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(54) Title: METHODS FOR THE IDENTIFICATION OF COMPOUNDS USEFUL FOR THE SUPPRESSION OF CHRONIC NEUROPATHIC PAIN AND COMPOSITIONS THEREOF

(57) Abstract: The invention relates to a method of screening for a compound able to modulate the activity of an N-type voltage-dependent calcium channel consisting of the Ca_v2.2 (α1B), α2δ1, β1 and γ4 subunits; wherein said compound may be in one embodiment e.g. an antibody or an antisense nucleotide sequence.



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Methods for the identification of compounds useful for the suppression of chronic neuropathic pain and compositions thereof

1. FIELD OF THE INVENTION

This invention provides methods for identifying agents useful for the suppression of chronic neuropathic pain in mammals, in particular humans, by screening for the ability of a candidate compound to modulate the activity and/or expression of N-type voltage-dependent calcium channel (VDCC) consisting of the Cav2.2 ($\alpha 1B$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits. The invention also provides said agents, which are nucleic acids, ribozymes, and antibodies.

2. DESCRIPTION OF THE RELATED ART

Compounds that regulate N-type VDCC activities, or are specific inhibitors of N-type VDCC are known. These include ω -conotoxin GVIA, SNX-111 (zincinotide), SNX-159, SNX 239, SNX-124 (Prado WA (2001) Braz J Med Biol Res 34: 449-461; Vanegas H, Schaible H (2000) Pain 85: 9-18). Gabapentin binds with high affinity to $\alpha 2\delta 1$ and $\alpha 2\delta 2$ subunits and it may exert its analgesic/anti-convulsant effect through modulation of VDCC currents, although this is controversial (Gong et al., J Memb Biol 184(1) 35-43 (2001); Sutton et al., Br J Pharmacol 135: 257-265 (2002)).

3. DESCRIPTION OF THE INVENTION

"Antisense" refers to nucleotide sequences that are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method, including synthesis by ligating the gene(s) of interest in a reverse orientation to a viral promoter that permits the synthesis of a complementary strand. Once introduced into a cell, this transcribed strand combines natural sequences produced by the cell to form duplexes. These duplexes then block either the further transcription or translation. The designation "negative" is sometimes used in reference to the

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antisense strand, and "positive" is sometimes used in reference to the sense strand.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains the essential properties thereof. A typical variant of a polynucleotide differs in nucleotide sequence from the reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from the reference polypeptide. Generally, alterations are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, insertions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. Typical conservative substitutions include Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln-I Ser, Thr; Lys, Arg; and Phe and Tyr. A variant of a polynucleotide or polypeptide may be naturally occurring such as an allele, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis. Also included as variants are polypeptides having one or more post-translational modifications, for instance glycosylation, phosphorylation, methylation, ADIP ribosylation and the like. Embodiments include methylation of the N-terminal amino acid, phosphorylations of serines and threonines and modification of C- terminal glycines. Also included as variants are polynucleotides having SNP (see below) and encode for polypeptides with one or more amino acid exchange. Also included as variants are polynucleotides which are so called splice variants (see below) and therefore encode altered polypeptides.

"Polymorphism" refers to a variation in nucleotide sequence (and encoded polypeptide sequence, if relevant) at a given position in the genome within a population.

"Single Nucleotide Polymorphism" (SNP) refers to the occurrence of nucleotide variability at a single nucleotide position in the genome, within a population. An SNP may occur within a gene or within intergenic regions of the genome. SNPs can be

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assayed using Allele Specific Amplification (ASA). For the process at least 3 primers are required. A common primer is used in reverse complement to the polymorphism being assayed. This common primer can be between 50 and 1500 bps from the polymorphic base. The other two (or more) primers are identical to each other except that the final 3' base wobbles to match one of the two (or more) alleles that make up the polymorphism. Two (or more) PCR reactions are then conducted on sample DNA, each using the common primer and one of the Allele Specific Primers.

"Splice Variant" as used herein refers to cDNA molecules produced from RNA molecules initially transcribed from the same genomic DNA sequence but which have undergone alternative RNA splicing. Alternative RNA splicing occurs when a primary RNA transcript undergoes splicing, generally for the removal of introns, which results in the production of more than one mRNA molecule each of that may encode different amino acid sequences. The term splice variant also refers to the proteins encoded by the above cDNA molecules.

"Identity" reflects a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, determined by comparing the sequences. In general, identity refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of the two polynucleotide or two polypeptide sequences, respectively, over the length of the sequences being compared.

"% Identity" - For sequences where there is not an exact correspondence, a "% identity" may be determined. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may be determined over the whole length of each of the sequences being compared (so-called global alignment), that is particularly suitable for sequences of the same or very similar length, or over shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length.

"Similarity" is a further, more sophisticated measure of the relationship between two polypeptide sequences. In general, "similarity" means a comparison between the amino acids of two polypeptide chains, on a residue by residue basis, taking into account not only exact correspondences between a between pairs of residues, one from each of the sequences being compared (as for identity) but also, where there is not an exact

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correspondence, whether, on an evolutionary basis, one residue is a likely substitute for the other. This likelihood has an associated "score" from which the "% similarity" of the two sequences can then be determined.

Methods for comparing the identity and similarity of two or more sequences are well known in the art. Thus for instance, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux J et al, Nucleic Acids Res, 12, 387-395, 1984, available from Genetics Computer Group, Madison, Wisconsin, USA), for example the programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity and the % similarity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of Smith and Waterman (J Mol Biol, 147,195-197, 1981, Advances in Applied Mathematics, 2, 482-489, 1981) and finds the best single region of similarity between two sequences. BESTFIT is more suited to comparing two polynucleotide or two polypeptide sequences that are dissimilar in length, the program assuming that the shorter sequence represents a portion of the longer. In comparison, GAP aligns two sequences, finding a "maximum similarity", according to the algorithm of Neddleman and Wunsch (J Mol Biol, 48, 443-453, 1970). GAP is more suited to comparing sequences that are approximately the same length and an alignment is expected over the entire length.

Preferably, the parameters "Gap Weight" and "Length Weight" used in each program are 50 and 3, for polynucleotide sequences and 12 and 4 for polypeptide sequences, respectively. Preferably, % identities and similarities are determined when the two sequences being compared are optimally aligned.

Other programs for determining identity and/or similarity between sequences are also known in the art, for instance the BLAST family of programs (Altschul S F et al, J Mol Biol, 215, 403-410, 1990, Altschul S F et al, Nucleic Acids Res., 25:389-3402, 1997, available from the National Center for Biotechnology Information (NCBI), Bethesda, Maryland, USA and accessible through the home page of the NCBI at www.ncbi.nlm.nih.gov) and FASTA (Pearson W R, Methods in Enzymology, 183, 63-99, 1990; Pearson W R and Lipman D J, Proc Nat Acad Sci USA, 85, 2444-2448,1988, available as part of the Wisconsin Sequence Analysis Package).

Preferably, the BLOSUM62 amino acid substitution matrix (Henikoff S and Henikoff J G, Proc. Nat. Acad Sci. USA, 89, 10915-10919, 1992) is used in polypeptide sequence

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comparisons including where nucleotide sequences are first translated into amino acid sequences before comparison.

Preferably, the program BESTFIT is used to determine the % identity of a query polynucleotide or a polypeptide sequence with respect to a reference polynucleotide or a polypeptide sequence, the query and the reference sequence being optimally aligned and the parameters of the program set at the default value, as hereinbefore described. **"Identity Index"** is a measure of sequence relatedness which may be used to compare a candidate sequence (polynucleotide or polypeptide) and a reference sequence. Thus, for instance, a candidate polynucleotide sequence having, for example, an Identity Index of 0.95 compared to a reference polynucleotide sequence is identical to the reference sequence except that the candidate polynucleotide sequence may include on average up to five differences per each 100 nucleotides of the reference sequence. Such differences are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion. These differences may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between these terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. In other words, to obtain a polynucleotide sequence having an Identity Index of 0.95 compared to a reference polynucleotide sequence, an average of up to 5 - 25 in every 100 of the nucleotides of the in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies mutatis mutandis for other values of the Identity Index, for instance 0.96, 0.97, 0.98 and 0.99.

Similarly, for a polypeptide, a candidate polypeptide sequence having, for example, an Identity Index of 0.95 compared to a reference polypeptide sequence is identical to the reference sequence except that the polypeptide sequence may include an average of up to five differences per each 100 amino acids of the reference sequence. Such differences are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion. These differences may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between these terminal positions, interspersed either individually among the amino acids in the reference sequence or in

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one or more contiguous groups within the reference sequence. In other words, to obtain a polypeptide sequence having an Identity Index of 0.95 compared to a reference polypeptide sequence, an average of up to 5 in every 100 of the amino acids in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies mutatis mutandis for other values of the Identity Index, for instance 0.96, 0.97, 0.98 and 0.99.

The relationship between the number of nucleotide or amino acid differences and the Identity Index may be expressed in the following equation:

$$n_a \leq x_a - (x_a \bullet I)$$

in which:

n_a is the number of nucleotide or amino acid differences,

x_a is the total number of nucleotides or amino acids for any given sequence (e.g. 596 for SEQ ID NO: 1),

I is the Identity Index,

$\bullet$ is the symbol for the multiplication operator, and in which any non-integer product of x_a and I is rounded down to the nearest integer prior to subtracting it from x_a .

"**Homolog**" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a reference sequence. Such relatedness may be quantified by determining the degree of identity and/or similarity between the two sequences as hereinbefore defined. Falling within this generic term are the terms "ortholog", and "paralog". "Ortholog" refers to a polynucleotide or polypeptide that is the functional equivalent of the polynucleotide or polypeptide in another species. "Paralog" refers to a polynucleotide or polypeptide that within the same species which is functionally similar.

"**Fusion protein**" refers to a protein encoded by two, unrelated, fused genes or fragments thereof. Examples have been disclosed in US 5541087, 5726044. Employing an immunoglobulin Fc region as a part of a fusion protein is advantageous for performing the functional expression of the protein of interest, to improve pharmacokinetic properties of such a fusion protein when used for therapy and to generate a dimeric fusion protein. The fusion protein DNA construct may comprise in 5' to 3' direction, a secretion cassette, i.e. a signal sequence that triggers export from a mammalian cell, DNA encoding an immunoglobulin Fc region fragment, as a fusion

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partner, and a DNA encoding a protein of interest or fragments thereof. In some uses it would be desirable to be able to alter the intrinsic functional properties (complement binding, Fc-Receptor binding) by mutating the functional Fc sides while leaving the rest of the fusion protein untouched or delete the Fc part completely after expression.

The present invention provides a method for screening compounds that inhibit, modulate, down-regulate or immobilize (in the cell) one or more Pain VDCC. It has been found that not only the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$) and $\alpha 2\delta 1$ subunits, but also the $\beta 1$ and $\gamma 4$ subunits of the VDCC are up-regulated in animal models of chronic neuropathic pain (e.g. Seltzer et al., (1990) Pain 43: 205-218, Chronic Construction Injury model (G.J and Xie, Y.K. Pain (1988) 33: 87-107), or Chung model (Kim, S.O. and Chung, J.M. Pain (1992) 50:355-363)), e.g. it was found that its messenger RNA is expressed in DRGs of those animals (see Table 2 comparing up-regulation of different subunits in different neuropathic pain models). Pain VDCC consisting of $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits or variants (including e.g. splice variants, SNPs) of said subunits of the VDCC can be used to screen drugs for the treatment of chronic neuropathic pain states associated with diseases including but not limited to the following: osteoarthritis, rheumatoid arthritis, cancer, diabetes, mechanical nerve injuries, postherpetic neuralgia, chronic lower back pain, abdominal pain and spinal stenosis. Such variants have more than 80% identity, more preferentially more than 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity. Levels of expression of the Pain VDCC or subunits thereof can be assayed from a biological sample, e.g., tissue (e.g. Dorsal Root Ganglion, DRG) sections, cell lysate, tissue lysate or white blood cell lysate, by any known methods, including in situ hybridization, quantitative PCR, immunoassays and electrophoresis assays (see also example 1). Test compounds which can be used in 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

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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.). The currently available VDCC blockers have unacceptable side effects which are due, at least in part, to non-specific channel blocking (other ion channel, other VDCCs). The Pain VDCC is expected to exist in a very limited number of tissues, and it may be the case that splice variants result in total tissue specificity. The $Ca_v2.2$ subunit is only expressed in neuronal tissue and splice variants distinguish brain and peripheral channels. The $\alpha 2\delta 1$ subunit is widely expressed, but at least 5 alternative splice variants have been identified. The $\beta 1$ subunit is predominantly expressed in skeletal muscle, but two brain specific isoforms exist. The $\gamma 4$ subunit is only expressed in neuronal tissue. It is expected that the same combination of splice variants won't exist outside of DRG neuronal sub-populations which should reduce side effects, but would mean that the efficacy of treatment could not be monitored by, for example, by blood sampling. In one embodiment, a screening assay comprises contacting a recombinant cell, which expresses the Pain VDCC, with a test compound, e.g. from above-mentioned libraries. One further determines then the ability of the test compound to modulate (by e.g. stimulating or inhibiting the Pain VDCC or by up/down-regulation of the expression of Pain VDCC or by immobilization or mobilization of Pain VDCC from intracellular pools) VDCC activity. Compounds identified by the above-described method can further be used and its activity confirmed in an appropriate animal model, e.g. the animal models described above (Seltzer model, Chronic Construction Injury model, or Chung model). In another embodiment of this invention, it relates to the monitoring of effects during clinical trials to evaluate a treatment, both in basic drug screening and in clinical trials. For example, the

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effectiveness of a compound determined by a screening assay as described herein to inhibit the Pain VDCC activity can be monitored in clinical trials of subjects exhibiting chronic neuropathic pain. In a preferred embodiment, the present invention provides a method for evaluating, e.g., monitoring, the effectiveness of treatment of a subject with a compound (e.g., peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) evaluating pre-administration levels of Pain VDCC activity in a subject prior to administration of a compound using detection methods known in the art, (ii) administering a compound to the subject; (iii) evaluating the level of Pain VDCC activity post-administration of the compound; and (iv) comparing the levels prior to and subsequent to the administration of the compound. According to such an embodiment, the levels of Pain VDCC of the subject may be used as an indicator of the effectiveness of a compound.

