

Use of Cathepsin C

5 Present invention concerns the use of Cathepsin C. Other aspects of the invention concern methods for screening pharmaceuticals, for diagnosing pain susceptibility and for the treatment of pain.

10 In the western world, chronic pain is a major unsolved health problem undermining the health and welfare of millions of citizens. Chronic pain severely afflicts the well-being of the individual experiencing it and it is frequently accompanied or followed by vegetative signs, which often result in depression. Chronic pain results in individual suffering and social economic costs of tremendous extent. Existing pharmacological pain therapies are widely unsatisfying both in terms of efficacy and of safety.

15 In light of the severe drawbacks connected with state of the art pain treatments, there is a great need for novel options for treatment of ongoing pain, and for diagnosis and prognosis concerning the potential development of chronic pain. Especially in light of the vast gap between the fast advancing understanding of the neurobiology of pain and the unmet clinical need to provide effective treatments without the drawbacks of state of the art treatments, efforts need to be directed to the discovery of new targets for novel classes of analgesics.

20 Thus, it is the object of the present invention to provide a new means for the development and provision of new classes of pain modulating drugs.

25 This object is solved by the use of Cathepsin C or functional fragments or derivatives thereof for identifying compounds that modulate pain.

30 The invention is based on the surprising finding of the inventors, demonstrating for the first time that Cathepsin C expression closely correlates with pain susceptibility in mouse models of neuropathic pain.

Pain is, per definition of the international association for the study of pain, an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is normally the consequence of an activation of the nociceptive nervous system, that is specialized to detect and encode damage or potential

35

damage of tissue. Pain is thus part of a warning system of the body to initiate reactions for minimizing actual or potential damage to the body. Pain can be the primary symptom of a medical condition or can be secondary effect of a diseased state, often without any biological meaning.

5

Pain may be acute or chronic. Acute pain is a physiological signal indicating a potential or actual injury. It occurs accompanying tissue damage, infection, inflammation or other acute causes, alerting the individual after bodily damage or malfunction. If acute pain is not treated properly, it may lead to chronic pain.

10

Chronic pain is a diseased state with varying origin, duration, intensity and specific symptoms.

Chronic pain may be of nociceptive origin, inflammatory or neuropathic. Nociceptive pain is judged to be commensurate with ongoing activation of somatic or visceral pain-sensitive nerve fibers. Neuropathic pain is pain resulting from any kind of damage to peripheral or central neuronal tissue; it is believed to be sustained by aberrant somatosensory processes in the peripheral nervous system, the CNS, or both. (For an overview of pain mechanisms, see for example Scholz and Woolf, 2002; Julius and Basbaum, 2001, Woolf and Mannion, 1999; Wood, J.D., 2000; Woolf and Salter, 2000.)

20

Chronic neuropathic pain is variable from patient to patient. Recent data indicate that individual pain susceptibility plays an important role for the amount of individual suffering, i.e. there is an important heritable predisposition to pain, particularly to the development of neuropathic pain. Present invention is based on extensive studies of the inventors which aimed to identify pain susceptibility genes (i.e. genes that determine the amount of pain felt in the presence of a given, fixed degree of tissue injury) in rodent models of chronic pain. The rodent models and experimental settings used by the inventors allowed for experimental conditions where among the different individuals a) nature and uniformity of the neural lesion can be precisely controlled and b) genetic and environmental variability can be minimized.

25
30

Cathepsin C (CTSC; alternative titles: dipeptidyl (amino)peptidase I, DPPI; (EC 3.4.14.1)), is a lysosomal protease.

The gene locus of Cathepsin C is on chromosome 11q14.1-q14.3 (see Rao et al., 1997). The genomic sequence of Cathepsin C is publicly available at the NCBI nucleotide database under:

NW_925129 (genomic sequence comprising the sequence of human Cathepsin C, SEQ ID NO:3) or NW_001030863 (genomic DNA sequence comprising mouse Cathepsin C)

The coding polynucleotide sequence of Cathepsin C is publicly available at the NCBI nucleotide database under several accession numbers, such as: NM_001814 (Homo sapiens cathepsin C transcript variant 1 mRNA), BC113897 (complete coding sequence (cds) of Homo sapiens cathepsin C, transcript variant 2, mRNA), BC109386 (Homo sapiens cathepsin C, mRNA, complete cds), NM_009982 (mus musculus cathepsin C mRNA). The skilled person knows how to retrieve further coding sequences of cathepsin C from the NCBI database (cathepsin C of other species; mutants or different isoforms of cathepsin C, if existing). If in the following, it is referred to the cathepsin C coding sequence, this can mean any of the above mRNA or coding sequences; preferably, the sequence according to reference numbers NM_001814 (human) (SEQ ID NO: 1) or NM_009982 (mouse) is meant.

The protein sequence of Cathepsin C is publicly available at the NCBI protein database, e.g. under the following accession numbers: homo sapiens (hs): complete cds: CAA6067, AAL48191, AAL48192, AAL38195, AAH54028, AAQ08887, AAI00893, AAI00894, AAI00892, AAI00895, AAI09387, AAI10072, AAI13851; hs isoform CRA_b: EAW59364; hs isoform CRA_a: EAW59363; hs cathepsin C isoform a preproprotein: NP_001805; hs isoform b precursor: AAI13898 or NP_680475. Murine (mus musculus) cathepsin C: AAH67063; preproprotein: NP_034112; isoform CRA-b (mus musculus): EDL06796; isoforms CRA-a (mus musculus): EDL06795. Moreover the protein sequence is publicly available at the UniProtKB database (www.beta.uniprot.org), under accession numbers: P53634 (HUMAN_CATC, human Cathepsin C, SEQ ID NO:2), or P97821 (CATC_MOUSE, murine Cathepsin C). If in the following, it is referred to the cathepsin C protein or amino acid sequence, this can mean any of the above protein sequences; preferably, the sequence according to reference numbers P53634 (for the human sequence) or P97821 (for the murine sequence).

NCBI is the national centre for biotechnology information (postal address: National Centre for Biotechnology Information, National Library of Medicine, Building 38A, Bethesda, MD 20894, USA; web-address: www.ncbi.nlm.nih.gov). More sequences (e.g. sequences carrying SwissProt or EMBL accession numbers) can be retrieved in the UniProtKB database under www.beta.uniprot.org.

The cloning of hs Cathepsin C is published by Paris et al., 1995, the cloning of murine cathepsin C is published by Pham et. al, 1997. The 5'flanking region (promoter/enhancer) is published by Rao et. al, 1997 (see especially page 10263, figure 4 and the detailed text on the lower part of said page, which is incorporated herein by reference; e.g. the gene sequence starting from -1127 up to +1). Cathepsin C is expressed at high levels in lung, kidney and placenta, and to a lesser extent in a variety of other organs, including cells of the immune system (Rao et. al., 1997).

Cathepsin C is a lysosomal protease capable of removing dipeptides from the amino terminus of protein substrates. Cathepsin C is involved in the activation of granule serine proteases, which are expressed in bone marrow-derived effector cells of the immune system; and it is involved for processing and activation of the T-lymphocyte granzymes A (GZMA) and B (GZMB) and granzyme C; further functions are the processing of different lysosomal cathepsins, the activation of different serine proteases (chymotrypsin-like serine proteases) by removal of an inhibitory N-terminal dipeptide residue (such as the above granzymes, Cathepsin C itself, neutrophil elastase, proteinase-3, mast cell chymase and tryptase (Toomes et al., 1999, Pham and Ley 1999, Turk et al, 2000, Henningssson et al, 2003; McGuire et al., 1993; Adkison et al., 2002; Wolters et al, 2001; Pham et al, 2004; DeHaar et al, 2004; Sheth et al, 2003, Methot et al., 2007). Its functions in general comprise: protease activity, peptidase activity, e.g. dipeptidylpeptidase, exopeptidase or endopeptidase activity; activation of serine proteases (e.g. one or more of the following: elastase, cathepsin G and granzymes A and B, neuraminidase and factor XIII).

Cathepsin C is a 200kD tetrameric protein (Paris et al., 1995), which stands in contrast to other cathepsins (e.g. cathepsin B, H, L and S) being small monomeric enzymes. The cathepsin C protein is processed from a prepro-form to a proteolytically active enzyme. The mature monomeric form of cathepsin C consists of a heavy chain, a light chain and a propeptide remaining associated with the active enzyme (Wolters et al., 1998; Cigic et al., 2000); four of such cathepsin C monomers forming the proteolytically active, 200kD cathepsin C tetramer.

The generation of cathepsin C knock-out mice (Dppi-knock out mice) is published by Pham and Ley, 1999 (see materials and methods part, from p. 8627 go 8629 and esp. p. 8627, second half of right column to p. 8628, first half of left column for construction of the DPPI targeting vector and generation of DPPI -/- mice; see as well Heusel et al, 1994; Mansour et

al., 1988 and Soriano et al., 1991, to which Pham and Ley, 1999, refers for embryonic stem cell generation).

The use according to present invention allows for the identification of novel substances for the prevention and/or treatment of pain, especially neuropathic pain. The use according to present invention comprises the identification of compounds with desired characteristics (i.e. lowering the pain sensation) as well as the identification of compounds with undesired characteristics (i.e. increasing the pain sensation). Moreover, present invention allows for the further characterisation of compounds already identified of being useful for the prevention and/or treatment of any disease or diseased state. In this case, present invention can e.g. be used for excluding identified active compounds having unwanted side-effects (i.e. the increase of pain sensation): Candidate compounds for a given disease can e.g. be profiled for their influence on Cathepsin C (protein and/or nucleic acid, expression and/or function, etc.).

A compound / test compound / active compound as to be employed for the different aspects of present invention can be any biological or chemical substance or natural product extract, either purified, partially purified, synthesized or manufactured by means of biochemical or molecular biological methods.

A compound considered as being active in modulating pain in the sense of the different aspects of present invention can be any substance having an influence on one of the functions of Cathepsin C or on the Cathepsin C amount (protein or nucleic acid) in a cell, on Cathepsin C expression, posttranslational modification (e.g. N-glycosilation or processing (e.g. cleavage of the exclusion domain, e.g. at position 58 or 61)), oligomerization of the monomers, protein folding or activation.

