

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
3 March 2011 (03.03.2011)

(10) International Publication Number
WO 2011/023786 A1

(51) International Patent Classification:
G01N 33/68 (2006.01) *C12Q 1/37* (2006.01)
G01N 33/573 (2006.01)

(74) Agent: KÖRNER, Kathrin; K 801, c/o Sanofi-Aventis Deutschland GmbH, Patents Germany, Industriepark Höchst, Geb. K 801, 65926 Frankfurt (DE).

(21) International Application Number:
PCT/EP2010/062525

(22) International Filing Date:
27 August 2010 (27.08.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09290659.3 31 August 2009 (31.08.2009) EP

(71) Applicant (for all designated States except US):
SANOFI-AVENTIS [FR/FR]; 174 avenue de France,
F-75013 Paris (FR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): GEBAUER, Matthias [DE/DE]; c/o Sanofi-Aventis Deutschland GmbH, 65926 Frankfurt am Main (DE). MICHAELIS, Martin [DE/DE]; 65926 Frankfurt (DE). DING-PFENNIGDORFF, Danping [DE/DE]; c/o Sanofi-Aventis Deutschland GmbH, 65926 Frankfurt am Main (DE). SCHULTE, Anke M. [DE/DE]; c/o Sanofi-Aventis Deutschland GmbH, 65926 Frankfurt am Main (DE). METZ-WEIDMANN, Christiane [DE/DE]; c/o Sanofi-Aventis Deutschland GmbH, 65926 Frankfurt am Main (DE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))



WO 2011/023786 A1

(54) Title: USE OF CATHESPIN H

(57) Abstract: Present invention concerns the use of Cathepsin H. Other aspects of the invention concern methods for screening pharmaceuticals, for diagnosing pain susceptibility and for the treatment of pain.

Use of Cathepsin H

Present invention concerns the use of Cathepsin H. Other aspects of the invention

- 5 concern methods for screening pharmaceuticals, for diagnosing pain susceptibility and for the treatment of pain.

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-

- 10 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
20 state of the art treatments, efforts need to be directed to the discovery of new targets for novel classes of analgesics.

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 H or functional fragments or derivatives thereof for identifying compounds that modulate pain and especially neuropathic pain.

- 30 The invention is based on the surprising finding of the inventors, demonstrating for the first time that Cathepsin H 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 damage of tissue. Pain is thus part of a warning system of the body to initiate reactions for minimizing actual or potential
5 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.

Pain may be acute or chronic. Acute pain is a physiological signal indicating a potential or actual injury. It occurs accompanying tissue damage, infection,
10 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.

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

15 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
20 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.)

25 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
30 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.

- Cathepsin H (CTSH; alternative titles according to the Atlas of Genetics and Cytogenetics in Oncology and Haematology (<http://atlasgeneticsoncology.org>): ACC-4, ACC-5, CPSB, DKFZp686B24257, EC 3.4.22.16, MGC1519, aleurain, minichain) is a lysosomal protease.

The gene locus of human Cathepsin H is on chromosome 15q24-125 (see e.g. Atlas of Genetics and Cytogenetics in Oncology and Haematology). The gene contains 12 exons spanning over 23 kb genomic sequences. The nucleotide sequence of the human preprocathepsin H was e.g. published by Fuchs and Gassen, 1989, Nucleic Acids Res. 17: 9471-9471. The gene structure of rat cathepsin H was published e.g. by Ishidoh et al, FEBS Letters, 1989, Vol. 253, number 1,2, 103-107. The genomic sequence of homo sapiens CTS defense 15 can e.g. be retrieved at the NCBI homepage under accession number NG_009614.1 (SEQ ID NO.1).

The gene of Cathepsin H has a TATA- and CAAT-less promoter and upstream of exon 1 one GC bo. Two different forms of cathepsin H cDNA have been detected in prostate tissues and cancer cell lines: a full-length form (CTSH) and a truncated form with deletion of 12 amino acids at the signal peptide region (CTSHdelta 10-21) (see Atlas of Genetics and Cytogenetics in Oncology and Haematology). The length of the preproenzyme transcript is 1005 bp. The coding polynucleotide sequence of Cathepsin H is publicly available at the NCBI nucleotide database under several accession numbers, such as: BC006878 (CTSH, complete coding sequence, mus musculus, SEQ ID NO.4, NM_004390.3 (Homo sapiens CTS defense transcript variant1 mRNA (SEQ ID NO.2), NM_148979.2 (Homo sapiens CTS defense transcript variant 2 mRNA (SEQ ID NO.3)),. The skilled person knows how to retrieve further coding or genomic sequences of Cathepsin H from the NCBI database (Cathepsin H of other species; mutants or different isoforms of Cathepsin H, if existing). If in the following, it is referred to the Cathepsin H coding sequence, this can mean any of the above mRNA or coding sequences; with sequences according to SEQ ID. NOs. 2, 3 and 4 being preferred examples.

- The protein sequence of Cathepsin H is publicly available at the NCBI protein database, e.g. under the following accession numbers: homo sapiens (hs) Cathepsin H isoform a preproprotein: NP_004381 (SEQ ID No.8); hs isoform b precursor: NP:683880 (SEQ ID NO.9); murine (mus musculus) Cathepsin H: preproprotein:
- 5 NP_031827; mus musculus isoform CRA_a: EDL20900, mus musculus isoform CRA_b: EDL20901, rat (Rattus norvegicus) Cathepsin H: NP_037071; cathepsin H isoform CRA-a (Rattus norvegicus): EDL77562, isoform CRA-b (Rattus norvegicus) of Cathepsin H: EDL77563, cathepsin H, Moreover the protein sequence is publicly available at the UniProtKB database (www.beta.uniprot.org), under accession
- 10 numbers: P09668 (HUMAN_CATH, human Cathepsin H, SEQ ID NO.5) If in the following, it is referred to the Cathepsin H protein or amino acid sequence, this can mean any of the above protein sequences or translated protein sequences from the above listed coding sequences; such as the following sequences: SEQ ID NO.s 5, 6, 7, 8 or 9.
- 15 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-adress: 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.
- 20 Cathepsin H is a lysosomal protease with aminopeptidase and weaker endopeptidase function. It belongs to the family of C1 (papain-like) cystein proteases. Cathepsin H protein is synthesized as a proenzyme of 335 amino acids and proteolytically processed into an active single chain inside of endosomes or lysosomes. In addition to the heavy and light chain (together denominated long
- 25 chain), Cathepsin H contains a so-called mini chain "EPQNCSAT" that is derived from the propeptide. (For the structure of Cathepsin H, see also Turk et al., Biological Chemistry, 1997). The mini chain appears to be involved in the aminopeptidase activity of Cathepsin H and play a key role in substrate recognition. A recombinant form of human Cathepsin H lacking the mini-chain was shown to be an
- 30 endopeptidase (Valiljeva et al, 2003). Endopeptidase substrates of Cathepsin H are e.g.: Bz-Arg+NHNap, Bz-Arg+NH-Mec, Bz-Phe-Val-Arg+NHMec,. It has dipeptidyl-peptidase activity for the substrates: Pro-Gly+Phe and Pro-Arg+NHNap. It has

aminopeptidase activity for the substrates: H-Arg-NH-Mec, Bz-Arg-NH-Mec, Bz-Arg-NH-Mec and H-Cit-NH-Mec (see e.g. Rothe and Dodt, 1992; Bz=benzoyl, NH-Mec = e-methylcoumaryl-7-amide; Cit= citrulline). Naturally occurring inhibitors of Cathepsin H are the cystatins, alpha2-macroglobulin as well as antigens from mouse cytotoxic lymphocytes CTLA-2beta.

Cathepsin H is ubiquitously expressed with highest level in the kidney. Its expression may be increased in some cancerous tissues. Cathepsin is predominantly located in the endosomal-lysosomal compartment, but also secreted to some extent and circulating in the blood.

- 10 The functions of Cathepsin H comprise in general: protease activity, e.g hydrolase activity, peptidase activity, e.g. aminopeptidase, dipeptidylpeptidase, exopeptidase or endopeptidase activity, transacylase activity (see Koga et al., 1991); cleavage of the above-listed substrates; cleavage of native C5 and generation of chemotaxin C5a, processing of hydrophobic surfactant-associated protein C, cleavage and/or
- 15 degradation of fibronectin and fibrinogen. More specifically, it is involved as a lysosomal cysteine protease in the intracellular protein degradation. It is involved in the generation of chemotaxin C5a by cleavage of native C5. Cathepsin H is involved in the processing of hydrophobic surfactant-associated protein C. It furthermore appears to be involved in the development of unstable atherosclerotic plaques, and
- 20 possibly also in early atherogenesis.

The use according to present invention allows for the identification of novel substances for the prevention and/or treatment of pain and especially of 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

25 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 H (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 H or on the Cathepsin H amount (protein or nucleic acid) in a cell, on Cathepsin H expression, posttranslational modification (e.g. N-glycosilation or processing (e.g. cleavage), protein folding or activation.

