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(54) Title: METHODS AND COMPOSITIONS TO TREAT PAIN AND PAINFUL DISORDERS USING 577, 20739 OR 57145

(57) Abstract: The present invention relates to methods for the diagnosis and treatment of pain or painful disorders. Specifically, the present invention identifies the differential expression of 577, 20739 OR 57145 genes in tissues relating to pain sensation, relative to their expression in normal, or non-painful disease states, and/or in response to manipulations relevant to pain. The present invention describes methods for the diagnostic evaluation and prognosis of various cardiovascular diseases, and for the identification of subjects exhibiting a predisposition to such conditions. The invention also provides methods for identifying a compound capable of modulating pain or painful disorders. The present invention also provides methods for the identification and therapeutic use of compounds as treatments of pain and painful disorders.

METHODS AND COMPOSITIONS TO TREAT PAIN AND PAINFUL DISORDERS USING 577, 20739 OR 57145

This application claims priority to U.S. provisional application number, 60/333,073, filed November 6, 2001, the entire contents of which are incorporated herein by reference.

The sensation of pain can be categorized into two types, peripheral and central pain. Peripheral pain can be classified into three broad areas, nociceptive pain, inflammatory pain and neuropathic pain. Nociceptive pain is also referred to as physiological pain and serves as a defense mechanism throughout the animal kingdom. Inflammatory pain, arising from severe wounds and/or associated with inflammatory infiltrates, can be well controlled by non-steroidal anti-inflammatory drugs (NSAID)-like drugs, steroids and opiates. However, the etiology and management of neuropathic pain is not well understood. Neuropathic pain is thought to arise from inherent defects in sensory and as a consequence in sympathetic neurons and can be secondary to trauma.

Peripheral pain is mediated by two types of primary sensory neuron classes, the Ad- and C-fibers, whose cell bodies lie within the dorsal root ganglion. Although the mechanisms of generation of neuropathic pain are poorly understood it is clear that several factors influence the perception and transmission of the painful stimulus, namely, alterations in chemical environment, ectopic generation of sensory neuron firing and sympathetic discharge. Some of the most common syndromes associated with neuropathic pain arise from destruction of small sensory fibers (or possibly the alteration in ratios of small to large fibers) as it is common in post-traumatic situations. Other etiologies of pain arise from small fiber damage due to diabetic neuropathy, drug induced damage (chemotherapy drugs), alcoholism, damage due to cancer, and a variety of hereditary small- and large-fiber neuropathies. We rationalize that targets derived from the peripheral nervous system may be of strategic benefit in that candidate compounds do not need to cross the blood-brain barrier, they can act on the initiation site of pain without inducing central side effects.

It has long been established that central mechanisms are involved in the perception and modulation of pain. Electrical stimulation of the periaqueductal gray (PAG) area produces analgesia without loss of other sensory modalities. Descending pain pathways emanating from PAG and the nucleus raphe magnus impinge on dorsal spinal cord regions where primary nociceptive afferents terminate. Also, stimulation of regions such as the

paragigantocellularis nucleus in the medulla oblongata result in analgesia. Finally, opiate receptors, when stimulated by opioid alkaloids and opioid peptides, mediate analgesia and these sites are located in key "pain centers" within the brain including PAG, thalamic nuclei and cortical regions. Identification of genes in these CNS regions and the spinal thalamic tract from animal models of pain may elucidate important targets for pain modulation.

The present invention provides methods and compositions for the diagnosis and treatment of a subject experiencing pain or suffering from a painful disorders. Preferably, the subject is a human, e.g., a patient with pain or a pain-associated disorder disclosed herein. For example, the subject can be a patient with pain elicited from tissue injury, e.g., inflammation, infection, ischemia; pain associated with musculoskeletal disorders, e.g., joint pain; tooth pain; headaches, e.g., migrane; pain associated with surgery; pain related to inflammation, e.g., irritable bowel syndrome; or chest pain. The subject can be a patient with complex regional pain syndrome (CRPS), reflex sympathetic dystrophy (RSD), causalgia, neuralgia, central pain and dysesthesia syndrome, carotidynia, neurogenic pain, refractory cervicobrachial pain syndrome, myofascial pain syndrome, craniomandibular pain dysfunction syndrome, chronic idiopathic pain syndrome, Costen's pain-dysfunction, acute chest pain syndrome, gynecologic pain syndrome, patellofemoral pain syndrome, anterior knee pain syndrome, recurrent abdominal pain in children, colic, low back pain syndrome, neuropathic pain, phantom pain from amputation, phantom tooth pain, or pain asymbolia. The subject can be a cancer patient, e.g., a patient with brain cancer, bone cancer, or prostate cancer. In other embodiments, the subject is a non-human animal, e.g., an experimental animal, e.g., an arthritic rat model of chronic pain, a chronic constriction injury (CCI) rat model of neuropathic pain, or a rat model of unilateral inflammatory pain by intraplantar injection of Freund's complete adjuvant (FCA).

"Treatment", as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving or affecting the disease or disorder, the symptoms of disease or disorder or the predisposition toward a disease or disorder. A therapeutic agent includes, but is not limited to, the small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides described herein.

The present invention is based, at least in part, on the discovery that nucleic acid and protein molecules, (described infra), are differentially expressed in animal models of pain and in peripheral and central nervous system tissues known to be associated with pain (e.g. dorsal root ganglion (DRG)). The modulators of the molecules of the present invention, identified according to the methods of the invention can be used to modulate (*e.g.*, inhibit, treat, or prevent) pain and painful conditions.

"Differential expression", as used herein, includes both quantitative as well as qualitative differences in the temporal and/or tissue expression pattern of a gene. Thus, a differentially expressed gene may have its expression activated or inactivated in normal versus painful disease conditions (for example, in an experimental pain model system such as in an animal model for pain). The degree to which expression differs in normal versus treated or control versus experimental states need only be large enough to be visualized via standard characterization techniques, *e.g.*, quantitative PCR, Northern analysis, subtractive hybridization. The expression pattern of a differentially expressed gene may be used as part of a prognostic or diagnostic, evaluation, or may be used in methods for identifying compounds useful for the treatment of pain and painful disorders. In addition, a differentially expressed gene involved in cardiovascular disease may represent a target gene such that modulation of the level of target gene expression or of target gene product activity may act to ameliorate a painful disease condition. Compounds that modulate target gene expression or activity of the target gene product can be used in the treatment of pain or painful conditions. Although the genes described herein may be differentially expressed with respect to pain, and/or their products may interact with gene products important to pain, the genes may also be involved in mechanisms important to additional cell processes.

Molecules of the Present Invention

Molecules of the present invention include, but are not limited to ion channels (eg. Potassium channels), transporters (e.g. amino acid transporters), receptors (e.g. G protein coupled receptors) and enzymes (e.g. kinases)

Transmembrane ion channel proteins that selectively mediate the conductance of sodium, potassium, calcium and chloride ions directly modulate the electrical activity of sensory neurons and are, thus, important in nociception. In particular, potassium channels are main players in regulating the frequency and pattern of neuronal firing. The expression and peak currents of potassium channels has been shown to be regulated after different models of

inflammatory and chronic pain. Additionally, calcium ions serve important intracellular signaling roles including modulation of other ion channels and regulation of protein kinases and other enzymatic activity. As cell surface proteins with established three-dimensional structures and modes of action, the pore-forming alpha subunits of ion channels make ideal drug targets. In addition to alpha subunits, these channels may consist of beta subunits and other interacting proteins which modulate channel activity and are good targets for pharmacological manipulation of the channels. Therefore, ion channels are useful in treating pain and painful conditions.

Endogenous soluble factors mediate pain sensation by binding to specific transmembrane receptors either on the peripheral terminals of nociceptive neurons or on central neurons receiving input from these nociceptors. These soluble factors include, but are not limited to serotonine, histamine, bradykinin, tachykinins (substance P and neurokinin A), opioids, eicosanoids (leukotrienes, prostaglandins, thromboxanes), purines, excitatory amino acids and different proteins. In addition a growing body of evidence, including clinical trials in man, indicates that IL-1, TNFa, and members of the neurotrophin family are involved at several stages in the transmission of painful stimuli. Hydrogen ions (protons) may mediate pain associated with inflammation (and also acid taste) by activating vanilloid receptor calcium channels or amiloride-sensitive sodium channels. Additionally, numerous exogenous agents modulate pain by mimicking endogenous soluble factors. For instance the opiate drugs of abuse exert analgesic effects by binding to receptors for the endogenous opioids and capsaicin stimulates pain sensation by binding to vanilloid receptors. The receptors for these soluble factors are linked to several signal transduction mechanisms including tyrosine kinase activity (e.g. neurotrophin receptors), recruitment of cytoplasmic tyrosine kinases (e.g. cytokine receptors for TNFa and IL-1), ion channel opening, and G-protein coupled receptors. These cell surface receptors are ideal drug targets due to their transmembrane location, and the goal is to discover G-protein coupling receptors with known ligands or with surrogate ligands that may be important players in regulating pain mechanisms.

Intracellular kinases such as protein kinase A and protein kinase C are involved in the response to pain in sensory neurons. Similarly, enzymes such as cyclooxygenase(s) and thromboxane synthetase are know to be critical in the production of prostaglandins, leukotrienes and thromboxanes. Although these particular targets may be more important in inflammatory pain, the role of this gene family in long term or neuropathic pain is of importance.

Gene ID 577

The human 577 sequence (SEQ ID NO:1), (GI:1839269, known also as sodium dependent proline transporter) which is approximately 1908 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1911 nucleotides, including the termination codon (nucleotides indicated as coding of SEQ ID NO:1, SEQ ID NO:3). The coding sequence encodes a 636 amino acid protein (SEQ ID NO:3, 6, 9) (GI:8176779).

As assessed by TaqMan analysis, 577 is expressed in the brain and dorsal root ganglion (DRG). Therefore, it is involved in nociception and would be a potential target to discover therapeutics directed toward the treatment of pain and painful disorders.

Gene ID 20739

The human 20739 sequence (SEQ ID NO:4), (GI:3608385), known also as P21-activated kinase 3 (PAK-3)) which is approximately 1635 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1632 nucleotides, including the termination codon (nucleotides indicated as coding of SEQ ID NO:4, SEQ ID NO:6). The coding sequence encodes a 544 amino acid protein (SEQ ID NO:5) (GI3608386).

As assessed by TaqMan analysis, 20739 is expressed in the brain, dorsal root ganglion (DRG), superior cervical ganglion (SCG), ureter, testes, ovary and spinal cord. Additional TaqMan analyses indicated that 20739 mRNA was upregulated in two rat models of pain, CFA injection and axomomy. In situ hybridization indicated that the expression of 20739 in the DRG was restricted to neurons of all sizes. Due to the expression of 20739 in DRG, SCG and brain, along with its regulated in two animal models of pain, 20739 is a potential target to discover therapeutics directed toward the treatment of pain and painful disorders.

Gene ID 57145

The human 57145 sequence (SEQ ID NO:7), (known also as OCT-5) which is approximately 2561 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1644 nucleotides, including the termination codon (nucleotides indicated as coding of SEQ ID NO:7, SEQ ID NO:9). The coding sequence encodes a 548 amino acid protein (SEQ ID NO:8).

As assessed by TaqMan analysis, 57145 is expressed in the brain, dorsal root ganglion (DRG), superior cervical ganglion (SCG), ureter, testes, ovary and spinal cord. Therefore, it is involved in nociception and would be a potential target to discover therapeutics directed toward the treatment of pain and painful disorders.

Various aspects of the invention are described in further detail in the following subsections:

I. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules (organic or inorganic) or other drugs) which bind to 577, 20739 OR 57145 proteins, have a stimulatory or inhibitory effect on, for example, 577, 20739 OR 57145 expression or 577, 20739 OR 57145 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 577, 20739 OR 57145 substrate. Compounds identified using the assays described herein may be useful for treating pain and painful conditions.

These assays are designed to identify compounds that bind to a 577, 20739 OR 57145 protein, bind to other intracellular or extracellular proteins that interact with a 577, 20739 OR 57145 protein, and interfere with the interaction of the 577, 20739 OR 57145 protein with other intercellular or extracellular proteins. For example, in the case of the 577, 20739 OR 57145 protein, which is a transmembrane receptor-type protein, such techniques can identify ligands for such a receptor. A 577, 20739 OR 57145 protein ligand or substrate can, for example, be used to ameliorate pain and painful conditions. Such compounds may include, but are not limited to peptides, antibodies, or small organic or inorganic compounds. Such compounds may also include other cellular proteins.

Compounds identified via assays such as those described herein may be useful, for example, for treating pain and painful conditions. In instances whereby a painful condition results from an overall lower level of 577, 20739 OR 57145 gene expression and/or 577, 20739 OR 57145 protein in a cell or tissue, compounds that interact with the 577, 20739 OR 57145 protein may include compounds which accentuate or amplify the activity of the bound 577, 20739 OR 57145 protein. Such compounds would bring about an effective increase in the level of 577, 20739 OR 57145 protein activity, thus ameliorating symptoms.