The invention further provides substances that inhibit the expression of $\beta 1$ or $\gamma 4$ subunits of the Pain VDCC at the nucleic acid level. Such molecules include ribozymes, antisense oligonucleotides, triple helix DNA, RNA aptamers and/or double stranded RNA directed to an appropriate nucleotide sequence of $\beta 1$ or $\gamma 4$ nucleic acid. These inhibitory molecules may be created using conventional techniques by one of skill in the art without undue burden or experimentation. For example, modifications (e.g. inhibition) of gene expression can be obtained by designing antisense molecules, DNA or RNA, to the control regions of the genes encoding the polypeptides discussed herein, i.e. to promoters, enhancers, and introns. For example, oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site may be used. Notwithstanding, all regions of the gene may be used to design an antisense molecule in order to create those which gives strongest hybridization to the mRNA and such suitable antisense oligonucleotides may be produced and identified by standard assay procedures familiar to one of skill in the art. Typically, oligonucleotides between about 5 and 50 nucleotides in length are used. More preferably, oligonucleotides between about 5 and 35 nucleotides are used. Even more preferably, oligonucleotides about 20 nucleotides in length are used. An antisense oligonucleotide can be constructed using chemical synthesis procedures known in the art. An oligonucleotide can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological

stability of the oligonucleotide or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids. For example, the use of phosphorothioate, methyl phosphonate and ethyl phosphotriester antisense oligonucleotides (reviewed in Stein, C.A. and Cheng Y-C. (1993) Science 226:1004-1012) is within the scope of the invention. Additionally, acridine substituted nucleotides can be incorporated into the antisense oligonucleotides used in the present invention. A preferred antisense nucleic acid of the invention is an antisense to a coding or regulatory region of one of the subunit genes of the Pain VDCC (e.g. $\beta 1$ or $\gamma 4$ gene). As contemplated herein, antisense oligonucleotides, triple helix DNA, RNA aptamers, ribozymes and double stranded RNA are directed to a nucleic acid sequence of $\beta 1$ or $\gamma 4$ such that the chosen nucleotide sequence of $\beta 1$ or $\gamma 4$ will produce gene-specific inhibition of $\beta 1$ or $\gamma 4$ gene expression. For example, knowledge of the $\beta 1$ or $\gamma 4$ nucleotide sequence may be used to design an antisense molecule that gives strongest hybridization to the mRNA. Similarly, ribozymes can be synthesized to recognize specific nucleotide sequences of $\beta 1$ or $\gamma 4$ and cleave it (Cech. J. Amer. Med Assn. 260:3030 (1988)). Techniques for the design of such molecules for use in targeted inhibition of gene expression is well known to one of skill in the art. Gene specific inhibition of gene expression may also be achieved using conventional double stranded RNA technologies. A description of such technology may be found in WO 99/32619 which is hereby incorporated by reference in its entirety. Antisense molecules, triple helix DNA, RNA aptamers and ribozymes of the present invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding the genes of the polypeptides discussed herein. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues. Vectors may be introduced into cells or tissues by many available means, and may be used in vivo, in vitro or ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using methods that are well

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known in the art. It is contemplated herein that one can inhibit the function and/or expression of a gene for a related regulatory protein or protein modified by $\beta 1$ or $\gamma 4$ as a way to treat chronic pain by designing, for example, antibodies to these proteins and/or designing inhibitory antisense oligonucleotides, triple helix DNA, ribozymes and RNA aptamers targeted to the genes for such proteins according to conventional methods. Pharmaceutical compositions comprising such inhibitory substances for the treatment of chronic pain are also contemplated. Antisense oligonucleotides, or an antisense recombinant expression vector can be designed based upon the known nucleotide sequence of one of the subunit cDNAs of the Pain VDCC (e.g. $\beta 1$ or $\gamma 4$ cDNAs) known in the art. The nucleotide sequence of a human $\beta 1$ subunit cDNA is available from GenbankTM (Accession Number NM_000723), whereas the nucleotide sequence of a human $\gamma 4$ subunit cDNA is available from GenbankTM (Accession Number NM_014405). In addition, the nucleotide sequence of a rat $\beta 1$ subunit cDNA is available from GenbankTM (Accession Number NM_017346), and the nucleotide sequence of a rat $\gamma 4$ subunit cDNA is available from GenbankTM (Accession Number AF361341). To inhibit the activity of the Pain VDCC in a cell from another species, antisense oligonucleotides are designed which are complimentary to nucleotide sequences that are conserved among the different subunit genes in different species (e.g., based upon comparison of the known subunit sequences, including the human and murine sequences, to identify conserved regions). Antisense oligonucleotides can be used to inhibit the activity of Pain VDCC in a cell by genetic therapy and/or exogenously administering them to a subject at an amount and for a time period sufficient to inhibit transcription of a gene of one the subunits of Pain VDCC (e.g. $\beta 1$ or $\gamma 4$ gene) or translation of the mRNA of one the subunits of Pain VDCC (e.g. $\beta 1$ or $\gamma 4$ gene) in the cell. In one embodiment, an antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been sub-cloned in an antisense orientation (i.e., nucleic acid transcribed from the inserted sequence will be in an antisense orientation relative to a target nucleic acid of interest). The antisense expression vector is introduced into cells, for example, 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 of the vector, the activity of which can be determined by the cell type into which the vector is introduced.

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Preferably, the recombinant expression vector is a recombinant viral vector, such as a retroviral, adenoviral or adeno-associated viral vector. Protocols for producing recombinant retroviruses and for infecting cells in vitro or in vivo with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F.M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM that are well known to those skilled in the art. Examples of suitable packaging virus lines include WCrip, yCre, y2 and yAm. Adenoviral vectors are described in Berkner et al. (1988) BioTechniques 6:616; Rosenfeld et al. (1991) Science 252:431-434; and Rosenfeld et al. (1992) Cell 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 d1324 or other strains of adenovirus (e.g., Ad2, Ad3, Adz etc.) are well known to those skilled in the art. Adeno-associated vectors (AAV) are reviewed in Muzyczka et al. Curr. Topics in Micro. and Immunol. (1992) 158:97-129). An example of a suitable AAV vector is described in Tratschin et al. (1985) Mol. Cell. Biol. 5:3251-3260. A recombinant expression vector containing a nucleic acid in an antisense orientation is introduced into a cell to generate antisense nucleic acids in the cell to thereby inhibit the activity of the Pain VDCC in the cell. The vector can be introduced into a cell by a conventional method for introducing nucleic acid into a cell. When a viral vector is used, the cell can be infected with the vector by standard techniques. Cells can be infected in vitro or in vivo. When a non-viral vector, e.g., a plasmid, is used, the vector can be introduced into the cell by, for example, calcium phosphate precipitation, DEAE-dextran transfection, electroporation or other suitable method for transfection of the cell.

Additionally, the present invention relates to a method for identifying a compound useful for the treatment of chronic neuropathic pain, the method comprising: a) contacting a ligand of an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits with an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits in the presence and absence of a test compound; and b) determining whether the test compound alters the binding of the ligand to the N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits to an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits. Optionally, said method further comprises

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the steps of: c) adding a compound identified that alters binding of the ligand to the N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits to an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits in step (b); d) determining whether the compound alleviates chronic neuropathic pain; and e) identifying a compound that alleviates chronic neuropathic pain in step (d) as a compound useful for the treatment of chronic neuropathic pain.

In yet another embodiment, a nucleic acid used to inhibit the Pain VDCC activity in a cell is a ribozyme which is capable of cleaving a single- stranded nucleic acid encoding one of the subunits of the Pain VDCC, such as an mRNA transcript. A catalytic RNA (ribozyme) having ribonuclease activity can be designed which has specificity for an mRNA encoding one of the subunits of the Pain VDCC. For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the base sequence of the active site is complementary to the base sequence to be cleaved in a pain VDCC mRNA. See for example Cech et al. U.S. Patent No. 4,987,071; Cech et al. U.S. Patent No. 5,116,742 for descriptions of designing ribozymes. Alternatively, a RNA of one of the subunits of the Pain VDCC (e.g. $\beta 1$ or $\gamma 1$ subunit) can be used to select a catalytic RNA having specific ribonuclease activity against RNA of one of the subunits of the Pain VDCC from a pool of RNA molecules. See for example, Bartel, D. and Szostak, J.W. Science 261:1411-1418 (1993) for a description of selecting ribozymes. A ribozyme can be introduced into a cell by constructing a recombinant expression vector (e.g., a viral vector as discussed above) containing nucleic acid which, when transcribed, produces the ribozyme (i.e., DNA encoding the ribozyme is cloned into a recombinant expression vector by conventional techniques).

In certain additional preferred embodiments of the invention there is provided an antibody or a fragment thereof which specifically binds to the Pain VDCC or to the $\gamma 4$ subunit. Antibodies are commercially available to the subunits $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$. Described herein are methods for the production of antibodies capable of specifically recognizing one or more differentially expressed gene epitopes of the Pain VDCC. Such antibodies may include, but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')_2 fragments, fragments produced by a Fab expression library, anti-

idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Such antibodies may be used, for example, in the detection of a fingerprint, Pain VDCC gene in a biological sample, or, alternatively, as a method for the inhibition of abnormal Pain VDCC activity. Thus, such antibodies may be utilized for the suppression of chronic neuropathic pain in human and veterinary patients. Chronic neuropathic pain results from damage to nerves by trauma, by diseases such as diabetes, herpes zoster, or late-stage cancer, or by chemical injury (e.g. some anti-HIV drugs). It may also develop after amputation (including mastectomy), and is involved in some low-back pain (Portenoy RK. Neuropathic pain. In: Portenoy RK, Kanner RM, (Eds). Pain Management: Theory and Practice. Philadelphia: FA Davis, 1996, pp 83-125). For the production of antibodies to a differentially expressed gene, various host animals may be immunized by injection with a differentially expressed gene protein, or a portion thereof. Such host animals may include but are not limited to rabbits, mice, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals such as those described above, may be immunized by injection with differentially expressed gene product supplemented with adjuvants as also described above. Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and Milstein, (e.g. U.S. Pat. No. 4,376,110), the human B-cell hybridoma technique (e.g. Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (e.g. Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be

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cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production. In addition, techniques developed for the production of "chimeric antibodies" (e.g. Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable or hypervariable region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (e.g. U.S. Pat. No. 4,946,778) can be adapted to produce differentially expressed gene-single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Most preferably, techniques useful for the production of "humanized antibodies" can be adapted to produce antibodies to the polypeptides, fragments, derivatives, and functional equivalents disclosed herein. Such techniques are disclosed e.g. in U.S. Patent Nos. 5,770,429, the disclosures of which are incorporated by reference herein in their entirety.

Antibody fragments which recognize specific epitopes may be generated by known techniques. Preferred antibodies of the present invention have at least one activity of Pain VDCC.

Additionally, the present invention pertains to the use of an antibody as disclosed herein or a compound that binds to the $\beta 1$ and $\gamma 4$ subunits of an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits in medicine and, in particular, for the manufacture of a medicament for the treatment of chronic neuropathic pain.

A compound that binds to the $\beta 1$ and $\gamma 4$ subunits of an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits can be applied in vitro in the form of solutions, e.g. preferably aqueous solutions or suspensions, and in vivo either enterally or parenterally, advantageously orally, e.g. as a suspension or in aqueous solution, or as a solid capsule or tablet formulation.

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Hence, the present invention furthermore provides

- (a) a method for the treatment of chronic neuropathic pain which comprises administering an effective amount of a compound that binds to the $\beta 1$ and $\gamma 4$ subunits of an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits to a patient in need of such treatment; and
- (b) a pharmaceutical composition for the treatment of chronic neuropathic pain which comprises a compound that binds to the $\beta 1$ and $\gamma 4$ subunits of an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits, and a pharmaceutically acceptable carrier or diluent.

The following examples further illustrate the present invention, and the examples are provided for illustration purposes and are not intended to be limiting the invention.

EXAMPLE 1: Expression Profiling of VDCC subunits in naïve and animal models of neuropathic hyperalgesia.

(1) *VDCC subunit gene expression in naïve rat DRG:* Initially, all the primer sets described in Table 1 are used to test naïve DRG total RNA for gene expression. Total RNA samples are prepared from dissected DRG tissues from ten naïve rats using Tri Reagent (Sigma) as per manufacturers instructions. Two μg of total RNA is treated with 0.2 units of RNase-free Dnase I (Roche Diagnostics) at 37 °C for 5 minutes and purified using a RNA-easy column (Qiagen) as per manufacturers instructions. The concentration of RNA is determined by OD. 2 μg of DNase treated RNA is transcribed into cDNA using First strand cDNA synthesis kit (Amersham Pharmacia) in a 66 μl volume (2x scaled reaction) as per manufacturers instructions. The cDNA samples are diluted to 25 ng / μl RNA equivalent cDNA and 50 ng used in a standard RT-PCR reaction using Qiagen Hot Start Taq Polymerase (Qiagen) with annealing temperatures of 50 – 60 °C.

Gene	Accession number	Primers used for PCR (Sequence 5'-3')	Product (bp)
$\alpha 2\delta 1$	R: NM_012919	CAG ACA TAC TCC AGA TTG GC (SEQ ID	529

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	(SEQ ID NO:6) H: XM_004914 (SEQ ID NO:7)	NO:12) TAG TGT CTG CTG CCA GAT AC (SEQ ID NO:13)	
$\alpha 2\delta 2$	M: AF247139	CAG CCT GGA TAA CCA TGG TT (SEQ ID NO:14) AGC TGT GAT AGA TGA GAC CA (SEQ ID NO:15)	604
$\alpha 2\delta 3$	M: NM_009785	AAC AAG GAG AGC AGT GAC AG (SEQ ID NO:16) AGA AGA GAC TCG AAA CCA GG (SEQ ID NO:17)	521
$\beta 1$	R: NM_017346 (SEQ ID NO:1) H: NM_000723 (SEQ ID NO:2)	ACA TGA TGC AGA AGG CGT TG (SEQ ID NO:18) CCA GCT CAT TCT TAT TGC GC (SEQ ID NO:19)	1025
$\beta 2$	R: M80545	CTT ACG ATG TGG TAC CAT CC (SEQ ID NO:20) TGC ACT CAT TGT GGT CTT GG (SEQ ID NO:21)	1040
$\beta 3$	R: NM_012828	ACA GAC ATG ATG CAG AAG GC (SEQ ID NO:22) ATC TAA CTC CAA CCT GCC TG (SEQ ID NO:23)	952
$\beta 4$	R: L02315	TGA TTG GTG GAT AGG AAG GC (SEQ ID NO:24) AAT CTT CTT CCA CCA GAG GG (SEQ ID NO:25)	1072
Ca _v 2. 1 $\alpha 1A$	R: NM_012918	CAG GAG ATG TTC CAG AAG AC (SEQ ID NO:26)	448

		TGG TCA TGC TCA GAT CTG TC (SEQ ID NO:27)	
Ca _v 2. 2 α 1B	R: AF055477 (SEQ ID NO:8) H: AF055477 (SEQ ID NO:9)	ACC TAC AAG ACG GCC AAT TC (SEQ ID NO:28) GAG AGG ACC ATA TCC CTC TA (SEQ ID NO:29)	624
Ca _v 1. 2 α 1C	R: M67515	GAC ATC TCT CAG AAG ACA GC (SEQ ID NO:30) ACA GGT TGC TGA CAT AGG AC (SEQ ID NO:31)	400
Ca _v 1. 3 α 1D	R: NM_017298	ACC TTA TGC AGC AGC AGA TC (SEQ ID NO:32) CAA AGT CCT GGA GCT CAT AG (SEQ ID NO:33)	549
Ca _v 2. 3 α 1E	R: L15453	TTA CCG AGT CTT CCA ACT CC (SEQ ID NO:34) GTG AGA GAA GAG CAT GCA TC (SEQ ID NO:35)	359
Ca _v 1. 4 α 1F	H: NM_005183 M: NM_019582	TTC ACC ATC CAG TGT CTG CA (SEQ ID NO:36) CAT CTG CAA TCT CCT GCT TG (SEQ ID NO:37)	375
Ca _v 3. 1 α 1G	R: AF027984	AGA GAC CAG AAG CAG CTT AG (SEQ ID NO:38) CAT GTC TGT TGG GTC AGA AG (SEQ ID NO:39)	495
Ca _v 3. 2 α 1H	M: AF226868 H: AF051946	GGA ACA ACA ACC TGA CCT TC (SEQ ID NO:40) GAT CAT GAA GAA GGA GCC CA (SEQ	431