To this end, the substance can modulate any of the functions of Cathepsin H (e.g. those as listed above or below). Cathepsin H protein activity can be modulated by the substance e.g by direct interaction and interference of Cathepsin H polypeptide/protein or fragments thereof. The substance can also modulate Cathepsin H 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 the active protein), as well as protein folding etc.

of Cathepsin H. 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 H function / protein activity / expression etc.)

Functions of Cathepsin H 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 or peptide substrates, such those listed above, the ability to be inhibited by one of the inhibitors as listed above.

Functions of Cathepsin H comprise also generally the ability of Cathepsin H protein or nucleic acid or fragments thereof to interact with other molecules (comprising, but not limited to: proteins, peptides, nucleic acids, synthetic molecules) and preferably 5 concern its capability of interacting and cleaving protein substrates.

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 those listed 10 above), and which are also capable of being modified by the respective enzyme.

The modulation of pain and especially neuropathic pain can be either a decrease or an increase.

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

A fragment of a protein or polypeptide is a protein or polypeptide that carries one or 20 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 Examples of Cathepsin H; fragments comprise, e.g. the domains and/or fragments as listed in the description of Figure 7 (see below).

25 A functional fragment of Cathepsin H 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.

A fragment of a polynucleotide acid is a polynucleotide acid or an oligonucleotide 30 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 H 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).

The term derivative of Cathepsin H comprises any type of modification of Cathepsin

H in comparison to the naturally-occurring form, and especially in comparison to
5 Cathepsin H according to SEQ IDs NO: 5, 6, 7, 8 or 9, that is not a deletion. A
functional derivative of Cathepsin H 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
10 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 phosphoorothioate 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
15 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).

20

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

25

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

30

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).

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 5 heterologous expression can be constitutive or 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 10 transgenic animal heterologously expressing Cathepsin H or a functional fragment thereof as a model system for enhanced pain sensitivity, especially neuropathic pain sensitivity.

Yet another aspect of present invention concerns the use of a non-human Cathepsin 15 H knock-out animal for identifying or analyzing compounds that modulate pain and especially neuropathic pain.

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 20 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 H – knock out mice is described in Pham and Ley, 1999.

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

The use of a cell heterologously expressing Cathepsin H or a functional fragment 30 thereof for identifying compounds that modulate pain, especially neuropathic 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 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 prokaryotic cell culture, preferably Pichia or

- 5 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.

According to one embodiment, a modified cell, having a lower Cathepsin H activity as compared to its unmodified state, is used. This way, it can e.g. be tested, if the

- 10 compounds to be tested are able to enhance or restore the lowered or totally abolished Cathepsin H activity. Or it can be tested whether the substances are able to perform their 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.

- 15 The modification can be any type of modification (stable or transient, preferably stable), that leads to a decrease of Cathepsin H activity , Cathepsin H transcript steady state level (i.e. by activation of Cathepsin H transcription or transcript stabilisation) or Cathepsin H protein steady state level (i.e. by activation of Cathepsin H translation or its posttranslational processing; by modulation of Cathepsin H

- 20 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 H, antisense oligonucleotides, RNAi constructs of Cathepsin H, by generating functional or genomic Cathepsin H knock outs (which can e.g. be inducible) or other suitable techniques known within the state of the art. For an

- 25 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 and Function, 2000; Antisense Therapeutics, 1996; Scherr et al, 2003.

- 30 According to one embodiment, a Cathepsin H 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 generation of Cathepsin H (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).

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

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

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

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

The use of a cell heterologously expressing a reporter gene expressibly linked to the Cathepsin H promoter and/or enhancer for identifying or analyzing compounds that modulate pain, especially neuropathic pain, is another embodiment of present set of
25 related inventions.

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
30 promoter is active. The construct is 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 prokaryotic 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 H promoter is a part of the Cathepsin H 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. The cathepsin H promoter is part of the genomic upstream of 5'-sequence of the Cathepsin H gene that steers transcriptional activation of the downstream, transcribed region and can e.g. be derived from Ishidoh et al., FEBS letters, 1989 or Ishidoh et al, Biomed. Biochim. Acta, 1991, 50(4): 541-7.

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

- 5 HeLA, CHO, COS, SF9 or 3T3 cells) or single cell organisms such as yeast (e.g. s. pombe or s. cerevisiae), or a prokaryotic 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.

- 10 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 H promoter. This can e.g. mean that the reporter gene or functional fragment thereof is not operationally coupled to a functional Cathepsin H promoter (i.e. either totally

- 15 devoid of a Cathepsin H promotor, comprises a non functional Cathepsin H 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 H promoter (e.g.

- SV40 or another standard promoter). The functional vector and the control vector can 20 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.

- 25 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 30 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 5 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 10 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 15 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; ALPHAScreenTM) for 20 measuring the interaction of a labeled member with a non-labeled member (e.g. the interaction of labeled proteins with their 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. 25 These assays comprise e.g. reporter gene assays, wherein the regulation of a certain promotor or a 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 30 control of a defined promotor that is to be investigated itself or that is regulated by the signaling cascade under investigation. Suitable reporter 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.

- 5 using a 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 H under suitable conditions to allow for the
10 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.

- 15 One assay for the detection of cathepsin H activity in cells can e.g. be gained from Rüttger et. Al., BioTechniques, 2006.

Assays according to present invention concern e.g.:

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

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

30 A method for identifying or analyzing compounds that modulate and/or prevent pain, preferably neuropathic pain, comprising:

- a. Contacting a Cathepsin H protein or functional fragment or derivative thereof with a test compound; and

- b. Determining whether the test compound modulates the activity of the Cathepsin H protein or functional fragment or derivative thereof.

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

- a. Contacting a cell, which has a detectable amount or activity of Cathepsin H 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 H or the functional fragment or derivative thereof present in the cell.

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

- a. Contacting a nucleic acid coding for a Cathepsin H 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 H 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 H protein or functional fragment or derivative present in said system.

25 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 cell extracts) commercially available.

30 A method for identifying compounds or analyzing compounds that modulate and/or prevent pain, preferably neuropathic pain, comprising:

- a. Providing a cell transfected with a nucleic acid vector comprising the promoter of a Cathepsin H 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 H promoter;
- 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.

5

A method for identifying or analyzing a compound that modulates pain, preferably neuropathic pain comprising

- a. Selecting a compound that modulates the activity of Cathepsin H as a test compound, and
- b. Administering said test compound to a subject in sensation of pain to determine whether the pain is modulated.

10

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

- 20 c. Assaying a biological activity of Cathepsin H 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 H, and
- d. Testing one or more of the modulating compounds for their ability to reduce pain (especially neuropathic pain) and/or pain sensation (especially neuropathic pain sensation) and/ or pain sensitivity (especially neuropathic pain sensitivity) in a subject.

25

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:

30

- A method for analyzing the pain (especially neuropathic pain) threshold in an individual comprising analyzing the amount of Cathepsin H in a taken sample of said individual in comparison to one or more reference samples as to whether the amount of Cathepsin H mRNA and/or protein present in said sample is different from that of 5 one or more reference samples, wherein the presence of a higher amount indicates an increased pain (especially neuropathic pain) sensitivity and the presence of a lower amount indicates a decreased pain (especially neuropathic pain) sensitivity in said individual.
- 10 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 H 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 15 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 H in the taken sample of the individual is indicative of a need for a higher dose and a lower amount of Cathepsin H in the sample of the individual is indicative of a need for a lower dose.
- 20 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 25 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 prokaryotic cell culture, preferably Pichia or E.coli. Cells and samples derived from tissue can be 30 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 5 corresponding in certain characteristics (e.g. its level of Cathepsin H 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 10 detection of Cathepsin H for determining enhanced pain sensitivity (especially neuropathic pain sensitivity) in an individual by analyzing a biological sample taken from the body of an individual to be examined.

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

15 A means to detect Cathepsin H protein or polypeptide can be any means able to specifically detect either wildtype Cathepsin H protein/polypeptide and can also be a means to detect specifically Cathepsin H protein/polypeptide harbouring one or more mutations 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 20 specifically detect Cathepsin H protein, e.g. for use in immunohistological or immunohistochemical techniques (e.g. detection of Cathepsin H protein or certain mutations thereof in histological tissue sections or Cathepsin H protein immobilized on suitable carriers like membranes, chips, ELISA plates etc.).

The means to detect Cathepsin H nucleic acid can e.g. be a means to detect 25 Cathepsin H mRNA /cDNA or genomic DNA, either wildtype or also harbouring one or more mutations regarding their length or their nucleic acid sequence in comparison to a wild type Cathepsin H nucleic acid. The means can e.g. be a means to specifically detect and/or quantify Cathepsin H mRNA and preferably comprises or is a specific Cathepsin H nucleic acid probe or a primer set capable of amplifying 30 Cathepsin H DNA or, e.g. for use in PCR sequencing (for the detection of Mutations in the nucleotide sequence) or capable of amplifying Cathepsin H cDNA, e.g. for use in RT PCR (for the detection and/or quantification of Cathepsin H mRNA expression).