In other instances, mutations within the 577, 20739 OR 57145 gene may cause aberrant types or excessive amounts of 577, 20739 OR 57145 proteins to be made which have a deleterious effect that leads to a pain. Similarly, physiological conditions may cause an excessive increase in 577, 20739 OR 57145 gene expression leading pain. In such cases, compounds that bind to a 577, 20739 OR 57145 protein may be identified that inhibit the activity of the 577, 20739 OR 57145 protein. Assays for testing the effectiveness of compounds identified by techniques such as those described in this section are discussed herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 577, 20739 OR 57145 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 577, 20739 OR 57145 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a 577, 20739 OR 57145 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate 577, 20739 OR 57145 activity is determined. Determining the ability of the test compound to modulate 577, 20739 OR 57145 activity can be accomplished by monitoring, for example, intracellular calcium, IP_3, cAMP, or diacylglycerol concentration, the phosphorylation profile of intracellular proteins, cell proliferation and/or migration, gene expression of, for example, cell surface adhesion molecules or genes associated with analgesia, or the activity of a 577, 20739 OR 57145 -regulated transcription factor. The cell can be of mammalian origin, *e.g.*, a neural cell. In one embodiment, compounds that interact with a receptor domain can be screened for their ability to function as ligands, *i.e.*, to bind to the receptor and modulate a signal transduction pathway. Identification of ligands, and measuring the activity of the ligand-receptor complex, leads to the identification of modulators (*e.g.*, antagonists) of this interaction. Such modulators may be useful in the treatment of pain and painful conditions.

The ability of the test compound to modulate 577, 20739 OR 57145 binding to a substrate or to bind to 577, 20739 OR 57145 can also be determined. Determining the ability of the test compound to modulate 577, 20739 OR 57145 binding to a substrate can be accomplished, for example, by coupling the 577, 20739 OR 57145 substrate with a radioisotope or enzymatic label such that binding of the 577, 20739 OR 57145 substrate to 577, 20739 OR 57145 can be determined by detecting the labeled 577, 20739 OR 57145 substrate in a complex. 577, 20739 OR 57145 could also be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 577, 20739 OR 57145 binding to a 577, 20739 OR 57145 substrate in a complex. Determining the ability of the test compound to bind 577, 20739 OR 57145 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to 577, 20739 OR 57145 can be determined by detecting the labeled 577, 20739 OR 57145 compound in a complex. For example, compounds (*e.g.*, 577, 20739 OR 57145 ligands or substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Compounds can further be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, a 577, 20739 OR 57145 ligand or substrate) to interact with 577, 20739 OR 57145 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with 577, 20739 OR 57145 without the labeling of either the compound or the 577, 20739 OR 57145 (McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 577, 20739 OR 57145 .

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a 577, 20739 OR 57145 target molecule (*e.g.*, a 577, 20739 OR 57145 substrate) with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 577, 20739 OR 57145 target molecule. Determining the ability of the test compound to modulate the activity of a 577, 20739 OR 57145 target molecule can be accomplished, for example, by determining the ability of the 577, 20739 OR 57145 protein to bind to or interact with the 577, 20739 OR 57145 target molecule.

Determining the ability of the 577, 20739 OR 57145 protein or a biologically active fragment thereof, to bind to or interact with a 577, 20739 OR 57145 target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the 577, 20739 OR 57145 protein to bind to or interact with a 577, 20739 OR 57145 target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.*, intracellular Ca^{2+}, diacylglycerol, IP_3, cAMP), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response (*e.g.*, gene expression).

In yet another embodiment, an assay of the present invention is a cell-free assay in which a 577, 20739 OR 57145 protein or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to bind to the 577, 20739 OR 57145 protein or biologically active portion thereof is determined. Preferred biologically

active portions of the 577, 20739 OR 57145 proteins to be used in assays of the present invention include fragments which participate in interactions with non-577, 20739 OR 57145 molecules, *e.g.*, fragments with high surface probability scores. Binding of the test compound to the 577, 20739 OR 57145 protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the 577, 20739 OR 57145 protein or biologically active portion thereof with a known compound which binds 577, 20739 OR 57145 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 577, 20739 OR 57145 protein, wherein determining the ability of the test compound to interact with a 577, 20739 OR 57145 protein comprises determining the ability of the test compound to preferentially bind to 577, 20739 OR 57145 or biologically active portion thereof as compared to the known compound. Compounds that modulate the interaction of 577, 20739 OR 57145 with a known target protein may be useful in regulating the activity of a 577, 20739 OR 57145 protein, especially a mutant 577, 20739 OR 57145 protein.

In another embodiment, the assay is a cell-free assay in which a 577, 20739 OR 57145 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 577, 20739 OR 57145 protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a 577, 20739 OR 57145 protein can be accomplished, for example, by determining the ability of the 577, 20739 OR 57145 protein to bind to a 577, 20739 OR 57145 target molecule by one of the methods described above for determining direct binding. Determining the ability of the 577, 20739 OR 57145 protein to bind to a 577, 20739 OR 57145 target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA) (Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In another embodiment, determining the ability of the test compound to modulate the activity of a 577, 20739 OR 57145 protein can be accomplished by determining the ability of the 577, 20739 OR 57145 protein to further modulate the activity of a downstream effector of a 577, 20739 OR 57145 target molecule. For example, the activity

of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a 577, 20739 OR 57145 protein or biologically active portion thereof with a known compound which binds the 577, 20739 OR 57145 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the 577, 20739 OR 57145 protein, wherein determining the ability of the test compound to interact with the 577, 20739 OR 57145 protein comprises determining the ability of the 577, 20739 OR 57145 protein to preferentially bind to or modulate the activity of a 577, 20739 OR 57145 target molecule.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either 577, 20739 OR 57145 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 577, 20739 OR 57145 protein, or interaction of a 577, 20739 OR 57145 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/577, 20739 OR 57145 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 577, 20739 OR 57145 protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 577, 20739 OR 57145 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a 577, 20739 OR 57145 protein or a 577, 20739 OR 57145 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated 577, 20739 OR 57145 protein or target molecules can be

prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with 577, 20739 OR 57145 protein or target molecules but which do not interfere with binding of the 577, 20739 OR 57145 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or 577, 20739 OR 57145 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 577, 20739 OR 57145 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 577, 20739 OR 57145 protein or target molecule.

In another embodiment, modulators of 577, 20739 OR 57145 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of 577, 20739 OR 57145 mRNA or protein in the cell is determined. The level of expression of 577, 20739 OR 57145 mRNA or protein in the presence of the candidate compound is compared to the level of expression of 577, 20739 OR 57145 mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of 577, 20739 OR 57145 expression based on this comparison. For example, when expression of 577, 20739 OR 57145 mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 577, 20739 OR 57145 mRNA or protein expression. Alternatively, when expression of 577, 20739 OR 57145 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 577, 20739 OR 57145 mRNA or protein expression. The level of 577, 20739 OR 57145 mRNA or protein expression in the cells can be determined by methods described herein for detecting 577, 20739 OR 57145 mRNA or protein.

In yet another aspect of the invention, the 577, 20739 OR 57145 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 577, 20739 OR 57145 ("577, 20739 OR 57145 -binding proteins" or "577, 20739 OR 57145 -bp") and are involved in 577, 20739 OR 57145 activity. Such 577,

20739 OR 57145 -binding proteins are also likely to be involved in the propagation of signals by the 577, 20739 OR 57145 proteins or 577, 20739 OR 57145 targets as, for example, downstream elements of a 577, 20739 OR 57145 -mediated signaling pathway. Alternatively, such 577, 20739 OR 57145 -binding proteins are likely to be 577, 20739 OR 57145 inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 577, 20739 OR 57145 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming a 577, 20739 OR 57145 -dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 577, 20739 OR 57145 protein.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a 577, 20739 OR 57145 protein can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for pain, as described herein.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a 577, 20739 OR 57145 modulating agent, an antisense 577, 20739 OR 57145 nucleic acid molecule, a 577, 20739 OR 57145 -specific antibody, or a 577, 20739 OR 57145 -binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains

to uses of novel agents identified by the above-described screening assays for treatments as described herein.

Any of the compounds, including but not limited to compounds such as those identified in the foregoing assay systems, may be tested for the ability to ameliorate pain. Cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate pain are described herein.

In addition, animal-based models of pain, such as those described herein, may be used to identify compounds capable of treating pain and painful conditions. Such animal models may be used as test substrates for the identification of drugs, pharmaceuticals, therapies, and interventions which may be effective in treating pain. For example, animal models may be exposed to a compound, suspected of exhibiting an ability to treat pain, at a sufficient concentration and for a time sufficient to elicit such an amelioration of pain in the exposed animals. The response of the animals to the exposure may be monitored by assessing the reversal of the symptoms of pain before and after treatment.

With regard to intervention, any treatments which reverse any aspect of pain (i.e. have an analgesic effect) should be considered as candidates for human pain therapeutic intervention. Dosages of test agents may be determined by deriving dose-response curves.

Additionally, gene expression patterns may be utilized to assess the ability of a compound to ameliorate pain. For example, the expression pattern of one or more genes may form part of a "gene expression profile" or “transcriptional profile” which may be then be used in such an assessment. "Gene expression profile" or “transcriptional profile”, as used herein, includes the pattern of mRNA expression obtained for a given tissue or cell type under a given set of conditions. Gene expression profiles may be generated, for example, by utilizing a differential display procedure, Northern analysis and/or RT-PCR. In one embodiment, 577, 20739 OR 57145 gene sequences may be used as probes and/or PCR primers for the generation and corroboration of such gene expression profiles.

Gene expression profiles may be characterized for known states, either cardiovascular disease or normal, within the cell- and/or animal-based model systems. Subsequently, these known gene expression profiles may be compared to ascertain the effect a test compound has to modify such gene expression profiles, and to cause the profile to more closely resemble that of a more desirable profile.

For example, administration of a compound may cause the gene expression profile of a pain disease model system to more closely resemble the control system. Administration of a compound may, alternatively, cause the gene expression profile of a

control system to begin to mimic pain or a painful disease state. Such a compound may, for example, be used in further characterizing the compound of interest, or may be used in the generation of additional animal models.

II. Cell- and Animal-Based Model Systems

Described herein are cell- and animal-based systems which act as models for pain. These systems may be used in a variety of applications. For example, the cell- and animal-based model systems may be used to further characterize differentially expressed genes associated with cardiovascular disease, *e.g.*, 577, 20739 OR 57145 . In addition, animal- and cell-based assays may be used as part of screening strategies designed to identify compounds which are capable of ameliorating pain, as described, below. Thus, the animal- and cell-based models may be used to identify drugs, pharmaceuticals, therapies and interventions which may be effective in treating cardiovascular disease. Furthermore, such animal models may be used to determine the LD50 and the ED50 in animal subjects, and such data can be used to determine the *in vivo* efficacy of potential pain treatments.

A. Animal-Based Systems

Animal-based model systems of pain may include, but are not limited to, non-recombinant and engineered transgenic animals.

Non-recombinant animal models for pain may include, for example, genetic models.

Additionally, animal models exhibiting pain may be engineered by using, for example, 577, 20739 OR 57145 gene sequences described above, in conjunction with techniques for producing transgenic animals that are well known to those of skill in the art. For example, 577, 20739 OR 57145 gene sequences may be introduced into, and overexpressed in, the genome of the animal of interest, or, if endogenous 577, 20739 OR 57145 gene sequences are present, they may either be overexpressed or, alternatively, be disrupted in order to underexpress or inactivate 577, 20739 OR 57145 gene expression.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which 577, 20739 OR 57145 -coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous 577, 20739 OR 57145 sequences have been introduced into their genome or homologous recombinant animals in which endogenous 577, 20739 OR

57145 sequences have been altered. Such animals are useful for studying the function and/or activity of a 577, 20739 OR 57145 and for identifying and/or evaluating modulators of 577, 20739 OR 57145 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous 577, 20739 OR 57145 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal used in the methods of the invention can be created by introducing a 577, 20739 OR 57145 -encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The 577, 20739 OR 57145 cDNA sequence can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human 577, 20739 OR 57145 gene, such as a mouse or rat 577, 20739 OR 57145 gene, can be used as a transgene. Alternatively, a 577, 20739 OR 57145 gene homologue, such as another 577, 20739 OR 57145 family member, can be isolated based on hybridization to the 577, 20739 OR 57145 cDNA sequences and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a 577, 20739 OR 57145 transgene to direct expression of a 577, 20739 OR 57145 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a 577, 20739 OR 57145 transgene in its genome

and/or expression of 577, 20739 OR 57145 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 577, 20739 OR 57145 protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a 577, 20739 OR 57145 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the 577, 20739 OR 57145 gene. The 577, 20739 OR 57145 gene can be a human gene but more preferably, is a non-human homologue of a human 577, 20739 OR 57145 gene. For example, a rat 577, 20739 OR 57145 gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous 577, 20739 OR 57145 gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous 577, 20739 OR 57145 gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous 577, 20739 OR 57145 gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous 577, 20739 OR 57145 protein). In the homologous recombination nucleic acid molecule, the altered portion of the 577, 20739 OR 57145 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the 577, 20739 OR 57145 gene to allow for homologous recombination to occur between the exogenous 577, 20739 OR 57145 gene carried by the homologous recombination nucleic acid molecule and an endogenous 577, 20739 OR 57145 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking 577, 20739 OR 57145 nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced 577, 20739 OR 57145 gene has homologously recombined with the endogenous 577, 20739 OR 57145 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then injected into a

blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-human animals for use in the methods of the invention can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_o phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

The 577, 20739 OR 57145 transgenic animals that express 577, 20739 OR 57145 mRNA or a 577, 20739 OR 57145 peptide (detected immunocytochemically, using antibodies directed against 577, 20739 OR 57145 epitopes) at easily detectable levels should then be further evaluated to identify those animals which display characteristic pain.