		ID NO:41)	
Ca _v 3. 3 α 1I	R: AF08627	ACA GGC GAT AAC TGG AAT GG (SEQ ID NO:42) GAT GAA GAC CTG TCT CTT GC (SEQ ID NO:43)	584
γ 1	R: NM_019255	TGA CCC TCT TCT TCA TCC TG (SEQ ID NO:44) GAT CTA GTG CTC TGA CTC AG (SEQ ID NO:45)	643
γ 2	M: NM_007583 H: NM_006078	GCT GTT TGA TCG AGG TGT TC (SEQ ID NO:46) TGT ACA TGG AGA TCT CCG TG (SEQ ID NO:47)	808
γ 3	M: NM_019430 H: NM_006539	ATG AGG ATG TGT GAC AGA GG (SEQ ID NO:48) TGT GGA AGC CTT TGC TGA TG (SEQ ID NO:49)	776
γ 4	M: NM_019431 (SEQ ID NO: 5) H: NM_014405 (SEQ ID NO: 4) R: AF361341 SEQ ID NO: 3)	ACC AAC CTG ACC ATG GAC GAC (SEQ ID NO:50) TAC CTG TAG CTC GGC ATC CTG (SEQ ID NO:51)	583
γ 5	H: NM_014404	TGA GCA GTG TCT TTG CTG TC (SEQ ID NO:52) ATC CAT ACT TAG GGT GAC GG (SEQ ID NO:53)	562
γ 5*	M: NM_019432	AAG CTG TTG GGC CTT AAG AG (SEQ ID NO:54)	597

		AAG TCC CAT CAT AAC CTC GC (SEQ ID NO:55)	
$\gamma 6$	H: AF288386	GGG TGG AGC TCA ACA CCT AC (SEQ ID NO:56) GCC CAG GGA CCA GGA GTA (SEQ ID NO:57) ATA CAG AGG CAC CCC AAG G (SEQ ID NO:58)	230 859
$\gamma 7$	H: AF288387	CGG TCA GCA CTG ACT ACT GG (SEQ ID NO:59) ATG ACC TCG TCG TTG ATG C (SEQ ID NO:60)	625
$\gamma 8$	H: AF288388	ATG ACC ATC GCC ATC ATC AGC (SEQ ID NO:61) CGC CCA GAA TGA TGT TCC (SEQ ID NO:62)	900

Table 1. PCR primers designed to VDCC subunit genes. Accession numbers prefixed with R, M or H correspond to rat, mouse or human cDNA sequences respectively. PCR primers are designed to these sequences. * Two $\gamma 5$ subunits, with different sequences, have been deposited in Genbank. The $\text{Ca}_v1.1$ ($\alpha 1\text{S}$) gene is not analysed since this is thought to be skeletal muscle specific, however subsequent reports have reported low levels of neuronal expression of this gene.

Genes are regarded as being expressed in naïve rat DRG if products of the correct size are clearly visible after standard agarose gel electrophoresis. The primers for the β , γ and $\alpha 2\delta$ and $\text{Ca}_v2.2$ ($\alpha 1\text{B}$) subunits are then optimised for quantitative RT-PCR analysis using the LightCycler Technology (Roche Diagnostics). Naïve rat DRG cDNA is amplified using conditions suggested with the LightCycler Faststart DNA Master SYBR Green I kit (Roche Diagnostics). Annealing temperatures are determined empirically. Once optimized, the conditions are used to screen a panel of cDNAs from neuropathic

pain models and controls as described below. Four animal models of neuropathic pain are used:

1.) *Seltzer model*: In the Seltzer model (Seltzer et al., (1990) Pain 43: 205-218) rats are anaesthetized and a small incision made mid-way up one thigh (usually the left) to expose the sciatic nerve. The nerve is carefully cleared of surrounding connective tissue at a site near the trochanter just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. A silk 7-0 silk suture is inserted into the nerve with a 3/8 curve, reverved-cutting mini-needle, and tightly ligated so that the dorsal 1/3 to 1/2 of the nerve thickness is held within the ligature. The muscle and skin are closed with sutures and clips and the wound dusted with antibiotic powder. In sham animals the sciatic nerve is exposed but not ligated and the wound closed as in non-sham animals.

2.) *Chronic Constriction Injury (CCI) model*: In the CCI model (Bennett, G.J. and Xie, Y.K. Pain (1988)_33:87-107) rats are anaesthetized and a small incision is made mid-way up one thigh (usually the left) to expose the sciatic nerve. The nerve is cleared of surrounding connective tissue and four ligatures of 4/0 chromic gut are loosely tied around the nerve with approximately 1mm between each, so that the ligatures just barely constrict the surface of the nerve. The wound is closed with sutures and clips as described above. In sham animals the sciatic nerve is exposed but not ligated and the wound closed as in non-sham animals.

3.) *Axotomy model*: The axotomy model involves complete cut and ligation of the sciatic nerve. The nerve endings form neuromas but there is no behavioural correlate in this model as the nerve is not allowed to regenerate, and the foot is permanently denervated (Wall et al., (1979) Pain: 7:103-113).

4.) *Chung model*: In contrast to the Seltzer and CCI models that involve damage to peripheral nerves, the Chung model involves ligation of the spinal nerve (Kim, S.O. and Chung, J.M. Pain (1992): 50: 355-363). In this model, rats are anesthetized and placed in a prone position and an incision is made to the left of the spine at the L4-S2 level. A deep dissection through the paraspinal muscles and separation of the muscles from the spinal processes at the L4-S2 level will reveal part of the sciatic nerve as it branches to form the L4, L5 and L6 spinal nerves. The L6 transverse process is carefully removed

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with a small rongeur enabling visualisation of these spinal nerves. The L5 spinal nerve is isolated and tightly ligated with 7-0 silk suture. The wound is closed with a single muscle suture (6-0) silk and one or two skin closure clips and dusted with antibiotic powder. In sham animals the L5 nerve is exposed as before but not ligated and the wound closed as before.

Validation of the pain models: In all chronic pain models mechanical hyperalgesia is assessed by measuring paw withdrawal thresholds of both hindpaws to an increasing pressure stimulus using an Analgesymeter (Ugo-Baile, Milan). Mechanical allodynia is assessed by measuring withdrawal thresholds to non-noxious mechanical stimuli applied with von Frey hairs to the plantar surface of both hindpaws. Thermal hyperalgesia is assessed by measuring withdrawal latencies to a noxious thermal stimulus applied to the underside of each hindpaw. With all models, mechanical hyperalgesia and allodynia and thermal hyperalgesia develop within 1 - 3 days following surgery and persist for at least 50 days. In the experiments disclosed herein, male Wistar rats are employed in the pain models described above. Rats weigh approximately 120 – 140 grams at the time of surgery. All surgery is performed under enflurane/O₂ inhalation of anaesthesia. In all cases, the wound is closed after the procedure and the animal allowed to recover. In all but the axotomy model, a marked mechanical and thermal hyperalgesia develops in which there is a lowering of pain threshold and an enhanced reflex withdrawal response of the hind-paw to touch, pressure of thermal stimuli. After surgery, the animals also exhibit characteristic changes to the affected paw. In the majority of animals the toes of the affected hindpaw are held together and the foot turned slightly to one side; in some rats the toes are also curled under. The gait of the ligated rat varied, but limping is uncommon. Some rats are seen to raise the affected hindpaw from the cage floor and to demonstrate an unusual rigid extension of the hindlimb when held. The rats tend to be very sensitive to touch and may vocalise. Otherwise the general health and condition of the rats is good.

RNA Extraction from Dorsal root ganglia taken from animal models: L4 and L5 Dorsal Root Ganglia, ipsilateral to the nerve injury, are dissected at days 14, 21, 28 and 50 after surgery from rat models of neuropathic pain (described above). Contralateral L4 and L5 DRGs are taken for control samples as are DRGs from sham operated animals.

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Total RNA samples are prepared from the dissected DRG tissues using Tri Reagent (Sigma) as per manufacturers instructions. Two μg of total RNA is treated with 0.2 units of RNase-free Dnase I (Roche Diagnostics) at 37 °C for 5 minutes and purified using a RNA-easy column (Qiagen) as per manufacturers instructions. The concentration of RNA is determined by OD. 2 μg of DNase treated RNA is transcribed into cDNA using First strand cDNA synthesis kit (Amersham Pharmacia) in a 66 μl volume (2x scaled reaction) as per manufacturers instructions. The cDNA samples are diluted to 12.5 ng / μl RNA equivalent cDNA for LightCycler analysis. A standard curve of known concentrations of RNA equivalent cDNA from naïve rat DRG (50 – 1 ng) is run alongside the panel of samples. The relative cross-over points in the linear range of amplification are determined and used to quantify the sample message levels. Each run is performed in triplicate and all values averaged and standard error determined. Levels of b-actin mRNA are also determined using commercially available primers (Ambion, catalog number 1720) and the LightCycler Faststart DNA Master SYBR Green I kit (Roche Diagnostics). The VDCC subunit gene levels are normalized to b-actin to account for different efficiencies in cDNA synthesis. Standard errors are calculated using a propagation of error formula (Miller and Miller, 2000; Statistics and Chemometrics for Analytical Chemistry. Published by Prentice Hall (Harlow). Data from experimental and control samples are compared statistically using Kruskal-Wallis ANOVA with Dunn's multiple comparisons post-test.

The sham and contra mRNA levels are compared to the neuropathic pain model mRNA levels for each gene. It is apparent that the Cav2.2 ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits show an increased level of expression in the Seltzer, axotomy, and CCI samples compared to sham and contra (Table 2). The other genes encoding for the other types of VDCC channel subunits show lower levels of gene regulation. The subunits that are consistently regulated in the Seltzer, CCI and axotomy models are then further tested in the Chung model samples.

Table 2 shows a summary of the results:

Gene	Seltzer	CCI	Axotomy	Chung
$\alpha 2\delta 1$	Increased	Increased	Increased	increased in ligated and non-

				ligated neurons
$\alpha 2\delta 2$	Not consistently regulated	Not consistently regulated	Not consistently regulated	Not determined
$\alpha 2\delta 3$	Not consistently regulated	Not consistently regulated	Not consistently regulated	Not determined
$\beta 1$	Increased	Increased	Not changed	Increased in ligated neurons
$\beta 3$	Not consistently regulated	Not consistently regulated	Not consistently regulated	Not determined
$\beta 4$	Not consistently regulated	Not consistently regulated	Not consistently regulated	Not determined
$\gamma 2$	Not consistently regulated	Not consistently regulated	Not consistently regulated	Not determined
$\gamma 3$	Not consistently regulated	Not consistently regulated	Not consistently regulated	Not determined
$\gamma 4$	Increased	Increased	Not changed	Increased in injured and uninjured neurons
$\gamma 7$	Not consistently regulated	Not consistently regulated	Not consistently regulated	Not determined
$\gamma 8$	Not consistently regulated	Not consistently regulated	Not consistently regulated	Not determined
$\text{Ca}_v 2.2$ ($\alpha 1\text{B}$)	Increased	Decreased	Not changed	Increased in injured neurons

Example 2: Screening Methods

Compounds that modulate Pain VDCC activity are screened for their ability to modulate calcium flow through the Pain VDCC, e.g. by measuring the change of intracellular calcium levels by determining ^{45}Ca -uptake or by a fluorometric determination of intracellular calcium with a calcium sensitive dye (Fluorescence assay). This is demonstrated e.g. with the following 2 screening assays.

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Calcium uptake assay: Cultures of Chinese Hamster Ovary (ICN Pharmaceuticals Ltd., Basingstoke, Hampshire, U.K.) cells (therein referred to as recombinant cell) expressing human N-type voltage-dependent calcium channel (VDCC) consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits are prepared using multiple cassettes in tandem, e.g. clone the cDNA for two subunits in tandem with IRES (Chappell SA et al, PNAS, 97: 1536-1541), repeat with the cDNA for the other two subunits, clone both pairs into two multiple cloning site regions in a pBudCE4 vector (Invitrogen, California, U.S.) and transfect into the cell line. In addition, the cells are transfected with a potassium channel (e.g. Kir2.1 channel, Genbank Accession number AF011904) using standard protocols [McIntyre et al., J. British Journal of Pharmacology 132:1084-1094 (2001)]. The following primers are used to amplify the coding sequence of human Kir 2.1 by RT-PCR from total RNA samples prepared from human blood eosinophils (DD10Forward: ATG GGC AGT GTG CGA ACC AAC CGC TAC (SEQ ID NO: 10); DD10Reverse: TCA GTC ATA TCT CCG ATC CTC GCC GTA (SEQ ID NO: 11)). PCR products are cloned into the pCR4-TOPO vector (Invitrogen, Carlsbad, CA) and sequenced according to conventional methods). Cells are plated at a density of 25000 per well on 96 well plates cultured at 37°C in 5 % CO_2 in MEM medium overnight. On the day of the assay, the cells are washed four times with calcium/magnesium free HBSS plus 10 mM HEPES, pH7.4. All steps are carried out at RT. After washing the wells contain approximately 50 μl of buffer. The compound to be tested is added in 25 μl of buffer. The calcium channel is activated by eliciting depolarisation of the membrane by increasing the extracellular potassium concentration to ~ 80mM in $\text{Ca}^{2+}/\text{Mg}^{2+}$ free buffer containing 370 KBq of $^{45}\text{Ca}^{2+}/\text{ml}$. For negative control, potassium is omitted. Samples are incubated at RT for 15 min, then washed five times with HBSS/10 mM HEPES pH 7.4 . The remaining buffer is removed from the wells and replaced with 25 μl of 0.3 % SDS. After about 10 min, 200 μl of Microscint 40 scintillant is added and samples are counted on a Packard Topcount.

Compounds of interest effectively block Ca^{2+} -uptake (= IC_{50}) in the range from 1nM to 10 μM . Alternatively, the ability to block Ca^{2+} -uptake in a cell expressing Pain VDCC is compared with a cell not expressing Pain VDCC.

Fluorescence assay: Cultures of Chinese Hamster Ovary (CHO) cells expressing the human N-type voltage-dependent calcium channel (VDCC) consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits are prepared using multiple cassettes in tandem (other

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method, same as above). In addition, the cells are transfected with a potassium channel (Kir2.1 channel).