Another means can e.g. be a nucleic acid probe able to specifically hybridise to Cathepsin H 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

- 5 the standard laboratory stock for a given organism. According to one preferred embodiment, the wildtype sequences of Cathepsin H are the sequences according to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8 and/or 9.

The design and synthesis of suitable primers is well known in the art (see also above). Primer sets for the detection of Cathepsin H could for example be the

- 10 following:

Set 1: (Product length for detection of human CTS defense transcript variant 1 = 154 nucleotides, product length for detection of human CTS defense transcript variant 2=118 nucleotides):

Forward primer 5'-GCGCTCCCAGTTGACGCTCT-3' (SEQ ID NO.10)

- 15 Reverse primer 5'-CACGCACAGTTGGCGGC-3' (SEQ ID NO.11)

Set 2: (Product length for detection of human CTS defense transcript variant 1 = 153 nucleotides, product length for detection of human CTS defense transcript variant 2=117 nucleotides):

Forward primer 5'-CGCTCCCAGTTGACGCTCTGG-3' (SEQ ID NO.12)

- 20 Reverse primer 5'-CACGCACAGTTGGCGGC-3' (SEQ ID NO.13).

According to one embodiment of present invention, the means is a primer set for the amplification of Cathepsin H nucleic acid, and preferably a set of primers comprising at least one of the primers according to SEQ ID NOs. 10, 11 (together set 1), 12 and/or 13 (together set 2).

- 25 According to a further preferred embodiment of present invention, the means is a probe for the detection of Cathepsin H nucleic acid. The design and synthesis of suitable probes is well known in the art (see also standard literature below).

According to yet another preferred embodiment of present invention, the means is an antibody for the specific detection of Cathepsin H 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

- 5 H 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*: 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
10 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 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
15 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-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,
20 WO 88/01649, WO 93/06213 or WO 98/24884). Cathepsin H antibodies are also commercially available, such as Goat Anti-Mouse Cathepsin H, Catalog# BAF1013, R&D Systems (Minneapolis, USA), Rat Anti-Mouse Cathepsin H, Catalog# MAB1013, R&D Systems (Minneapolis, USA), Goat Anti-Mouse Cathepsin H, Catalog# AF1013 or Rabbit Anti-Human Cathepsin H, Catalog# ABIN285430 (antibodies-online GmbH,
25 Germany, see <http://www.antikoerper-online.de>) or Mouse Anti-Human Cathepsin H, Catalog#ABIN165388 (antibodies-online GmbH, Germany).

The production of Cathepsin H antibodies and the detection of human Cathepsin H from human tissue cytosols and sera is e.g. described in detail in Schweiger et al., *Journal of Immunological Methods*, 2001, p. 165-172(see e.g. p. 166-167 for the materials and methods).

Another aspect of present invention concerns a diagnostic kit for determining the pain and especially neuropathic pain sensitivity in an individual, which test kit comprises at

least one means for the detection of Cathepsin H in a biological sample and its use.

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

5 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 H (e.g. amount/or mutation) in a biological sample, suitably together with suitable buffers and/or reagents for performing a detection reaction (e.g.

10 immunological detection of Cathepsin H by means of an antibody, an enzymatic reaction for assaying Cathepsin H 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:

15

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 H in said subject. This can be the amount or activity of Cathepsin H altogether or in a certain tissue, e.g. in neural 20 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 (especially with respect to neuropathic pain) in the individual.

25

A method for lowering the pain (and especially neuropathic 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 H in said subject (e.g. in lymphatic or neural tissue or cells of the immune system), 30 concerns yet another aspect of present invention.

Moreover, present invention concerns a method for modulating the pain (and especially neuropathic pain) sensitivity in an offspring from a non-human female subject comprising transferring (e.g. electroporating) a nucleic acid conferring a

modulated Cathepsin H expression into Zygotes, transferring the Zygotes into a non-human foster mother and electing offspring according to its Cathepsin H expression characteristics (lowered or abolished Cathepsin H expression in comparison with wild type subjects, such as mice).

5

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

- 10 Inhibitors of Cathepsin H are known in the art, such as E-64d (see e.g. Rüttger et al., BioTechniques, 41: 469-473, 2006) and Kirschke, H et al, 1995, Protein Profile 2: 1581-1643).

15 For the production of the medicament the modulators of Cathepsin H of the present invention 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.

20 depending on the kind of administration.

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 as, for example, bovine or human serum albumin. Detergents, polyols and/or zwitterionic compounds are preferred.

25 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. 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 depends on the desired buffer molarity. Phosphate buffer is suitable, for example, for injection and infusion solutions.

5

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 example the nose or the oral cavity, in the form of dispositories implanted under the skin, by means of injections, infusions or gels

10 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 therapeutic system (TTS), which makes possible a temporally controlled release of the

15 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.

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.

20 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 themselves noticeable or only make 25 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

30 obtained. An 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 H, the derivative or fragment thereof can be used as an isolated molecule.

In the context of this invention, the term "isolated molecule", especially with respect

- 5 to Cathepsin H, refers to Cathepsin H 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).

The preparation of recombinant polypeptide or polynucleotide molecules and the

- 10 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).

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

- 15 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.

- 20 Shorter molecules of this kind are referred to as peptides. The term „protein“ refers to molecules comprising at least one 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“ as well.

25

30

35

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

10 they differ significantly 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 PicoPureTM RNA

Isolation Kit (Arcturus) following the manufacturer's instructions. RNA quality was

15 assessed using the 2100 Bioanalyzer and RNA 6000 Nano LabChipTM kit (Agilent).

Affymetrix GeneChipTM Microarrays:

First-strand cDNA synthesis was performed using 500ng total RNA with a 100pM T7-(dT)24 oligomer (GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGC GG-dT₂₄

20 SEQ ID NO:14) according to Baugh, L.R, Hill, A.A., Brown, E.L. and Hunter, C.P.

(2001) *Nucleic Acids Res.* 29, 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

25 sample using the BioArray High Yield RNA Transcription Labeling kit (Enzo) according to the manufacturer's instructions. Transcription reactions were incubated at 37°C for 16h. cRNA was purified using the RNeasyTM 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,

30 150mM MgOAc, pH 8.1). Hybridization and staining of Mouse Genome 430 2.0

GeneChipsTM (Affymetrix) was performed according to the manufacturer's instructions. The microarrays were scanned using a GeneChipTM 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

- 5 transection surgery, however, the L5 spinal nerve was not transected (see DeLeo et al. 2000)..

Determination of paw withdrawal threshold:

Paw withdrawal thresholds (PWTs) were assessed using a dynamic plantar

- 10 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
15 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

- 20 Correlational analysis:

For correlational analysis, the "pain phenotype" was defined for each nerve-transsected animal (Chung animal) as $C1 - S1$, where

$C1 = \ln(\text{ipsilateral PWT} / \text{contralateral PWT})$ and

$S1 = \text{mean}_{\text{all sham animals within same strain}} \ln(\text{ipsilateral PWT} / \text{contralateral PWT})$.

- 25 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 $\ln(C2 / S2)$,
30 where $C2 = \text{Chung expression intensity}$ and $S2 = \text{mean}_{\text{all sham animals within same strain}} \text{Sham expression intensity}$.

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-value< 0.001. 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 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.

10

Legend to the Figures:

Figure 1: Cathepsin H – Correlation Plot

Figure 1 shows for every individual mouse its neuropathic pain phenotype (mechanical hypersensitivity, X-axis) and the corresponding gene regulation of

15 Cathepsin H (log ratio(Chung vs. Sham control), Y-axis) in the L5 DRG. Mouse data are colour-coded 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 H gene regulation. This means for individual mice that the higher the L5 DRG expression of Cathepsin H in Chung-operated neuropathic
20 mice was, the more pronounced the mechanical hyperalgesia as exhibited in the behavioral test.

This significant correlation indicates a causal relationship of Cathepsin H gene expression for the induction of the neuropathic pain phenotype.

25

Figure 2: Cathepsin H – Intensity data

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

30 Figure 3: Genomic DNA sequence of homo sapiens Cathepsin H on chromosome 15 according to NCBI Reference Sequence: NG_009614.1 (SEQ ID NO. 1).

Figure 4: Coding sequence of homo sapiens Cathepsin H transcript variant 1 (SEQ ID NO:2) according to NM_004390.3 having a length of 1494 bp and encoding the longer isoform A of Cathepsin H.

- 5 Figure 5: Coding sequence of homo sapiens Cathepsin H transcript variant 2 (SEQ ID NO:3) according to NM_148979.2 having a length of 1458 bp and encoding the shorter isoform B of Cathepsin H. According to the above NCBI entry, this transcript variant lacks an alternative in-frame segment compared to variant 1 resulting in a shorter protein (isoform B) when compared to isoform A encoded by transcript variant
10 1. This may result in a protein (isoform B) that may more likely be a secreted than a lysosomal protein

Figure 6: coding sequence of mus musculus Cathepsin H (SEQ ID NO.4) according to NCBI entry BC006878.1.