B. Cell-Based Systems

Cells that contain and express 577, 20739 OR 57145 gene sequences which encode a 577, 20739 OR 57145 protein, and, further, exhibit cellular phenotypes associated with nociception, may be used to identify compounds that exhibit analgesic effect. Such cells may include non-recombinant monocyte cell lines, such as U937 (ATCC# CRL-1593), THP-1 (ATCC#TIB-202), and P388D1 (ATCC# TIB-63); endothelial cells such as human umbilical vein endothelial cells (HUVECs), human microvascular endothelial cells (HMVEC), and bovine aortic endothelial cells (BAECs); as well as generic mammalian cell lines such as HeLa cells and COS cells, *e.g.*, COS-7 (ATCC# CRL-1651), and neural cell lines.. Further, such cells may include recombinant, transgenic cell lines. For example, the pain animal models of the invention, discussed above, may be used to generate cell lines, containing one or more cell types involved in nociception, that can be used as cell culture models for this disorder. While primary cultures derived from the pain model transgenic animals of the invention may be utilized, the generation of continuous cell lines is preferred. For examples of techniques which may be used to derive a continuous cell line from the transgenic animals, see Small *et al.*, (1985) *Mol. Cell Biol.* 5:642-648.

Alternatively, cells of a cell type known to be involved in nociception may be transfected with sequences capable of increasing or decreasing the amount of 577, 20739 OR 57145 gene expression within the cell. For example, 577, 20739 OR 57145 gene sequences may be introduced into, and overexpressed in, the genome of the cell of interest, or, if endogenous 577, 20739 OR 57145 gene sequences are present, they may be either overexpressed or, alternatively disrupted in order to underexpress or inactivate 577, 20739 OR 57145 gene expression.

In order to overexpress a 577, 20739 OR 57145 gene, the coding portion of the 577, 20739 OR 57145 gene may be ligated to a regulatory sequence which is capable of driving gene expression in the cell type of interest, *e.g.*, an endothelial cell. Such regulatory regions will be well known to those of skill in the art, and may be utilized in the

absence of undue experimentation. Recombinant methods for expressing target genes are described above.

For underexpression of an endogenous 577, 20739 OR 57145 gene sequence, such a sequence may be isolated and engineered such that when reintroduced into the genome of the cell type of interest, the endogenous 577, 20739 OR 57145 alleles will be inactivated. Preferably, the engineered 577, 20739 OR 57145 sequence is introduced via gene targeting such that the endogenous 577, 20739 OR 57145 sequence is disrupted upon integration of the engineered 577, 20739 OR 57145 sequence into the cell's genome. Transfection of host cells with 577, 20739 OR 57145 genes is discussed, above.

Cells treated with compounds or transfected with 577, 20739 OR 57145 genes can be examined for phenotypes associated with nociception.

Transfection of 577, 20739 OR 57145 nucleic acid may be accomplished by using standard techniques (described in, for example, Ausubel (1989) *supra*). Transfected cells should be evaluated for the presence of the recombinant 577, 20739 OR 57145 gene sequences, for expression and accumulation of 577, 20739 OR 57145 mRNA, and for the presence of recombinant 577, 20739 OR 57145 protein production. In instances wherein a decrease in 577, 20739 OR 57145 gene expression is desired, standard techniques may be used to demonstrate whether a decrease in endogenous 577, 20739 OR 57145 gene expression and/or in 577, 20739 OR 57145 protein production is achieved.

III. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining 577, 20739 OR 57145 protein and/or nucleic acid expression as well as 577, 20739 OR 57145 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, *e.g.*, endothelial cells, or tissue, *e.g.*, vascular tissue) to thereby determine whether an individual is afflicted with a predisposition or is experiencing pain. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a painful disorder. For example, mutations in a 577, 20739 OR 57145 gene can be assayed for in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby phophylactically treat an individual prior to the onset of a painful disorder.

Another aspect of the invention pertains to monitoring the influence of 577, 20739 OR 57145 modulators (*e.g.*, anti-577, 20739 OR 57145 antibodies or 577, 20739 OR 57145 ribozymes) on the expression or activity of 577, 20739 OR 57145 in clinical trials.

These and other agents are described in further detail in the following sections.

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A. Diagnostic Assays

To determine whether a subject is afflicted with a disease, a biological sample may be obtained from a subject and the biological sample may be contacted with a compound or an agent capable of detecting a 577, 20739 OR 57145 protein or nucleic acid (*e.g.*,
10 mRNA or genomic DNA) that encodes a 577, 20739 OR 57145 protein, in the biological sample. A preferred agent for detecting 577, 20739 OR 57145 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to 577, 20739 OR 57145 mRNA or genomic DNA. The nucleic acid probe can be, for example, the 577, 20739 OR 57145 nucleic acid set forth in SEQ ID NO:1, 4 or 7, or a portion thereof, such as an
15 oligonucleotide of at least 15, 20, 25, 30, 25, 40, 45, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 577, 20739 OR 57145 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting 577, 20739 OR 57145 protein in a sample is an
20 antibody capable of binding to 577, 20739 OR 57145 protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')2) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the
25 probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

The term "biological sample" is intended to include tissues, cells, and biological
30 fluids isolated from a subject, as well as tissues, cells, and fluids present within a subject. That is, the detection method of the invention can be used to detect 577, 20739 OR 57145 mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of 577, 20739 OR 57145 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of

577, 20739 OR 57145 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of 577, 20739 OR 57145 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of 577, 20739 OR 57145 protein include introducing into a subject a labeled anti-577, 20739 OR 57145 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting 577, 20739 OR 57145 protein, mRNA, or genomic DNA, such that the presence of 577, 20739 OR 57145 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of 577, 20739 OR 57145 protein, mRNA or genomic DNA in the control sample with the presence of 577, 20739 OR 57145 protein, mRNA or genomic DNA in the test sample.

B. Prognostic Assays

The present invention further pertains to methods for identifying subjects having or at risk of developing a disease associated with aberrant 577, 20739 OR 57145 expression or activity.

As used herein, the term "aberrant" includes a 577, 20739 OR 57145 expression or activity which deviates from the wild type 577, 20739 OR 57145 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant 577, 20739 OR 57145 expression or activity is intended to include the cases in which a mutation in the 577, 20739 OR 57145 gene causes the 577, 20739 OR 57145 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional 577, 20739 OR 57145 protein or a protein which does not function in a wild-type fashion, *e.g.*, a protein which does not interact with a 577, 20739 OR 57145 substrate, or one which interacts with a non-577, 20739 OR 57145 substrate.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be used to identify a subject having or at risk of developing a disease. A biological sample may be obtained from a subject and tested for the presence or absence of a genetic alteration. For example, such genetic alterations can be detected by

ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 577, 20739 OR 57145 gene, 2) an addition of one or more nucleotides to a 577, 20739 OR 57145 gene, 3) a substitution of one or more nucleotides of a 577, 20739 OR 57145 gene, 4) a chromosomal rearrangement of a 577, 20739 OR 57145 gene, 5) an alteration in the level of a messenger RNA transcript of a 577, 20739 OR 57145 gene, 6) aberrant modification of a 577, 20739 OR 57145 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 577, 20739 OR 57145 gene, 8) a non-wild type level of a 577, 20739 OR 57145 -protein, 9) allelic loss of a 577, 20739 OR 57145 gene, and 10) inappropriate post-translational modification of a 577, 20739 OR 57145 -protein.

As described herein, there are a large number of assays known in the art which can be used for detecting genetic alterations in a 577, 20739 OR 57145 gene. For example, a genetic alteration in a 577, 20739 OR 57145 gene may be detected using a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in a 577, 20739 OR 57145 gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method includes collecting a biological sample from a subject, isolating nucleic acid (*e.g.*, genomic DNA, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 577, 20739 OR 57145 gene under conditions such that hybridization and amplification of the 577, 20739 OR 57145 gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are

especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a 577, 20739 OR 57145 gene from a biological sample can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in 577, 20739 OR 57145 can be identified by hybridizing biological sample derived and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7:244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2:753-759). For example, genetic mutations in 577, 20739 OR 57145 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al.* (1996) *supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential, overlapping probes. This step allows for the identification of point mutations. This step is followed by a second hybridization array that allows for the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 577, 20739 OR 57145 gene in a biological sample and detect mutations by comparing the sequence of the 577, 20739 OR 57145 in the biological sample with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert (1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger (1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve, C. W. (1995) *Biotechniques* 19:448-53), including sequencing by mass spectrometry (see, *e.g.*, PCT International

Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the 577, 20739 OR 57145 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type 577, 20739 OR 57145 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digest the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397 and Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 577, 20739 OR 57145 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a 577, 20739 OR 57145 sequence, *e.g.*, a wild-type 577, 20739 OR 57145 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 577, 20739 OR 57145 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad.*

Sci USA: 86:2766; see also Cotton (1993) *Mutat. Res.* 285:125-144 and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 577, 20739 OR 57145 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to ensure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce

polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered a 577, 20739 OR 57145 modulator (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, or small molecule) to effectively treat a disease.

C. Monitoring of Effects During Clinical Trials

The present invention further provides methods for determining the effectiveness of a 577, 20739 OR 57145 modulator (*e.g.*, a 577, 20739 OR 57145 modulator identified herein) in treating a disease. For example, the effectiveness of a 577, 20739 OR 57145 modulator in increasing 577, 20739 OR 57145 gene expression, protein levels, or in upregulating 577, 20739 OR 57145 activity, can be monitored in clinical trials of subjects exhibiting decreased 577, 20739 OR 57145 gene expression, protein levels, or downregulated 577, 20739 OR 57145 activity. Alternatively, the effectiveness of a 577, 20739 OR 57145 modulator in decreasing 577, 20739 OR 57145 gene expression, protein levels, or in downregulating 577, 20739 OR 57145 activity, can be monitored in clinical trials of subjects exhibiting increased 577, 20739 OR 57145 gene expression, protein levels, or 577, 20739 OR 57145 activity. In such clinical trials, the expression or activity of a 577, 20739 OR 57145 gene, and preferably, other genes that have been implicated in nociception can be used as a "read out" or marker of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including 577, 20739 OR 57145 , that are modulated in cells by treatment with an agent which modulates 577, 20739 OR 57145 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents which modulate 577, 20739 OR 57145 activity on subjects suffering from a painful disorder in, for example, a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of 577, 20739 OR 57145 and other genes implicated in the painful disorder. The levels of gene expression

(*e.g.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods described herein, or by measuring the levels of activity of 577, 20739 OR 57145 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent which modulates 577, 20739 OR 57145 activity. This response state may be determined before, and at various points during treatment of the individual with the agent which modulates 577, 20739 OR 57145 activity.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent which modulates 577, 20739 OR 57145 activity (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, or small molecule identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a 577, 20739 OR 57145 protein, mRNA, or genomic DNA in the pre-administration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the 577, 20739 OR 57145 protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the 577, 20739 OR 57145 protein, mRNA, or genomic DNA in the pre-administration sample with the 577, 20739 OR 57145 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of 577, 20739 OR 57145 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of 577, 20739 OR 57145 to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, 577, 20739 OR 57145 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

IV. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject, *e.g.*, a human, at risk of (or susceptible to) a disease. With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of

pharmacogenomics. "Pharmacogenomics," as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers to the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype").

