gillespie E, Leeman SE, Watts LA, et al. Truncated neurokinin-1 receptor is increased in colonic epithelial cells from patients with colitis-associated cancer. *Proc Natl Acad Sci U S A*. 2011;108(42):17420-17425.
122. Lu Z, Xu J, Rossi GC, Majumdar S, Pasternak GW, Pan Y-X. Mediation of opioid analgesia by a truncated 6-transmembrane GPCR [published online ahead of print May 26, 2015]. *J Clin Invest*. http://dx.doi.org/10.1172/JCI81070.