- 15 Figure 7: human Cathepsin H protein sequence according to UniProtKB/Swiss-Prot P09668 (SEQ ID NO.5) comprising 335 amino acids and constituting the preproform of Human Cathepsin H (isoform A). This sequence is further processed into a mature form; it is cleaved into the following 3 chains: Cathepsin H mini chain Cathepsin H
20 heavy chain, Cathepsin H light chain (light and heavy chain together may be referred to as large chain); all chains are held together by disulfide bonds. Amino Acids 1-22 constitute the signal peptide (22 aa long), amino acids 23-97 constitute the activation peptide (75 aa long), aminoacids 98-105 constitute the Cathepsin H mini chain (8 aa long), amino acids 106-115 constitute the propeptide (10 aa long), amino acids 116-
25 335 constitute the Cathepsin H long chain (220 aa long) consisting of the heavy and light chain held together by disulfide bonds, amino acids 116-292 constitute the Cathepsin H heavy chain (177 aa long), amino acids 293-335 constitute the Cathepsin H light chain (43 aa long).

- 30 Figure 8: Human isoform A prepro protein according to NM_004390.3 (SEQ ID NO:6; translated amino acid sequence of SEQ ID NO.2).

Figure 9: Human isoform B prepro protein according to NM_148979.2 (SEQ ID NO:7; translated amino acid sequence of SEQ ID NO:3).

Figure 10: protein sequence of human Cathepsin H isoform a preproprotein according to NP_004381.2 (SEQ ID NO.8).

- 5 Figure 11: protein sequence of human Cathepsin H isoform b precursor protein according to NP_683880.1 (SEQ ID NO.9).

References:

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

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.* 314:111-119.

10

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

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 pf*

20 *Physiological Gastrointestinal Liver Physiology*, 2000, volume 278, G507-G512;

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

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

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

30 dipeptidyl peptidase I” *J. Biol. Chem.* 272: 10695-10703, 1997.

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.

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 to non-selectable genes", *Nature* 336, 348-352.

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.

10

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

Turk B., Turk V. and Turk D., 1997, Structural and Functional Aspects of Papain-Like Cysteine Proteinases and Their Protein Inhibitors, *Biological Chemistry*, Vol. 378, pp. 141-150;

Turk V., Turk. B and Turk. D, 2001, Lysosomal Cysteine Proteases: Facts and Opportunities, *The EMBO Journal*, Vol. 20, No.17 pp. 4629-4633.

20

Rüttger, A, Mollenhauer, J., Löser, R., Gütschow, M., and Wiederanders, B., Microplate assay for quantitative determination of cathepsin activities in viable cells using derivatives of 4-methoxy-β-naphthylamide, *BioTechniques* 41: 469-473 (October 2006);

Kirschke, H., A.J. Barrett, and N.D. Rawlings, 1995, Proteinases 1: lysosomal cysteine proteinases: Protein Profile 2: 1581-1643).

Ishidoh K., Suzuki, K., Katunuma, N., and Kominami, E., Gene Structures of Rat 30 Cathepsins H and L., *Biomedica and Biochimica Acta*, 1991; 50 (4-6): 541-7.

Ishidoh K., Kominami EI, Katunuma N. and Suzuki K., 1989, Gene structure of rat cathepsin H, *FEBS Letters Volume 253, number 1,2, 103-107;*

Rothe M, and Dodt J, 1992, Studies on the Aminopeptidase Activity of Rat Cathepsin H, European Journal of Biochemistry, 210, 759-764

Vasiljeva O., Dolinar M., Turk V and Turk B., 2003, Recombinant Human Cathepsin H Lacking the Mini Chain Is an Endopeptidase, Biochemistry 2003, 42, 13522-13528;

Schweiger A, Stabuc B., Popovic T, Turk V and Kos J., 1997, Enzyme-linked immunosorbent assay for the detection of total cathepsin H in human tissue cytosols and sera, Journal of Immunological Methods 201 (1997) 165-172;

10

Koga, H., Mori N., Yamada H., Nishimura Y, Kazuo T., Kato K. and Imoto T., 1991, Rat Cathepsin H-Catalyzed Transacylation: Comparisons of the Mechanism and the Specificity with Papain-Superfamily Proteases, Journal of Biochemistry 110, 939-944.

Literature for standard laboratory methods

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

20 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 E. Kingston, David D. Moore, J.G. Seidman, John A. Smith, Kevin Struhl.

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.

30

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;

Short Protocols in Molecular Biology, 5th edition, by Frederick M. Ansuel (Editor), Roger Brent (Editor), Robert E. Kingston (Editor), David D. Moore (Editor), J.G.

10 Seidman (Editor), John A. Smith (Editor), Kevin Struhl (Editor), October 2002, John Wiley & Sons, Inc., New York“

Transgenic Animal Technology A Laboratory Handboook. 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 University Press, New York;

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

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

Standard Literature for Laboratory Methods:

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

30

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;

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.

Current Protocols in Human Genetics; regularly uptdated, 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.

10 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.

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

Remington´s Pharmaceutical Sciences, Edition 17, 1985.

Claims

1. The use of Cathepsin H for identifying compounds that modulate neuropathic pain
2. The use of a non-human transgenic animal heterologously expressing Cathepsin H for identifying or analyzing compounds that modulate neuropathic pain.
3. The use of a non-human transgenic animal heterologously expressing Cathepsin H as a model system for enhanced neuropathic pain sensitivity.
- 10 4. The use of a non-human Cathepsin H knock-out animal for identifying or analyzing compounds that modulate neuropathic pain.
5. The use of a non-human Cathepsin H knock-out animal as a model system for lowered neuropathic pain sensitivity.
6. The use of a cell heterologously expressing Cathepsin H or a functional fragment thereof for identifying compounds that modulate neuropathic pain.
7. The use of a cell heterologously expressing Cathepsin H or a functional fragment thereof as a model system for enhanced neuropathic pain sensitivity.
8. The use of a Cathepsin knock-out cell for identifying or analyzing compounds that modulate neuropathic pain.
- 20 9. The use of a Cathepsin knock-out cell as a model system for lowered neuropathic pain sensitivity.
10. The use of a cell heterologously expressing a reporter gene expressibly linked to the Cathepsin H promoter and/or enhancer or a functional fragment thereof for identifying or analyzing compounds that modulate neuropathic pain.
11. A method of identifying or analyzing compounds modulating and/or preventing neuropathic pain, comprising the steps
 - f. Providing at least two samples;
 - g. Contacting one sample containing Cathepsin H or a functional fragment or derivative thereof with a compound,
 - 30 h. determining the activity of Cathepsin H in the presence of compound,
 - i. determining the activity of Cathepsin H in the absence of compound, and
 - j. comparing the activity of Cathepsin H according to c) with that according to d).
12. A method for identifying or analyzing compounds that modulate and/or prevent

neuropathic pain comprising:

- c. Contacting a Cathepsin H protein or functional fragment or derivative thereof with a test compound; and
- d. Determining whether the test compound modulates the activity of the Cathepsin H protein or functional fragment or derivative thereof.

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

- a. Contacting a cell, which has a detectable amount or activity of Cathepsin H 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 H or the functional fragment or derivative thereof present in the cell.

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

- a. Contacting a nucleic acid coding for a Cathepsin H 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 H 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 H protein or functional fragment or derivative present in said system.

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

- a. Providing a cell transfected with a nucleic acid vector comprising the promoter of a Cathepsin H 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 H promoter;
- 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.

16. A method for identifying or analyzing a compound that modulates neuropathic pain comprising

- c. Selecting a compound that modulates the activity of Cathepsin H as a test compound, and
- d. Administering said test compound to a subject in sensation of pain to determine whether the pain is modulated.

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

- a. Assaying a biological activity of Cathepsin H 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 H, and

- b. Testing one or more of the modulating compounds for their ability to reduce pain, pain sensation or pain sensitivity in a subject.