Thus, another aspect of the invention provides methods for tailoring an subject's prophylactic or therapeutic treatment with either the 577, 20739 OR 57145 molecules of the present invention or 577, 20739 OR 57145 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

A. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease by administering to the subject an agent which modulates 577, 20739 OR 57145 expression or 577, 20739 OR 57145 activity. Subjects at risk for a cardiovascular disease, *e.g.*, atherosclerosis and/or thrombosis, can be identified by, for example, any or a combination of the diagnostic or prognostic assays described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of aberrant 577, 20739 OR 57145 expression or activity, such that a disease is prevented or, alternatively, delayed in its progression. Depending on the type of 577, 20739 OR 57145 aberrancy, for example, a 577, 20739 OR 57145 , 577, 20739 OR 57145 agonist or 577, 20739 OR 57145 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

B. Therapeutic Methods

Described herein are methods and compositions whereby pain may be ameliorated. Certain painful disorders are brought about, at least in part, by an excessive level of a gene product, or by the presence of a gene product exhibiting an abnormal or excessive activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of pain. Techniques for the reduction of gene expression levels or the activity of a protein are discussed below.

Alternatively, certain other painful disorders are brought about, at least in part, by the absence or reduction of the level of gene expression, or a reduction in the level of a

protein's activity. As such, an increase in the level of gene expression and/or the activity of such proteins would bring about the amelioration of pain.

In some cases, the up-regulation of a gene in a disease state reflects a protective role for that gene product in responding to the disease condition. Enhancement of such a gene's expression, or the activity of the gene product, will reinforce the protective effect it exerts. Some pain states may result from an abnormally low level of activity of such a protective gene. In these cases also, an increase in the level of gene expression and/or the activity of such gene products would bring about the amelioration of pain. Techniques for increasing target gene expression levels or target gene product activity levels are discussed herein.

Accordingly, another aspect of the invention pertains to methods of modulating 577, 20739 or 57145 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a 577, 20739 or 57145 or agent that modulates one or more of the activities of 577, 20739 or 57145 protein activity associated with the cell (*e.g.*, an endothelial cell or an ovarian cell). An agent that modulates 577, 20739 or 57145 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 577, 20739 or 57145 protein (*e.g.*, a 577, 20739 or 57145 ligand or substrate), a 577, 20739 or 57145 antibody, a 577, 20739 or 57145 agonist or antagonist, a peptidomimetic of a 577, 20739 or 57145 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more 577, 20739 or 57145 activities. Examples of such stimulatory agents include active 577, 20739 or 57145 protein and a nucleic acid molecule encoding 577, 20739 or 57145 that has been introduced into the cell. In another embodiment, the agent inhibits one or more 577, 20739 or 57145 activities. Examples of such inhibitory agents include antisense 577, 20739 or 57145 nucleic acid molecules, anti-577, 20739 or 57145 antibodies, and 577, 20739 or 57145 inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 577, 20739 or 57145 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) 577, 20739 or 57145 expression or activity. In another embodiment, the method involves administering a

577, 20739 or 57145 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 577, 20739 or 57145 expression or activity.

Stimulation of 577, 20739 or 57145 activity is desirable in situations in which 577, 20739 or 57145 is abnormally downregulated and/or in which increased 577, 20739 or 57145 activity is likely to have a beneficial effect. Likewise, inhibition of 577, 20739 or 57145 activity is desirable in situations in which 577, 20739 or 57145 is abnormally upregulated and/or in which decreased 577, 20739 or 57145 activity is likely to have a beneficial effect.

(i) Methods for Inhibiting Target Gene Expression, Synthesis, or Activity

As discussed above, genes involved in cardiovascular disorders may cause such disorders via an increased level of gene activity. In some cases, such up-regulation may have a causative or exacerbating effect on the disease state. A variety of techniques may be used to inhibit the expression, synthesis, or activity of such genes and/or proteins.

For example, compounds such as those identified through assays described above, which exhibit inhibitory activity, may be used in accordance with the invention to ameliorate pain. Such molecules may include, but are not limited to, small organic molecules, peptides, antibodies, and the like.

For example, compounds can be administered that compete with endogenous ligand for the 577, 20739 or 57145 protein. The resulting reduction in the amount of ligand-bound 577, 20739 or 57145 protein will modulate endothelial cell physiology. Compounds that can be particularly useful for this purpose include, for example, soluble proteins or peptides, such as peptides comprising one or more of the extracellular domains, or portions and/or analogs thereof, of the 577, 20739 or 57145 protein, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins. (For a discussion of the production of Ig-tailed fusion proteins, see, for example, U.S. Pat. No. 5,116,964). Alternatively, compounds, such as ligand analogs or antibodies, that bind to the 577, 20739 or 57145 receptor site, but do not activate the protein, (*e.g.*, receptor-ligand antagonists) can be effective in inhibiting 577, 20739 or 57145 protein activity.

Further, antisense and ribozyme molecules which inhibit expression of the 577, 20739 or 57145 gene may also be used in accordance with the invention to inhibit aberrant 577, 20739 or 57145 gene activity. Still further, triple helix molecules may be utilized in inhibiting aberrant 577, 20739 or 57145 gene activity.

The antisense nucleic acid molecules used in the methods of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 577, 20739 or 57145 protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, an antisense nucleic acid molecule used in the methods of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid used in the methods of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave 577, 20739 or 57145 mRNA transcripts to thereby inhibit translation of 577, 20739 or 57145 mRNA. A ribozyme having specificity for a 577, 20739 or 57145-encoding nucleic acid can be designed based upon the nucleotide sequence of a 577, 20739 or 57145 cDNA disclosed herein (*i.e.*, SEQ ID NO:1 or 3). For example, a derivative of a

Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 577, 20739 or 57145-encoding mRNA (see, for example, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742). Alternatively, 577, 20739 or 57145 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (see, for example, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418).

577, 20739 or 57145 gene expression can also be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 577, 20739 or 57145 (*e.g.*, the 577, 20739 or 57145 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 577, 20739 or 57145 gene in target cells (see, for example, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15).

Antibodies that are both specific for the 577, 20739 or 57145 protein and interfere with its activity may also be used to modulate or inhibit 577, 20739 or 57145 protein function. Such antibodies may be generated using standard techniques described herein, against the 577, 20739 or 57145 protein itself or against peptides corresponding to portions of the protein. Such antibodies include but are not limited to polyclonal, monoclonal, Fab fragments, single chain antibodies, or chimeric antibodies.

In instances where the target gene protein is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin liposomes may be used to deliver the antibody or a fragment of the Fab region which binds to the target epitope into cells. Where fragments of the antibody are used, the smallest inhibitory fragment which binds to the target protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the antibody that binds to the target gene protein may be used. Such peptides may be synthesized chemically or produced via recombinant DNA technology using methods well known in the art (described in, for example, Creighton (1983), *supra*; and Sambrook *et al.* (1989) *supra*). Single chain neutralizing antibodies which bind to intracellular target gene epitopes may also be administered. Such single chain antibodies may be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as those described in Marasco *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

In some instances, the target gene protein is extracellular, or is a transmembrane protein, such as the 577, 20739 or 57145 protein. Antibodies that are specific for one or more extracellular domains of the 577, 20739 or 57145 protein, for example, and that interfere with its activity, are particularly useful in treating pain or a painful disorder. Such antibodies are especially efficient because they can access the target domains directly from the bloodstream. Any of the administration techniques described below which are appropriate for peptide administration may be utilized to effectively administer inhibitory target gene antibodies to their site of action.

(ii) Methods for Restoring or Enhancing Target Gene Activity

Genes that cause pain may be underexpressed within cardiovascular disease situations. Alternatively, the activity of the protein products of such genes may be decreased, leading to the development of pain. Such down-regulation of gene expression or decrease of protein activity might have a causative or exacerbating effect on the disease state.

In some cases, genes that are up-regulated in the disease state might be exerting a protective effect. A variety of techniques may be used to increase the expression, synthesis, or activity of genes and/or proteins that exert a protective effect in response to pain conditions.

Described in this section are methods whereby the level 577, 20739 or 57145 activity may be increased to levels wherein pain are ameliorated. The level of 577, 20739 or 57145 activity may be increased, for example, by either increasing the level of 577, 20739 or 57145 gene expression or by increasing the level of active 577, 20739 or 57145 protein which is present.

For example, a 577, 20739 or 57145 protein, at a level sufficient to ameliorate pain may be administered to a patient exhibiting such symptoms. Any of the techniques discussed below may be used for such administration. One of skill in the art will readily know how to determine the concentration of effective, non-toxic doses of the 577, 20739 or 57145 protein, utilizing techniques such as those described below.

Additionally, RNA sequences encoding a 577, 20739 or 57145 protein may be directly administered to a patient exhibiting pain, at a concentration sufficient to produce a level of 577, 20739 or 57145 protein such that pain are ameliorated. Any of the techniques discussed below, which achieve intracellular administration of compounds, such as, for example, liposome administration, may be used for the administration of such RNA

molecules. The RNA molecules may be produced, for example, by recombinant techniques such as those described herein.

Further, subjects may be treated by gene replacement therapy. One or more copies of a 577, 20739 or 57145 gene, or a portion thereof, that directs the production of a normal 577, 20739 or 57145 protein with 577, 20739 or 57145 function, may be inserted into cells using vectors which include, but are not limited to adenovirus, adeno-associated virus, and retrovirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes. Additionally, techniques such as those described above may be used for the introduction of 577, 20739 or 57145 gene sequences into human cells.

Cells, preferably, autologous cells, containing 577, 20739 or 57145 expressing gene sequences may then be introduced or reintroduced into the subject at positions which allow for the amelioration of pain. Such cell replacement techniques may be preferred, for example, when the gene product is a secreted, extracellular gene product.

C. Pharmaceutical Compositions

Another aspect of the invention pertains to methods for treating a subject suffering from a disease. These methods involve administering to a subject an agent which modulates 577, 20739 OR 57145 expression or activity (*e.g.*, an agent identified by a screening assay described herein), or a combination of such agents. In another embodiment, the method involves administering to a subject a 577, 20739 OR 57145 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 577, 20739 OR 57145 expression or activity.

Stimulation of 577, 20739 OR 57145 activity is desirable in situations in which 577, 20739 OR 57145 is abnormally downregulated and/or in which increased 577, 20739 OR 57145 activity is likely to have a beneficial effect. Likewise, inhibition of 577, 20739 OR 57145 activity is desirable in situations in which 577, 20739 OR 57145 is abnormally upregulated and/or in which decreased 577, 20739 OR 57145 activity is likely to have a beneficial effect.

The agents which modulate 577, 20739 OR 57145 activity can be administered to a subject using pharmaceutical compositions suitable for such administration. Such compositions typically comprise the agent (*e.g.*, nucleic acid molecule, protein, or antibody) and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption

delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition used in the therapeutic methods of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol,

sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the agent that modulates 577, 20739 OR 57145 activity (*e.g.*, a fragment of a 577, 20739 OR 57145 protein or an anti-577, 20739 OR 57145 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of

nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The agents that modulate 577, 20739 OR 57145 activity can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the agents that modulate 577, 20739 OR 57145 activity are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the agent that modulates 577, 20739 OR 57145 activity and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an agent for the treatment of subjects.

Toxicity and therapeutic efficacy of such agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. Agents which exhibit large therapeutic indices are preferred. While agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such 577, 20739 OR 57145 modulating agents lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the therapeutic methods of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e*,. including heteroorganic and organometallic compounds)

having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram). It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone,

glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules used in the methods of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see, *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

D. Pharmacogenomics

In conjunction with the therapeutic methods of the invention, pharmacogenomics (*i.e.*, the study of the relationship between a subject's genotype and that subject's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer an agent which modulates 577, 20739 OR 57145 activity, as well as tailoring the dosage and/or therapeutic regimen of treatment with an agent which modulates 577, 20739 OR 57145 activity.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp.Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate aminopeptidase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants). Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach" can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug target is known (*e.g.*, a 577, 20739 OR 57145 protein used in the methods of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and the cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently

experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling" can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a 577, 20739 OR 57145 molecule or 577, 20739 OR 57145 modulator used in the methods of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of a subject. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and, thus, enhance therapeutic or prophylactic efficiency when treating a subject suffering from a cardiovascular disease, *e.g.*, atherosclerosis, with an agent which modulates 577, 20739 OR 57145 activity.

V. Recombinant Expression Vectors and Host Cells Used in the Methods of the Invention

The methods of the invention (*e.g.*, the screening assays described herein) include the use of vectors, preferably expression vectors, containing a nucleic acid encoding a 577, 20739 OR 57145 protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as

"expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors to be used in the methods of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel (1990) *Methods Enzymol.* 185:3-7. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, 577, 20739 OR 57145 proteins, mutant forms of 577, 20739 OR 57145 proteins, fusion proteins, and the like).

The recombinant expression vectors to be used in the methods of the invention can be designed for expression of 577, 20739 OR 57145 proteins in prokaryotic or eukaryotic cells. For example, 577, 20739 OR 57145 proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel (1990) *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in 577, 20739 OR 57145 activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to generate antibodies specific for 577, 20739 OR 57145 proteins. In a preferred embodiment, a 577, 20739 OR 57145 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six weeks).

In another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid).