To this end, the substance can modulate any of the functions of Cathepsin C (e.g. those as listed above or below). Cathepsin C protein activity can be modulated by the substance e.g. by direct interaction and interference of Cathepsin C polypeptide/protein or fragments thereof. The substance can also modulate Cathepsin C expression, e.g. on the level of transcription (initiation, elongation, processing, etc), transcript stability, translation. Moreover it can modulate the posttranslational modification, the processing from the inactive to the active form (cleavage of the prepro-form to the three polypeptides forming the active monomer) and/or the oligomerization from the monomeric to the tetrameric form, as well as protein folding etc. of Cathepsin C. The substance can exert the above effects directly or indirectly (indirectly

meaning i.e. by interfering (positively or negatively) with natural signalling cascades having influence on Cathepsin C function / protein activity / expression etc.)

- 5 Functions of Cathepsin C comprise those as listed above, e.g. protease activity; the ability of removing dipeptides from the amino terminus of one or more protein substrates; the ability to interact specifically with one or more protein substrates (protein-protein interaction), such as those listed above; the ability to cleave and/or activate one or more protein substrates, such as those listed above; the ability to process protein substrates, such those listed above.
- 10 Functions of Cathepsin C comprise also generally the ability of Cathepsin C protein or nucleic acid or fragments thereof to interact with other molecules (comprising, but not limited to: proteins, nucleic acids, synthetic molecules) and preferably concern its capability of interacting and cleaving protein substrates.

- 15 Substrate of an enzyme is understood, within the terms of present application, to be any molecule that can be modified by the enzyme. Naturally occurring substrates in the scope of present invention are molecules that correspond to the form in which they occur in the natural physiological or pathological context (such as granule serine proteases, GZMA, GZMB), and which are also capable of being modified by the respective enzyme.

- 20 The modulation of pain can be either a decrease or an increase.

According to one aspect of present group of inter-related inventions, a fragment or derivative of Cathepsin C can be used. A fragment can be a fragment of a protein, polypeptide or polynucleic acid.

25

A fragment of a protein or polypeptide is a protein or polypeptide that carries one or more end-terminal (n- and/or c-terminal) and/or internal deletions of one, two or more amino acids, when compared to the full-length Cathepsin C; fragments comprise, e.g.

- 30 1. a Cathepsin C fragment carrying an n-terminal deletion of the dipeptide Xaa-Yaa, Zaa- in its Amino Acid chain, especially except when Xaa is Arg or Lys, or Yaa or Zaa is Pro;
2. a Cathepsin C fragment in which the Signal peptide (Amino Acids 1-24 of the Amino Acid chain) is deleted or Cathepsin C fragment consisting of amino acids 25-463
3. a Cathepsin C fragment comprising or consisting of positions 25-134 of the Amino Acid chain according to SEQ ID NO:2 (dipeptidyl-peptidase 1 exclusion domain chain)

4. a Cathepsin C fragment in which the propeptide (Amino Acids 135-230 is deleted)
5. a Cathepsin C fragment comprising or consisting of the dipeptidyl peptidase 1 heavy chain (Amino Acids 231-394);
6. a Cathepsin C fragment comprising or consisting of the dipeptidyl peptidase 1 light chain (Amino acids 395-463)
7. a Cathepsin C fragment comprising the dipeptidyl peptidase 1 light chain (Amino acids 395-463) and heavy chain (Amino Acids 231-394);
8. a Cathepsin C fragment comprising the dipeptidyl peptidase 1 light chain (Amino acids 395-463) and heavy chain (Amino Acids 231-394);
- 10 The positions of the above fragments refer to SEQ ID NO:2; preferred examples of the above fragments concern fragments of the protein according to SEQ ID NO:2.

A functional fragment of Cathepsin C protein is any fragment of this protein having at least one or more of the functional characteristics of the full-length protein, especially as listed above.

15

A fragment of a polynucleotide acid is a polynucleotide acid or an oligonucleotide carrying one or more end-terminal (5'- and/or 3'-) and/or internal deletions of one, two or more nucleotides, when compared to the full-length genomic or coding sequence. A functional fragment of Cathepsin C nucleic acid is any fragment having at least one or more of the functional characteristics of the full-length polynucleic acid (mRNA, genomic or coding sequence).

20

The term derivative of Cathepsin C comprises any type of modification of Cathepsin C in comparison to the naturally-occurring form, and especially in comparison to Cathepsin C according to SEQ ID NO:1 or SEQ ID NO2, that is not a deletion. A functional derivative of Cathepsin C is any derivative of this protein having at least one and preferably two or more of the functional characteristics of the unmodified protein. Derivatives comprise, e.g. modifications of the amino acid or nucleotide sequence or any other kind of modification such as a chemical or biological modification leading e.g. to the stabilization of the polypeptide or polynucleotide (such as phosphorothioate modifications of the nucleic acid backbone or of exchanges of the bonds between amino acids, etc), or enabling a specific targeting of the polypeptide or polynucleotide to certain cells or facilitating its entry into or its uptake by cells (such as cell-permeant phosphopeptides, ortho coupling to cell-permeant peptide vectors, e.g. based on the antennapedia/penetrating, TAT and signal-peptide based sequences; or coupling to parts of ligands for specific transporters or importers).

35

Present invention also comprises functional derivatives of fragments of Cathepsin C.

Another aspect of present invention concerns the use of a non-human transgenic animal heterologously expressing Cathepsin C or a functional fragment thereof for identifying or
5 analyzing compounds that modulate pain.

The non human animal can be any non human animal. Preferred are rodents, such as rats or mice.

10 A transgenic animal is an animal that carries in its genome foreign DNA, which has deliberately been transferred thereto. The introduction of the foreign DNA into the animal genome can be performed according to standard procedures (see e.g. Transgenic Animal Technology A Laboratory Handboook. C.A. Pinkert, editor; Academic Press Inc., San Diego, California, 1994 (ISBN: 0125571658)).

15 The term heterologous expression refers to an expression differing from the normal gene expression in the host organism (concerning steady state level, amount, timing or tissue distribution of the expressed gene or concerning the type of expressed gene (i.e. the gene is normally not expressed in the host at all)). The heterologous expression can be constitutive or
20 inducible. Suitable inducible expression systems are well known in the art (e.g. the Tetracycline inducible system or the like). The organism can be a cell or a non-human animal.

According to another aspect, present invention concerns the use of a non-human transgenic animal heterologously expressing Cathepsin C or a functional fragment thereof as a model
25 system for enhanced pain sensitivity.

Yet another aspect of present invention concerns the use of a non-human Cathepsin C knock-out animal for identifying or analyzing compounds that modulate pain.

30 A knock-out organism (such as an animal or a cell) refers to an organism in which the expression or function of a gene is partially or completely deleted and comprises genomic as well as functional knock outs, inducible as well as constitutive knock outs. The generation of knock out organisms is well known in the art, as well as cells or animals which can be used for generating knock out organisms. The generation of cathepsin C – knock out mice is described
35 in Pham and Ley, 1999.

A further aspect of present invention concerns use of a non-human Cathepsin C knock-out animal as a model system for lowered pain sensitivity.

- 5 The use of a cell heterologously expressing Cathepsin C or a functional fragment thereof for identifying compounds that modulate pain, is another aspect of present invention.

The cell can be any prokaryotic or eucaryotic cell, such as cells capable of being transfected with a nucleic acid vector and of expressing a reporter gene. These comprise principally
10 primary cells and cells from a cell culture, such as a eukaryotic cell culture comprising cells derived either from multicellular organisms and tissue (such as HeLA, CHO, COS, SF9 or 3T3 cells) or single cell organisms such as yeast (e.g. *S. pombe* or *S. cerevisiae*), or a procaryotic cell culture, preferably *Pichia* or *E. coli*. Cells and samples derived from tissue can be gained by well-known techniques, such as taking of blood, tissue puncture or surgical techniques.

15 According to one embodiment, a modified cell, having a lower Cathepsin C activity as compared to its unmodified state, is used. This way, it can e.g. be tested, if the chemical compounds to be tested are able to enhance or restore the lowered or totally abolished Cathepsin C activity. Or it can be tested whether the substances are able to perform their
20 function (e.g. pain modulation or even a function in the context of another diseased state or disease) in the context of lowered pain sensitivity.

The modification can be any type of modification (stable or transient, preferably stable), that leads to a decrease of Cathepsin C activity, Cathepsin C transcript steady state level (i.e. by
25 activation of Cathepsin C transcription or transcript stabilisation) or Cathepsin C protein steady state level (i.e. by activation of Cathepsin C translation or its posttranslational processing; by modulation of Cathepsin C posttranslational modification or by activation of its stabilisation or by inhibition of its degradation). This can for example be achieved by using dominant negative mutants of Cathepsin C, antisense oligonucleotides, RNAi constructs of
30 Cathepsin C, by generating functional or genomic Cathepsin C knock outs (which can e.g. be inducible) or other suitable techniques known within the state of the art. For an overview of the above techniques, see for example: Current protocols in Molecular biology (2000) J.G. Seidman, Chapter 23, Supplement 52, John Wiley and Sons, Inc.; Gene Targeting: a practical approach (1995), Editor: A.L. Joyner, IRL Press; Genetic Manipulation of Receptor Expression
35 and Function, 2000; Antisense Therapeutics, 1996; Scherr et al, 2003.

According to one embodiment, a Cathepsin C knock-out cell is used. Suitable cell lines for the generation of knock-outs are well known in the state of the art, see e.g., Current protocols in Molecular Biology (2000) J.G. Seidman, Chapter 23, Supplement 52, John Wiley and Sons, Inc; or Gene Targeting a practical approach. (1995) Ed. A.L. Joyner, IRL Press. The
5 generation of Cathepsin C (DPPI) knock-out cells is also published in Pham and Ley, 1999 (the generation of DPPI murine embryonic stem cell knock out clones, see page 8628, left column, upper half).

Another aspect of the invention concerns thus the use of a Cathepsin knock-out cell for
10 identifying or analyzing compounds that modulate pain.

Furthermore, the use of a Cathepsin knock-out cell as a model system for lowered pain sensitivity, is another aspect of present group of inter-related inventions.

15 According to another embodiment of present invention, the cell can have a higher amount of Cathepsin C as compared to a reference cell (e.g. the same cell in its unmodified state). This cellular system can serve to mimick a state of enhanced pain sensitivity, as the amount of Cathepsin C expression is related to pain sensitivity.

20 Present invention relates thus also to the use of a cell heterologously expressing Cathepsin C or a functional fragment thereof as a model system for enhanced pain sensitivity.

The use of a cell heterologously expressing a reporter gene expressibly linked to the Cathepsin C promoter and/or enhancer or a functional fragment thereof for identifying or
25 analyzing compounds that modulate pain.