Figure 1 Cathepsin H: Correlation plot

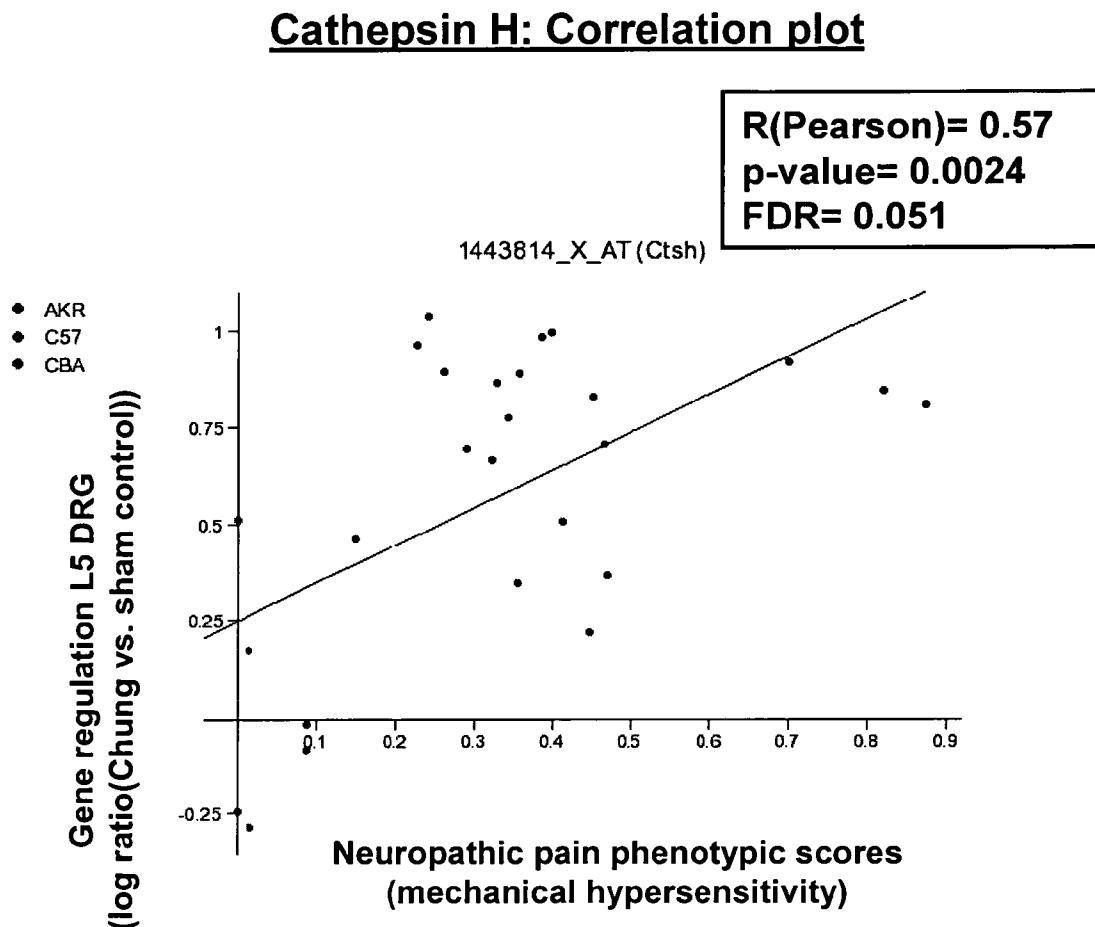


Figure 2

Cathepsin H: Intensity data, L5 DRG, 3d p.o.

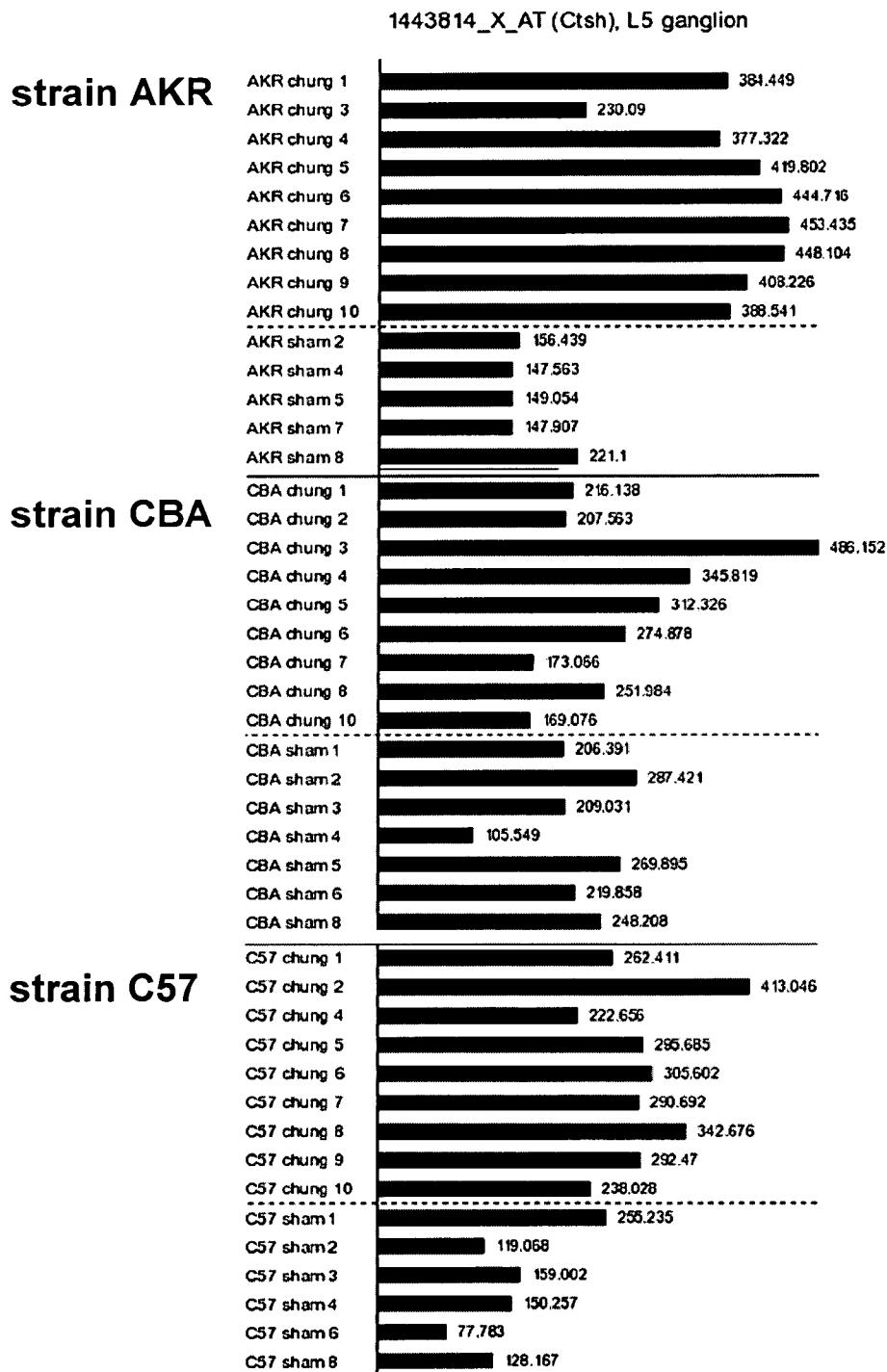
Cathepsin H: Intensity data, L5 DRG, 3d p.o.

Figure 3: genomic DNA sequence of Cathepsin H (SEQ ID NO:1)