The activity of test compounds are investigated using a fluorescence assay utilizing calcium sensitive dyes to measure intracellular changes of $[Ca^{2+}]_i$. The cells are plated at a density of 25,000 per well on 96 well Costar black, clear bottomed plates cultured at 37°C in 5% CO₂ in MEM medium overnight. On the day of the assay, cells are incubated in either 2µM fura-2/AM or 2µM fura-6F (Molecular Probes) made up in assay buffer [Hank's Balanced Salt Solution (HBSS, Invitrogen) containing 10mM N-2-(hydroxyethylpiperazine-N'-[2-ethanesulfonic acid) (HEPES), pH 7.4] containing 0.01% pluronic F-127 for 30min at room temperature. After washing twice with assay buffer 100µl assay buffer containing the compound to be tested (range from 1nM to 10 µM final) or assay buffer alone where appropriate, are added to each well and the plate placed in a Molecular Devices Flexstation. The fluorescence is measured over 1min at 4s intervals using excitation wavelengths of 340 and 380nm and emission of 520nm. The N type calcium channels are activated by adding 20µl of 480mM KCl in HBSS to increase the extracellular potassium concentration to elicit a membrane depolarisation. The ratio of fluorescence intensities following excitation at 340 and 380nm is calculated for each time point. The potassium-evoked response is calculated as the mean of the ratios in the four time-points following stimulation minus the basal ratio. Compounds of interest effectively reduce the ratio of the fluorescence intensity following excitation at 340 and 380nm (= IC₅₀) in the range from 1nM to 10 µM. Alternatively, the ability to reduce the ratio of the fluorescence intensity in a cell expressing Pain VDCC can be compared with a cell not expressing Pain VDCC.

Example 3: Antisense Oligonucleotides to β1 or γ4

Synthesis of Antisense Oligonucleotides: Antisense oligonucleotides (ASOs) useful to inhibit gene expression, including the expression of β1 or γ4, may be made according to conventional methods. For example, ASOs against β1 or γ4 may be fully or partially phosphorothioated or fully or partially phosphodiester18-mers with nucleotides at both ends modified with MOE (methoxy ethoxy) groups. These may be synthesized using phosphoramidite chemistry, HPLC-purified and characterized by electrospray mass spectrometry and capillary gel electrophoresis according to conventional methods. ASOs, each with a GC content between 38 and 72%, may be selected and synthesized

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complementary to parts of the coding region of, for example, rat or human $\beta 1$ or $\gamma 4$. For mismatch-containing control oligonucleotides, the approximate base composition of the match oligonucleotides may be maintained. Additionally, two control ASOs may be selected, e.g., one for rat GAPDH coding regions and a second random synthetic ASO. The format of the anti-rat-GAPDH oligonucleotide may be the same as for anti- $\beta 1$ or $\gamma 4$ oligonucleotides; the synthetic oligonucleotide may have its MOE ribonucleotide modifications at both ends of the sequence with phosphorothioate or phosphodiester DNA residues in the middle.

In vitro selection of $\beta 1$ or $\gamma 4$ ASOs: Using methods familiar to one of skill in the art, optimal ASOs may be selected from a collection of ASO candidates in vitro in order to identify the most active ASO for subsequent analyses (e.g. in vivo target validation). Such ASO candidates may be tested in comparison with mismatched ASOs (i.e. otherwise identical ASOs bearing conservative inactivating mutations), vehicle, and/or untreated controls. Once an optimal candidate ASO sequence has been identified as a target for antisense, chemical derivatives and formats, more suited for in vivo applications, and based on the identical optimal target sequence, may then be synthesized and subsequently administered in vivo.

Transfection protocol: Transfection of ASOs may be performed according to methods familiar to one of skill in the art. For example, twenty four hours before transfection, 2×10^5 cells e.g., Chinese Hamster Ovary cells (ICN Pharmaceuticals Ltd., Basingstoke, Hampshire, U.K.) in a volume of 2 ml per well (F12 Nutrient mix (DMEM), 100unit/millilitre Penicillin, 100 micrograms per millilitre streptomycin, 2millimolar L-Glutamine, 10% fetal bovine serum (GIBCO-BRL, Rockville, MD)) may be plated into 6-well plates and cultured in 5% CO₂ to yield 70-80% confluency. On the day of transfection, a 2 fold stock transfection solution may then be prepared by diluting Lipofectin™ into serum-free OptiMEM (GIBCO-BRL, Rockville, MD) (3 microliters Lipofectin™ per 100 nM desired final oligonucleotide concentration into 1 ml OptiMEM) and incubating for 15 minutes at room temperature. This solution may then be combined 1:1 with a 2 fold ASO-solution containing twice the desired final amount of ASO in OptiMEM. After incubating the transfection mixture for 15 minutes at room temperature to form the transfection complex, 2 ml may then be added to each of the previously aspirated well of cells. A Lipofectin™ reagent-only control and a normal cell

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control (untreated) may also be included. After incubation for 4 hours at 37°C, 500 microlitres of 50% FBS in MEM (Invitrogen, Carlsbad, CA) may then be added to each well to obtain a final FBS concentration of 10%. The cultures may then be incubated at 37°C in a humidified incubator with 5% CO₂ for 24 hours for mRNA harvest or 48 hours for protein harvest and electrophysiology.

Real-time quantitative PCR mRNA analysis may be performed according to methods standard in the art. For example, total RNA may be isolated with the RNeasy 96 Kit (Qiagen, GmBH, Germany) according to the manufacturer's protocol. The RNA samples may be individually diluted to 1ng/L. Five nanograms of RNA for each sample may then be mixed with gene-specific detection primers (easily determined by one of skill in the art) and with the appropriate reagents from the real-time quantitative PCR reaction kit PLATINUM® Quantitative RT-PCR THERMOSCRIPT™ One-Step System (Gibco-BRL, Rockville, MD) and run according to manufacturer's protocol. The rat $\beta 1$ or $\gamma 4$ primers with the appropriate sequences may be purchased from PE Applied Biosystems, (Foster City, CA). GAPDH may be chosen as a control gene for comparisons. The same RNA samples may be run with rat GAPDH primers from the TaqMan® Rodent GAPDH Control Reagents Kit (PE Applied Biosystems, Foster City, CA). The sequence-specific fluorescent emission signal may be detected using the ABI PRISM™ 7700 Sequence Detector (PE Applied Biosystems, Foster City, CA). Along with the samples, a standard from dilutions of pure template mRNA may be run to obtain absolute concentrations per inserted amount of total RNA.

$\beta 1$ or $\gamma 4$ RNA analysis In vitro: dose response vs. mismatch: $\beta 1$ or $\gamma 4$ specific ASOs may be synthesized, along with mismatch controls each bearing mutations compared with the original match ASOs. Briefly, for example, a fully phosphodiester 18-mer with 5 nucleotides at the 3' and 5'-ends modified with 2'-O-(2-methoxyethyl) (MOE) groups, may be synthesized using phosphoramidite chemistry (Martin, P and Natt, F. EP 99-119768, US 98-168447, CAN 132:279477, AN 2000:240734) HPLC-purified, and characterised by electrospray mass spectrometry and capillary gel electrophoresis. The ASO that are most efficient at inhibiting $\beta 1$ or $\gamma 4$ mRNA levels, as determined by real time quantitative PCR in an in vitro assay performed on a cell line(s) that expresses relatively high levels of endogenous $\beta 1$ or $\gamma 4$ mRNA may then be determined. Subsequently, these may be tested against the appropriate mismatch controls, in a dose response experiment again in the cell line(s).

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In vivo assay: Based on the dose response data generated in vitro, a fully or partially phosphodiester version of the ASO and missense oligonucleotide (MSO) 18-mer with 5 nucleotides at the 3' and 5'-ends modified with 2'-O-(2-methoxyethyl) (MOE) groups, for example, may be used in vivo. ASO, MSO or vehicle may then be delivered to rats (e.g. Wistar) for up to 7 days at a desired concentration to allow cell bodies within the spinal cord and the dorsal root ganglia to take up the oligonucleotides or vehicle. ASO and MSO may be administered intrathecally via an indwelling cannula, inserted 24 h prior to or 14 days following sciatic nerve ligation, or 24 h prior to CFA injection. Rats may be anaesthetised and an incision made in the dorsal skin just lateral to the midline and approximately 10 mm caudal to the ventral iliac spines. A sterile catheter (polyethylene PE10 tubing) may be inserted via a guide cannula (20 gauge needle) and advanced 3 cm cranially in the intrathecal space approximately to the L1 level. The catheter may then be connected to an osmotic mini-pump (Alza corporation, Palo Alto, CA) delivering ASO, MSO or saline (1 μ l / h, 7 days) which may be inserted subcutaneously in the left or right flank. The incision may then be closed with wound clips and dusted with antibiotic powder. Preliminary experiments may then be carried out to establish maximal tolerated dose. Mechanical hyperalgesia, allodynia may be measured in the following way to assess the effect of β 1 or γ 4 antisense oligonucleotides in reversal of hyperalgesia. Mechanical hyperalgesia may be assessed by measuring paw withdrawal thresholds of both hindpaws to an increasing pressure stimulus using an Analgesymeter (Ugo-Basile, Milan). The cut-off may be set at 250 g and the end-point taken as paw withdrawal, vocalisation or overt struggling. Paw withdrawal at pressure stimuli below 65 g is not observed after surgery. Mechanical allodynia may be assessed by measuring withdrawal thresholds to non-noxious mechanical stimuli applied with custom made von Frey hairs to the plantar surface of both hindpaws. Animals may be placed individually into wire-mesh bottom cages, with groups of 6 tested concurrently, and allowed to acclimatize for approximately 30 min. Von Frey hairs may then tested in ascending order of force with a single trial of up to 6 s for each hair until a withdrawal response was established, with a 20.6 g cut-off. This may be confirmed as the withdrawal threshold by testing a lack of response to hair with the next lowest force. Each animal may be tested only once, in random order. The statistical significance of mechanical hyperalgesia and allodynia data may be obtained from the different experimental animal groups analysed using ANOVA followed by Tukey's HSD test.

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SEQUENCE LISTING

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- 32 -

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Lys His Arg Phe Asp Gly Arg Ile Ser Ile Thr Arg Val Thr Ala Asp						
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Ile Ser Leu Ala Lys Arg Ser Val Leu Asn Asn Pro Ser Lys His Ile						
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Ile Ile Glu Arg Ser Asn Thr Arg Ser Ser Leu Ala Glu Val Gln Ser						
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Val Gln Ile Ala Ala Ser Glu Lys Leu Ala Gln Cys Pro Pro Glu Met						
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Leu Ala Glu Tyr Leu Glu Ala Tyr Trp Lys Ala Thr His Pro Pro Ser						
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Arg Thr Pro Pro Asn Pro Leu Leu Asn Arg Thr Met Ala Thr Ala Ala						
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Thr Glu Pro Gly Asp Ser Cys Val Asp Met Glu Thr Asp Pro Ser Glu		
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Lys	Arg	Lys	Gly	Arg	Phe	Lys	Arg	Ser	Asp	Gly	Ser	Thr	Ser	Ser	Asp
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Thr	Thr	Ser	Asn	Ser	Phe	Val	Arg	Gln	Gly	Ser	Ala	Glu	Ser	Tyr	Thr
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Pro	Ser	Pro	Gly	Asp	Glu	Val	Pro	Val	Gln	Gly	Val	Ala	Ile	Thr	Phe
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Glu	Pro	Lys	Asp	Phe	Leu	His	Ile	Lys	Glu	Lys	Tyr	Asn	Asn	Asp	Trp
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Trp	Ile	Gly	Arg	Leu	Val	Lys	Glu	Gly	Cys	Glu	Val	Gly	Phe	Ile	Pro
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Arg Gln Asn Arg Leu Gly Ser Ser Lys Ser Gly Asp Asn Ser Ser Ser
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Leu Ala Pro Ile Ile Val Tyr Ile Lys Ile Thr Ser Pro Lys Val Leu
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Ser	Glu	Tyr	Leu	Leu	Arg	Ile	Val	Arg	Ala	Ser	Ser	Val	Phe	Pro	Ile
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Gly Leu Trp Arg Val Cys Cys Ile Glu Gly Ile Tyr Lys Gly His Cys
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Phe Val Ala Ala Gly Leu Ser Asn Ile Ile Gly Ile Ile Val Tyr Ile
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Asn His Tyr Asn Tyr Gly Trp Ser Phe Tyr Phe Gly Ala Leu Ser Phe
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Ile Val Ala Glu Thr Val Gly Val Leu Ala Val Asn Ile Tyr Ile Glu
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Phe Val Ala Ala Gly Leu Ser Asn Ile Ile Gly Ile Ile Val Tyr Ile
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Asn His Tyr Asn Tyr Gly Trp Ser Phe Tyr Phe Gly Ala Leu Ser Phe
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Tyr Gln His Ala Ala Val His Ile Pro Thr Asp Ile Tyr Glu Gly Ser
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His Leu Val Gln Ala Asn Val Arg Asn Lys Lys Val Leu Lys Asp Ala
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Val Asn Asn Ile Thr Ala Lys Gly Ile Thr Asp Tyr Lys Lys Gly Phe
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Asn Lys Ser Gly Pro Gly Ala Tyr Glu Ser Gly Ile Met Val Ser Lys
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Ala Val Glu Leu Tyr Ile Gln Gly Lys Leu Leu Lys Pro Ala Val Val
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Gly Ile Lys Ile Asp Val Asn Ser Trp Ile Glu Asn Phe Thr Lys Thr
 805 810 815

Ser	Ile	Arg	Asp	Pro	Cys	Ala	Gly	Pro	Val	Cys	Asp	Cys	Lys	Arg	Asn
			820					825					830		
Ser	Asp	Val	Met	Asp	Cys	Val	Ile	Leu	Asp	Asp	Gly	Gly	Phe	Leu	Leu
		835					840					845			
Met	Ala	Asn	His	Asp	Asp	Tyr	Thr	Asn	Gln	Ile	Gly	Arg	Phe	Phe	Gly
	850					855					860				
Glu	Ile	Asp	Pro	Arg	Met	Met	Arg	His	Leu	Val	Asn	Ile	Ser	Leu	Tyr
865					870					875					880
Ala	Phe	Asn	Lys	Ser	Tyr	Asp	Tyr	Gln	Ser	Val	Cys	Asp	Pro	Gly	Ala
				885					890					895	
Ala	Pro	Lys	Gln	Gly	Ala	Gly	His	Arg	Ser	Ala	Tyr	Val	Pro	Ser	Ile
			900					905					910		
Thr	Asp	Ile	Leu	Gln	Ile	Gly	Trp	Trp	Ala	Thr	Ala	Ala	Ala	Trp	Ser
		915					920					925			
Ile	Leu	Gln	Gln	Leu	Leu	Leu	Ser	Leu	Thr	Phe	Pro	Arg	Leu	Leu	Glu
	930						935				940				
Ala	Val	Glu	Met	Glu	Glu	Asp	Asp	Phe	Thr	Ala	Ser	Leu	Ser	Lys	Gln
945					950					955					960
Ser	Cys	Ile	Thr	Glu	Gln	Thr	Gln	Tyr	Phe	Phe	Lys	Asn	Asp	Thr	Lys
				965					970					975	
Ser	Phe	Ser	Gly	Leu	Leu	Asp	Cys	Gly	Asn	Cys	Ser	Arg	Ile	Phe	His
			980					985					990		
Val	Glu	Lys	Leu	Met	Asn	Thr	Asn	Leu	Val	Phe	Ile	Met	Val	Glu	Ser
		995					1000					1005			
Lys	Gly	Thr	Cys	Pro	Cys	Asp	Thr	Arg	Leu	Leu	Met	Gln	Ala	Glu	
	1010					1015					1020				