The above aspect of present invention is based on a typical reporter gene assay commonly known in the art. To this end, the promoter of choice is inserted into an expression vector suitable for the type of host cell chosen, upstream of the reporter gene of choice in such a way as to allow for an expression of the reporter gene if the promoter is active. The construct is
30 subsequently introduced into the host cell of choice. Suitable methods for transformation or transfection are well known in the art as well as conditions for cell cultivation and detection of reporter gene expression (see e.g. standard literature listed below). Suitable conditions are well known in the art as well as vectors, reporter genes and necessary reagents, which are also commercially available.

A vector is a circular or linear polynucleotide molecule, e.g. a DNA plasmid, bacteriophage or cosmid, by aid of which polynucleotide fragments (e.g. cut out from other vectors or amplified by PCR and inserted in the cloning vector) can specifically be amplified in suitable cells or organisms. Expression vectors enable the heterologous expression of a gene of interest (e.g. a reporter gene), in the host cell or organism. The type of cell or organism largely depends on the aim and the choice lies within the knowledge of the skilled artisan. Suitable organisms for the amplification of a nucleic acid are e.g. mostly single cell organisms with high proliferation rates, like e.g. bacteria or yeast. Suitable organisms can also be cells isolated and cultivated from multicellular tissues, like e.g. cell lines generated from diverse organisms (e.g. SF9 cells from *Spodoptera Frugiperda*, etc.). Suitable cloning vectors are known in the art and commercially available at diverse biotech suppliers like, e.g. Roche Diagnostics, New England Biolabs, Promega, Stratagene and many more. Suitable cell lines are e.g. commercially available at the American Type Culture Collection (ATCC).

For the heterologous expression of a protein or polypeptide, the cell can be any prokaryotic or eucaryotic cell capable of being transfected with a nucleic acid vector and of expressing the gene of interest, e.g. a reporter gene. These comprise principally primary cells and cells from a cell culture, preferably an eukaryotic cell culture comprising cells derived either from multicellular organisms and tissue (such as HEK293, RIN-5F, HeLA, CHO, COS, SF9 or 3T3 cells) or single cell organisms such as yeast (e.g. *S. pombe* or *S. cerevisiae*), or a procaryotic cell culture, preferably *Pichia* or *E. coli*. Cells and samples derived from tissue can be gained by well-known techniques, such as taking of blood, tissue puncture or surgical techniques.

Within the context of present application, the term "transfection" refers to the introduction of a nucleic acid vector into a host cell (either prokaryotic or eucaryotic) and comprises thus the term "transformation".

The transfection can be a stable or transient transfection.

The Cathepsin C promoter is a part of the Cathepsin C gene able to drive expression of a gene product of interest if introduced into a suitable expression vector upstream of the coding sequence of the gene product. Preferably, the Cathepsin C promoter comprises or consists of the sequence according to nucleotides -1127 to +1 of the sequence as published by Rao et al., 1997, page 10263, figure 4. A functional fragment of the Cathepsin C promoter is any fragment of the Cathepsin C promoter that is able to drive expression of a gene product of interest if introduced into a suitable expression vector upstream of the coding sequence of the gene

product. Preferable fragments comprise functional fragments of the Cathepsin C promoter as published by Rao et al., 1997, p. 10263, figure 4.

A reporter gene can be any gene that allows for an easy quantification of its gene product. A vast variety of reporter genes for eukaryotic or prokaryotic hosts as well as detection methods and necessary reagents are known in the art and commercially available. These comprise e.g. the genes of beta Lactamase (lacZ), Luciferase, Green or Blue fluorescent protein (GFP or BFP), DsRed, HIS3, URA3, TRP1 or LEU2 or beta Galactosidase. These genes encode proteins which can be easily detected by means of a visible (colour or luminescent) reaction (e.g. lacZ, Luciferase). These comprise gene-products which can be easily detected by means of a visible (colour or luminescent) reaction or gene-products conferring resistance towards antibiotics like Ampicillin or Kanamycin when expressed. Other reporter gene-products enable the expressing cells to grow under certain conditions like e.g. auxotrophic genes.

A functional fragment of a reporter gene is any fragment of a given reporter gene that allows for an easy quantification of its gene product.

A functional fragment of a reporter gene is any fragment of a given reporter gene that allows for an easy quantification of its gene product.

Within the context of present application, the term "transfection" refers to the introduction of a nucleic acid vector into a host cell (either prokaryotic or eucaryotic) and comprises thus the term "transformation".

The transfection can be a stable or transient transfection.

The cell can be any prokaryotic or eucaryotic cell capable of being transfected with a nucleic acid vector and of expressing a reporter gene. These comprise principally primary cells and cells from a cell culture, preferably a eukaryotic cell culture comprising cells derived either from multicellular organisms and tissue (such as HeLA, CHO, COS, SF9 or 3T3 cells) or single cell organisms such as yeast (e.g. *s. pombe* or *s. cerevisiae*), or a procaryotic cell culture, preferably *Pichia* or *E.coli*. Cells and samples derived from tissue can be gained by well-known techniques, such as taking of blood, tissue punction or surgical techniques.

Within the context of the above aspect of present invention the control vector can be any suitable vector which comprises a reporter gene or functional fragment thereof, but wherein

reporter gene expression is not driven by a (functional) Cathepsin C promoter. This can e.g. mean that the reporter gene or functional fragment thereof is not operationally coupled to a functional Cathepsin C promoter (i.e. either totally devoid of a Cathepsin C promoter, comprises a non functional Cathepsin C promoter or promoter fragment or wherein the coupling of promoter and reporter gene is not functional). Another possibility is that the reporter gene or functional fragment thereof is operationally coupled to another promoter than the Cathepsin C promoter (e.g. SV40 or another standard promoter). The functional vector and the control vector can also be transfected to the same cell, but in which case the reporter genes need to be different.

The identification of compounds according to the above uses can e.g. be performed according to assays as described below or as known in the art.

An assay is any type of analytical method or system to monitor a biological process. Suitably, molecular cascades and mechanisms representing parts of physiological metabolic pathways but also of pathological conditions are reproduced in cellular or biochemical (in vitro) systems. The pharmacological activity of a potential pharmaceutical compound can thus be determined according to its capability of interfering with or modulating these cascades or mechanisms.

For the use in drug screening, especially the high throughput screening for novel pharmaceutical compounds, the assay needs to be reproducible and is preferably also scalable and robust. In the scope of present invention, high throughput screen means, that a method according to present invention is performed in a very small scale, e.g. on 96, 386 or 1536 well plates in samples of very small volume in the range of few millilitres down to few nanoliters or even less. Thus, a very large amount of samples can be analysed in a short time.

High throughput screening mostly comprises the screening of approximately 500.000 different compounds for a certain ability by means of one single assay. The assay is preferably suitable for high throughput screening of chemical substances for their ability of modulating the activity of the target molecule under investigation. The type of assay depends e.g. on the type of target molecule used (e.g. polypeptide or polynucleotide) and the "read out", i.e. the parameter, according to which the activity of the target molecule is determined (see below).

Different types of such assays are commonly known in the state of the art and commercially available from commercial suppliers.

Suitable assays for different purposes encompass radioisotopic or fluorescent assays, for example fluorescence polarization assays (such as those offered commercially by Panvera) or Packard BioScience (HTRF; ALPHAScreen™) for measuring the interaction of a labeled member with a non-labeled member (e.g. the interaction of labeled proteins with their
5 unlabeled protein-ligands).

More examples include cell based assays, wherein a cell line stably (inducibly or not; chromosomal or episomal) or transiently expresses a recombinant protein of interest. These assays comprise e.g. reporter gene assays, wherein the regulation of a certain promotor or a
10 signal transduction pathway of a member of a signal transduction cascade is measured according to the activity of a reporter enzyme, the expression of which is under the control of said certain promotor. For this type of assay, a recombinant cell line has to be constructed containing the reporter gene under the control of a defined promotor that is to be investigated itself or that is regulated by the signaling cascade under investigation. Suitable reporter
15 enzymes are commonly known within the state of the art and comprise firefly luciferase, renilla luciferase (e.g. commercially available by Packard reagents), β -Galactosidase. Suitable cell lines depend on the aim of the assay but comprise mostly cell lines that are easy to transfect and easy to cultivate, such as, e.g. HeLA, COS, CHO, NIH-3T3, etc.

For determination of protease activity, typical protease assay formats are known: e.g. using a
20 substrate carrying a reporter tag (e.g. a luminescent/fluorescent or other signal emitting protein/peptide or entity) at one position of the substrate and a quencher (an entity (e.g. another peptide inhibiting the signal emission of the reporter tag as long as the substrate is intact/uncleaved) at another position; the substrate is incubated with Cathepsin C under
25 suitable conditions to allow for the cleavage of the substrate leading to the emission of a detectable signal (e.g. light-emission), because of the separation of quencher and reporter tag.

Other types of assays and other types of "read out" are well known in the state of the art.

30 Assays according to present invention concern:

A method of identifying or analyzing compounds modulating and/or preventing pain, comprising the steps

- a. Providing at least two samples;

- b. Contacting one sample containing Cathepsin C or a functional fragment or derivative thereof with a compound,
- c. determining the activity of Cathepsin C in the presence of compound,
- d. determining the activity of Cathepsin C in the absence of compound, and
- 5 e. comparing the activity of Cathepsin C according to c) with that according to d).

A method for identifying or analyzing compounds that modulate and/or prevent pain comprising:

- 10 a. Contacting a Cathepsin C protein or functional fragment or derivative thereof with a test compound; and
- b. Determining whether the test compound modulates the activity of the Cathepsin C protein or functional fragment or derivative thereof.

15 A method for identifying or analyzing compounds that modulate and/or prevent pain comprising:

- a. Contacting a cell, which has a detectable amount or activity of Cathepsin C or of a functional fragment or derivative thereof, with a test compound;
- b. Determining whether the test compound is able to modulate the amount or activity of Cathepsin C or the functional fragment or derivative thereof present in the cell.

20 A method for identifying or analyzing compounds that modulate and/or prevent pain comprising:

- a. Contacting a nucleic acid coding for a Cathepsin C protein or a functional fragment or derivative thereof with a test compound in a transcriptionally active system, and
- 25 b. Determining the amount of mRNA coding for the Cathepsin C protein or the functional fragment or derivative thereof present in said system in presence of said compound, and
- c. Determining whether the compound is capable of modulating the amount of mRNA coding for the Cathepsin C protein or functional fragment or derivative present in
- 30 said system.