The methods of the invention may further use a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to 577, 20739 OR 57145 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes, see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to the use of host cells into which a 577, 20739 OR 57145 nucleic acid molecule of the invention is introduced, *e.g.*, a 577, 20739 OR 57145 nucleic acid molecule within a recombinant expression vector or a 577, 20739 OR 57145 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 577, 20739 OR 57145 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

A host cell used in the methods of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a 577, 20739 OR 57145 protein. Accordingly, the invention further provides methods for producing a 577, 20739 OR 57145 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a 577, 20739 OR 57145 protein has been introduced) in a suitable medium such that a 577, 20739 OR 57145 protein is produced. In another embodiment, the method further comprises isolating a 577, 20739 OR 57145 protein from the medium or the host cell.

VI. Isolated Nucleic Acid Molecules Used in the Methods of the Invention

The methods of the invention include the use of isolated nucleic acid molecules that encode 577, 20739 OR 57145 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify 577, 20739 OR 57145 -encoding nucleic acid molecules (e.g., 577, 20739 OR 57145 mRNA) and fragments for use as PCR primers for the amplification or mutation of 577, 20739 OR 57145 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

A nucleic acid molecule used in the methods of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, 4, or 7 , or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of

SEQ ID NO:1, 4 or 7, as a hybridization probe, 577, 20739 OR 57145 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, 4 or 7 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, 4 or 7.

A nucleic acid used in the methods of the invention can be amplified using cDNA, mRNA or, alternatively, genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. Furthermore, oligonucleotides corresponding to 577, 20739 OR 57145 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In a preferred embodiment, the isolated nucleic acid molecules used in the methods of the invention comprise the nucleotide sequence shown in SEQ ID NO:1, 4 or 7, a complement of the nucleotide sequence shown in SEQ ID NO:1, 4 or 7, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, 4 or 7, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 4 or 7 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, 4 or 7 thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule used in the methods of the present invention comprises a nucleotide sequence which is at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1, 4 OR 7, or a portion of any of this nucleotide sequence.

Moreover, the nucleic acid molecules used in the methods of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, 4 OR 7, , for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a 577, 20739 OR 57145 protein, *e.g.*, a biologically active portion of a 577, 20739 OR 57145 protein. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a

sense sequence of SEQ ID NO:1, 4 OR 7, of an anti-sense sequence of SEQ ID NO:1, 4 OR 7, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 4 OR 7, . In one embodiment, a nucleic acid molecule used in the methods of the present invention comprises a nucleotide sequence which is greater than 100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, 4 OR 7, .

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70° C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is

determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(°C) = 2(\#\text{ of A + T bases}) + 4(\#\text{ of G + C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(°C) = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - (600/N)$, where N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the hybridization buffer ($[Na^+]$ for 1xSSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4, 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH_2PO_4, 1% SDS at 65°C, see *e.g.*, Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or alternatively 0.2X SSC, 1% SDS).

In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a 577, 20739 OR 57145 protein, such as by measuring a level of a 577, 20739 OR 57145 -encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting 577, 20739 OR 57145 mRNA levels or determining whether a genomic 577, 20739 OR 57145 gene has been mutated or deleted.

The methods of the invention further encompass the use of nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 4 OR 7, due to degeneracy of the genetic code and thus encode the same 577, 20739 OR 57145 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, 4 OR 7, . In another embodiment, an isolated nucleic acid molecule included in the methods of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:3, 6, 9 .

The methods of the invention further include the use of allelic variants of human 577, 20739 OR 57145 , *e.g.*, functional and non-functional allelic variants. Functional allelic variants are naturally occurring amino acid sequence variants of the human 577, 20739 OR 57145 protein that maintain a 577, 20739 OR 57145 activity. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of

SEQ ID NO:3, 6, 9 , or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human 577, 20739 OR 57145 protein that do not have a 577, 20739 OR 57145 activity. Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:3, 6, 9 , or a substitution, insertion or deletion in critical residues or critical regions of the protein.

The methods of the present invention may further use non-human orthologues of the human 577, 20739 OR 57145 protein. Orthologues of the human 577, 20739 OR 57145 protein are proteins that are isolated from non-human organisms and possess the same 577, 20739 OR 57145 activity.

The methods of the present invention further include the use of nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1, 4 OR 7, or a portion thereof, in which a mutation has been introduced. The mutation may lead to amino acid substitutions at "non-essential" amino acid residues or at "essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 577, 20739 OR 57145 (*e.g.*, the sequence of SEQ ID NO:3, 6, 9) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the 577, 20739 OR 57145 proteins of the present invention are not likely to be amenable to alteration.

Mutations can be introduced into SEQ ID NO:1, 4 OR 7, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted

nonessential amino acid residue in a 577, 20739 OR 57145 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 577, 20739 OR 57145 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 577, 20739 OR 57145 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, 4 OR 7, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using the assay described herein.

Another aspect of the invention pertains to the use of isolated nucleic acid molecules which are antisense to the nucleotide sequence of SEQ ID NO:1, 4 OR 7, . An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire 577, 20739 OR 57145 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a 577, 20739 OR 57145 . The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 577, 20739 OR 57145 . The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding 577, 20739 OR 57145 disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of 577, 20739 OR 57145 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 577, 20739 OR 57145 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 577, 20739 OR 57145 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an

antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest). Antisense nucleic acid molecules used in the methods of the invention are further described above, in section IV.

In yet another embodiment, the 577, 20739 OR 57145 nucleic acid molecules used in the methods of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide

synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* (1996) *Proc. Natl. Acad. Sci.* 93:14670-675.

PNAs of 577, 20739 OR 57145 nucleic acid molecules can be used in the therapeutic and diagnostic applications described herein. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 577, 20739 OR 57145 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. *et al.* (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* (1996) *supra*).

In another embodiment, PNAs of 577, 20739 OR 57145 can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of 577, 20739 OR 57145 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNAse H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. *et al.* (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. *et al.* (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide used in the methods of the invention may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*

(1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

VII. Isolated 577, 20739 OR 57145 Proteins and Anti-577, 20739 OR 57145 Antibodies Used in the Methods of the Invention

The methods of the invention include the use of isolated 577, 20739 OR 57145 proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-577, 20739 OR 57145 antibodies. In one embodiment, native 577, 20739 OR 57145 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, 577, 20739 OR 57145 proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a 577, 20739 OR 57145 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

As used herein, a "biologically active portion" of a 577, 20739 OR 57145 protein includes a fragment of a 577, 20739 OR 57145 protein having a 577, 20739 OR 57145 activity. Biologically active portions of a 577, 20739 OR 57145 protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the 577, 20739 OR 57145 protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:3, 6, 9 , which include fewer amino acids than the full length 577, 20739 OR 57145 proteins, and exhibit at least one activity of a 577, 20739 OR 57145 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 577, 20739 OR 57145 protein (*e.g.*, the N-terminal region of the 577, 20739 OR 57145 protein that is believed to be involved in the regulation of apoptotic activity). A biologically active portion of a 577, 20739 OR 57145 protein can be a polypeptide which is, for example, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300 or more amino acids in length. Biologically active portions of a 577, 20739 OR 57145 protein can be used as targets for developing agents which modulate a 577, 20739 OR 57145 activity.

In a preferred embodiment, the 577, 20739 OR 57145 protein used in the methods of the invention has an amino acid sequence shown in SEQ ID NO:3, 6, 9 . In other embodiments, the 577, 20739 OR 57145 protein is substantially identical to SEQ ID NO:3, 6, 9 , and retains the functional activity of the protein of SEQ ID NO:3, 6, 9 , yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection V above. Accordingly, in another embodiment, the 577, 20739 OR 57145 protein used in the methods of the invention is a protein which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:3, 6, 9 .

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the 577, 20739 OR 57145 amino acid sequence of SEQ ID NO:3, 6, 9 having 500 amino acid residues, at least 75, preferably at least 150, more preferably at least 225, even more preferably at least 300, and even more preferably at least 400 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blosum 62 matrix or a PAM250 matrix, and a gap

weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The methods of the invention may also use 577, 20739 OR 57145 chimeric or fusion proteins. As used herein, a 577, 20739 OR 57145 "chimeric protein" or "fusion protein" comprises a 577, 20739 OR 57145 polypeptide operatively linked to a non-577, 20739 OR 57145 polypeptide. An "577, 20739 OR 57145 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a 577, 20739 OR 57145 molecule, whereas a "non-577, 20739 OR 57145 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 577, 20739 OR 57145 protein, *e.g.*, a protein which is different from the 577, 20739 OR 57145 protein and which is derived from the same or a different organism. Within a 577, 20739 OR 57145 fusion protein the 577, 20739 OR 57145 polypeptide can correspond to all or a portion of a 577, 20739 OR 57145 protein. In a preferred embodiment, a 577, 20739 OR 57145 fusion protein comprises at least one biologically active portion of a 577, 20739 OR 57145 protein. In another preferred embodiment, a 577, 20739 OR 57145 fusion protein comprises at least two biologically active portions of a 577, 20739 OR 57145 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the 577, 20739 OR 57145 polypeptide and the non-577, 20739 OR 57145 polypeptide are fused in-frame to each other. The non-577, 20739 OR 57145 polypeptide can be fused to the N-terminus or C-terminus of the 577, 20739 OR 57145 polypeptide.

For example, in one embodiment, the fusion protein is a GST-577, 20739 OR 57145 fusion protein in which the 577, 20739 OR 57145 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 577, 20739 OR 57145 .

In another embodiment, this fusion protein is a 577, 20739 OR 57145 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*,

mammalian host cells), expression and/or secretion of 577, 20739 OR 57145 can be increased through use of a heterologous signal sequence.

The 577, 20739 OR 57145 fusion proteins used in the methods of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 577, 20739 OR 57145 fusion proteins can be used to affect the bioavailability of a 577, 20739 OR 57145 substrate. Use of 577, 20739 OR 57145 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 577, 20739 OR 57145 protein; (ii) mis-regulation of the 577, 20739 OR 57145 gene; and (iii) aberrant post-translational modification of a 577, 20739 OR 57145 protein.

Moreover, the 577, 20739 OR 57145 -fusion proteins used in the methods of the invention can be used as immunogens to produce anti-577, 20739 OR 57145 antibodies in a subject, to purify 577, 20739 OR 57145 ligands and in screening assays to identify molecules which inhibit the interaction of 577, 20739 OR 57145 with a 577, 20739 OR 57145 substrate.

Preferably, a 577, 20739 OR 57145 chimeric or fusion protein used in the methods of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A 577, 20739 OR 57145 -encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 577, 20739 OR 57145 protein.

The present invention also pertains to the use of variants of the 577, 20739 OR 57145 proteins which function as either 577, 20739 OR 57145 agonists (mimetics) or as 577, 20739 OR 57145 antagonists. Variants of the 577, 20739 OR 57145 proteins can be

generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a 577, 20739 OR 57145 protein. An agonist of the 577, 20739 OR 57145 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a 577, 20739 OR 57145 protein. An antagonist of a 577, 20739 OR 57145 protein can inhibit one or more of the activities of the naturally occurring form of the 577, 20739 OR 57145 protein by, for example, competitively modulating a 577, 20739 OR 57145 -mediated activity of a 577, 20739 OR 57145 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 577, 20739 OR 57145 protein.

In one embodiment, variants of a 577, 20739 OR 57145 protein which function as either 577, 20739 OR 57145 agonists (mimetics) or as 577, 20739 OR 57145 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a 577, 20739 OR 57145 protein for 577, 20739 OR 57145 protein agonist or antagonist activity. In one embodiment, a variegated library of 577, 20739 OR 57145 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of 577, 20739 OR 57145 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential 577, 20739 OR 57145 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of 577, 20739 OR 57145 sequences therein. There are a variety of methods which can be used to produce libraries of potential 577, 20739 OR 57145 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential 577, 20739 OR 57145 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of a 577, 20739 OR 57145 protein coding sequence can be used to generate a variegated population of 577, 20739 OR 57145 fragments for screening and subsequent selection of variants of a 577, 20739 OR 57145

protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a 577, 20739 OR 57145 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the 577, 20739 OR 57145 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of 577, 20739 OR 57145 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 577, 20739 OR 57145 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

The methods of the present invention further include the use of anti-577, 20739 OR 57145 antibodies. An isolated 577, 20739 OR 57145 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind 577, 20739 OR 57145 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length 577, 20739 OR 57145 protein can be used or, alternatively, antigenic peptide fragments of 577, 20739 OR 57145 can be used as immunogens. The antigenic peptide of 577, 20739 OR 57145 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:3, 6, 9 and encompasses an epitope of 577, 20739 OR 57145 such that an antibody raised against the peptide forms a specific immune complex with the 577, 20739 OR 57145 protein. Preferably, the antigenic peptide comprises at least 10 amino

acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of 577, 20739 OR 57145 that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

A 577, 20739 OR 57145 immunogen is typically used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse, or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed 577, 20739 OR 57145 protein or a chemically synthesized 577, 20739 OR 57145 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic 577, 20739 OR 57145 preparation induces a polyclonal anti-577, 20739 OR 57145 antibody response.