4501 gcacattcag ggagatgggg gtgtgagggt gagacaggca gggacatagg gggagcggagg
 4561 tggggacagg cagggagatg gagggtgcaa ggtgaagaca ggcagggaca tggtgtgagg
 4621 tggggacagg aaggacatg aggggtgca gttgggata ggcagggaga tgggggggtg
 4681 cgaggtgggg acacgcaggg agatggggga cgtgggatg ggcacaggct gggagaggg
 4741 gaggccaggg tgagacctc cccgcgggtg ccagccagtc caggcagatc caggactcg
 4801 ccaggctga tacccagata agcccttct cccagaggag ataaggagc gacgcggccg
 4861 gcccacagg gaactagac tggcggatg tgcggcccg cccctcccg cgagccggc
 4921 ctccccggg taccgcac cccgcggccg gtcgcctcg cccgcgtc gtcgcctcc
 4981 ttgcgcctc ccgcgcctc ccacgcctgt ggcgcctccc cccgcgtc ccagtggac
 5041 ctctggccg ccacctcgc ggaccttag cgcggacggc aagccgcac cgctcgatg
 5101 tgggccacgc tgccgctgt ctgcgcggg gcctggctcc tggggatccc cgtctcggt
 5161 gcccggaaac tggcgtgaa ctcccttaggt acgcagaggg cgccggccccc gagagggtgc
 5221 tagcaggccc tagagtggac gctgggagaa cttggccagg ggcgcactgg gctccggagg
 5281 aacggggcag agctttcccc ggtgagggc cagaactct gctgtggc acggggaggg
 5341 gttaactgca ttataggcg gggtttcgg agggtggggc gtttgtagga ccagaggac
 5401 agatgggtg agtgtccagg gtcaccccta gccagggtgc tccctagcc agaataccaa
 5461 aggagccgc agtccttcc gccttcctca actggggaca cagagtgtca cacagaggac
 5521 tctgatctgt ccctgcca ggtgagcaag gacgtcccag cttctctag cagttcccc
 5581 cgggacttgg cacacagggg atccccgtag tgtttggga gtcccatctg tagagtaggt
 5641 tggacgggct gtttggaca gtgaatccc gtgcacacgc accaggaaaa gcaactcagac
 5701 caacacgcg ttaaacagca ccccgaggcc catggcgtca ggtggagccc caggactcct
 5761 ctttgtggc agattgggtg cgcactgtc agttctgtc tcggcttc tccctgtcac
 5821 tgccgggct caggcactc ccctggctga aggcaaggag cttgtcttc cctactgtc
 5881 ccctcccttc ctactgtccc ttgtcccgac ctcagccac aggctctgaa cttaaagacc
 5941 acctggccccc ttcaccgaga aaagtggcca agaaaacaacc caaggagcca agaacctagg
 6001 gaggggggggt gcccctgagct ctggagggac gggacagggc ttcccccag cttagggctc
 6061 aggggatggg acagagatca ctggggctga atctggggca gagggttgc tcattccctc
 6121 ccacattttt attttttttt cttcccgac ttggcaaccc cttttttttt cttttttttt
 6181 gtcagtccccc gcctaaaggaa aacctgtc cccggacggc gggctggctg gagaaggctg
 6241 gcgctgagag gctgtctgaa ttccagagca gggctggca aacttttttct gtggaaagacc
 6301 agacggtaaa taacttaggc tttatgggtg tctctgtc aactactaac tctggcatgg
 6361 tagtgtgata gcaaccatag attgtatgt aagtggatc tggtaactggg ttctataaaa
 6421 tgatttttat ggccactgaa atttcgtttt caagggattt ttgggtgcta cggaaatatgt
 6481 ttcttctaaat tggtaaaaaac cttttttttt cttttttttt cttttttttt cttttttttt
 6541 ggagtccagg accggctcg gtcggctca cctgttaatct cggaaatttt ggagaccgg
 6601 gcagtcggat cacttttagt cggggatc agggggatc ggcaacatgg tggaaacccct
 6661 gtctctacta aaaataca aattttttttt tttttttttt tttttttttt tttttttttt
 6721 tactcaggag gctggggcag gagaatcgct tggtaactggat aggtggcgat cactccagcc
 6781 tgggtgacag agtaagactc catctaaaac aaacaaacaa acaaacaggd gaataactgt
 6841 ctttcctgaa acctgtctgaa aatccaggca aggtatttttgc gacttttttgc agtcctgact
 6901 agatggaaat ggttctttt tagggtaagg cccgggttcc cgggggggggggggggggggggg
 6961 aaaaataatct cttccggggc aaaaatgggttcaattttttttt tttttttttt tttttttttt
 7021 aacttt
 7081 taatcaagat ctggatccccctt ctgg
 7141 ttcccccgggg aaaaacggca tagattggtc tctgggggggggggggggggggggggggggg
 7201 gatcttt
 7261 gaagatatt
 7321 tttcccaatgt tccttt
 7381 ttccctctca ttcaatctgaa acctgggggggggggggggggggggggggggggggggggggg
 7441 aaaaagacaa aacggtaaaat aatccggatc atccggatc atccggatc atccggatc
 7501 gaatttgg
 7561 ggg
 7621 ggtggatcac ctggggatc aagtt
 7681 ttt
 7741 tgg
 7801 gtggccactgc cttccgg
 7861 aaaaatgg
 7921 cacagaaaggaa aatgg
 7981 ctgg
 8041 att
 8101 ggg
 8161 ttacttt
 8221 ctt
 8281 ttt
 8341 ctgg
 8401 gactgg
 8461 ggg
 8521 aggg
 8581 ttt
 8641 ttt
 8701 gatcttt
 8761 caatgg
 8821 gagatgg
 8881 tgg
 8941 aataatgg
 9001 atgg
 9061 aacaagaaaaa aaaaaactgtc tataatatct gacttttttttttttttttttttttttt
 9121 agatgg

5/12

13861 agagcgagac tctgtctcaa aaaaaaaaaaaa aaaaaaaaaaga aaagaaaaaga aaaagcagaa
 13921 atgatcaac atccctgtg gggctgtga gagaccaaat taggcagca gttggaaaggc
 13981 ccctggcacc agtgagatgg ttctttaacct agctatccat cagactcagc agggggatgt
 14041 ctaaaaaaagt aagtccccag gccccacccc agaccaactg cattagcatc tctggagatc
 14101 ggcgggaca tctataccat tgaaaaaagct cccaggtat tttggcagca ctgtaaaggc
 14161 tgattgaat atctactgaat ttatataaa tacaaaaact taaaggatgg ggggtgcagt
 14221 gtttctaaat acgacacagg ttaaacaggag gtttcatcac tttttcttgg ttcttcatta
 14281 gtgattcaact aaccattttt gttttgggtt tttttcttc ttttttagtgc gcaactaacc
 14341 aatttcaga catgagctt gctgaaataa aacacaagta tctctgtca gaggcctagg
 14401 taggtataat tcattgcaac caaatctgaa tcctttact gaaacctttt gaataaaattt
 14461 ggagcctgt ctgtttctag gcagatttag tcccatcccc agatgcagct gagggtatcc
 14521 ccagattttt tgggggaaacc agaagagtca gtttctcagca gtttctcagat ccaggccat
 14581 ggccttagca ttgtggaccc ttaactttag gtttctcagat taaaatataat atactcatt
 14641 aaaaatgtat ttcaagatata caacaagtaa ttcttttagca taagtatattt ctaaatattt
 14701 cacaggatata acttactaca caaaggatattt tttttttttt ctgttttttca aattttacta
 14761 ggtgagctgt attttttttt gtttctttagt tttttttttt ctttttttttca ttttttttttca
 14821 aggggacatg tcatttttagca ctggggacca gtagacaggg gggggaggt ttttttttttca
 14881 gcagcctttt ttatttcttc ttgggttattt ctttttttttca ttttttttttca ttttttttttca
 14941 agaaagggtt ttgggtttttt ttatttttttca gaaaatataat ttttttttttca ttttttttttca
 15001 gctcagccad caaaaatgtat taccccttcgag gtttttttttca ttttttttttca ttttttttttca
 15061 ggccggaaaaaa agggaaattttt gtttctttagt ttttttttttca gtttttttttca ttttttttttca
 15121 tcctctccctt gcaaaaacccgc cacactccca gacaatcac ctttttttttca ttttttttttca
 15181 gtcccacaaaaa ccaccacaaag cacatccctg ctttttttttca ttttttttttca ttttttttttca
 15241 ctcttcctttt gggggccag ggaggtcagc acttctttagt ttttttttttca ttttttttttca
 15301 agtttcttctt ttttttttttca aagtttactt ctttttttttca ctttttttttca ttttttttttca
 15361 ttgggtcccaag gtttcttcttca ctttttttttca ttttttttttca ttttttttttca ttttttttttca
 15421 acaccacaaat cccatgtttt ctttttttttca gtttcttcttca ttttttttttca ttttttttttca
 15481 agecccccttgc ctggctggcc gtttcttcttca ttttttttttca ttttttttttca ttttttttttca
 15541 ttcttcatggc agggggcttgc ctttttttttca ttttttttttca ttttttttttca ttttttttttca
 15601 gtgagaggcc ctttttttttca ctttttttttca ttttttttttca ttttttttttca ttttttttttca
 15661 gtcccaagaga gtttcttcttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 15721 agcaggaaac ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 15781 tgaggtgaag ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 15841 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 15901 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 15961 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16021 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16081 acagggtggaa aagagaggcc ctttttttttca ttttttttttca ttttttttttca ttttttttttca
 16141 gccatcaggc aggacgcaggc ctttttttttca ttttttttttca ttttttttttca ttttttttttca
 16201 agatgtgtgc ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16261 gcttccatca gtttcttcttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16321 aggggggtt gtttcttcttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16381 gtttcttcttca ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16441 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16501 agggccgtcc ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16561 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16621 agttagaaatgtt gtttcttcttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16681 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16741 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16801 aagccaaggc ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16861 gtttcttcttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16921 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 16981 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17041 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17101 agtttcttcttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17161 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17221 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17281 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17341 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17401 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17461 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17521 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17581 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17641 ctttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17701 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17761 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17821 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17881 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 17941 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 18001 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 18061 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 18121 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 18181 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 18241 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 18301 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 18361 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 18421 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca
 18481 ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca ttttttttttca

18541 tggcaggta ctgggttgtg tccttcttcc aggcggaaaca gcagctggtg gactgcgcc
 18601 aggacttcaa taatcacggc tgccaagggt acgttctga ccagaagggg cccaaaactt
 18661 cctcatttcc cactgaccgc ttgcacagga tccagtgtgt gctgggtctg ccacagat
 18721 aaccaccac tgccctggc gatggcaaaa tggctctgt tgcacagggg tggcacctc
 18781 acatggccag gtctcatagc caggacaggg cagagctgga atctgagtcc aggcctctgt
 18841 cctcttcera ctgtatcacc tggcttcca cagtgccgtt ggcctcacc cccaccaagg
 18901 cctctggggat tggccaaaaa agaatatta agatgtggca acacaccatt acacatcatc
 18961 ctccaaaccc gagatccaca cacagataca gggttgcct ttagatgaac tcactaaagt
 19021 cccagctcc caagcttac gcgcctgtg agggtgaggt ggctgtgt aagggtgt
 19081 ctttccttgg acctgtttc ctggcccact gtaaagcagg ttgcccaggaa tttgtgaca
 19141 tttctacagt gactctagcc ctcccttcat gcagtgtct gagacatttc tgggtt
 19201 gtcacactgg cacaatgggt gcataaaagcc aggttcttgg ggtgcgtgc ctcgactccc
 19261 ctccacactt ccggggcaca tccctctccc agaccccacg ccctctgagc tggctt
 19321 ctctgcaggg agccgtgtc cagccaaatgc ctggggaccg aggggtgacg tcgtacgt
 19381 ctctcagatc caccatcatc ctcaatgtc ctttttttgc ggtctgttcc cactccacag
 19441 ctttagaaacc caacccataa caaagatctg acatggggc actctgggtt cccaccgtt
 19501 ttatttttta ttatttttt tattttttt tttatttttt tttttttttt ttgagatgga
 19561 gtctcgctt gttggccagg ctggagtgca gtggcgcgtt ctcagctca tcagactcc
 19621 gcctcccggtt ttcacccat ttcctgttcc cagccatcca gggagctggg attacaggcg
 19681 tgcgcacca caccgttca atttttgtat ttttagtaga gatgggggtt tcaccatgtt
 19741 ggccagggtt gtctcgaccc cctggacttca ggtgatccac ccaccttggc ctcccaaaatg
 19801 gctggattt caggtgttagt ccaccatc cagctgttcc ttc当地aggag tctatgg
 19861 ttgggttccagg agacttctt ctttttcatc tccatataaca aatatacagc agcaccgt
 19921 gtgtgttaggc cctggggata cagcgcttag cagaacttggg agagccccca ttctggcagg
 19981 ctcagagaga ccacagtcgaa ggcttagacta ggtcaggccg tgccgaatgc tatggagaga
 20041 aacagagaag ggacggggat tgctggccgg ctgtctgatgg tttttttt ctgggaggcc
 20101 acatgtatgaa aaaaagaccc actttttggg ccacttccca gcttttagctg tggccctg
 20161 gccagggtt ttcacccat ttccttccggc agtgcacggc tccaccacac agggtcgaca
 20221 cgagggttca gggagtgatc cttggggaaac acctggccagg gggctcggtt gttggcgt
 20281 cagtgtctg ggtgtatgtt gttgaccatg gtgtggggct gtgcacttcc gacagacgg
 20341 ctggccttaa ggggtctcta tcccaacttgc gcatgtttgg gctggcttcc ggactccgg
 20401 ggcctcatct gagtttgcgt agtcttatcc caaggctggg cctctcagag accccctct
 20461 ccactggggac atgcccacc aagtccccag cttggccaggc cagatatctc gccacccgg
 20521 tccccggggg ccccgccctt atcaggacac cccagggtgg gtcacactgt gtgtactt
 20581 tgcagggttcc tcccccggc ggcttgcgttatacttgc tccacaaaggg gatcatgg
 20641 gaagacaccc accccctacca gggcaaggta actccacacg cctccacttctt gtttttgg
 20701 cgtacagcat gcttagtcc acctccccc cagcttggg agacatccct tctccccc
 20761 cttccctccc gccggaaatc tccaaattgt ctgttcttcc caaatttcc tttccctccc
 20821 tccacccacc tggccactt gctcttacca gtccaggggc cttttatca ttctcc
 20881 ataccccgcc tgcggccacca tcttttcttcc tcccgagcc gacttctcag gaccccttcc
 20941 tccccccacc tggcccttcc tcttgcgttcc tccccccttcc gccacggcc ccatccctc
 21001 gcttctgttcc atgcgttgg ctttttgcgttcc caaggccatcc tttttttttt accccc
 21061 ctaccctatc aaggctgtgtt ggcctcccaatcc catcacccttcc gcccacccctt gccacacca
 21121 agtgccttgc tttttccca gacgttgc tcccttta tttttcccttcc tgggtatgg
 21181 cagcaacttgc tcccttcaga atttccagg tggccatgtt ccttgcgttcc cggcccc
 21241 ctccacccca cccttgcggc agcctgtgttcc caaccacaga cctccatgtt cccttgcgtt
 21301 ccatctgtgtt cgttgcaccac tccggccggg gctccatcc agaccccttcc ggggtggcc
 21361 ccccgccggc ctccaaaccctt gcttgcaccac cccggccgttcc tccatccatcc
 21421 gcttgcgttcc gcttgcggg gaaagcccg actccacactt agtctccctt tttttttt
 21481 tccccccttcc tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 21541 ggctggatgtt caatggccca atctccggcctt actgtcaaccc tccgttcccttcc agttcaagca
 21601 gttatctgc tccatccctt gatgtatgtt gatgtacggc accccggccacc acggcc
 21661 aactttttat atttttatgatgatgtt gatgtatgtt gatgtatgtt gatgtatgtt gatgtatgtt
 21721 tccctggccctt gatgtatgtt gatgtatgtt gatgtatgtt gatgtatgtt gatgtatgtt
 21781 accgtggcccg gccaacttcc ctttctttaac ctcacggccca gggccggccatcatgtt
 21841 cccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 21901 tggcccttccca gggctcggtt tcccttccca tccatccatcc tccatccatcc tccatccatcc
 21961 gatgtatgtt tggcccttccca tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22021 tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22081 accatgtgttcc cccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22141 tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22201 acatgtgttcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22261 cataacatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22321 tggcccttccca tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22381 cccttgcggc tggccatgtt gatgtatgtt gatgtatgtt gatgtatgtt gatgtatgtt
 22441 gtggggcccg caaggccggc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22501 tggcccttccca tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22561 aggcaggccccc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22621 tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22681 gcttgcgttcc gatgtatgtt gatgtatgtt gatgtatgtt gatgtatgtt gatgtatgtt
 22741 gatgtatgttcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22801 ggcacttccgtt ccgttccgtt tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22861 cccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22921 agccggcccg tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 22981 ccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 23041 gatgtatgttcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 23101 tatgtatgttcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc
 23161 atgtatgttcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc tccatccatcc