A transcriptionally active system is any biochemical or cellular system which at least has the ability to perform a transcription reaction of a transcription unit. Such systems are well known in the art and comprise cells as well as in vitro transcription systems or kits (e.g. on basis of

35 cell extracts) commercially available.

A method for identifying compounds or analyzing compounds that modulate and/or prevent pain comprising:

- 5 a. Providing a cell transfected with a nucleic acid vector comprising the promoter of a Cathepsin C gene or a functional fragment thereof operationally coupled to a reporter gene or a functional fragment thereof;
- b. Providing a cell transfected with a control vector which comprises a reporter gene or a functional fragment thereof not being operationally coupled to a functional Cathepsin C promoter;
- 10 c. Determining the reporter gene activity of the cell according to a) and b) in the presence of a test compound;
- d. Determining the reporter gene activity of the cell according to a) and b) in absence of the test compound.

- 15 A method for identifying or analyzing a compound that modulates pain comprising
- a. Selecting a compound that modulates the activity of Cathepsin C as a test compound, and
 - b. Administering said test compound to a subject in sensation of pain to determine whether the pain is modulated.

20

A method of identifying or analyzing a compound that modulates and/or prevents pain in a subject comprising:

- c. Assaying a biological activity of Cathepsin C or a functional fragment or derivative thereof in the presence of one or more test compounds to identify one or more
- 25 modulating compounds that modulate the biological activity of Cathepsin C, and
- d. Testing one or more of the modulating compounds for their ability to reduce pain, pain sensation or pain sensitivity in a subject.

30 Further aspects of present invention concern pharmacogenomic methods for classifying patient groups and assisting the physician to adapt/improve his treatment of individual patients, such as:

A method for analyzing the pain threshold in an individual comprising analyzing the amount of Cathepsin C in a taken sample of said individual in comparison to one or more reference samples as to whether the amount of Cathepsin C mRNA and/or protein present in said sample is different from that of one or more reference samples, wherein the presence of a higher amount indicates an increased pain sensitivity and the presence of a lower amount indicates a decreased pain sensitivity in said individual.

A method for adapting the dosage of a pharmaceutical for the prevention and/or treatment of pain in an individual, which method comprises examining a taken sample of an individual as to whether the amount of Cathepsin C mRNA and/or protein present in said sample is different from that of one or more reference samples, said dosage being adapted depending on whether the amount of protein and/or mRNA in the taken sample of the individual is different from that of the one or more reference samples, wherein a higher amount of Cathepsin C in the taken sample of the individual is indicative of a need for a higher dose and a lower amount of Cathepsin C in the sample of the individual is indicative of a need for a lower dose.

The term "taken sample" as used herein, refers to a biological sample taken/separated from the body of one or more individual beings (humans or non-human animals). Biological material and biological samples comprise, e.g. cells, preparations or parts of tissue or organs (e.g. brain, blood, liver, spleen, kidney, heart, blood vessels, etc.), preferably if derived from a vertebrate, and more preferably from a mammal including a human. Comprised are also cells from a cell culture, preferably a eukaryotic cell culture comprising cells derived either from multicellular organisms and tissue (such as HeLA, CHO, COS, SF9 or 3T3 cells) or single cell organisms such as yeast (e.g. *s. pombe* or *s. cerevisiae*), or a procaryotic cell culture, preferably *Pichia* or *E.coli*. Cells and samples derived from tissue can be gained by well-known techniques, such as taking of blood, tissue puncture or surgical techniques. The preparation of recombinant molecules and the purification of naturally occurring molecules from cells or tissue, as well as the preparation of cell- or tissue extracts is well known to the person of skill in the art (see e.g. also the standard literature listed below).

The term "reference sample" refers to a biological sample taken from one or more individuals with a known given pain phenotype or to an *in vitro* biological sample (e.g. a sample stemming from *in vitro* cell or tissue culture (e.g. cultivated cells)) and corresponding in certain characteristics (e.g. its level of Cathepsin C activity, amount or expression) to a given pain phenotype (e.g. high pain sensitivity or low pain sensitivity).

Yet another aspect of present invention concerns the use of a means for the detection of Cathepsin C for determining enhanced pain sensitivity in an individual by analyzing a biological sample taken from the body of an individual to be examined.

- 5 The means for the detection of Cathepsin C can be any means able to specifically detect Cathepsin C polypeptide/protein or nucleic acid present in a biological sample.

A means to specifically detect Cathepsin C protein or polypeptide can be any means able to specifically detect either wildtype Cathepsin C protein/polypeptide and can also be a means to detect specifically Cathepsin C protein/polypeptide harbouring one or more mutations
10 regarding the size or the amino acid sequence in comparison to a wild type polypeptide/protein. A preferred example of such a means is an antibody able to specifically detect Cathepsin C protein, e.g. for use in immunohistological or immunohistochemical techniques (e.g. detection of Cathepsin C protein or certain mutations thereof in histological tissue sections or cathepsin C protein immobilized on suitable carriers like membranes, chips, ELISA plates etc.).
15 CathepsinC antibodies are commercially available, such as Goat Anti-Human Cathepsin C, Catalog# AF1071, R&D Systems (Minneapolis, USA), Goat Anti-Mouse Cathepsin C, Catalog# BAF1034, R&D Systems (Minneapolis, USA).

The means to specifically detect Cathepsin C nucleic acid can e.g be a means to detect Cathepsin C mRNA /cDNA or genomic DNA, either wildtype or also harbouring one or more
20 mutations regarding their length or their nucleic acid sequence in comparison to a wild type Cathepsin C nucleic acid. The means can e.g. be a means to specifically detect and/or quantify Cathepsin C mRNA and preferably comprises or is a specific Cathepsin C nucleic acid probe or a primer set capable of amplifying Cathepsin C DNA or, e.g. for use in PCR sequencing (for the detection of Mutations in the nucleotide sequence) or capable of amplifying
25 Cathepsin C cDNA, e.g. for use in RT PCR (for the detection and/or quantification of Cathepsin C mRNA expression). Another means can e.g. be a nucleic acid probe able to specifically hybridise to Cathepsin C mRNA or cDNA under standard conditions, e.g. for use in Northern Blot or Chip hybridisation techniques.

The term wild type refers to the genotype or phenotype that is found in nature or in the
30 standard laboratory stock for a given organism. According to one preferred embodiment, the wildtype sequences of Cathepsin C are the sequences according to SEQ ID NOs: 1, 2, 3.

The design and synthesis of suitable primers is well known in the art (see also above). According to a preferred embodiment of present invention, the means is a primer set for the amplification of Cathepsin C nucleic acid, such as human Cathepsin C nucleic acid, preferably a set of primers comprising at least one of the primers according to SEQ ID No.4 and/or 5.

- 5 According to a further preferred embodiment of present invention, the means is a probe for the detection of Cathepsin C nucleic acid and preferably a probe having the sequence according to SEQ ID No.6. The design and synthesis of suitable probes is well known in the art (see also standard literature below).

- 10 According to yet another preferred embodiment of present invention, the means is an antibody for the specific detection of Cathepsin C protein or polypeptide. The preparation of suitable antibodies or functional fragments thereof is well known in the art as well, e.g. by immunizing a mammal, for example a rabbit, with Cathepsin C protein or a fragment thereof, where appropriate in the presence of, for example, Freund's adjuvant and/or aluminium hydroxide gels (see, for example, Diamond, B.A. et al. (1981) The New England Journal of Medicine:
15 1344-1349). The polyclonal antibodies which are formed in the animal as a result of an immunological reaction can subsequently be isolated from the blood using well-known methods and, for example, purified by means of column chromatography. Monoclonal antibodies can, for example, be prepared in accordance with the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) Nature, 349, 293-299). Suitable procedures to
20 produce monoclonal antibodies are well known in the art as well (see e.g. literature for standard methods listed below). In the context of present invention, the term antibody or antibody fragment comprises also antibodies or antigen-binding parts thereof, which have been prepared recombinantly and, where appropriate, modified, such as chimaeric antibodies, humanized antibodies, multifunctional antibodies, bispecific or oligospecific antibodies, single-
25 stranded antibodies and F(ab) or F(ab)₂ fragments (see, for example, EP-B1-0 368 684, US 4,816,567, US 4,816,397, WO 88/01649, WO 93/06213 or WO 98/24884).

Another aspect of present invention concerns a diagnostic kit for determining the pain sensitivity in an individual, which test kit comprises at least one means for the detection of Cathepsin C in a biological sample.

30

In the context of the present invention, a test kit is understood to be any combination of the components identified in this application, which are combined, coexisting spatially, to a functional unit, and which can contain further components.

In the context of present invention, a test kit comprises at least a means for detection of Cathepsin C (e.g. amount/or mutation) in a biological sample, suitably together with suitable buffers and/or reagents for performing a detection reaction (e.g. immunological detection of Cathepsin C by means of an antibody, an enzymatic reaction for assaying Cathepsin C activity or the like), and/or sample preparation, and optionally a handling manual for performing the respective detection technique.

Other aspects of present invention concern methods of treatment, such as:

A method for treating pain in a subject that is experiencing pain comprising administering to said subject a therapeutically effective amount of a composition lowering the amount or activity of Cathepsin C in said subject. This can be the amount or activity of Cathepsin C altogether or in a certain tissue, e.g. in neural tissue, in lymphatic tissue or cells of the immune system such as mast cells, macrophages, neutrophils, T-cells (such as CD8+ T-cells), etc. wherein a therapeutically effective amount comprises an amount sufficient to ameliorate the pain sensation or sensitivity in the individual.

A method for lowering the pain sensitivity in a subject comprising administering to said subject a therapeutically effective amount of a composition lowering the amount (e.g. expression, half life) or activity of Cathepsin C in said subject (e.g. in lymphatic or neural tissue or cells of the immune system), concerns yet another aspect of present invention.

Moreover, present invention concerns a method for modulating the pain sensitivity in an offspring from a non-human female subject comprising transferring (e.g. electroporating) a nucleic acid conferring a modulated Cathepsin C expression into Zygotes, transferring the Zygotes into a non-human foster mother and electing offspring according to its Cathepsin C expression characteristics (lowered or abolished cathepsin C expression in comparison with wild type subjects, such as mice).

Another aspect of present invention concerns a compound that is able to lower Cathepsin C activity and/or expression for the treatment of pain.

Inhibitors of Cathepsin C are known in the art, such as peptide nitrile inhibitors (see e.g. Methot et al., 2007, which is incorporated herein by reference, see especially page 20839,

figure 1 for structures of different Cathepsin C-inhibitors, such as Gly-Phe-DMK, Gly4-(I)Phe-DMK and compound 1* and compound 2* of said figure).