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Gln Thr Ser Asp Gly Pro Asp Pro Cys Asp Met Val Lys Gln Pro
 1025 1030 1035

Arg Tyr Arg Lys Gly Pro Asp Val Cys Phe Asp Asn Asn Val Leu
 1040 1045 1050

Glu Asp Tyr Thr Asp Cys Gly Gly Val Ser Gly Leu Asn Pro Ser
 1055 1060 1065

Leu Trp Ser Ile Phe Gly Leu Gln Phe Ile Leu Leu Trp Leu Val
 1070 1075 1080

Ser Gly Ser Arg His Tyr Leu Trp
 1085 1090

<210> 7

<211> 533

<212> PRT

<213> Homo sapiens

<220>

<221> PEPTIDE

<222> (1) .. (533)

<223>

<400> 7

Met Ile Asp Gly Glu Ser Gly Glu Lys Thr Phe Arg Thr Leu Val Lys
 1 5 10 15

Ser Gln Asp Glu Arg Tyr Ile Asp Lys Gly Asn Arg Thr Tyr Thr Trp
 20 25 30

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Thr Pro Val Asn Gly Thr Asp Tyr Ser Leu Ala Leu Val Leu Pro Thr
 35 40 45

Tyr Ser Phe Tyr Tyr Ile Lys Ala Lys Leu Glu Glu Thr Ile Thr Gln
 50 55 60

Ala Arg Ser Lys Lys Gly Lys Met Lys Asp Ser Glu Thr Leu Lys Pro
 65 70 75 80

Asp Asn Phe Glu Glu Ser Gly Tyr Thr Phe Ile Ala Pro Arg Asp Tyr
 85 90 95

Cys Asn Asp Leu Lys Ile Ser Asp Asn Asn Thr Glu Phe Leu Leu Asn
 100 105 110

Phe Asn Glu Phe Ile Asp Arg Lys Thr Pro Asn Asn Pro Ser Cys Asn
 115 120 125

Ala Asp Leu Ile Asn Arg Val Leu Leu Asp Ala Gly Phe Thr Asn Glu
 130 135 140

Leu Val Gln Asn Tyr Trp Ser Lys Gln Lys Asn Ile Lys Gly Val Lys
 145 150 155 160

Ala Arg Phe Val Val Thr Asp Gly Gly Ile Thr Arg Val Tyr Pro Lys
 165 170 175

Glu Ala Gly Glu Asn Trp Gln Glu Asn Pro Glu Thr Tyr Glu Asp Ser
 180 185 190

Phe Tyr Lys Arg Ser Leu Asp Asn Asp Asn Tyr Val Phe Thr Ala Pro
 195 200 205

Tyr Phe Asn Lys Ser Gly Pro Gly Ala Tyr Glu Ser Gly Ile Met Val
 210 215 220

Ser Lys Ala Val Glu Ile Tyr Ile Gln Gly Lys Leu Leu Lys Pro Ala
 225 230 235 240

Val	Val	Gly	Ile	Lys	Ile	Asp	Val	Asn	Ser	Trp	Ile	Glu	Asn	Phe	Thr
				245					250					255	
Lys	Thr	Ser	Ile	Arg	Asp	Pro	Cys	Ala	Gly	Pro	Val	Cys	Asp	Cys	Lys
			260					265					270		
Arg	Asn	Ser	Asp	Val	Met	Asp	Cys	Val	Ile	Leu	Asp	Asp	Gly	Gly	Phe
		275					280					285			
Leu	Leu	Met	Ala	Asn	His	Asp	Asp	Tyr	Thr	Asn	Gln	Ile	Gly	Arg	Phe
	290					295					300				
Phe	Gly	Glu	Ile	Asp	Pro	Ser	Leu	Met	Arg	His	Leu	Val	Asn	Ile	Ser
305					310					315					320
Val	Tyr	Ala	Phe	Asn	Lys	Ser	Tyr	Asp	Tyr	Gln	Ser	Val	Cys	Glu	Pro
				325					330					335	
Gly	Ala	Ala	Pro	Lys	Gln	Gly	Ala	Gly	His	Arg	Ser	Ala	Tyr	Val	Pro
			340					345					350		
Ser	Val	Ala	Asp	Ile	Leu	Gln	Ile	Gly	Trp	Trp	Ala	Thr	Ala	Ala	Ala
		355					360					365			
Trp	Ser	Ile	Leu	Gln	Gln	Phe	Leu	Leu	Ser	Leu	Thr	Phe	Pro	Arg	Leu
	370					375					380				
Leu	Glu	Ala	Val	Glu	Met	Glu	Asp	Asp	Asp	Phe	Thr	Ala	Ser	Leu	Ser
385					390					395					400
Lys	Gln	Ser	Cys	Ile	Thr	Glu	Gln	Thr	Gln	Tyr	Phe	Phe	Asp	Asn	Asp
				405					410					415	
Ser	Lys	Ser	Phe	Ser	Gly	Val	Leu	Asp	Cys	Gly	Asn	Cys	Ser	Arg	Ile
			420					425					430		
Phe	His	Gly	Glu	Lys	Leu	Met	Asn	Thr	Asn	Leu	Ile	Phe	Ile	Met	Val
		435					440					445			

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Glu Ser Lys Gly Thr Cys Pro Cys Asp Thr Arg Leu Leu Ile Gln Ala
 450 455 460

Glu Gln Thr Ser Asp Gly Pro Asn Pro Cys Asp Met Val Lys Gln Pro
 465 470 475 480

Arg Tyr Arg Lys Gly Pro Asp Val Cys Phe Asp Asn Asn Val Leu Glu
 485 490 495

Asp Tyr Thr Asp Cys Gly Gly Val Ser Gly Leu Asn Pro Ser Leu Trp
 500 505 510

Tyr Ile Ile Gly Ile Gln Phe Leu Leu Leu Trp Leu Val Ser Gly Ser
 515 520 525

Thr His Arg Leu Leu
 530

<210> 8

<211> 2333

<212> PRT

<213> Rattus norvegicus

<220>

<221> PEPTIDE

<222> (1) .. (2333)

<223>

<400> 8

Met Val Arg Phe Gly Asp Glu Leu Gly Gly Arg Tyr Gly Gly Thr Gly
 1 5 10 15

Gly Gly Glu Arg Ala Arg Gly Gly Gly Ala Gly Gly Ala Gly Gly Pro
 20 25 30

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Gly Gln Gly Gly Leu Pro Pro Gly Gln Arg Val Leu Tyr Lys Gln Ser
 35 40 45

Ile Ala Gln Arg Ala Arg Thr Met Ala Leu Tyr Asn Pro Ile Pro Val
 50 55 60

Lys Gln Asn Cys Phe Thr Val Asn Arg Ser Leu Phe Val Phe Ser Glu
 65 70 75 80

Asp Asn Val Val Arg Lys Tyr Ala Lys Arg Ile Thr Glu Trp Pro Pro
 85 90 95

Phe Glu Tyr Met Ile Leu Ala Thr Ile Ile Ala Asn Cys Ile Val Leu
 100 105 110

Ala Leu Glu Gln His Leu Pro Asp Gly Asp Lys Thr Pro Met Ser Glu
 115 120 125

Arg Leu Asp Asp Thr Glu Pro Tyr Phe Ile Gly Ile Phe Cys Phe Glu
 130 135 140

Ala Gly Ile Lys Ile Ile Ala Leu Gly Phe Val Phe His Lys Gly Ser
 145 150 155 160

Tyr Leu Arg Asn Gly Trp Asn Val Met Asp Phe Val Val Val Leu Thr
 165 170 175

Gly Ile Leu Ala Thr Ala Gly Thr Asp Phe Asp Leu Arg Thr Leu Arg
 180 185 190

Ala Val Arg Val Leu Arg Pro Leu Lys Leu Val Ser Gly Ile Pro Ser
 195 200 205

Leu Gln Val Val Leu Lys Ser Ile Met Lys Ala Met Val Pro Leu Leu
 210 215 220

Gln Ile Gly Leu Leu Leu Phe Phe Ala Ile Leu Met Phe Ala Ile Ile
 225 230 235 240

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Gly	Leu	Glu	Phe	Tyr	Met	Gly	Lys	Phe	His	Lys	Ala	Cys	Phe	Pro	Asn
				245					250						255
Ser	Thr	Asp	Ala	Glu	Pro	Val	Gly	Asp	Phe	Pro	Cys	Gly	Lys	Glu	Ala
			260					265					270		
Pro	Ala	Arg	Leu	Cys	Asp	Ser	Asp	Thr	Glu	Cys	Arg	Glu	Tyr	Trp	Pro
		275					280					285			
Gly	Pro	Asn	Phe	Gly	Ile	Thr	Asn	Phe	Asp	Asn	Ile	Leu	Phe	Ala	Ile
	290					295					300				
Leu	Thr	Val	Phe	Gln	Cys	Ile	Thr	Met	Glu	Gly	Trp	Thr	Asp	Ile	Leu
305					310					315					320
Tyr	Asn	Thr	Asn	Asp	Ala	Ala	Gly	Asn	Thr	Trp	Asn	Trp	Leu	Tyr	Phe
				325					330					335	
Ile	Pro	Leu	Ile	Ile	Ile	Gly	Ser	Phe	Phe	Met	Leu	Asn	Leu	Val	Leu
			340					345					350		
Gly	Val	Leu	Ser	Gly	Glu	Phe	Ala	Lys	Glu	Arg	Glu	Arg	Val	Glu	Asn
		355					360					365			
Arg	Arg	Ala	Phe	Leu	Lys	Leu	Arg	Arg	Gln	Gln	Gln	Ile	Glu	Arg	Glu
	370					375					380				
Leu	Asn	Gly	Tyr	Leu	Glu	Trp	Ile	Phe	Lys	Ala	Glu	Glu	Val	Met	Leu
385					390					395					400
Ala	Glu	Glu	Asp	Lys	Asn	Ala	Glu	Glu	Lys	Ser	Pro	Leu	Asp	Val	Leu
				405					410					415	
Lys	Arg	Ala	Ala	Thr	Lys	Lys	Ser	Arg	Asn	Asp	Leu	Ile	His	Ala	Glu
			420					425					430		
Glu	Gly	Glu	Asp	Arg	Phe	Val	Asp	Leu	Cys	Ala	Ala	Gly	Ser	Pro	Phe
		435					440					445			

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Ala Arg Ala Ser Leu Lys Ser Gly Lys Thr Glu Ser Ser Ser Tyr Phe
 450 455 460

Arg Arg Lys Glu Lys Met Phe Arg Phe Leu Ile Arg Arg Met Val Lys
 465 470 475 480

Ala Gln Ser Phe Tyr Trp Val Val Leu Cys Val Val Ala Leu Asn Thr
 485 490 495

Leu Cys Val Ala Met Val His Tyr Asn Gln Pro Gln Arg Leu Thr Thr
 500 505 510

Ala Leu Tyr Phe Ala Glu Phe Val Phe Leu Gly Leu Phe Leu Thr Glu
 515 520 525

Met Ser Leu Lys Met Tyr Gly Leu Gly Pro Arg Ser Tyr Phe Arg Ser
 530 535 540

Ser Phe Asn Cys Phe Asp Phe Gly Val Ile Val Gly Ser Ile Phe Glu
 545 550 555 560

Val Val Trp Ala Ala Ile Lys Pro Gly Thr Ser Phe Gly Ile Ser Val
 565 570 575

Leu Arg Ala Leu Arg Leu Leu Arg Ile Phe Lys Val Thr Lys Tyr Trp
 580 585 590

Asn Ser Leu Arg Asn Leu Val Val Ser Leu Leu Asn Ser Met Lys Ser
 595 600 605

Ile Ile Ser Leu Leu Phe Leu Leu Phe Leu Phe Ile Val Val Phe Ala
 610 615 620

Leu Leu Gly Met Gln Leu Phe Gly Gly Gln Phe Asn Phe Gln Asp Glu
 625 630 635 640

Thr Pro Thr Thr Asn Phe Asp Thr Phe Pro Ala Ala Ile Leu Thr Val
 645 650 655

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Phe Gln Ile Leu Thr Gly Glu Asp Trp Asn Ala Val Met Tyr His Gly
 660 665 670

Ile Glu Ser Gln Gly Gly Val Ser Lys Gly Met Phe Ser Ser Phe Tyr
 675 680 685

Phe Ile Val Leu Thr Leu Phe Gly Asn Tyr Thr Leu Leu Asn Val Phe
 690 695 700

Leu Ala Ile Ala Val Asp Asn Leu Ala Asn Ala Gln Glu Leu Thr Lys
 705 710 715 720

Asp Glu Glu Glu Met Glu Glu Ala Ala Asn Gln Lys Leu Ala Leu Gln
 725 730 735

Lys Ala Lys Glu Val Ala Glu Val Ser Pro Met Ser Ala Ala Asn Ile
 740 745 750

Ser Ile Ala Ala Arg Gln Gln Asn Ser Ala Lys Ala Arg Ser Val Trp
 755 760 765

Glu Gln Arg Ala Ser Gln Leu Arg Leu Gln Asn Leu Arg Ala Ser Cys
 770 775 780

Glu Ala Leu Tyr Ser Glu Met Asp Pro Glu Glu Arg Leu Arg Tyr Ala
 785 790 795 800

Ser Thr Arg His Val Arg Pro Asp Met Lys Thr His Met Asp Arg Pro
 805 810 815

Leu Val Val Glu Pro Gly Arg Asp Gly Leu Arg Gly Pro Ala Gly Asn
 820 825 830

Lys Ser Lys Pro Glu Gly Thr Glu Ala Thr Glu Gly Ala Asp Pro Pro
 835 840 845

Arg Arg His His Arg His Arg Asp Arg Asp Lys Thr Ser Ala Ser Thr
 850 855 860

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Pro Ala Gly Gly Glu Gln Asp Arg Thr Asp Cys Pro Lys Ala Glu Ser
 865 870 875 880