The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a 577, 20739 OR 57145 . Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind 577, 20739 OR 57145 molecules. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of 577, 20739 OR 57145 . A monoclonal antibody composition thus typically displays a single binding affinity for a particular 577, 20739 OR 57145 protein with which it immunoreacts.

Polyclonal anti-577, 20739 OR 57145 antibodies can be prepared as described above by immunizing a suitable subject with a 577, 20739 OR 57145 immunogen. The anti-577, 20739 OR 57145 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized 577, 20739 OR 57145 . If desired, the antibody molecules directed against 577, 20739 OR 57145 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-577, 20739 OR 57145 antibody titers are highest, antibody-producing cells can be obtained

from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally Kenneth, R. H. in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); Lerner, E. A. (1981) *Yale J. Biol. Med.* 54:387-402; Gefter, M. L. *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a 577, 20739 OR 57145 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds 577, 20739 OR 57145 .

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-577, 20739 OR 57145 monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* (1977) *supra*; Lerner (1981) *supra*; and Kenneth (1980) *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal

antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind 577, 20739 OR 57145 , *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-577, 20739 OR 57145 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with 577, 20739 OR 57145 to thereby isolate immunoglobulin library members that bind 577, 20739 OR 57145 . Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* (1990) *Nature* 348:552-554.

Additionally, recombinant anti-577, 20739 OR 57145 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the methods of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-

1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-577, 20739 OR 57145 antibody can be used to detect 577, 20739 OR 57145 protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the 577, 20739 OR 57145 protein. Anti-577, 20739 OR 57145 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figure and the Sequence Listing is incorporated herein by reference.

EXAMPLES

EXAMPLE 1: TISSUE DISTRIBUTION OF USING TAQMAN™ ANALYSIS

This example describes the TaqMan™ procedure. The Taqman™ procedure is a quantitative, reverse transcription PCR-based approach for detecting mRNA. The RT-

PCR reaction exploits the 5' nuclease activity of AmpliTaq Gold™ DNA Polymerase to cleave a TaqMan™ probe during PCR. Briefly, cDNA was generated from the samples of interest, *e.g.*, heart, kidney, liver, skeletal muscle, and various vessels, and used as the starting material for PCR amplification. In addition to the 5' and 3' gene-specific primers, a gene-specific oligonucleotide probe (complementary to the region being amplified) was included in the reaction (*i.e.*, the Taqman™ probe). The TaqMan™ probe includes the oligonucleotide with a fluorescent reporter dye covalently linked to the 5' end of the probe (such as FAM (6-carboxyfluorescein), TET (6-carboxy-4,7,2',7'-tetrachlorofluorescein), JOE (6-carboxy-4,5-dichloro-2,7-dimethoxyfluorescein), or VIC) and a quencher dye (TAMRA (6-carboxy-N,N,N',N'-tetramethylrhodamine) at the 3' end of the probe.

During the PCR reaction, cleavage of the probe separates the reporter dye and the quencher dye, resulting in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'-3' nucleolytic activity of the AmpliTaq™ Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. RNA was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA. cDNA was synthesized using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no detectable PCR amplification of the control gene confirms efficient removal of genomic DNA contamination.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1. A method for identifying a compound capable of treating a pain disorder, comprising assaying the ability of the compound to modulate 577, 20739 OR 57145 nucleic acid expression or 577, 20739 OR 57145 polypeptide activity, thereby identifying a compound capable of treating a pain disorder.

2. A method for identifying a compound capable of modulating a pain signaling mechanism comprising:

a) contacting a cell which expresses 577, 20739 OR 57145 with a test compound; and

b) assaying the ability of the test compound to modulate the expression of a 577, 20739 OR 57145 nucleic acid or the activity of a 577, 20739 OR 57145 polypeptide, thereby identifying a compound capable of modulating pain signalling.

3. A method for modulating a pain signaling mechanism in a cell comprising contacting a cell with a 577, 20739 OR 57145 modulator, thereby modulating a pain signaling mechanism in the cell.

4. The method of claim 2, wherein the cell is a brain cell, neuron, or cell derived from spinal cord or dorsal root ganglion.

5. The method of claim 3, wherein the 577, 20739 OR 57145 modulator is a small organic molecule, peptide, antibody or antisense nucleic acid molecule.

6. The method of claim 3, wherein the 577, 20739 OR 57145 modulator is capable of modulating 577, 20739 OR 57145 polypeptide activity.

7. The method of claim 6, wherein the 577, 20739 OR 57145 modulator is a small organic molecule, peptide, antibody or antisense nucleic acid molecule.

8. The method of claim 6, wherein the 577, 20739 OR 57145 modulator is capable of modulating 577, 20739 OR 57145 nucleic acid expression.

9. A method for treating a subject having a pain disorder characterized by aberrant 577, 20739 OR 57145 polypeptide activity or aberrant 577, 20739 OR 57145

nucleic acid expression comprising administering to the subject a 577, 20739 OR 57145 modulator, thereby treating said subject having a pain disorder.

10. The method of claim 9, wherein said pain disorder includes inflammatory pain, chronic pain, neuropathic pain, causalgia, fibromyalgia, cancer pain, migraine/headache pain and tissue pain.

11. The method of claim 9, wherein said 577, 20739 OR 57145 modulator is administered in a pharmaceutically acceptable formulation.

12. The method of claim 9, wherein the 577, 20739 OR 57145 modulator is a small organic molecule, peptide, antibody or antisense nucleic acid molecule.

13. The method of claim 9, wherein the 577, 20739 OR 57145 modulator is capable of modulating 577, 20739 OR 57145 polypeptide activity.

SEQUENCE LISTING

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        595                 600                 605
Arg Lys Tyr Gly Gly Ile Thr Ser Phe Glu Asn Thr Ala Ile Glu Val
    610                 615                 620
Asp Arg Glu Ile Ala Glu Glu Glu Glu Ser Met Met
625                 630                 635
```

<210> 4
<211> 1635
<212> DNA
<213> Homo Sapien

<400> 4

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aacaaccggg attcttcagc actcaaccac agctccaaac cacttcccat ggcccctgaa 120
gagaagaata agaaagccag gcttcgctct atcttcccag gaggagggga taaaaccaat 180
aagaagaagg agaaagagcg cccagagatc tctcttcctt cagactttga gcatacgatt 240
catgtggggt ttgatgcagt caccggggaa ttcactggaa ttccagagca atgggcacga 300
ttactccaaa cttccaacat aacaaaattg gaacagaaga agaacccaca agctgttcta 360
gatgttctca aattctatga ttccaaagaa acagtcaaca accagaaata catgagcttt 420
acatcaggag ataaaagtgc acatggatac atagcagccc atccttcgag tacaaaaaca 480
gcatctgagc ctccattggc ccctcctgtg tctgaagaag aagatgaaga ggaagaagaa 540
gaagaagatg aaaatgagcc accaccagtt atcgcaccaa gaccagagca tacaaaatca 600
atctatactc gttctgtggt tgaatccatt gcttcaccag cagtaccaaa taaagaggtc 660
acaccaccct ctgctgaaaa tgccaattcc agtactttgt acaggaacac agatcggcaa 720
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gttggggacc caaagaaaaa atacacaaga tttgaaaaaa ttggtcaagg ggcatcaggt 840
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gttaaattga ctgactttgg gttctgtgcc cagatcactc ctgagcaaag taaacgaagc 1260
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ccttacctta atgaaaatcc actcagggca ttgtatctga tagccactaa tggaactcca 1440
gagctccaga atcctgagag actgtcagct gtattccgtg acttttaaa tcgctgtctt 1500
gagatggatg tggataggcg aggatctgcc aaggagcttt tgcagcatcc atttttaaaa 1560
ttagccaagc ctctctccag cctgactcct ctgattatcg ctgcaaagga agcaattaag 1620
aacagcagcc gctaa                                                  1635
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<210> 5
<211> 1635
<212> DNA
<213> Homo Sapien

<220>
<221> mRNA
<222> (0)...(0)

<221> CDS
<222> (1)...(1635)

<400> 5

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atg tct gac ggt ctg gat aat gaa gag aaa ccc ccg gct cct cca ctg   48
Met Ser Asp Gly Leu Asp Asn Glu Glu Lys Pro Pro Ala Pro Pro Leu
 1               5                  10                  15
```

```
agg atg aat agt aac aac cgg gat tct tca gca ctc aac cac agc tcc   96
Arg Met Asn Ser Asn Asn Arg Asp Ser Ser Ala Leu Asn His Ser Ser
             20                  25                  30

aaa cca ctt ccc atg gcc cct gaa gag aag aat aag aaa gcc agg ctt   144
Lys Pro Leu Pro Met Ala Pro Glu Glu Lys Asn Lys Lys Ala Arg Leu
         35                  40                  45

cgc tct atc ttc cca gga gga ggg gat aaa acc aat aag aag aag gag   192
Arg Ser Ile Phe Pro Gly Gly Gly Asp Lys Thr Asn Lys Lys Lys Glu
     50                  55                  60

aaa gag cgc cca gag atc tct ctt cct tca gac ttt gag cat acg att   240
Lys Glu Arg Pro Glu Ile Ser Leu Pro Ser Asp Phe Glu His Thr Ile
 65                  70                  75                  80

cat gtg ggg ttt gat gca gtc acc ggg gaa ttc act gga att cca gag   288
His Val Gly Phe Asp Ala Val Thr Gly Glu Phe Thr Gly Ile Pro Glu
                 85                  90                  95

caa tgg gca cga tta ctc caa act tcc aac ata aca aaa ttg gaa cag   336
Gln Trp Ala Arg Leu Leu Gln Thr Ser Asn Ile Thr Lys Leu Glu Gln
            100                 105                 110

aag aag aac cca caa gct gtt cta gat gtt ctc aaa ttc tat gat tcc   384
Lys Lys Asn Pro Gln Ala Val Leu Asp Val Leu Lys Phe Tyr Asp Ser
        115                 120                 125

aaa gaa aca gtc aac aac cag aaa tac atg agc ttt aca tca gga gat   432
Lys Glu Thr Val Asn Asn Gln Lys Tyr Met Ser Phe Thr Ser Gly Asp
    130                 135                 140

aaa agt gca cat gga tac ata gca gcc cat cct tcg agt aca aaa aca   480
Lys Ser Ala His Gly Tyr Ile Ala Ala His Pro Ser Ser Thr Lys Thr
145                 150                 155                 160

gca tct gag cct cca ttg gcc cct cct gtg tct gaa gaa gaa gat gaa   528
Ala Ser Glu Pro Pro Leu Ala Pro Pro Val Ser Glu Glu Glu Asp Glu
                165                 170                 175

gag gaa gaa gaa gaa gaa gat gaa aat gag cca cca cca gtt atc gca   576
Glu Glu Glu Glu Glu Glu Asp Glu Asn Glu Pro Pro Pro Val Ile Ala
            180                 185                 190

cca aga cca gag cat aca aaa tca atc tat act cgt tct gtg gtt gaa   624
Pro Arg Pro Glu His Thr Lys Ser Ile Tyr Thr Arg Ser Val Val Glu
        195                 200                 205

tcc att gct tca cca gca gta cca aat aaa gag gtc aca cca ccc tct   672
Ser Ile Ala Ser Pro Ala Val Pro Asn Lys Glu Val Thr Pro Pro Ser
    210                 215                 220

gct gaa aat gcc aat tcc agt act ttg tac agg aac aca gat cgg caa   720
Ala Glu Asn Ala Asn Ser Ser Thr Leu Tyr Arg Asn Thr Asp Arg Gln
225                 230                 235                 240

aga aaa aaa tcc aag atg aca gat gag gag atc tta gag aag cta aga   768
Arg Lys Lys Ser Lys Met Thr Asp Glu Glu Ile Leu Glu Lys Leu Arg
                245                 250                 255
```

```
agc att gtg agt gtt ggg gac cca aag aaa aaa tac aca aga ttt gaa   816
Ser Ile Val Ser Val Gly Asp Pro Lys Lys Lys Tyr Thr Arg Phe Glu
            260                 265                 270

aaa att ggt caa ggg gca tca ggt act gtt tat aca gca cta gac att   864
Lys Ile Gly Gln Gly Ala Ser Gly Thr Val Tyr Thr Ala Leu Asp Ile
        275                 280                 285

gca aca gga caa gag gtg gcc ata aag cag atg aac ctt caa cag caa   912
Ala Thr Gly Gln Glu Val Ala Ile Lys Gln Met Asn Leu Gln Gln Gln
    290                 295                 300

ccc aag aag gaa tta att att aat gaa att ctg gtc atg agg gaa aat   960
Pro Lys Lys Glu Leu Ile Ile Asn Glu Ile Leu Val Met Arg Glu Asn
305                 310                 315                 320

aag aac cct aat att gtt aat tat tta gat agc tac ttg gtg ggt gat   1008
Lys Asn Pro Asn Ile Val Asn Tyr Leu Asp Ser Tyr Leu Val Gly Asp
                325                 330                 335

gaa cta tgg gta gtc atg gaa tac ttg gct ggt ggc tct ctg act gat   1056
Glu Leu Trp Val Val Met Glu Tyr Leu Ala Gly Gly Ser Leu Thr Asp
            340                 345                 350

gtg gtc aca gag acc tgt atg gat gaa gga cag ata gca gct gtc tgc   1104
Val Val Thr Glu Thr Cys Met Asp Glu Gly Gln Ile Ala Ala Val Cys
        355                 360                 365

aga gag tgc ctg caa gct ttg gat ttc ctg cac tca aac cag gtg atc   1152
Arg Glu Cys Leu Gln Ala Leu Asp Phe Leu His Ser Asn Gln Val Ile
    370                 375                 380

cat aga gat ata aag agt gac aat att ctt ctc ggg atg gat ggc tct   1200
His Arg Asp Ile Lys Ser Asp Asn Ile Leu Leu Gly Met Asp Gly Ser
385                 390                 395                 400

gtt aaa ttg act gac ttt ggg ttc tgt gcc cag atc act cct gag caa   1248
Val Lys Leu Thr Asp Phe Gly Phe Cys Ala Gln Ile Thr Pro Glu Gln
                405                 410                 415

agt aaa cga agc act atg gtg gga acc cca tat tgg atg gca cct gag   1296
Ser Lys Arg Ser Thr Met Val Gly Thr Pro Tyr Trp Met Ala Pro Glu
            420                 425                 430

gtg gtg act cga aaa gct tat ggt ccg aaa gtt gat atc tgg tct ctt   1344
Val Val Thr Arg Lys Ala Tyr Gly Pro Lys Val Asp Ile Trp Ser Leu
        435                 440                 445

gga att atg gca att gaa atg gtg gaa ggt gaa ccc cct tac ctt aat   1392
Gly Ile Met Ala Ile Glu Met Val Glu Gly Glu Pro Pro Tyr Leu Asn
    450                 455                 460

gaa aat cca ctc agg gca ttg tat ctg ata gcc act aat gga act cca   1440
Glu Asn Pro Leu Arg Ala Leu Tyr Leu Ile Ala Thr Asn Gly Thr Pro
465                 470                 475                 480

gag ctc cag aat cct gag aga ctg tca gct gta ttc cgt gac ttt tta   1488
Glu Leu Gln Asn Pro Glu Arg Leu Ser Ala Val Phe Arg Asp Phe Leu
                485                 490                 495

aat cgc tgt ctt gag atg gat gtg gat agg cga gga tct gcc aag gag   1536
```

```
Asn Arg Cys Leu Glu Met Asp Val Asp Arg Arg Gly Ser Ala Lys Glu
            500                 505                 510

ctt ttg cag cat cca ttt tta aaa tta gcc aag cct ctc tcc agc ctg   1584
Leu Leu Gln His Pro Phe Leu Lys Leu Ala Lys Pro Leu Ser Ser Leu
        515                 520                 525

act cct ctg att atc gct gca aag gaa gca att aag aac agc agc cgc   1632
Thr Pro Leu Ile Ile Ala Ala Lys Glu Ala Ile Lys Asn Ser Ser Arg
    530                 535                 540

taa                                                               1635
 *
```