For the production of the medicament the modulators of Cathepsin C of the present invention
5 can be formulated with suitable additives or auxiliary substances, such as physiological buffer solution, e.g. sodium chloride solution, demineralized water, stabilizers, such as protease or nuclease inhibitors, preferably aprotinin, ϵ -aminocaproic acid or pepstatin A or sequestering agents such as EDTA, gel formulations, such as white vaseline, low-viscosity paraffin and/or yellow wax, etc. depending on the kind of administration.

10 Suitable further additives are, for example, detergents, such as, for example, Triton X-100 or sodium deoxycholate, but also polyols, such as, for example, polyethylene glycol or glycerol, sugars, such as, for example, sucrose or glucose, zwitterionic compounds, such as, for example, amino acids such as glycine or in particular taurine or betaine and/or a protein, such
15 as, for example, bovine or human serum albumin. Detergents, polyols and/or zwitterionic compounds are preferred.

The physiological buffer solution preferably has a pH of approx. 6.0-8.0, especially a pH of approx. 6.8-7.8, in particular a pH of approx. 7.4, and/or an osmolarity of approx.
20 200-400 milliosmol/liter, preferably of approx. 290-310 milliosmol/liter. The pH of the medicament is in general adjusted using a suitable organic or inorganic buffer, such as, for example, preferably using a phosphate buffer, tris buffer (tris(hydroxymethyl)aminomethane), HEPES buffer ([4-(2-hydroxyethyl)piperazino]ethanesulphonic acid) or MOPS buffer (3-morpholino-1-propanesulphonic acid). The choice of the respective buffer in general
25 depends on the desired buffer molarity. Phosphate buffer is suitable, for example, for injection and infusion solutions.

The medicament can be administered in a conventional manner, e.g. by means of oral dosage forms, such as, for example, tablets or capsules, by means of the mucous membranes, for
30 example the nose or the oral cavity, in the form of dispositories implanted under the skin, by means of injections, infusions or gels which contain the medicaments according to the invention. It is further possible to administer the medicament topically and locally in order to treat the particular joint disease as described above, if appropriate, in the form of liposome complexes. Furthermore, the treatment can be carried out by means of a transdermal
35 therapeutic system (TTS), which makes possible a temporally controlled release of the

medicaments. TTS are known for example, from EP 0 944 398 A1, EP 0 916 336 A1, EP 0 889 723 A1 or EP 0 852 493 A1.

5 Injection solutions are in general used if only relatively small amounts of a solution or suspension, for example about 1 to about 20 ml, are to be administered to the body. Infusion solutions are in general used if a larger amount of a solution or suspension, for example one or more litres, are to be administered. Since, in contrast to the infusion solution, only a few millilitres are administered in the case of injection solutions, small differences from the pH and from the osmotic pressure of the blood or the tissue fluid in the injection do not make
10 themselves noticeable or only make themselves noticeable to an insignificant extent with respect to pain sensation. Dilution of the formulation according to the invention before use is therefore in general not necessary. In the case of the administration of relatively large amounts, however, the formulation according to the invention should be diluted briefly before administration to such an extent that an at least approximately isotonic solution is obtained. An
15 example of an isotonic solution is a 0.9% strength sodium chloride solution. In the case of infusion, the dilution can be carried out, for example, using sterile water while the administration can be carried out, for example, via a so-called bypass.

According to one preferred embodiment of the different aspects of present invention, Cathepsin C, the derivative or fragment thereof can be used as an isolated molecule.
20

In the context of this invention, the term "isolated molecule", especially with respect to Cathepsin C, refers to Cathepsin C polynucleotides or polypeptides purified from natural sources as well as purified recombinant molecules (wherein the term purified comprises a partial purification as well as a complete purification).
25

The preparation of recombinant polypeptide or polynucleotide molecules and the purification of naturally occurring molecules from cells or tissue, as well as the preparation of cell- or tissue extracts is well known to the person of skill in the art (see e.g. also the standard literature listed below).

30 These comprise e.g. amplifying polynucleotides of desired length via the polymerase chain reaction (PCR) on the basis of the published genomic or coding polynucleotide sequences and the subsequent cloning of the produced polynucleotides in host cells (see e.g. standard literature listed below).

In the context of present invention, the term „polypeptide“ refers to a molecule comprising amino acids bound to each other by peptide bonds containing at least 50 amino acids coupled to each other in a linear mode to form a polypeptide chain. Shorter molecules of this kind are referred to as peptides. The term „protein“ refers to molecules comprising at least one
5 polypeptide chain but can refer also to molecules comprising more than one polypeptide chains associated or bound to each other. Thus, the term „protein“ comprises the term „polypeptide“.

In the following, the invention is explained in more detail by means of examples without meaning to be limited by them.

Examples:

Materials and Methods:

5 **Mouse strains used:**

Five different inbred mouse strains were used: AKR/J (AKR), CBA/J (CBA), C3H/HeJ (C3H), C57BL/6J (B6) and C58/J (C58). Mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). For these mice strains it has been shown that they differ significantly
10 concerning several in vivo measures of pain (Mogil et al 1999)

Total RNA Isolation:

Total RNA from DRGs (dorsal root ganglia) was isolated with the PicoPure™ RNA Isolation Kit (Arcturus) following the manufacturer's instructions. RNA quality was assessed using the 2100
15 Bioanalyzer and RNA 6000 Nano LabChip™ kit (Agilent).

Affymetrix GeneChip™ Microarrays:

First-strand cDNA synthesis was performed using 500ng total RNA with a 100pM T7-(dT)₂₄ oligomer (GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG-dT₂₄ SEQ ID NO:4) according to Baugh, L.R, Hill, A.A., Brown, E.L. and Hunter, C.P. (2001) *Nucleic Acids Res.* **29**,
20 e29 and SuperScript II Reverse Transcriptase following the manufacturer's instructions. Double-stranded cDNA was synthesized and then extracted using phenol-chloroform followed by an ethanol precipitation step. An *in vitro* transcription reaction was performed with the doublestranded cDNA sample using the BioArray High Yield RNA Transcription Labeling kit
25 (Enzo) according to the manufacturer's instructions. Transcription reactions were incubated at 37°C for 16h. cRNA was purified using the RNeasy™ Mini kit (Qiagen) protocol for RNA cleanup and quantified by a spectrophotometer. The biotin-labeled cRNA was fragmented using a RNA fragmentation buffer (200mM Tris-acetate, 500mM KOAc, 150mM MgOAc, pH 8.1). Hybridization and staining of Mouse Genome 430 2.0 GeneChips™ (Affymetrix) was
30 performed according to the manufacturer's instructions. The microarrays were scanned using a GeneChip™ 3000 Scanner, and the scanned data were imported and analyzed using Resolver v5.1 expression data analysis software (Rosetta Biosoftware).

L5 spinal nerve transection and sham surgical procedures:

In anesthetized mice, the left L5 spinal nerve was exposed and the transverse process was then partially removed. After separation from the L4 spinal nerve, the L5 spinal nerve was transected. Sham surgery was identical to the L5 spinal nerve transection surgery, however, the L5 spinal nerve was not transected (see DeLeo et al. 2000).

5

Determination of paw withdrawal threshold:

Paw withdrawal thresholds (PWTs) were assessed using a dynamic plantar aesthesiometer (see Szabo et al. 2005). After acclimation in a compartment with metal mesh floor, the stimulator was positioned under the animal's hindpaw, a straight metal filament driven by an electrodynamic actuator touched the plantar surface and exerted an increasing upward force until the animal removed the paw (paw withdrawal threshold, PWT). PWTs were assessed for hindpaws of the ipsilateral, operated side and of the contralateral side. Each animal was used at one occasion only. In all animal experiments the ethics guidelines for investigations in conscious animals were obeyed, and the procedures were approved by the local Ethics Committee

15

Correlational analysis:

For correlational analysis, the "pain phenotype" was defined for each nerve-transected animal (Chung animal) as $C1 - S1$, where $C1 = \ln(\text{ipsilateral PWT} / \text{contralateral PWT})$ and $S1 = \text{mean}_{\text{all sham animals within same strain}} \ln(\text{ipsilateral PWT} / \text{contralateral PWT})$.

20

Two measures of differential transcriptional regulation were defined for each Chung animal and each measured gene based on its intensity expression data. The "raw intensity measure" was taken as the intensity measure computed by the Resolver expression data analysis software (v5.1) for the respective gene and animal. The "log ratio measure" was computed for a specific gene and Chung animal as $\ln(C2 / S2)$, where $C2 = \text{Chung expression intensity}$ and $S2 = \text{mean}_{\text{all sham animals within same strain}} \text{Sham expression intensity}$.

25

Before correlations were computed, the set of genes was filtered to exclude genes that were expressed below noise level and without significant Chung vs. sham regulation. Eligible genes must be regulated in at least 60% of Chung animals with an absolute fold-change ≥ 1.5 or in at least 20% of Chung animals with an absolute fold-change ≥ 2.0 . Also, corresponding gene expression had to be detectable ("present") in at least five animals as defined by a respective intensity $p\text{-value} < 0.001$.

30

Pearson correlation coefficients for each gene between the pain phenotypic scores and one of the defined measures of transcriptional regulation were computed using the R software package (<http://www.r-project.org/>). Based on these, p-values of statistical significance and

35

corresponding false-discovery rates (FDRs) were generated following the method of Storey et al. (2002). Genes with $FDR < 0.05$ under "log ratio measure" or "intensity measure" were considered significantly correlated.

5 **Legend to the Figures:**

Figure 1: CathepsinC – Correlation Plot

Figure 1 shows for every individual mouse its neuropathic pain phenotype (mechanical hypersensitivity, X-axis) and the corresponding gene regulation of Cathepsin C (log ratio(Chung vs. Sham control), Y-axis) in the L5 DRG. Mouse data are colour-coded
10 depending on the used strain. A Pearson correlation analysis has been performed and revealed a significant positive correlation of the two parameters pain phenotype and Cathepsin C gene regulation. This means for individual mice that the higher the L5 DRG expression of Cathepsin C in Chung-operated neuropathic mice was, the more pronounced the mechanical
15 hyperalgesia as exhibited in the behavioral test. This significant correlation indicates a causal relationship of Cathepsin C gene expression for the induction of the neuropathic pain phenotype.

Figure 2: Cathepsin C – Intensity data

20 Absolute values of Cathepsin C expression in L5 ganglia of the individual mice of the strains AKR, CBA and C57 after chung or sham surgery.