Thr Glu Thr Gly Ala Arg Glu Glu Arg Ala Arg Pro Arg Arg Ser His
 885 890 895

Ser Lys Glu Ala Pro Gly Ala Asp Thr Gln Val Arg Cys Glu Arg Ser
 900 905 910

Arg Arg His His Arg Arg Gly Ser Pro Glu Glu Ala Thr Glu Arg Glu
 915 920 925

Pro Arg Arg His Arg Ala His Arg His Ala Gln Asp Ser Ser Lys Glu
 930 935 940

Gly Lys Glu Gly Thr Ala Pro Val Leu Val Pro Lys Gly Glu Arg Arg
 945 950 955 960

Ala Arg His Arg Gly Pro Arg Thr Gly Pro Arg Glu Thr Glu Asn Ser
 965 970 975

Glu Glu Pro Thr Arg Arg His Arg Ala Lys His Lys Val Pro Pro Thr
 980 985 990

Leu Glu Pro Pro Glu Arg Glu Val Ala Glu Lys Glu Ser Asn Val Val
 995 1000 1005

Glu Gly Asp Lys Glu Thr Arg Asn His Gln Pro Lys Glu Pro Arg
 1010 1015 1020

Cys Asp Leu Glu Ala Ile Ala Val Thr Gly Val Gly Ser Leu His
 1025 1030 1035

Met Leu Pro Ser Thr Cys Leu Gln Lys Val Asp Glu Gln Pro Glu
 1040 1045 1050

Asp Ala Asp Asn Gln Arg Asn Val Thr Arg Met Gly Ser Gln Pro
 1055 1060 1065

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Ser	Asp	Pro	Ser	Thr	Thr	Val	His	Val	Pro	Val	Thr	Leu	Thr	Gly
1070						1075					1080			
Pro	Pro	Gly	Glu	Ala	Thr	Val	Val	Pro	Ser	Ala	Asn	Thr	Asp	Leu
1085						1090					1095			
Glu	Gly	Gln	Ala	Glu	Gly	Lys	Lys	Glu	Ala	Glu	Ala	Asp	Asp	Val
1100						1105					1110			
Leu	Arg	Arg	Gly	Pro	Arg	Pro	Ile	Val	Pro	Tyr	Ser	Ser	Met	Phe
1115						1120					1125			
Cys	Leu	Ser	Pro	Thr	Asn	Leu	Leu	Arg	Arg	Phe	Cys	His	Tyr	Ile
1130						1135					1140			
Val	Thr	Met	Arg	Tyr	Phe	Glu	Met	Val	Ile	Leu	Val	Val	Ile	Ala
1145						1150					1155			
Leu	Ser	Ser	Ile	Ala	Leu	Ala	Ala	Glu	Asp	Pro	Val	Arg	Thr	Asp
1160						1165					1170			
Ser	Phe	Arg	Asn	Asn	Ala	Leu	Lys	Tyr	Met	Asp	Tyr	Ile	Phe	Thr
1175						1180					1185			
Gly	Val	Phe	Thr	Phe	Glu	Met	Val	Ile	Lys	Met	Ile	Asp	Leu	Gly
1190						1195					1200			
Leu	Leu	Leu	His	Pro	Gly	Ala	Tyr	Phe	Arg	Asp	Leu	Trp	Asn	Ile
1205						1210					1215			
Leu	Asp	Phe	Ile	Val	Val	Ser	Gly	Ala	Leu	Val	Ala	Phe	Ala	Phe
1220						1225					1230			
Ser	Gly	Ser	Lys	Gly	Lys	Asp	Ile	Asn	Thr	Ile	Lys	Ser	Leu	Arg
1235						1240					1245			
Val	Leu	Arg	Val	Leu	Arg	Pro	Leu	Lys	Thr	Ile	Lys	Arg	Leu	Pro
1250						1255					1260			

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Lys Leu	Lys Ala Val Phe Asp	Cys Val Val Asn Ser	Leu Lys Asn
1265	1270	1275	
Val Leu	Asn Ile Leu Ile Val	Tyr Met Leu Phe Met	Phe Ile Phe
1280	1285	1290	
Ala Val	Ile Ala Val Gln Leu	Phe Lys Gly Lys Phe	Phe Tyr Cys
1295	1300	1305	
Thr Asp	Glu Ser Lys Glu Leu	Glu Arg Asp Cys Arg	Gly Gln Tyr
1310	1315	1320	
Leu Asp	Tyr Glu Lys Glu Glu	Val Glu Ala Gln Pro	Arg Gln Trp
1325	1330	1335	
Lys Lys	Tyr Asp Phe His Tyr	Asp Asn Val Leu Trp	Ala Leu Leu
1340	1345	1350	
Thr Leu	Phe Thr Val Ser Thr	Gly Glu Gly Trp Pro	Met Val Leu
1355	1360	1365	
Lys His	Ser Val Asp Ala Thr	Tyr Glu Glu Gln Gly	Pro Ser Pro
1370	1375	1380	
Gly Phe	Arg Met Glu Leu Ser	Ile Phe Tyr Val Val	Tyr Phe Val
1385	1390	1395	
Val Phe	Pro Phe Phe Phe Val	Asn Ile Phe Val Ala	Leu Ile Ile
1400	1405	1410	
Ile Thr	Phe Gln Glu Gln Gly	Asp Lys Val Met Ser	Glu Cys Ser
1415	1420	1425	
Leu Glu	Lys Asn Glu Arg Ala	Cys Ile Asp Phe Ala	Ile Ser Ala
1430	1435	1440	
Lys Pro	Leu Thr Arg Tyr Met	Pro Gln Asn Lys Gln	Ser Phe Gln
1445	1450	1455	

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Tyr	Lys	Thr	Trp	Thr	Phe	Val	Val	Ser	Pro	Pro	Phe	Glu	Tyr	Phe
1460						1465					1470			
Ile	Met	Ala	Met	Ile	Ala	Leu	Asn	Thr	Val	Val	Leu	Met	Met	Lys
1475						1480					1485			
Phe	Tyr	Asp	Ala	Pro	Tyr	Glu	Tyr	Glu	Leu	Met	Leu	Lys	Cys	Leu
1490						1495					1500			
Asn	Ile	Val	Phe	Thr	Ser	Met	Phe	Ser	Leu	Glu	Cys	Ile	Leu	Lys
1505						1510					1515			
Ile	Ile	Ala	Phe	Gly	Val	Leu	Asn	Tyr	Phe	Arg	Asp	Ala	Trp	Asn
1520						1525					1530			
Val	Phe	Asp	Phe	Val	Thr	Val	Leu	Gly	Ser	Ile	Thr	Asp	Ile	Leu
1535						1540					1545			
Val	Thr	Glu	Ile	Ala	Glu	Thr	Asn	Asn	Phe	Ile	Asn	Leu	Ser	Phe
1550						1555					1560			
Leu	Arg	Leu	Phe	Arg	Ala	Ala	Arg	Leu	Ile	Lys	Leu	Leu	Arg	Gln
1565						1570					1575			
Gly	Tyr	Thr	Ile	Arg	Ile	Leu	Leu	Trp	Thr	Phe	Val	Gln	Ser	Phe
1580						1585					1590			
Lys	Ala	Leu	Pro	Tyr	Val	Cys	Leu	Leu	Ile	Ala	Met	Leu	Phe	Phe
1595						1600					1605			
Ile	Tyr	Ala	Ile	Ile	Gly	Met	Gln	Val	Phe	Gly	Asn	Ile	Ala	Leu
1610						1615					1620			
Asp	Asp	Gly	Thr	Ser	Ile	Asn	Arg	His	Asn	Asn	Phe	Arg	Thr	Phe
1625						1630					1635			
Leu	Gln	Ala	Leu	Met	Leu	Leu	Phe	Arg	Ser	Ala	Thr	Gly	Glu	Ala
1640						1645					1650			

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Trp	His	Glu	Ile	Met	Leu	Ser	Cys	Leu	Gly	Asn	Arg	Ala	Cys	Asp
1655						1660					1665			
Pro	His	Ala	Asn	Ala	Ser	Glu	Cys	Gly	Ser	Asp	Phe	Ala	Tyr	Phe
1670						1675					1680			
Tyr	Phe	Val	Ser	Phe	Ile	Phe	Leu	Cys	Ser	Phe	Leu	Met	Leu	Asn
1685						1690					1695			
Leu	Phe	Val	Ala	Val	Ile	Met	Asp	Asn	Phe	Glu	Tyr	Leu	Thr	Arg
1700						1705					1710			
Asp	Ser	Ser	Ile	Leu	Gly	Pro	His	His	Leu	Asp	Glu	Phe	Ile	Arg
1715						1720					1725			
Val	Trp	Ala	Glu	Tyr	Asp	Pro	Ala	Ala	Cys	Gly	Arg	Ile	Ser	Tyr
1730						1735					1740			
Asn	Asp	Met	Phe	Glu	Met	Leu	Lys	His	Met	Ser	Pro	Pro	Leu	Gly
1745						1750					1755			
Leu	Gly	Lys	Lys	Cys	Pro	Ala	Arg	Val	Ala	Tyr	Lys	Arg	Leu	Val
1760						1765					1770			
Arg	Met	Asn	Met	Pro	Ile	Ser	Asn	Glu	Asp	Met	Thr	Val	His	Phe
1775						1780					1785			
Thr	Ser	Thr	Leu	Met	Ala	Leu	Ile	Arg	Thr	Ala	Leu	Glu	Ile	Lys
1790						1795					1800			
Leu	Ala	Pro	Ala	Gly	Thr	Lys	Gln	His	Gln	Cys	Asp	Ala	Glu	Leu
1805						1810					1815			
Arg	Lys	Glu	Ile	Ser	Ser	Val	Trp	Ala	Asn	Leu	Pro	Gln	Lys	Thr
1820						1825					1830			
Leu	Asp	Leu	Leu	Val	Pro	Pro	His	Lys	Pro	Asp	Glu	Met	Thr	Val
1835						1840					1845			

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Gly	Lys	Val	Tyr	Ala	Ala	Leu	Met	Ile	Phe	Asp	Phe	Tyr	Lys	Gln
1850						1855					1860			
Asn	Lys	Thr	Thr	Arg	Asp	Gln	Thr	His	Gln	Ala	Pro	Gly	Gly	Leu
1865						1870					1875			
Ser	Gln	Met	Gly	Pro	Val	Ser	Leu	Phe	His	Pro	Leu	Lys	Ala	Thr
1880						1885					1890			
Leu	Glu	Gln	Thr	Gln	Pro	Ala	Val	Leu	Arg	Gly	Ala	Arg	Val	Phe
1895						1900					1905			
Leu	Arg	Gln	Lys	Ser	Ala	Thr	Ser	Leu	Ser	Asn	Gly	Gly	Ala	Ile
1910						1915					1920			
Gln	Thr	Gln	Glu	Ser	Gly	Ile	Lys	Glu	Ser	Leu	Ser	Trp	Gly	Thr
1925						1930					1935			
Gln	Arg	Thr	Gln	Asp	Val	Leu	Tyr	Glu	Ala	Arg	Ala	Pro	Leu	Glu
1940						1945					1950			
Arg	Gly	His	Ser	Ala	Glu	Ile	Pro	Val	Gly	Gln	Pro	Gly	Ala	Leu
1955						1960					1965			
Ala	Val	Asp	Val	Gln	Met	Gln	Asn	Met	Thr	Leu	Arg	Gly	Pro	Asp
1970						1975					1980			
Gly	Glu	Pro	Gln	Pro	Gly	Leu	Glu	Ser	Gln	Gly	Arg	Ala	Ala	Ser
1985						1990					1995			
Met	Pro	Arg	Leu	Ala	Ala	Glu	Thr	Gln	Pro	Ala	Pro	Asn	Ala	Ser
2000						2005					2010			
Pro	Met	Lys	Arg	Ser	Ile	Ser	Thr	Leu	Ala	Pro	Arg	Pro	His	Gly
2015						2020					2025			
Thr	Gln	Leu	Cys	Asn	Thr	Val	Leu	Asp	Arg	Pro	Pro	Pro	Ser	Gln
2030						2035					2040			

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Val	Ser	His	His	His	His	His	Arg	Cys	His	Arg	Arg	Arg	Asp	Lys
2045						2050					2055			
Lys	Gln	Arg	Ser	Leu	Glu	Lys	Gly	Pro	Ser	Leu	Ser	Val	Asp	Thr
2060						2065					2070			
Glu	Gly	Ala	Pro	Ser	Thr	Ala	Ala	Gly	Ser	Gly	Leu	Pro	His	Gly
2075						2080					2085			
Glu	Gly	Ser	Thr	Gly	Cys	Arg	Arg	Glu	Arg	Lys	Gln	Glu	Arg	Gly
2090						2095					2100			
Arg	Ser	Gln	Glu	Arg	Arg	Gln	Pro	Ser	Ser	Ser	Ser	Ser	Glu	Lys
2105						2110					2115			
Gln	Arg	Phe	Tyr	Ser	Cys	Asp	Arg	Phe	Gly	Ser	Arg	Glu	Pro	Pro
2120						2125					2130			
Gln	Pro	Lys	Pro	Ser	Leu	Ser	Ser	His	Pro	Ile	Ser	Pro	Thr	Ala
2135						2140					2145			
Ala	Leu	Glu	Pro	Gly	Pro	His	Pro	Gln	Gly	Ser	Gly	Ser	Val	Asn
2150						2155					2160			
Gly	Ser	Pro	Leu	Met	Ser	Thr	Ser	Gly	Ala	Ser	Thr	Pro	Gly	Arg
2165						2170					2175			
Gly	Gly	Arg	Arg	Gln	Leu	Pro	Gln	Thr	Pro	Leu	Thr	Pro	Arg	Pro
2180						2185					2190			
Ser	Ile	Thr	Tyr	Lys	Thr	Ala	Asn	Ser	Ser	Pro	Val	His	Phe	Ala
2195						2200					2205			
Glu	Gly	Gln	Ser	Gly	Leu	Pro	Ala	Phe	Ser	Pro	Gly	Arg	Leu	Ser
2210						2215					2220			
Arg	Gly	Leu	Ser	Glu	His	Asn	Ala	Leu	Leu	Gln	Lys	Glu	Pro	Leu
2225						2230					2235			

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Ser Gln Pro Leu Ala Ser Gly Ser Arg Ile Gly Ser Asp Pro Tyr
 2240 2245 2250

Leu Gly Gln Arg Leu Asp Ser Glu Ala Ser Ala His Asn Leu Pro
 2255 2260 2265

Glu Asp Thr Leu Thr Phe Glu Glu Ala Val Ala Thr Asn Ser Gly
 2270 2275 2280

Arg Ser Ser Arg Thr Ser Tyr Val Ser Ser Leu Thr Ser Gln Ser
 2285 2290 2295

His Pro Leu Arg Arg Val Pro Asn Gly Tyr His Cys Thr Leu Gly
 2300 2305 2310

Leu Ser Thr Gly Val Arg Ala Arg His Ser Tyr His His Pro Asp
 2315 2320 2325

Gln Asp His Trp Cys
 2330

<210> 9

<211> 2339

<212> PRT

<213> Homo sapiens

<220>

<221> PEPTIDE

<222> (1) .. (2339)

<223>

<400> 9

Met Val Arg Phe Gly Asp Glu Leu Gly Gly Arg Tyr Gly Gly Pro Gly
 1 5 10 15

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Gly Gly Glu Arg Ala Arg Gly Gly Gly Ala Gly Gly Ala Gly Gly Pro
 20 25 30