<210> 6
<211> 544
<212> PRT
<213> Homo Sapien

<400> 6

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Met Ser Asp Gly Leu Asp Asn Glu Glu Lys Pro Pro Ala Pro Pro Leu
 1               5                  10                  15
Arg Met Asn Ser Asn Asn Arg Asp Ser Ser Ala Leu Asn His Ser Ser
            20                  25                  30
Lys Pro Leu Pro Met Ala Pro Glu Glu Lys Asn Lys Lys Ala Arg Leu
        35                  40                  45
Arg Ser Ile Phe Pro Gly Gly Gly Asp Lys Thr Asn Lys Lys Lys Glu
    50                  55                  60
Lys Glu Arg Pro Glu Ile Ser Leu Pro Ser Asp Phe Glu His Thr Ile
65                  70                  75                  80
His Val Gly Phe Asp Ala Val Thr Gly Glu Phe Thr Gly Ile Pro Glu
                85                  90                  95
Gln Trp Ala Arg Leu Leu Gln Thr Ser Asn Ile Thr Lys Leu Glu Gln
            100                 105                 110
Lys Lys Asn Pro Gln Ala Val Leu Asp Val Leu Lys Phe Tyr Asp Ser
        115                 120                 125
Lys Glu Thr Val Asn Asn Gln Lys Tyr Met Ser Phe Thr Ser Gly Asp
    130                 135                 140
Lys Ser Ala His Gly Tyr Ile Ala Ala His Pro Ser Ser Thr Lys Thr
145                 150                 155                 160
Ala Ser Glu Pro Pro Leu Ala Pro Pro Val Ser Glu Glu Glu Asp Glu
                165                 170                 175
Glu Glu Glu Glu Glu Glu Asp Glu Asn Glu Pro Pro Pro Val Ile Ala
            180                 185                 190
Pro Arg Pro Glu His Thr Lys Ser Ile Tyr Thr Arg Ser Val Val Glu
        195                 200                 205
Ser Ile Ala Ser Pro Ala Val Pro Asn Lys Glu Val Thr Pro Pro Ser
    210                 215                 220
Ala Glu Asn Ala Asn Ser Ser Thr Leu Tyr Arg Asn Thr Asp Arg Gln
225                 230                 235                 240
Arg Lys Lys Ser Lys Met Thr Asp Glu Glu Ile Leu Glu Lys Leu Arg
                245                 250                 255
Ser Ile Val Ser Val Gly Asp Pro Lys Lys Lys Tyr Thr Arg Phe Glu
            260                 265                 270
Lys Ile Gly Gln Gly Ala Ser Gly Thr Val Tyr Thr Ala Leu Asp Ile
        275                 280                 285
Ala Thr Gly Gln Glu Val Ala Ile Lys Gln Met Asn Leu Gln Gln Gln
    290                 295                 300
Pro Lys Lys Glu Leu Ile Ile Asn Glu Ile Leu Val Met Arg Glu Asn
```