Figure 3: Fragment of mouse Cathepsin C mRNA, detected by the Affymetrix probe set 1416382_at (Mouse Genome 430 2.0 microarray) (SEQ ID NO.7)

25

Figure 4: Cathepsin C cDNA sequence according to NM_001814 (SEQ ID NO.1)

Figure 5: Cathepsin C protein sequence according to Swiss-Prot HUMAN_CATC P53634 (SEQ ID NO.2)

30

Figure 6: Primer set for Detection of human Cathepsin C cDNA according to SEQ ID NO. 1 (SEQ ID NOs 4 and 5).

Figure 7: Probe for detecting mouse Cathepsin C cDNA (SEQ ID NO.6).

References:

DeLeo JA et al (2000) Transgenic expression of TNF by astrocytes increases mechanical allodynia in a mouse neuropathy model. *Neuroreport* 11:599-602.

- 5 Storey JD. (2002) A direct approach to false discovery rates. *Journal of the Royal Statistical Society, Series B*, 64: 479-498.

- Szabo A et al. (2005) Role of transient receptor potential vanilloid 1 receptors in adjuvant-induced chronic arthritis: in vivo study using gene-deficient mice. *J. Pharmacol. Exp. Ther.*
10 314:111-119.

Julius and Basbaum "Molecular mechanisms of nociception", *Nature*, volume 413, 13. September 2001, pp. 203 – 209;

- 15 Scholz and Woolf "Can we conquer pain", *Nature neuroscience supplement*, volume 5, November 2002, pp. 1062 – 1067;

- Wood, J.D. "Pathobiology of Visceral Pain: Molecular Mechanisms and Therapeutic Implications II. genetic approaches to pain therapy", *American Journal of Physiological Gastrointestinal Liver Physiology*, 2000, volume 278, G507-G512;
20

Woolf and Mannion "Neuropathic pain: aetiology, symptoms mechanisms, and management", *The LANCET*, volume 353, June 5, 1999, pp. 1959 – 1964;

- 25 Woolf J. and Salter M.W. "Neuronal Plasticity: Increasing the Gain in Pain", *Science*, volume 288, June 9, 2000, pp. 1765-1768;

- Cigic, B.; Dahl, S. W.; Pain, R. H. "The residual pro-part of cathepsin C fulfills the criteria required for an intramolecular chaperone in folding and stabilizing the human proenzyme".
30 *Biochemistry* 39: 12382-12390, 2000

Paris, A.; Strukelj, B.; Pungercar, J.; Renko, M.; Dolenc, I.; Turk, V. "Molecular cloning and sequence analysis of human preprocathepsin C". *FEBS Lett.* 369: 326-330, 1995

Pham, C. T. N.; Armstrong, R. J.; Zimonjic, D. B.; Popescu, N. C.; Payan, D. G.; Ley, T. J. "Molecular cloning, chromosomal localization, and expression of murine dipeptidyl peptidase I" *J. Biol. Chem.* 272: 10695-10703, 1997.

- 5 Pham, C. T. N.; Ley, T. J. : "Dipeptidyl peptidase I is required for the processing and activation of granzymes A and B in vivo". *Proc. Nat. Acad. Sci.* 96: 8627-8632, 1999.

Rao, N. V.; Rao, G. V.; Hoidal, J. R. : "Human dipeptidyl-peptidase I". *J. Biol. Chem.* 272: 10260-10265, 1997.

10

Toomes, C.; James, J.; Wood, A. J.; Wu, C. L.; McCormick, D.; Lench, N.; Hewitt, C.; Moynihan, L.; Roberts, E.; Woods, C. G.; Markham, A.; Wong, M.; and 10 others : "Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis". *Nature Genet.* 23: 421-424, 1999.

15

Wolters, P. J.; Raymond, W. W.; Blount, J. L.; Caughey, G. H. : "Regulated expression, processing, and secretion of dog mast cell dipeptidyl peptidase I." *J. Biol. Chem.* 273: 15514-15520, 1998

20

Heusel, J.W., Wesselschmidt, R., Shresta, S., Russel, J & Ley, T.J. "Cytotoxic lymphocytes require granzyme B for the rapid induction of DNA fragmentation and apoptosis in allogeneic target cells", 1994, *Cell* 76, 977-987.

25

Manour, S., Thomas, K.R., and Capecchi, M.R., 1989, " disruption of the proto-oncogene *Int-2* in mouse embryo-derived stem cells: a general strategy for targeting mutations so non-selectable genes", *Nature* 336, 348-352.

30

Soriano, PI, Montgomery, C., Geske, R., and Bradley, A., 1991, "Targeted disruption of the *c-src* proto-oncogene leads to osteopetrosis in mice", *Cell* 65, 693-702.

Turk, B., Turk, D., and Turk, V., Lysosomal cysteine proteases: more than scavengers. *Biochim Biophys Acta.* 2000 Mar 7;1477(1-2):98-111.

McGuire, M., J., Lipsky, P.E., and Thiele, D. L. (1993) J. Biol. Chem., 268, 2458-2467, Generation of active myeloid and lymphoid granule serine proteases requires processing by the granule thiol protease dipeptidyl peptidase I.

- 5 Henningsson, F., wolters, P., Chapman, H.A., Caughey, G.H., and Pejler, G., (2003), Biol., Chem. 384, 1527-1531, Mast cell cathepsins C and S control levels of carboxypeptidase A and the chymase, mouse mast cell protease 5.

- 10 Adkison, A.M., Raptis, S.Z., Kelley, D.G., and Pham, C.T.N., (2002), J. Clin. Invest. 109, 363-371, Dipeptidyl peptidase I activates neutrophil-derived serine proteases and regulates the development of acute experimental arthritis.

- 15 Wolters, P.J., Pham, C.T.N., Muilenburg, D.J., ley, T.J., and Caughey, G.H., (20019 j: Biol: Chem., 276, 18551-18556, Dipeptidyl peptidase I is essential for activation of mast cell chymases, but not tryptases, in mice.

- 20 Pham, C.T.N., Ivanovich, M.L., Raptis, S.Z., Zehnbaauer, B., and Ley, T.J., (2004) J. Immunol., 173, 7277-7281, Papillon-Lefèvre syndrome: correlating the molecular, cellular, and clinical consequences of cathepsin C/dipeptidyl peptidase I deficiency in humans.

De Haar, S.F., Jansen, D.C., Schoenmaker, T., De Vree, H., Everts, V., and Beertsen, W. (2004) Hum. Mutat. 23, 524-524, Loss-of-function mutations in cathepsin C in two families with Papillon-Lefèvre syndrome are associated with deficiency of serine proteinases in PMNs.

- 25 Sheth, P.D., Pedersen, NM., Walls, A.F., and McEuen, A.R. (2003) Biochem., Pharmacol., 66, 2251-2262, Inhibition of dipeptidyl peptidase I in the human mast cell line HMC-1: blocked activation of tryptase, but not of the predominant chymotryptic activity.

Literature for standard laboratory methods

- 30 If not indicated otherwise, standard laboratory methods were or can be performed according to the following standard literature:

Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual. Second edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 545 pp;

Current Protocols in Molecular Biology; regularly updated, e.g. Volume 2000; Wiley & Sons, Inc; Editors: Fred M. Ausubel, Roger Brent, Robert Eg. Kingston, David D. Moore, J.G. Seidman, John A. Smith, Kevin Struhl.

5

Current Protocols in Human Genetics; regularly updated; Wiley & Sons, Inc; Editors: Nicholas C. Dracopoli, Honathan L. Haines, Bruce R. Korf, Cynthia C. Morton, Christine E. Seidman, J.G. Seigman, Douglas R. Smith.

10 Current Protocols in Protein Science; regularly updated; Wiley & Sons, Inc; Editors: John E. Coligan, Ben M. Dunn, Hidde L. Ploegh, David W. Speicher, Paul T. Wingfield.

Molecular Biology of the Cell; third edition; Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J.D.; Garland Publishing, Inc. New York & London, 1994;

15

Short Protocols in Molecular Biology, 5th edition, by Frederick M. Ausubel (Editor), Roger Brent (Editor), Robert E. Kingston (Editor), David D. Moore (Editor), J.G. Seidman (Editor), John A. Smith (Editor), Kevin Struhl (Editor), October 2002, John Wiley & Sons, Inc., New York"

20

Transgenic Animal Technology A Laboratory Handbook. C.A. Pinkert, editor; Academic Press Inc., San Diego, California, 1994 (ISBN: 0125571658)

Gene targeting: A Practical Approach, 2nd Ed., Joyner AL, ed. 2000. IRL Press at Oxford
25 University Press, New York;

Manipulating the Mouse Embryo: A Laboratory Manual. Nagy, A, Gertsenstein, M., Vintersten, K., Behringer, R., 2003, Cold Spring Harbor Press, New York;

30 Remington's Pharmaceutical Sciences, 17th Edition, 1985 (for physiologically tolerable salts (anorganic or organic), see esp. p. 1418)

Standard Literature for Laboratory Methods:

35

If not indicated otherwise, laboratory methods were or can be performed according to standard methods listed in the below standard literature:

- 5 Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual. Second edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 545 pp or Current Protocols in Molecular Biology;
- 10 Current Protocols in Molecular Biology; regularly updated, e.g. Volume 2000; John Wiley & Sons, Inc; Editors: Fred M. Ausubel, Roger Brent, Robert Eg. Kingston, David D. Moore, J.G. Seidman, John A. Smith, Kevin Struhl.
- 15 Current Protocols in Human Genetics; regularly updtated, e.g. Volume 2003; John Wiley & Sons, Inc; Editors: Nicholas C. Dracopoli, Honathan L. Haines, Bruce R. Korf, Cynthia C. Morton, Christine E. Seidman, J.G. Seigman, Douglas R. Smith.
- 20 Current Protocols in Protein Science; regularly updated, e.g. Volume 2003; John Wiley & Sons, Inc; Editors: John E. Coligan, Ben M. Dunn, Hidde L. Ploegh, David W. Speicher, Paul T. Wingfield.
- 25 Molecular Biology of the Cell; third edition; Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J.D.; Garland Publishing, Inc. New York & London, 1994;
- Gene Targeting: a practical approach (1995), Editor: A.L. Joyner, IRL Press
- 25 Remington's Pharmaceutical Sciences, Edition 17, 1985.