Gly Pro Gly Gly Leu Gln Pro Gly Gln Arg Val Leu Tyr Lys Gln Ser
 35 40 45

Ile Ala Gln Arg Ala Arg Thr Met Ala Leu Tyr Asn Pro Ile Pro Val
 50 55 60

Lys Gln Asn Cys Phe Thr Val Asn Arg Ser Leu Phe Val Phe Ser Glu
 65 70 75 80

Asp Asn Val Val Arg Lys Tyr Ala Lys Arg Ile Thr Glu Trp Pro Pro
 85 90 95

Phe Glu Tyr Met Ile Leu Ala Thr Ile Ile Ala Asn Cys Ile Val Leu
 100 105 110

Ala Leu Glu Gln His Leu Pro Asp Gly Asp Lys Thr Pro Met Ser Glu
 115 120 125

Arg Leu Asp Asp Thr Glu Pro Tyr Phe Ile Gly Ile Phe Cys Phe Glu
 130 135 140

Ala Gly Ile Lys Ile Ile Ala Leu Gly Phe Val Phe His Lys Gly Ser
 145 150 155 160

Tyr Leu Arg Asn Gly Trp Asn Val Met Asp Phe Val Val Val Leu Thr
 165 170 175

Gly Ile Leu Ala Thr Ala Gly Thr Asp Phe Asp Leu Arg Thr Leu Arg
 180 185 190

Ala Val Arg Val Leu Arg Pro Leu Lys Leu Val Ser Gly Ile Pro Ser
 195 200 205

Leu Gln Val Val Leu Lys Ser Ile Met Lys Ala Met Val Pro Leu Leu
 210 215 220

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Gln Ile Gly Leu Leu Leu Phe Phe Ala Ile Leu Met Phe Ala Ile Ile
 225 230 235 240

Gly Leu Glu Phe Tyr Met Gly Lys Phe His Lys Ala Cys Phe Pro Asn
 245 250 255

Ser Thr Asp Ala Glu Pro Val Gly Asp Phe Pro Cys Gly Lys Glu Ala
 260 265 270

Pro Ala Arg Leu Cys Glu Gly Asp Thr Glu Cys Arg Glu Tyr Trp Pro
 275 280 285

Gly Pro Asn Phe Gly Ile Thr Asn Phe Asp Asn Ile Leu Phe Ala Ile
 290 295 300

Leu Thr Val Phe Gln Cys Ile Thr Met Glu Gly Trp Thr Asp Ile Leu
 305 310 315 320

Tyr Asn Thr Asn Asp Ala Ala Gly Asn Thr Trp Asn Trp Leu Tyr Phe
 325 330 335

Ile Pro Leu Ile Ile Ile Gly Ser Phe Phe Met Leu Asn Leu Val Leu
 340 345 350

Gly Val Leu Ser Gly Glu Phe Ala Lys Glu Arg Glu Arg Val Glu Asn
 355 360 365

Arg Arg Ala Phe Leu Lys Leu Arg Arg Gln Gln Gln Ile Glu Arg Glu
 370 375 380

Leu Asn Gly Tyr Leu Glu Trp Ile Phe Lys Ala Glu Glu Val Met Leu
 385 390 395 400

Ala Glu Glu Asp Arg Asn Ala Glu Glu Lys Ser Pro Leu Asp Val Leu
 405 410 415

Lys Arg Ala Ala Thr Lys Lys Ser Arg Asn Asp Leu Ile His Ala Glu
 420 425 430

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Glu Gly Glu Asp Arg Phe Ala Asp Leu Cys Ala Val Gly Ser Pro Phe
 435 440 445

Ala Arg Ala Ser Leu Lys Ser Gly Lys Thr Glu Ser Ser Ser Tyr Phe
 450 455 460

Arg Arg Lys Glu Lys Met Phe Arg Phe Phe Ile Arg Arg Met Val Lys
 465 470 475 480

Ala Gln Ser Phe Tyr Trp Val Val Leu Cys Val Val Ala Leu Asn Thr
 485 490 495

Leu Cys Val Ala Met Val His Tyr Asn Gln Pro Arg Arg Leu Thr Thr
 500 505 510

Thr Leu Tyr Phe Ala Glu Phe Val Phe Leu Gly Leu Phe Leu Thr Glu
 515 520 525

Met Ser Leu Lys Met Tyr Gly Leu Gly Pro Arg Ser Tyr Phe Arg Ser
 530 535 540

Ser Phe Asn Cys Phe Asp Phe Gly Val Ile Val Gly Ser Val Phe Glu
 545 550 555 560

Val Val Trp Ala Ala Ile Lys Pro Gly Ser Ser Phe Gly Ile Ser Val
 565 570 575

Leu Arg Ala Leu Arg Leu Leu Arg Ile Phe Lys Val Thr Lys Tyr Trp
 580 585 590

Ser Ser Leu Arg Asn Leu Val Val Ser Leu Leu Asn Ser Met Lys Ser
 595 600 605

Ile Ile Ser Leu Leu Phe Leu Leu Phe Leu Phe Ile Val Val Phe Ala
 610 615 620

Leu Leu Gly Met Gln Leu Phe Gly Gly Gln Phe Asn Phe Gln Asp Glu
 625 630 635 640

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Thr Pro Thr Thr Asn Phe Asp Thr Phe Pro Ala Ala Ile Leu Thr Val
 645 650 655

Phe Gln Ile Leu Thr Gly Glu Asp Trp Asn Ala Val Met Tyr His Gly
 660 665 670

Ile Glu Ser Gln Gly Gly Val Ser Lys Gly Met Phe Ser Ser Phe Tyr
 675 680 685

Phe Ile Val Leu Thr Leu Phe Gly Asn Tyr Thr Leu Leu Asn Val Phe
 690 695 700

Leu Ala Ile Ala Val Asp Asn Leu Ala Asn Ala Gln Glu Leu Thr Lys
 705 710 715 720

Asp Glu Glu Glu Met Glu Glu Ala Ala Asn Gln Lys Leu Ala Leu Gln
 725 730 735

Lys Ala Lys Glu Val Ala Glu Val Ser Pro Met Ser Ala Ala Asn Ile
 740 745 750

Ser Ile Ala Ala Arg Gln Gln Asn Ser Ala Lys Ala Arg Ser Val Trp
 755 760 765

Glu Gln Arg Ala Ser Gln Leu Arg Leu Gln Asn Leu Arg Ala Ser Cys
 770 775 780

Glu Ala Leu Tyr Ser Glu Met Asp Pro Glu Glu Arg Leu Arg Phe Ala
 785 790 795 800

Thr Thr Arg His Leu Arg Pro Asp Met Lys Thr His Leu Asp Arg Pro
 805 810 815

Leu Val Val Glu Leu Gly Arg Asp Gly Ala Arg Gly Pro Val Gly Gly
 820 825 830

Lys Ala Arg Pro Glu Ala Ala Glu Ala Pro Glu Gly Val Asp Pro Pro
 835 840 845

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Arg Arg His His Arg His Arg Asp Lys Asp Lys Thr Pro Ala Ala Gly
850 855 860

Asp Gln Asp Arg Ala Glu Ala Pro Lys Ala Glu Ser Gly Glu Pro Gly
865 870 875 880

Ala Arg Glu Glu Arg Pro Arg Pro His Arg Ser His Ser Lys Glu Ala
885 890 895

Ala Gly Pro Pro Glu Ala Arg Ser Glu Arg Gly Arg Gly Pro Gly Pro
900 905 910

Glu Gly Gly Arg Arg His His Arg Arg Gly Ser Pro Glu Glu Ala Ala
915 920 925

Glu Arg Glu Pro Arg Arg His Arg Ala His Arg His Gln Asp Pro Ser
930 935 940

Lys Glu Cys Ala Gly Ala Lys Gly Glu Arg Arg Ala Arg His Arg Gly
945 950 955 960

Gly Pro Arg Ala Gly Pro Arg Glu Ala Glu Ser Gly Glu Glu Pro Ala
965 970 975

Arg Arg His Arg Ala Arg His Lys Ala Gln Pro Ala His Glu Ala Val
980 985 990

Glu Lys Glu Thr Thr Glu Lys Glu Ala Thr Glu Lys Glu Ala Glu Ile
995 1000 1005

Val Glu Ala Asp Lys Glu Lys Glu Leu Arg Asn His Gln Pro Arg
1010 1015 1020

Glu Pro His Cys Asp Leu Glu Thr Ser Gly Thr Val Thr Val Gly
1025 1030 1035

Pro Met His Thr Leu Pro Ser Thr Cys Leu Gln Lys Val Glu Glu
1040 1045 1050

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Gln Pro	Glu Asp Ala Asp Asn	Gln Arg Asn Val Thr	Arg Met Gly
1055	1060	1065	
Ser Gln	Pro Pro Asp Pro Asn	Thr Ile Val His Ile	Pro Val Met
1070	1075	1080	
Leu Thr	Gly Pro Leu Gly Glu	Ala Thr Val Val Pro	Ser Gly Asn
1085	1090	1095	
Val Asp	Leu Glu Ser Gln Ala	Glu Gly Lys Lys Glu	Val Glu Ala
1100	1105	1110	
Asp Asp	Val Met Arg Ser Gly	Pro Arg Pro Ile Val	Pro Tyr Ser
1115	1120	1125	
Ser Met	Phe Cys Leu Ser Pro	Thr Asn Leu Leu Arg	Arg Phe Cys
1130	1135	1140	
His Tyr	Ile Val Thr Met Arg	Tyr Phe Glu Val Val	Ile Leu Val
1145	1150	1155	
Val Ile	Ala Leu Ser Ser Ile	Ala Leu Ala Ala Glu	Asp Pro Val
1160	1165	1170	
Arg Thr	Asp Ser Pro Arg Asn	Asn Ala Leu Lys Tyr	Leu Asp Tyr
1175	1180	1185	
Ile Phe	Thr Gly Val Phe Thr	Phe Glu Met Val Ile	Lys Met Ile
1190	1195	1200	
Asp Leu	Gly Leu Leu Leu His	Pro Gly Ala Tyr Phe	Arg Asp Leu
1205	1210	1215	
Trp Asn	Ile Leu Asp Phe Ile	Val Val Ser Gly Ala	Leu Val Ala
1220	1225	1230	
Phe Ala	Phe Ser Gly Ser Lys	Gly Lys Asp Ile Asn	Thr Ile Lys
1235	1240	1245	

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Ser	Leu	Arg	Val	Leu	Arg	Val	Leu	Arg	Pro	Leu	Lys	Thr	Ile	Lys
	1250					1255					1260			
Arg	Leu	Pro	Lys	Leu	Lys	Ala	Val	Phe	Asp	Cys	Val	Val	Asn	Ser
	1265					1270					1275			
Leu	Lys	Asn	Val	Leu	Asn	Ile	Leu	Ile	Val	Tyr	Met	Leu	Phe	Met
	1280					1285					1290			
Phe	Ile	Phe	Ala	Val	Ile	Ala	Val	Gln	Leu	Phe	Lys	Gly	Lys	Phe
	1295					1300					1305			
Phe	Tyr	Cys	Thr	Asp	Glu	Ser	Lys	Glu	Leu	Glu	Arg	Asp	Cys	Arg
	1310					1315					1320			
Gly	Gln	Tyr	Leu	Asp	Tyr	Glu	Lys	Glu	Glu	Val	Glu	Ala	Gln	Pro
	1325					1330					1335			
Arg	Gln	Trp	Lys	Lys	Tyr	Asp	Phe	His	Tyr	Asp	Asn	Val	Leu	Trp
	1340					1345					1350			
Ala	Leu	Leu	Thr	Leu	Phe	Thr	Val	Ser	Thr	Gly	Glu	Gly	Trp	Pro
	1355					1360					1365			
Met	Val	Leu	Lys	His	Ser	Val	Asp	Ala	Thr	Tyr	Glu	Glu	Gln	Gly
	1370					1375					1380			
Pro	Ser	Pro	Gly	Tyr	Arg	Met	Glu	Leu	Ser	Ile	Phe	Tyr	Val	Val
	1385					1390					1395			
Tyr	Phe	Val	Val	Phe	Pro	Phe	Phe	Phe	Val	Asn	Ile	Phe	Val	Ala
	1400					1405					1410			
Leu	Ile	Ile	Ile	Thr	Phe	Gln	Glu	Gln	Gly	Asp	Lys	Val	Met	Ser
	1415					1420					1425			
Glu	Cys	Ser	Leu	Glu	Lys	Asn	Glu	Arg	Ala	Cys	Ile	Asp	Phe	Ala
	1430					1435					1440			

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Ile Ser Ala Lys Pro Leu Thr Arg Tyr Met Pro Gln Asn Arg Gln
 1445 1450 1455

Ser Phe Gln Tyr Lys Thr Trp Thr Phe Val Val Ser Pro Pro Phe
 1460 1465 1470

Glu Tyr Phe Ile Met Ala Met Ile Ala Leu Asn Thr Val Val Leu
 1475 1480 1485

Met Met Lys Phe Tyr Asp Ala Pro Tyr Glu Tyr Glu Leu Met Leu
 1490 1495 1500

Lys Cys Leu Asn Ile Val Phe Thr Ser Met Phe Ser Met Glu Cys
 1505 1510 1515

Val Leu Lys Ile Ile Ala Phe Gly Val Leu Asn Tyr Phe Arg Asp
 1520 1525 1530

Ala Trp Asn Val Phe Asp Phe Val Thr Val Leu Gly Ser Ile Thr
 1535 1540 1545

Asp Ile Leu Val Thr Glu Ile Ala Glu Thr Asn Asn Phe Ile Asn
 1550 1555 1560

Leu Ser Phe Leu Arg Leu Phe Arg Ala Ala Arg Leu Ile Lys Leu
 1565 1570 1575

Leu Arg Gln Gly Tyr Thr Ile Arg Ile Leu Leu Trp Thr Phe Val
 1580 1585 1590

Gln Ser Phe Lys Ala Leu Pro Tyr Val Cys Leu Leu Ile Ala Met
 1595 1600 1605

Leu Phe Phe Ile Tyr Ala Ile Ile Gly Met Gln Val Phe Gly Asn
 1610 1615 1620

Ile Ala Leu Asp Asp Asp Thr Ser Ile Asn Arg His Asn Asn Phe
 1625 1630 1635

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Arg	Thr	Phe	Leu	Gln	Ala	Leu	Met	Leu	Leu	Phe	Arg	Ser	Ala	Thr
1640						1645					1650			
Gly	Glu	Ala	Trp	His	Glu	Ile	Met	Leu	Ser	Cys	Leu	Ser	Asn	Gln
1655						1660					1665			
Ala	Cys	Asp	Glu	Gln	Ala	Asn	Ala	Thr	Glu	Cys	Gly	Ser	Asp	Phe
1670						1675					1680			
Ala	Tyr	Phe	Tyr	Phe	Val	Ser	Phe	Ile	Phe	Leu	Cys	Ser	Phe	Leu
1685						1690					1695			
Met	Leu	Asn	Leu	Phe	Val	Ala	Val	Ile	Met	Asp	Asn	Phe	Glu	Tyr
1700						1705					1710			
Leu	Thr	Arg	Asp	Ser	Ser	Ile	Leu	Gly	Pro	His	His	Leu	Asp	Glu
1715						1720					1725			
Phe	Ile	Arg	Val	Trp	Ala	Glu	Tyr	Asp	Pro	Ala	Ala	Cys	Gly	Arg
1730						1735					1740			
Ile	Ser	Tyr	Asn	Asp	Met	Phe	Glu	Met	Leu	Lys	His	Met	Ser	Pro
1745						1750					1755			
Pro	Leu	Gly	Leu	Gly	Lys	Lys	Cys	Pro	Ala	Arg	Val	Ala	Tyr	Lys
1760						1765					1770			
Arg	Leu	Val	Arg	Met	Asn	Met	Pro	Ile	Ser	Asn	Glu	Asp	Met	Thr
1775						1780					1785			
Val	His	Phe	Thr	Ser	Thr	Leu	Met	Ala	Leu	Ile	Arg	Thr	Ala	Leu
1790						1795					1800			
Glu	Ile	Lys	Leu	Ala	Pro	Ala	Gly	Thr	Lys	Gln	His	Gln	Cys	Asp
1805						1810					1815			
Ala	Glu	Leu	Arg	Lys	Glu	Ile	Ser	Val	Val	Trp	Ala	Asn	Leu	Pro
1820						1825					1830			