```
305                 310                 315                 320
Lys Asn Pro Asn Ile Val Asn Tyr Leu Asp Ser Tyr Leu Val Gly Asp
                325                 330                 335
Glu Leu Trp Val Val Met Glu Tyr Leu Ala Gly Gly Ser Leu Thr Asp
            340                 345                 350
Val Val Thr Glu Thr Cys Met Asp Glu Gly Gln Ile Ala Ala Val Cys
        355                 360                 365
Arg Glu Cys Leu Gln Ala Leu Asp Phe Leu His Ser Asn Gln Val Ile
    370                 375                 380
His Arg Asp Ile Lys Ser Asp Asn Ile Leu Leu Gly Met Asp Gly Ser
385                 390                 395                 400
Val Lys Leu Thr Asp Phe Gly Phe Cys Ala Gln Ile Thr Pro Glu Gln
                405                 410                 415
Ser Lys Arg Ser Thr Met Val Gly Thr Pro Tyr Trp Met Ala Pro Glu
            420                 425                 430
Val Val Thr Arg Lys Ala Tyr Gly Pro Lys Val Asp Ile Trp Ser Leu
        435                 440                 445
Gly Ile Met Ala Ile Glu Met Val Glu Gly Glu Pro Pro Tyr Leu Asn
    450                 455                 460
Glu Asn Pro Leu Arg Ala Leu Tyr Leu Ile Ala Thr Asn Gly Thr Pro
465                 470                 475                 480
Glu Leu Gln Asn Pro Glu Arg Leu Ser Ala Val Phe Arg Asp Phe Leu
                485                 490                 495
Asn Arg Cys Leu Glu Met Asp Val Asp Arg Arg Gly Ser Ala Lys Glu
            500                 505                 510
Leu Leu Gln His Pro Phe Leu Lys Leu Ala Lys Pro Leu Ser Ser Leu
        515                 520                 525
Thr Pro Leu Ile Ile Ala Ala Lys Glu Ala Ile Lys Asn Ser Ser Arg
    530                 535                 540
```

<210> 7
<211> 2520
<212> DNA
<213> Homo Sapien

<400> 7

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gctcgtcatc caccactacc atgtaagggc catgagaagg gctcatcctg gcgcagcgcg 180
gacatggagg aggacttatt ccagctaagg cagctgccgg ttgtgaaatt ccgtcgcaca 240
ggcgagagtg caaggtcaga ggacgacacg gcttcaggag agcatgaagt ccagattgaa 300
ggggtccacg tgggcctaga ggctgtggag ctggatgatg gggcagctgt gcccaaggag 360
tttgccaatc ccaccgatga tactttcatg gtggaagatg cagtggaagc cattggcttt 420
ggaaaatttc agtggaagct gtctgttctc actggcttgg cttggatggc tgatgccatg 480
gagatgatga tcctcagcat cctggcacca cagctgcatt gcgagtggag gctcccaagc 540
tggcaggtgg cattgctgac ctcggtggtc tttgtaggca tgatgtccag ctccacgctc 600
tggggaaata tctcagacca gtacggcagg aaaacagggc tgaagatcag cgtgctgtgg 660
actctgtact atggcatcct tagtgcattt gcgcccgtgt atagctggat cctggtgctc 720
cggggcctgg tgggcttcgg gatcggagga gttccccagt cggtgacgct gtatgccgag 780
ttccttccca tgaaagccag agctaaatgt attttgctga ttgaggtatt ctgggccatc 840
gggacagtgt tcgaggtcgt cctggctgtg ttcgtgatgc ccagcctggg ctggcgttgg 900
ctgctcatcc tctcagctgt cccgctcctc ctctttgccg tgctgtgttt ctggctgcct 960
gaaagtgcaa ggtatgatgt gctgtcaggg aaccaggaaa aggcaatcgc caccttaaag 1020
aggatagcaa ctgaaaacgg agctcccatg ccgctgggga aactcatcat ctccagacag 1080
gaagaccgag gcaaaatgag ggacctttte acaccccatt ttagatggac aactttgctg 1140
ctgtggttta tatggttttc caatgcattc tcttactacg ggttagttct actcaccaca 1200
gaactcttcc aggcaggaga tgtctgcggc atctccagtc ggaagaaggc tgtagaggca 1260
aaatgcagcc tggcctgcga gtacctgagt gaggaggatt acatggactt gctgtggacc 1320
accctctctg agtttccagg tgtccttgtg actctgtgga ttattgaccg cctggggcgc 1380
aagaagacca tggccctgtg ctttgtcatc ttctccttct gcagcctcct gctgtttatc 1440
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tgtgttggaa gaaatgtgct cactctgtta ctcttcattg caagagcgtt tatttctgga 1500
ggctttcaag cggcatatgt ttacacacct gaggtctacc ccacggcaac gcgggccctc 1560
ggcctgggca cctgcagcgg catggcaaga gtgggtgctc tcatcactcc gttcatcgcc 1620
caggtgatgc tggaatcctc tgtgtacctg actctggcag tttacagtgg ctgctgcctc 1680
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accaggtcga actctggctc tcaggaatag tgaccgatgg gggactgagc tggtctttga 1860
ggctgcagag cttgggggggc tggcaggccc caactggggc actgattgtc actgccgaca 1920
tcaagaactc acccaagagt atgacctgga ccaacagggt tttgtgtctt gactcagttt 1980
gctcatcttc attgaggtcc acccagggat ggggagatgt ttgctctagg gggttctctg 2040
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aggagggtct ggtaatgcca gcaaccaatc agacaccacc cagagtcacc cggccaaacc 2160
ctcagtgaac aaccaaaata tctctctgta gataccgtcc aggctcaggc ccatgtgaca 2220
cctgctgtcc acccaccgga cctgttcagt aggtttctcc cacacccaca gccccaggct 2280
ttcttctttg aaattgcagg cgatctaggt gtggtctgag cagctatttc ctggcagggg 2340
ccccccggtt tgcctcccta gagcctgacc agtggattct ctggcagatg gacatggtgc 2400
attcaaactg gagccacatg cccccaccca gcccctnttg gagttgcccg ttgttggcac 2460
caagagatcc agatgtgtcc tggggacagc tgggtcttgc accaggtgac aacctcaaaa 2520


<210> 8
<211> 2520
<212> DNA
<213> Homo Sapien

<220>
<221> mRNA
<222> (0)...(0)

<221> CDS
<222> (184)...(1830)

<400> 8
ccacgcgtcc gggctttgtc tcgtgggctg gtccccagcg gctccctccc cgaacagctg 60
ctgctccagg gaggaagcgg cgcgggtgct gtccagcttc ccggtgctga aaaccggagg 120
gctcgtcatc caccactacc atgtaagggc catgagaagg gctcatcctg gcgcagcgcg 180
gac atg gag gag gac tta ttc cag cta agg cag ctg ccg gtt gtg aaa   228
    Met Glu Glu Asp Leu Phe Gln Leu Arg Gln Leu Pro Val Val Lys
     1               5                  10                  15

ttc cgt cgc aca ggc gag agt gca agg tca gag gac gac acg gct tca   276
Phe Arg Arg Thr Gly Glu Ser Ala Arg Ser Glu Asp Asp Thr Ala Ser
                 20                  25                  30

gga gag cat gaa gtc cag att gaa ggg gtc cac gtg ggc cta gag gct   324
Gly Glu His Glu Val Gln Ile Glu Gly Val His Val Gly Leu Glu Ala
             35                  40                  45

gtg gag ctg gat gat ggg gca gct gtg ccc aag gag ttt gcc aat ccc   372
Val Glu Leu Asp Asp Gly Ala Ala Val Pro Lys Glu Phe Ala Asn Pro
         50                  55                  60

acc gat gat act ttc atg gtg gaa gat gca gtg gaa gcc att ggc ttt   420
Thr Asp Asp Thr Phe Met Val Glu Asp Ala Val Glu Ala Ile Gly Phe
     65                  70                  75

gga aaa ttt cag tgg aag ctg tct gtt ctc act ggc ttg gct tgg atg   468
Gly Lys Phe Gln Trp Lys Leu Ser Val Leu Thr Gly Leu Ala Trp Met
 80                  85                  90                  95

gct gat gcc atg gag atg atg atc ctc agc atc ctg gca cca cag ctg   516
```

```
Ala Asp Ala Met Glu Met Met Ile Leu Ser Ile Leu Ala Pro Gln Leu
                100                 105                 110

cat tgc gag tgg agg ctc cca agc tgg cag gtg gca ttg ctg acc tcg   564
His Cys Glu Trp Arg Leu Pro Ser Trp Gln Val Ala Leu Leu Thr Ser
            115                 120                 125

gtg gtc ttt gta ggc atg atg tcc agc tcc acg ctc tgg gga aat atc   612
Val Val Phe Val Gly Met Met Ser Ser Ser Thr Leu Trp Gly Asn Ile
        130                 135                 140

tca gac cag tac ggc agg aaa aca ggg ctg aag atc agc gtg ctg tgg   660
Ser Asp Gln Tyr Gly Arg Lys Thr Gly Leu Lys Ile Ser Val Leu Trp
    145                 150                 155

act ctg tac tat ggc atc ctt agt gca ttt gcg ccc gtg tat agc tgg   708
Thr Leu Tyr Tyr Gly Ile Leu Ser Ala Phe Ala Pro Val Tyr Ser Trp
160                 165                 170                 175

atc ctg gtg ctc cgg ggc ctg gtg ggc ttc ggg atc gga gga gtt ccc   756
Ile Leu Val Leu Arg Gly Leu Val Gly Phe Gly Ile Gly Gly Val Pro
                180                 185                 190

cag tcg gtg acg ctg tat gcc gag ttc ctt ccc atg aaa gcc aga gct   804
Gln Ser Val Thr Leu Tyr Ala Glu Phe Leu Pro Met Lys Ala Arg Ala
            195                 200                 205

aaa tgt att ttg ctg att gag gta ttc tgg gcc atc ggg aca gtg ttc   852
Lys Cys Ile Leu Leu Ile Glu Val Phe Trp Ala Ile Gly Thr Val Phe
        210                 215                 220

gag gtc gtc ctg gct gtg ttc gtg atg ccc agc ctg ggc tgg cgt tgg   900
Glu Val Val Leu Ala Val Phe Val Met Pro Ser Leu Gly Trp Arg Trp
    225                 230                 235

ctg ctc atc ctc tca gct gtc ccg ctc ctc ctc ttt gcc gtg ctg tgt   948
Leu Leu Ile Leu Ser Ala Val Pro Leu Leu Leu Phe Ala Val Leu Cys
240                 245                 250                 255

ttc tgg ctg cct gaa agt gca agg tat gat gtg ctg tca ggg aac cag   996
Phe Trp Leu Pro Glu Ser Ala Arg Tyr Asp Val Leu Ser Gly Asn Gln
                260                 265                 270

gaa aag gca atc gcc acc tta aag agg ata gca act gaa aac gga gct  1044
Glu Lys Ala Ile Ala Thr Leu Lys Arg Ile Ala Thr Glu Asn Gly Ala
            275                 280                 285

ccc atg ccg ctg ggg aaa ctc atc atc tcc aga cag gaa gac cga ggc  1092
Pro Met Pro Leu Gly Lys Leu Ile Ile Ser Arg Gln Glu Asp Arg Gly
        290                 295                 300

aaa atg agg gac ctt ttc aca ccc cat ttt aga tgg aca act ttg ctg  1140
Lys Met Arg Asp Leu Phe Thr Pro His Phe Arg Trp Thr Thr Leu Leu
    305                 310                 315

ctg tgg ttt ata tgg ttt tcc aat gca ttc tct tac tac ggg tta gtt  1188
Leu Trp Phe Ile Trp Phe Ser Asn Ala Phe Ser Tyr Tyr Gly Leu Val
320                 325                 330                 335

cta ctc acc aca gaa ctc ttc cag gca gga gat gtc tgc ggc atc tcc  1236
Leu Leu Thr Thr Glu Leu Phe Gln Ala Gly Asp Val Cys Gly Ile Ser
```

```
                340                 345                 350

agt cgg aag aag gct gta gag gca aaa tgc agc ctg gcc tgc gag tac   1284
Ser Arg Lys Lys Ala Val Glu Ala Lys Cys Ser Leu Ala Cys Glu Tyr
            355                 360                 365

ctg agt gag gag gat tac atg gac ttg ctg tgg acc acc ctc tct gag   1332
Leu Ser Glu Glu Asp Tyr Met Asp Leu Leu Trp Thr Thr Leu Ser Glu
        370                 375                 380

ttt cca ggt gtc ctt gtg act ctg tgg att att gac cgc ctg ggg cgc   1380
Phe Pro Gly Val Leu Val Thr Leu Trp Ile Ile Asp Arg Leu Gly Arg
    385                 390                 395

aag aag acc atg gcc ctg tgc ttt gtc atc ttc tcc ttc tgc agc ctc   1428
Lys Lys Thr Met Ala Leu Cys Phe Val Ile Phe Ser Phe Cys Ser Leu
400                 405                 410                 415

ctg ctg ttt atc tgt gtt gga aga aat gtg ctc act ctg tta ctc ttc   1476
Leu Leu Phe Ile Cys Val Gly Arg Asn Val Leu Thr Leu Leu Leu Phe
                420                 425                 430

att gca aga gcg ttt att tct gga ggc ttt caa gcg gca tat gtt tac   1524
Ile Ala Arg Ala Phe Ile Ser Gly Gly Phe Gln Ala Ala Tyr Val Tyr
            435                 440                 445

aca cct gag gtc tac ccc acg gca acg cgg gcc ctc ggc ctg ggc acc   1572
Thr Pro Glu Val Tyr Pro Thr Ala Thr Arg Ala Leu Gly Leu Gly Thr
        450                 455                 460

tgc agc ggc atg gca aga gtg ggt gct ctc atc act ccg ttc atc gcc   1620
Cys Ser Gly Met Ala Arg Val Gly Ala Leu Ile Thr Pro Phe Ile Ala
    465                 470                 475

cag gtg atg ctg gaa tcc tct gtg tac ctg act ctg gca gtt tac agt   1668
Gln Val Met Leu Glu Ser Ser Val Tyr Leu Thr Leu Ala Val Tyr Ser
480                 485                 490                 495

ggc tgc tgc ctc ctg gct gcc ctg gcc tcc tgc ttt ttg ccc att gag   1716
Gly Cys Cys Leu Leu Ala Ala Leu Ala Ser Cys Phe Leu Pro Ile Glu
                500                 505                 510

acc aaa ggc gga gga ctg cag gag tcc agc cac cgg gag tgg ggc cag   1764
Thr Lys Gly Gly Gly Leu Gln Glu Ser Ser His Arg Glu Trp Gly Gln
            515                 520                 525

gag atg gtc ggc cga gga atg cac ggt gca ggt gtt acc agg tcg aac   1812
Glu Met Val Gly Arg Gly Met His Gly Ala Gly Val Thr Arg Ser Asn
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tct ggc tct cag gaa tag tgaccgatgg gggactgagc tggtctttga          1860
Ser Gly Ser Gln Glu  *
    545

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<400> 9
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Glu His Glu Val Gln Ile Glu Gly Val His Val Gly Leu Glu Ala Val
35 40 45
Glu Leu Asp Asp Gly Ala Ala Val Pro Lys Glu Phe Ala Asn Pro Thr
50 55 60
Asp Asp Thr Phe Met Val Glu Asp Ala Val Glu Ala Ile Gly Phe Gly
65 70 75 80
Lys Phe Gln Trp Lys Leu Ser Val Leu Thr Gly Leu Ala Trp Met Ala
85 90 95
Asp Ala Met Glu Met Met Ile Leu Ser Ile Leu Ala Pro Gln Leu His
100 105 110
Cys Glu Trp Arg Leu Pro Ser Trp Gln Val Ala Leu Leu Thr Ser Val
115 120 125
Val Phe Val Gly Met Met Ser Ser Ser Thr Leu Trp Gly Asn Ile Ser
130 135 140
Asp Gln Tyr Gly Arg Lys Thr Gly Leu Lys Ile Ser Val Leu Trp Thr
145 150 155 160
Leu Tyr Tyr Gly Ile Leu Ser Ala Phe Ala Pro Val Tyr Ser Trp Ile
165 170 175
Leu Val Leu Arg Gly Leu Val Gly Phe Gly Ile Gly Gly Val Pro Gln
180 185 190
Ser Val Thr Leu Tyr Ala Glu Phe Leu Pro Met Lys Ala Arg Ala Lys
195 200 205
Cys Ile Leu Leu Ile Glu Val Phe Trp Ala Ile Gly Thr Val Phe Glu
210 215 220
Val Val Leu Ala Val Phe Val Met Pro Ser Leu Gly Trp Arg Trp Leu
225 230 235 240
Leu Ile Leu Ser Ala Val Pro Leu Leu Leu Phe Ala Val Leu Cys Phe
245 250 255
Trp Leu Pro Glu Ser Ala Arg Tyr Asp Val Leu Ser Gly Asn Gln Glu
260 265 270
Lys Ala Ile Ala Thr Leu Lys Arg Ile Ala Thr Glu Asn Gly Ala Pro
275 280 285
Met Pro Leu Gly Lys Leu Ile Ile Ser Arg Gln Glu Asp Arg Gly Lys
290 295 300
Met Arg Asp Leu Phe Thr Pro His Phe Arg Trp Thr Thr Leu Leu Leu
305 310 315 320
Trp Phe Ile Trp Phe Ser Asn Ala Phe Ser Tyr Tyr Gly Leu Val Leu
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Leu Thr Thr Glu Leu Phe Gln Ala Gly Asp Val Cys Gly Ile Ser Ser
340 345 350
Arg Lys Lys Ala Val Glu Ala Lys Cys Ser Leu Ala Cys Glu Tyr Leu
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Ser Glu Glu Asp Tyr Met Asp Leu Leu Trp Thr Thr Leu Ser Glu Phe
370 375 380
Pro Gly Val Leu Val Thr Leu Trp Ile Ile Asp Arg Leu Gly Arg Lys

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385                 390                 395                 400
Lys Thr Met Ala Leu Cys Phe Val Ile Phe Ser Phe Cys Ser Leu Leu
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Leu Phe Ile Cys Val Gly Arg Asn Val Leu Thr Leu Leu Leu Phe Ile
            420                 425                 430
Ala Arg Ala Phe Ile Ser Gly Gly Phe Gln Ala Ala Tyr Val Tyr Thr
        435                 440                 445
Pro Glu Val Tyr Pro Thr Ala Thr Arg Ala Leu Gly Leu Gly Thr Cys
    450                 455                 460
Ser Gly Met Ala Arg Val Gly Ala Leu Ile Thr Pro Phe Ile Ala Gln
465                 470                 475                 480
Val Met Leu Glu Ser Ser Val Tyr Leu Thr Leu Ala Val Tyr Ser Gly
                485                 490                 495
Cys Cys Leu Leu Ala Ala Leu Ala Ser Cys Phe Leu Pro Ile Glu Thr
            500                 505                 510
Lys Gly Gly Gly Leu Gln Glu Ser Ser His Arg Glu Trp Gly Gln Glu
        515                 520                 525
Met Val Gly Arg Gly Met His Gly Ala Gly Val Thr Arg Ser Asn Ser
    530                 535                 540
Gly Ser Gln Glu
545
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