Claims

- 5 1. The use of Cathepsin C or functional fragments or derivatives thereof for identifying compounds that modulate pain.
2. The use of a non-human transgenic animal heterologously expressing Cathepsin C or a functional fragment thereof for identifying or analyzing compounds that modulate pain.
- 10 3. The use of a non-human transgenic animal heterologously expressing Cathepsin C or a functional fragment thereof as a model system for enhanced pain sensitivity.
4. The use of a non-human Cathepsin C knock-out animal for identifying or analyzing compounds that modulate pain.
- 15 5. The use of a non-human Cathepsin C knock-out animal as a model system for lowered pain sensitivity.
6. The use of a cell heterologously expressing Cathepsin C or a functional fragment thereof for identifying compounds that modulate pain.
- 20 7. The use of a cell heterologously expressing Cathepsin C or a functional fragment thereof as a model system for enhanced pain sensitivity.
8. The use of a Cathepsin knock-out cell for identifying or analyzing compounds that modulate pain.
- 25 9. The use of a Cathepsin knock-out cell as a model system for lowered pain sensitivity.
- 30 10. The use of a cell heterologously expressing a reporter gene expressibly linked to the Cathepsin C promoter and/or enhancer or a functional fragment thereof for identifying or analyzing compounds that modulate pain.
- 35 11. A method of identifying or analyzing compounds modulating and/or preventing pain, comprising the steps

- a. Providing at least two samples;
- b. Contacting one sample containing Cathepsin C or a functional fragment or derivative thereof with a compound,
- c. determining the activity of Cathepsin C in the presence of compound,
- 5 d. determining the activity of Cathepsin C in the absence of compound, and
- e. comparing the activity of Cathepsin C according to c) with that according to d).

12. A method for identifying or analyzing compounds that modulate and/or prevent pain comprising:

- 10 a. Contacting a Cathepsin C protein or functional fragment or derivative thereof with a test compound; and
- b. Determining whether the test compound modulates the activity of the Cathepsin C protein or functional fragment or derivative thereof.

13. A Method for identifying or analyzing compounds that modulate and/or prevent pain comprising:

- a. Contacting a cell, which has a detectable amount or activity of Cathepsin C or of a functional fragment or derivative thereof, with a test compound;
- 20 b. Determining whether the test compound is able to modulate the amount or activity of Cathepsin C or the functional fragment or derivative thereof present in the cell.

14. A method for identifying or analyzing compounds that modulate and/or prevent pain comprising:

- 25 a. Contacting a nucleic acid coding for a Cathepsin C protein or a functional fragment or derivative thereof with a test compound in a transcriptionally active system, and
- b. Determining the amount of mRNA coding for the Cathepsin C protein or the functional fragment or derivative thereof present in said system in presence of said compound, and
- 30 c. Determining whether the compound is capable of modulating the amount of mRNA coding for the Cathepsin C protein or functional fragment or derivative present in said system.

15. A method for identifying compounds or analyzing compounds that modulate and/or prevent pain comprising:

- a. Providing a cell transfected with a nucleic acid vector comprising the promoter of a Cathepsin C gene or a functional fragment thereof operationally coupled to a reporter gene or a functional fragment thereof:
 - 5 b. Providing a cell transfected with a control vector which comprises a reporter gene or a functional fragment thereof not being operationally coupled to a functional Cathepsin C promoter;
 - c. Determining the reporter gene activity of the cell according to a) and b) in the presence of a test compound;
 - 10 d. Determining the reporter gene activity of the cell according to a) and b) in absence of the test compound.
16. A method of identifying or analyzing a compound that modulates and/or prevents pain in a subject comprising:
- 15 a. Assaying a biological activity of Cathepsin C or a functional fragment or derivative thereof in the presence of one or more test compounds to identify one or more modulating compounds that modulate the biological activity of Cathepsin C, and
- 20 b. Testing one or more of the modulating compounds for their ability to reduce pain, pain sensation or pain sensitivity in a subject.
17. A method for analyzing the pain threshold in an individual comprising analyzing the amount of Cathepsin C in a taken sample of said individual in comparison to one or more reference samples as to whether the amount of Cathepsin C mRNA and/or
- 25 protein present in said sample is different from that of one or more reference samples, wherein the presence of a higher amount indicates an increased pain sensitivity and the presence of a lower amount indicates a decreased pain sensitivity in said individual.
18. A method for adapting the dosage of a pharmaceutical for the prevention and/or
- 30

treatment of pain in an individual, which method comprises examining a taken sample of an individual as to whether the amount of Cathepsin C mRNA and/or protein present in said sample is different from that of one or more reference samples, said dosage being adapted depending on whether the amount of protein and/or mRNA in the taken sample of the individual is different from that of the one or more reference samples, wherein a higher amount of Cathepsin C in the taken sample of the individual is indicative of a need for a higher dose and a lower amount of Cathepsin C in the sample of the individual is indicative of a need for a lower dose.

19. The use of means for the detection of Cathepsin C for diagnosing enhanced pain sensitivity in an individual by analyzing a biological sample taken from the body of an individual to be examined.

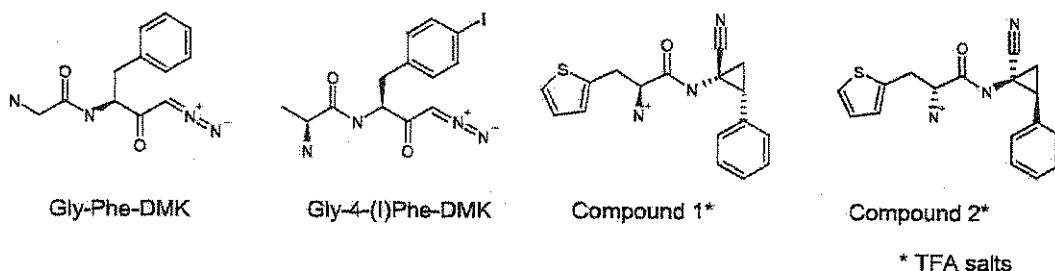
20. A test kit for diagnosing pain sensitivity in individuals, which test kit comprises at least one means for the detection of Cathepsin C in a biological samples.

21. Use of a composition lowering the amount or activity of Cathepsin C in a subject in the manufacture of a medicament for treating existing pain.

22. Use of a composition lowering the amount or activity of Cathepsin C in a subject in the manufacture of a medicament for lowering pain sensitivity.

23. A method for modulating the pain sensitivity in an offspring from a non-human female subject comprising transferring a nucleic acid conferring a modulated Cathepsin C expression into Zygotes Selecting offspring according to its Cathepsin C expression characteristics (modulated).

24. A compound able to lower Cathepsin C activity and/or expression for the treatment of pain, wherein said compound is selected from



25. The use according to one of the claims 1-3, 6, 8 and 10 or the method according to one of the claims 11- 16, wherein the modulation is a decrease, abolishment or prevention of pain.

26. The use according to claims one of the claims 1-3, 6, 8 and 10 or the method according to one of the claims 11- 16, wherein the modulation is an increase.

5 27. The use or the method according to claim 26 wherein the compounds are compounds for the prevention or treatment of pain.

28. The use, the test kit or the method according to one of the above claims, wherein Cathepsin C is mammalian and preferably human Cathepsin C.

10 29. The use, test kit, method or compound according to one of the above claims, wherein the pain is acute or chronic pain and preferably chronic pain.

30. The use, test kit, method or compound according to one of the above claims, wherein the pain is osteoarthritic, inflammatory or neuropathic pain.

15

31. The use according to one of the claims 1-3, 6, 8 and 10 or the method according to one of the claims 11- 16, wherein Cathepsin C is used as an isolated protein or polynucleotide.

20 32. The use one of the claims 1-3, 6, 8 and 10 or the method according to one of the claims 11- 16, wherein Cathepsin C is

- a. a polypeptide or functional fragment thereof comprising or consisting of the sequence according to SEQ ID NO.2, or
- 25 b. a polypeptide that is encoded by a polynucleotide comprising or consisting of the sequence according to SEQ ID NO 1, or
- c. a polypeptide that is encoded by a polynucleotide able to hybridize with a polynucleotide consisting of SEQ ID NO.1 under stringent conditions.

30 33. The use one of the claims 1-3, 6, 8 and 10 or the method according to one of the claims 11- 16, wherein Cathepsin C is

- a. a polynucleotide comprising or consisting of the sequence according to SEQ ID No.1, or

35

40

- b. a polynucleotide able to hybridize with a polynucleotide consisting of SEQ ID No.1 under stringent conditions, or
- c. a polynucleotide coding for Cathepsin C according to SEQ ID NO 2 or for a functional fragment thereof.

5

34. The method according to claim 13 or 14, wherein a cell expressing recombinant Cathepsin C is used.

10

35. The method according to one of the claims 11- 16, wherein the determination of Cathepsin C activity concerns its protease activity.

36. The use according to one of the claims 6-10 or the method according to one of the claims 13 or 14, wherein the cell is a mammalian and preferably a rodent cell.

15

37. The use according to one of the claims 2-5 or the method according to claim 23, wherein the non-human animal is a mammal and preferably a rodent and more preferably a mouse.

20

38. The use according to claim 10 or the method according to claim 15, wherein the reporter gene is.

39. The use according to claim 20 , the method according to claim 18 or 19 or the test kit as claimed in claim 21, wherein the sample is a mammalian and preferably a human sample.

25

40. The use according to claim 20 , the method according to claim 18 or 19 or the test kit as claimed in claim 21, wherein the sample is a histological sample, a biopsy sample, a cell extract, one or more cells or taken body fluid.

30

41. The use according to claim 20 or the test kit according to claim 21, wherein the means for the detection is a means for the detection of Cathepsin C DNA.

35

42. The use or test kit according to claim 41, wherein the means is a primer or set of primers, a suitable probe or a sequence-specific anti-DNA antibody.

43. The method as claimed in one of the claims 14, 18 or 19, wherein the change in the amount of mRNA is analyzed, preferably using one or more suitable primers for the amplification of Cathepsin C cDNA or one or more probes suitable for hybridization with Cathepsin C cDNA or mRNA at standard conditions.

5

44. The method according to claim 43, wherein the amount of mRNA is analysed by means of PCR, Northern Blot, Array- or Chip-hybridisation.

10

45. The use as claimed in claim 20 or the test kit as claimed in claim 20, wherein the means for detection is a means for detecting Cathepsin C mRNA and/or protein present in a biological sample.

15

46. The use or test kit as claimed in claim 45, wherein the means for detecting Cathepsin C protein is an antibody.

20

47. The method according to one of the claims 14, 18 or 19, wherein the change in the amount of Cathepsin C protein is determined.