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Gln	Lys	Thr	Leu	Asp	Leu	Leu	Val	Pro	Pro	His	Lys	Pro	Asp	Glu
1835						1840					1845			
Met	Thr	Val	Gly	Lys	Val	Tyr	Ala	Ala	Leu	Met	Ile	Phe	Asp	Phe
1850						1855					1860			
Tyr	Lys	Gln	Asn	Lys	Thr	Thr	Arg	Asp	Gln	Met	Gln	Gln	Ala	Pro
1865						1870					1875			
Gly	Gly	Leu	Ser	Gln	Met	Gly	Pro	Val	Ser	Leu	Phe	His	Pro	Leu
1880						1885					1890			
Lys	Ala	Thr	Leu	Glu	Gln	Thr	Gln	Pro	Ala	Val	Leu	Arg	Gly	Ala
1895						1900					1905			
Arg	Val	Phe	Leu	Arg	Gln	Lys	Ser	Ser	Thr	Ser	Leu	Ser	Asn	Gly
1910						1915					1920			
Gly	Ala	Ile	Gln	Asn	Gln	Glu	Ser	Gly	Ile	Lys	Glu	Ser	Val	Ser
1925						1930					1935			
Trp	Gly	Thr	Gln	Arg	Thr	Gln	Asp	Ala	Pro	His	Glu	Ala	Arg	Pro
1940						1945					1950			
Pro	Leu	Glu	Arg	Gly	His	Ser	Thr	Glu	Ile	Pro	Val	Gly	Arg	Ser
1955						1960					1965			
Gly	Ala	Leu	Ala	Val	Asp	Val	Gln	Met	Gln	Ser	Ile	Thr	Arg	Arg
1970						1975					1980			
Gly	Pro	Asp	Gly	Glu	Pro	Gln	Pro	Gly	Leu	Glu	Ser	Gln	Gly	Arg
1985						1990					1995			
Ala	Ala	Ser	Met	Pro	Arg	Leu	Ala	Ala	Glu	Thr	Gln	Pro	Val	Thr
2000						2005					2010			
Asp	Ala	Ser	Pro	Met	Lys	Arg	Ser	Ile	Ser	Thr	Leu	Ala	Gln	Arg
2015						2020					2025			

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Pro	Arg	Gly	Thr	His	Leu	Cys	Ser	Thr	Thr	Pro	Asp	Arg	Pro	Pro
2030						2035					2040			
Pro	Ser	Gln	Ala	Ser	Ser	His	His	His	His	His	Arg	Cys	His	Arg
2045						2050					2055			
Arg	Arg	Asp	Arg	Lys	Gln	Arg	Ser	Leu	Glu	Lys	Gly	Pro	Ser	Leu
2060						2065					2070			
Ser	Ala	Asp	Met	Asp	Gly	Ala	Pro	Ser	Ser	Ala	Val	Gly	Pro	Gly
2075						2080					2085			
Leu	Pro	Pro	Gly	Glu	Gly	Pro	Thr	Gly	Cys	Arg	Arg	Glu	Arg	Glu
2090						2095					2100			
Arg	Arg	Gln	Glu	Arg	Gly	Arg	Ser	Gln	Glu	Arg	Arg	Gln	Pro	Ser
2105						2110					2115			
Ser	Ser	Ser	Ser	Glu	Lys	Gln	Arg	Phe	Tyr	Ser	Cys	Asp	Arg	Phe
2120						2125					2130			
Gly	Gly	Arg	Glu	Pro	Pro	Lys	Pro	Lys	Pro	Ser	Leu	Ser	Ser	His
2135						2140					2145			
Pro	Thr	Ser	Pro	Thr	Ala	Gly	Gln	Glu	Pro	Gly	Pro	His	Pro	Gln
2150						2155					2160			
Gly	Ser	Gly	Ser	Val	Asn	Gly	Ser	Pro	Leu	Leu	Ser	Thr	Ser	Gly
2165						2170					2175			
Ala	Ser	Thr	Pro	Gly	Arg	Gly	Gly	Arg	Arg	Gln	Leu	Pro	Gln	Thr
2180						2185					2190			
Pro	Leu	Thr	Pro	Arg	Pro	Ser	Ile	Thr	Tyr	Lys	Thr	Ala	Asn	Ser
2195						2200					2205			
Ser	Pro	Ile	His	Phe	Ala	Gly	Ala	Gln	Thr	Ser	Leu	Pro	Ala	Phe
2210						2215					2220			

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Ser Pro Gly Arg Leu Ser Arg Gly Leu Ser Glu His Asn Ala Leu
 2225 2230 2235

Leu Gln Arg Asp Pro Leu Ser Gln Pro Leu Ala Pro Gly Ser Arg
 2240 2245 2250

Ile Gly Ser Asp Pro Tyr Leu Gly Gln Arg Leu Asp Ser Glu Ala
 2255 2260 2265

Ser Val His Ala Leu Pro Glu Asp Thr Leu Thr Phe Glu Glu Ala
 2270 2275 2280

Val Ala Thr Asn Ser Gly Arg Ser Ser Arg Thr Ser Tyr Val Ser
 2285 2290 2295

Ser Leu Thr Ser Gln Ser His Pro Leu Arg Arg Val Pro Asn Gly
 2300 2305 2310

Tyr His Cys Thr Leu Gly Leu Ser Ser Gly Gly Arg Ala Arg His
 2315 2320 2325

Ser Tyr His His Pro Asp Gln Asp His Trp Cys
 2330 2335

<210> 10

<211> 27

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1) .. (27)

<223> primers are used to amplify the coding sequence of human Kir 2.1

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<400> 10
atgggcagtg tgcgaaccaa ccgtac
27

<210> 11

<211> 27

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1)..(27)

<223> primers are used to amplify the coding sequence of human Kir 2.1

<400> 11
tcagtcatat ctccgatacct cgccgta
27

<210> 12

<211> 20

<212> DNA

<213> artificial

<220>

<221> primer_bind

<222> (1)..(20)

<223>

<400> 12
cagacatact ccagattggc
20

<210> 13

<211> 20

<212> DNA

<213> artificial

- 77 -

<220>

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 13
tagtgtctgc tgccagatac
20

<210> 14

<211> 20

<212> DNA

<213> artificial

<220>

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 14
cagcctggat aaccatgggtt
20

<210> 15

<211> 20

<212> DNA

<213> artificial

<220>

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 15
agctgtgata gatgagacca
20

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<210> 16

<211> 20

<212> DNA

<213> artificial

<220>

<221> primer_bind

<222> (1)..(20)

<223>

<400> 16

aacaaggaga gcagtgacag
20

<210> 17

<211> 20

<212> DNA

<213> artificial

<220>

<221> primer_bind

<222> (1)..(20)

<223>

<400> 17

agaagagact cgaaaccagg
20

<210> 18

<211> 20

<212> DNA

<213> artificial

<220>

- 79 -

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 18
acatgatgca gaaggcgttg
20

<210> 19

<211> 20

<212> DNA

<213> artificial

<220>

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<222> (1) .. (20)

<223>

<400> 19
ccagctcatt cttattgcgc
20

<210> 20

<211> 20

<212> DNA

<213> artificial

<220>

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<222> (1) .. (20)

<223>

<400> 20
cttacgatgt ggtaccatcc
20

<210> 21

- 80 -

<211> 20

<212> DNA

<213> artificial

<220>

<221> primer_bind

<222> (1)..(20)

<223>

<400> 21

tgcactcatt gtggtcttgg
20

<210> 22

<211> 20

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1)..(20)

<223>

<400> 22

acagacatga tgcagaaggc
20

<210> 23

<211> 20

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1)..(20)

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<223>

<400> 23
atctaactcc aacctgcctg
20

<210> 24

<211> 20

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 24
tgattggtgg ataggaaggg
20

<210> 25

<211> 20

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 25
aatcttcttc caccagaggg
20

<210> 26

<211> 20

<212> DNA

- 82 -

<213> Artificial

<220>

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 26
caggagatgt tccagaagac
20

<210> 27

<211> 20

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<213> Artificial

<220>

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<222> (1) .. (20)

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<400> 27
tggatcatgct cagatctgtc
20

<210> 28

<211> 20

<212> DNA

<213> Artificial

<220>

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<222> (1) .. (20)

<223>

<400> 28

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acctacaaga cggccaattc
20

<210> 29

<211> 20

<212> DNA

<213> Artificial

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<221> primer_bind

<222> (1) .. (20)

<223>

<400> 29
gagaggacca tatccctcta
20

<210> 30

<211> 20

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 30
gacatctctc agaagacagc
20

<210> 31

<211> 20

<212> DNA

<213> Artificial

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<220>

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 31
acagggttgct gacataggac
20

<210> 32

<211> 20

<212> DNA

<213> Artificial

<220>

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<222> (1) .. (20)

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<400> 32
accttatgca gcagcagatc
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<210> 33

<211> 20

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1) .. (20)

<223>

<400> 33
caaagtcctg gagctcatag
20

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<210> 34

<211> 20

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1)..(20)

<223>

<400> 34
ttaccgagtc ttccaactcc
20

<210> 35

<211> 20

<212> DNA

<213> Artificial

<220>

<221> primer_bind

<222> (1)..(20)

<223>

<400> 35
gtgagagaag agcatgcatc
20

<210> 36

<211> 20

<212> DNA

<213> Artificial

<220>

<221> primer_bind

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<222> (1) .. (20)

<223>

<400> 36
ttcaccatcc agtgtctgca
20

<210> 37

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- 96 -

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<400> 62

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18

CLAIMS:

1. A method of screening for a compound comprising the steps of
 - a) contacting a recombinant cell with a test compound, wherein said recombinant cell expresses at least an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits, provided that said cell does not express a functional calcium channel; and
 - b) determining the ability of said compound to modulate the calcium flow into said cell.
2. The method of claim 1, wherein said recombinant cell also expresses a potassium channel.
3. The method of claim 1, where the ability of said test compound is further compared with the ability of said test compound to modulate the calcium flow into said cell in a cell not comprising an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits.
4. A method for identifying a compound useful for the treatment of chronic neuropathic pain, the method comprising:
 - a) contacting a ligand of an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits with an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits in the presence and absence of a test compound; and
 - b) determining whether the test compound alters the binding of the ligand to the N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits to an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits.
5. The method of claim 4, said method further comprising the steps of:
 - c) adding a compound identified that alters binding of the ligand to the N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits to an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits in step (b);
 - d) determining whether the compound alleviates chronic neuropathic pain; and
 - e) identifying a compound that alleviates

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chronic neuropathic pain in step (d) as a compound useful for the treatment of chronic neuropathic pain.

6. The method according to any one of claims 1 to 5, wherein said $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits consist of:
 - a) SEQ ID NO: 8 or 9 or variants thereof with 80% sequence identity to SEQ ID NO: 8 or 9 (for $\text{Ca}_v2.2$ ($\alpha 1\text{B}$));
 - b) SEQ ID NO: 6 or 7 or variants thereof with 80% sequence identity to SEQ ID NO: 6 or 7 (for $\alpha 2\delta 1$);
 - c) SEQ ID NO: 1 or 2 or variants thereof with 80% sequence identity to SEQ ID NO: 1 or 2 (for $\beta 1$); and
 - d) SEQ ID NO: 3, 4 or 5 or variants thereof with 80% sequence identity to SEQ ID NO: 3, 4 or 5 (for $\gamma 4$).
7. The method according to any one of claims 1 to 6, wherein said test compound is an antisense nucleotide sequence.
8. A purified antibody or a fragment thereof which specifically binds to the Pain voltage-dependent calcium channel (VDCC); said Pain VDCC consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits.
9. The antibody of claim 8, wherein said $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits consist of:
 - a) SEQ ID NO: 8 or 9 or variants thereof with 80% sequence identity to SEQ ID NO: 8 or 9 (for $\text{Ca}_v2.2$ ($\alpha 1\text{B}$));
 - b) SEQ ID NO: 6 or 7 or variants thereof with 80% sequence identity to SEQ ID NO: 6 or 7 (for $\alpha 2\delta 1$);
 - c) SEQ ID NO: 1 or 2 or variants thereof with 80% sequence identity to SEQ ID NO: 1 or 2 (for $\beta 1$); and
 - d) SEQ ID NO: 3, 4 or 5 or variants thereof with 80% sequence identity to SEQ ID NO: 3, 4 or 5 (for $\gamma 4$).
10. The antibody fragment of claim 8 which is an Fab or F(ab')_2 fragment.

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11. The antibody of claim 8 which is a polyclonal, monoclonal, chimeric or single chain antibody.
12. The method according to any one of claims 1 to 5, wherein said test compound is an antibody of claim 6.
13. The use of an antibody according to any one of claims 8 to 11 in medicine.
14. The use of an antibody according to any one of claims 8 to 11 for the manufacture of a medicament for the treatment of chronic neuropathic pain.
15. The use of a compound that binds to the $\beta 1$ and $\gamma 4$ subunits of an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits for the manufacture of a medicament for the treatment of chronic neuropathic pain.
16. A method for the treatment of chronic neuropathic pain which comprises administering an effective amount of a compound that binds to the $\beta 1$ and $\gamma 4$ subunits of an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits to a patient in need of such treatment.
17. A pharmaceutical composition for the treatment of chronic neuropathic pain which comprises a compound that binds to the $\beta 1$ and $\gamma 4$ subunits of an N-type voltage-dependent calcium channel consisting of the $\text{Ca}_v2.2$ ($\alpha 1\text{B}$), $\alpha 2\delta 1$, $\beta 1$ and $\gamma 4$ subunits, and a pharmaceutically acceptable carrier or diluent.