48. The method according to claim 47, wherein the protein is detected with the aid of at least one antibody.

25

49. The use, method or test kit as claimed in claim 47 or 48, wherein the detection is carried out by way of ELISA, Western blot or protein chip.

50. The use, method or test kit as claimed in claim 47 or 48, wherein detection is carried out by means of immunohistochemical or immunoradiochemical detection methods.

30

51. The method according to one of the claims 16 or 17, wherein the amount of Cathepsin C is lowered.

52. The method according to claim 51, wherein the amount of Cathepsin C is lowered in neuronal tissue.

35

53. The method according to one of the claims 16 or 17, wherein the activity of Cathepsin C is lowered.

54. The method according to claim 53 wherein the lowered activity of Cathepsin C is the protease activity.

5 55. The method according to claim 53, wherein the Cathepsin C activity is lowered in neuronal tissue.

56. The method according to claim 18, wherein the expression is modulated in neuronal or lymphatic tissue.

10

57. The use according to one of the claims 21 or 22 or the compound according to claim 24, wherein the compound is a reversible peptide nitrile inhibitor of Cathepsin C and preferably a dipeptide alpha-aminoacetonitrile inhibitor of Cathepsin C.

15

DE2008-026.ST25.txt
SEQUENCE LISTING

<110> sanofi-aventis

<120> USE OF CATHEPSIN C

<130> DE2008/026

<150> 08290285.9

<151> 2008-03-26

<160> 6

<170> PatentIn version 3.3

<210> 1

<211> 1924

<212> DNA

<213> Homo sapiens

<400> 1

```

cgtagctatt tcaaggcgcg cgctctgtgg tggactcacc gctagcccg c agcgctcggc      60
ttcctggtaa ttcttcacct cttttctcag ctccctgcag catgggtgct gggccctcct      120
tgctgctcgc cgccctcctg ctgcttctct ccggcgacgg cgccgtgcgc tgcgacacac      180
ctgccaaactg cacctatctt gacctgctgg gcacctgggt cttccagggt ggctccagcg      240
gttcccagcg cgatgtcaac tgctcggtta tgggaccaca agaaaaaaaa gtagtggtgt      300
accttcagaa gctggataca gcatatgatg accttgcaa ttctggccat ttcaccatca      360
tttacaacca aggcctttgag attgtgttga atgactacaa gtggtttgcc ttttttaagt      420
ataaagaaga gggcagcaag gtgaccactt actgcaacga gacaatgact ggggtgggtgc      480
atgatgtgtt gggccggaac tgggcttgtt tcaccgaaa gaaggtggga actgcctctg      540
agaatgtgta tgtcaacata gcacacctta agaattctca ggaaaagtat tctaataaggc      600
tctacaagta tgatcacaac tttgtgaaag ctatcaatgc cattcagaag tcttggactg      660
caactacata catggaatat gagactctta ccctgggaga tatgattagg agaagtgggtg      720
gccacagtcg aaaaatccca aggcccaaac ctgcaccact gactgctgaa atacagcaaa      780
agattttgca ttgccaaca tcttgggact ggagaaatgt tcatggtatc aattttgtca      840
gtcctgttcg aaaccaagca tcctgtggca gctgctactc atttgcttct atgggtatgc      900
tagaagcgag aatccgtata ctaaccaaca attctcagac cccaatccta agccctcagg      960
aggttgtgtc ttgtagccag tatgctcaag gctgtgaagg cggcttccca taccttattg      1020
caggaaagta cgccaagat tttgggctgg tggagaagc ttgcttcccc tacacaggca      1080
ctgattctcc atgcaaaatg aaggaagact gctttcgtaa ttactctct gagtaccact      1140
atgtaggagg tttctatgga ggtgcaatg aagccctgat gaagcttgag ttggtccatc      1200
atggggcccat ggcagttgct tttgaagtat atgatgactt cctccactac aaaaagggga      1260
tctaccacca cactggtcta agagaccctt tcaaccctt tgagctgact aatcatgctg      1320
ttctgcttgt gggctatggc actgactcag cctctgggat ggattactgg attgttaaaa      1380
acagctgggg caccggctgg ggtgagaatg gctacttccg gatccgcaga ggaactgatg      1440

```

DE2008-026.ST25.txt

```

agtgtgcaat tgagagcata gcagtgccag ccacaccaat tcctaaattg taggggatgc 1500
cttccagtat ttcataatga tctgcatcag ttgtaaaggg gaattgggtat attcacagac 1560
tgtagacttt cagcagcaat ctcagaagct tacaaataga tttccatgaa gatatttgtc 1620
ttcagaatta aaactgccct taattttaat atacctttca atcggccact ggccattttt 1680
ttctaagtat tcaattaagt ggggaattttc tggaagatgg tcagctatga agtaatagag 1740
tttgcttaat catttgtaat tcaaacatgc tatatttttt aaaatcaatg tgaaaacata 1800
gacttatatt taaattgtac caatcacaag aaaataatgg caataattat caaaactttt 1860
aaaatagatg ctcatatttt taaaataaag ttttaaaaat aactgcaaaa aaaaaaaaaa 1920
aaaa 1924

```

```

<210> 2
<211> 463
<212> PRT
<213> Homo sapiens

```

```
<400> 2
```

```

Met Gly Ala Gly Pro Ser Leu Leu Leu Ala Ala Leu Leu Leu Leu Leu
1 5 10 15

```

```

Ser Gly Asp Gly Ala Val Arg Cys Asp Thr Pro Ala Asn Cys Thr Tyr
20 25 30

```

```

Leu Asp Leu Leu Gly Thr Trp Val Phe Gln Val Gly Ser Ser Gly Ser
35 40 45

```

```

Gln Arg Asp Val Asn Cys Ser Val Met Gly Pro Gln Glu Lys Lys Val
50 55 60

```

```

Val Val Tyr Leu Gln Lys Leu Asp Thr Ala Tyr Asp Asp Leu Gly Asn
65 70 75 80

```

```

Ser Gly His Phe Thr Ile Ile Tyr Asn Gln Gly Phe Glu Ile Val Leu
85 90 95

```

```

Asn Asp Tyr Lys Trp Phe Ala Phe Phe Lys Tyr Lys Glu Glu Gly Ser
100 105 110

```

```

Lys Val Thr Thr Tyr Cys Asn Glu Thr Met Thr Gly Trp Val His Asp
115 120 125

```

```

Val Leu Gly Arg Asn Trp Ala Cys Phe Thr Gly Lys Lys Val Gly Thr
130 135 140

```

```

Ala Ser Glu Asn Val Tyr Val Asn Thr Ala His Leu Lys Asn Ser Gln
145 150 155 160

```

```

Glu Lys Tyr Ser Asn Arg Leu Tyr Lys Tyr Asp His Asn Phe Val Lys
Seite 2

```

165

170

175

Ala Ile Asn Ala Ile Gln Lys Ser Trp Thr Ala Thr Thr Tyr Met Glu
 180 185 190

Tyr Glu Thr Leu Thr Leu Gly Asp Met Ile Arg Arg Ser Gly Gly His
 195 200 205

Ser Arg Lys Ile Pro Arg Pro Lys Pro Ala Pro Leu Thr Ala Glu Ile
 210 215 220

Gln Gln Lys Ile Leu His Leu Pro Thr Ser Trp Asp Trp Arg Asn Val
 225 230 235 240

His Gly Ile Asn Phe Val Ser Pro Val Arg Asn Gln Ala Ser Cys Gly
 245 250 255

Ser Cys Tyr Ser Phe Ala Ser Met Gly Met Leu Glu Ala Arg Ile Arg
 260 265 270

Ile Leu Thr Asn Asn Ser Gln Thr Pro Ile Leu Ser Pro Gln Glu Val
 275 280 285

Val Ser Cys Ser Gln Tyr Ala Gln Gly Cys Glu Gly Gly Phe Pro Tyr
 290 295 300

Leu Ile Ala Gly Lys Tyr Ala Gln Asp Phe Gly Leu Val Glu Glu Ala
 305 310 315 320

Cys Phe Pro Tyr Thr Gly Thr Asp Ser Pro Cys Lys Met Lys Glu Asp
 325 330 335

Cys Phe Arg Tyr Tyr Ser Ser Glu Tyr His Tyr Val Gly Gly Phe Tyr
 340 345 350

Gly Gly Cys Asn Glu Ala Leu Met Lys Leu Glu Leu Val His His Gly
 355 360 365

Pro Met Ala Val Ala Phe Glu Val Tyr Asp Asp Phe Leu His Tyr Lys
 370 375 380

Lys Gly Ile Tyr His His Thr Gly Leu Arg Asp Pro Phe Asn Pro Phe
 385 390 395 400

Glu Leu Thr Asn His Ala Val Leu Leu Val Gly Tyr Gly Thr Asp Ser
 405 410 415

Ala Ser Gly Met Asp Tyr Trp Ile Val Lys Asn Ser Trp Gly Thr Gly
 420 425 430

Trp Gly Glu Asn Gly Tyr Phe Arg Ile Arg Arg Gly Thr Asp Glu Cys
 435 440 445 450

435

DE2008-026.ST25.txt
440 445

Ala Ile Glu Ser Ile Ala Val Ala Ala Thr Pro Ile Pro Lys Leu
450 455 460

<210> 3 (Description ID No.4)

<211> 30

<212> DNA

<213> Homo sapiens (forward)

<400> 3

gctccctgca gcatgggtgc tgggccctcc

30

<210> 4 (Description ID No.5)

<211> 30

<212> DNA

<213> Homo sapiens (reverse)

<400> 4

gcatacccta caatttagga attggtgtgg

30

<210> 5 (Description ID No.6)

<211> 40

<212> DNA

<213> Mus musculus

<400> 5

ggccagtga ttgtaatacg actcactata gggaggcggg

40

<210> 6 (Description ID No.7)

<211> 360

<212> DNA

<213> Mus musculus

<220>

<221> misc_feature

<222> (157)..(157)

<223> n is a, c, g, or t

<400> 6

gctgttttgc ttgtgggcta tggaagagat ccagttactg ggatagaata ctggattata

60

aagaacagct ggggctctaa ctgggggggag agtggctact tccgtatccg cagaggaact

120

gatgaatgtg caattgagag tatagccgtg gcggccnata ccgattccta aattatagga

180

catagctccc agtgttacat acgggtcttt atcactcaca gaggatttta gtcacatgct

240

gaagactttt tcagagcaat atcagaagct taccactaag catctttaaa gaattttgtc

300

tttgaactta aaaccatcct tgattttttt cttttaatat cttccccatc aactactgaa

360