23221 aaccctcaat acgcactate ccctctagac ccgaactggc cctaggagat gagtatcctt
 23281 attacctcat ttgacaggtg agaaaacggg ggctccaagt ggttaagta tttgcccagg
 23341 gccacagagc taggaaatag atgctggatt tgaactaaga atgtgtcatt ccagaacccg
 23401 ggctcatttc cttgacaaca tacacggcaa atcctgtact caggatgtca ccctcttcag
 23461 aatgttgctg ctgtcaactca agctggctgc tctggccat gatacccgg gacagggtga
 23521 atttggccct caaaggatc ccttgcattt gtccccagga gagcctcattt ggtgacacct
 23581 gcagggccct gacgaccagg ccagaactag cattcagccc ttttggccag gcccatgggg
 23641 tggaaatggt gccacctgc gaagtctatc ccctgtgtc gggctggccct ctgtgcctc
 23701 catgtctcata atatgcctc acctgttggg ctcaaggc ctgtccaca taccaggaa
 23761 gagggcactc ctttgcattt cacctgtttt ctttcacag ctgggtctct gtccacccct
 23821 ctagggggcaggc aggactgagg gaggctggc gcccgtccc tcctgcactt gctttccctc
 23881 agtagggccc tggcctcagc acaggttaact tccccctca tttcagctcc cagaggctcc
 23941 ttggtagtgc cacttgaacc cagccacccc caacccagaa gctccaaaaa gtgcaatgc
 24001 ctgggacccca ccctgcagat actgttgtag ttgtgttgc gacagcctg gacttgggt
 24061 tttttttaagg ttctgcattt ggttctaccc gtcgtgtca cttggctctc gcaagcttgt
 24121 tccaaagctg cagccctccag gaaggccacc tgacttcccccc acacccatct tccccctgg
 24181 agggggacagg ttctactcag aagtcaacaca gcccctggat gggcttctc gttttccacg
 24241 ccatctggga ctttccctc catggtttat aacccctgtt ttctagcagg aaagctttgt
 24301 agactctggg ttatgggtt taggggttag agctgttgc ccaggagagg acctcaggga
 24361 atggatgtga atgactgaat gaggtagtcc acgtgttacc cctgtgtccc tatctcagag
 24421 ggtgcagggt ttcttaggag cagagttggg gagagggaaac agatggcc agacagaccc
 24481 agccagagac aggaagggg agaggccgc gggagctgc ttctggccat ccatgtgtct
 24541 ctgggctctc agcccttgc ttgaggccaa aggtgtatc gtgaaggag tggggagggt
 24601 acaggccatc tggttctgtc tgggttctgt ttccacagta tggaggagaa gcatgtgtgg
 24661 aggctgtggc cctctacaa cctgtgtact ttgccttgc ggtgactca gacttcatga
 24721 tttatagaac gggcatctac tccaggttagt tcagggtccc aggagccagg agggacagt
 24781 gggagggggct tgggtggccct cttcacaccc cactcgtggg acttggcttc gtggccacca
 24841 cttccatgtgc tcaagacccctt catgacacat gcaagggtgtt gggcggtttt cacatccctc
 24901 agttaaatcc cacaaggcc caaggcccg gaggcatggc cttttctcac agcaaaaggag
 24961 aggtggctc aagggtatc acagacacag gcaacccgtt tagtgagac tagggccaaa
 25021 atttgagac ttgtgttcc ttaacacaaatg agtttccatc agttttttt aaaccatgt
 25081 tcaggccagg cgtgggtgtt catgctgttca tcctccagcac tttggggggc cgaggccagg
 25141 ggttcaacttggt agggccaggat ttcaagacca gcttggccaa catggcgaaa ccccatctct
 25201 actaaaaata caaaaaaaaaaaaaaaagccca gctgtgtgtt gcatgtctat aatcccaact
 25261 actcggggagg ctgagacaca agaatgggtt gacacttagga ggtggaaagta gcaactaagcc
 25321 agatctgtgc cactcgttgc caacccctgtt gacagagacatc agtctgtct cagaaaaaaa
 25381 aaaaaaaaaaccatgtttaaa gatgttggaaatggat ggtggaaagat agtgccttc
 25441 acacacttggg atccatgtgtt gggagggttca ccccaaggctt ttccagaaaa accaagctt
 25501 agcctttaa ctatgggtt tctctaccc acacccccc gctgtgttgc tttgcagcc
 25561 catttccctc ctttcatcag ttgtgtccccc ttaacgttac tcaactaatgt cactcactgc
 25621 tttgtgttcc tttccctccccc tgaggctctg ccctggctac cattgttccctt ggacccccc
 25681 accccccccca cactgttccca gtttccactg ggagctgttcc gcttctgaa acactccct
 25741 tgccaaacggc aaccccttgc caacccatgtt tccctgtttt ccagggttcc taccctgagg
 25801 gggaccctca tgatctggc tttccctttt ttccacactc atttggccatc aaaccctggc
 25861 cccggccgttcc acccttgcacg ccccaaaaaa gcaacggcaga agggttcgca gttccatgg
 25921 cttttccctc cttccacaggg agccttccctt gacccaccc gcccacccctt cccccccccc
 25981 cccggccgttcc ggtatcccccc cacccttgc gggcttgc gccctccac tttttccct
 26041 ctcaggccac tgtagagaaatggc ctttccactt gcaacccctt gttttccatc
 26101 caaggggacacat ttatggatc tgccatggatc cccggccactt gcccggccg gacccatgg
 26161 cccacttata catcagcttca attcacccat gaccacttca gtttttctc ttgaatttct
 26221 tggatattcc ttatggggc tagtttactt ttgttacccat gggatggatc aaaaatgtt attttgc
 26281 taggtataaaa gtaatatttta ataaatgttca gtttccatc aaaaatgtt gtttttgc
 26341 gcaacccactt gtttccatc gtttccatc gtttccatc gtttccatc gtttccatc
 26401 gagggagtag aaagtgttca ggggttgc ggggttgc ggggttgc ggggttgc ggggttgc
 26461 cagaacccactt gaaatgggttca gtttccatc gtttccatc gtttccatc gtttccatc
 26521 ccacacttggg ggggggggg ggggggggg ggggggggg ggggggggg ggggggggg
 26581 tagccaaatcat gatgttggatc tttttttttt ggggggggg ggggggggg ggggggggg
 26641 tggccatgttcc tttttttttt ggggggggg ggggggggg ggggggggg ggggggggg
 26701 ggttccatc gtttccatc gtttccatc gtttccatc gtttccatc gtttccatc
 26761 gactccatc ctttccatc gtttccatc gtttccatc gtttccatc gtttccatc
 26821 ttatggatc tttttttttt ggggggggg ggggggggg ggggggggg ggggggggg
 26881 caaaaatggatc tttttttttt ggggggggg ggggggggg ggggggggg ggggggggg
 26941 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27001 gttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27061 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27121 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27181 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27241 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27301 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27361 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27421 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27481 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27541 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27601 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27661 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27721 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27781 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 27841 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt

27901 catgtgtggc ctggctgct gcgccttcata cccatcccct ctgggtgtgag ccgtggcagc
27961 cgcagcgcag actggcgagg aaggagagga acgggcagcc tgggcctggg tggaaatcc
28021 gcccctggagg aagtgtggg gagatccact ggaaacccca acattctgcc ctcacctctg
28081 tgcccagcct ggaaacctac agacaaggag gagttccacc atgagctcac ccgtgtctat
28141 gacgcaaaga tcaccagcca tggccttag tggccttctt aacagactca aaccacatgg
28201 accacgata ttcttcgtt ccagaaggc tacattccac atatagagct ccagggactg
28261 tctttctgtt attcgctgtt caataaacat tgagtggcga ctcggccaga tggagcatgc
28321 tggcctggg ccatgactca aaccaggctt gtcctcgcca tcagtc caggaggag
28381 gcatcagggg gtgtccaggg tctggctggg aggatggtgg ggggtgctggc ggtattatcc
28441 accagatggg gtatccagga tgaagagcag gctaattt agatcttgac tatttgtccc
28501 aaacctgagt ggagaggtgg ggaggaagga gaaaaacggg ccaagctgcg gcagaggcag
28561 gattcaaaacc cacaatgtca gcccagaagc ctgtacttct ctgtgtggcc tccccagct
28621 cagctgccta ctccactca gactctgccc cccacccca catgagactg cccacccctt
28681 cctcaccaac cccttattta ttcaaacgt gccagatgc tccctgtcggtt tccacacat
28741 cccctccctt atcttcgtt ttgagaaattt tgatctctg tttagaggc ggcctatgaag
28801 ttgctgctgc ttccctgaagc ctccggcgtc tggatgtct tggagacaag ctggagttca
28861 ccagctttt gtcctatgca tcacagactt tcttaatgtt aaaaagctgtc atcttattt
28921 ggcttcctga aaagctaaag gcttgagaat atttgaaaaa tatatatgtt catgataagg
28981 cttattttaga taatgcaaat atagttat ttatttagaa attcttagtaa cataagacca
29041 caactgtata agacaaaaaa caatcacctt gctaggcatg gtggctcaag cctataatcc
29101 cagcacccctt ggaggcccgag gcagggtggat cacctggat caggagttt agactagcct
29161 ggccaacatg tgaaacccc gtctctaata aaaataaaaaaattt tagccaggag
29221 tggtagtgca cacctgtatg cccagctact cggggagggtg aggcaagaga atcgcttaag
29281 cctggagat ggagttgca gtgagctgat atcatgccac tgcactccag cctaaaaaaaa
29341 aaaataataa taataataga cttcatttga tggaaattcta ttcttagattt aaaaaaaaaac
29401 taacatgaaa tgaaagcata atttctttt ctttctttt ttttttttt ttttagatgt
29461 agtctcaactc tgcaccccg gctggagtgc agtgggtgtca tctcagctca ctgcaaccc
29521 caccccccgg gttcaagcga ttctcctgccc tcagcctctt gaatagctgg gattacaggc
29581 atgcacccacc actccctggat aatttatgtt ttttttagtag agatggggtc tcaccatgtt
29641 ggtcaagctg gtctcgaact cctgacccca aagtgtggg attacaggag
29701 tgtgagcaac cgccacccgc ctgaaagcat aatttctgaa tttgacaaag attctcaat
29761 caatggtcag ttgtataaaaa ggattcccat tgaagtcaat ggtataaaaag gattccatt
29821 gaagatacat ggcaaaataag tctaaggta ccattcttgc ttacatctat ctgaaagatc
29881 tggctgctgt gataataaa cccaaattaa atttgcagac agcatgataa tgtggctaaa
29941 aacattcagg agaatcaaca aaacttagaa ctaatggaaag agttctgaaa gtggctggat
30001 accagaaaaaa aaaatcaaaa actcagaaaaa aatacactat tttttgttac tattgcaact
30061 ttactattgc aacaaaaacc gcaagcttagga ataaccacca aaaagtctta tatgaagaaa
30121 attactaaat taataacact ataaaggaag acttagataa atggagagcc accatattct
30181 tttgttggtgg tttgttggatc agagtcac actgttgcggc gggctggagt gcaatggcat
30241 gatcttggct cactgcaacc tccgcctccc gggttcacgt gattctctg cctcagccac
30301 ccgagtagct gggattacag gtgcccacc

Figure 4: Cathepsin H human mRNA sequence, transcript variant 1 (SEQ ID NO.2)

1 ccacgctcggt gcccgtcccc ccccgcgctc ccagttgacg ctctggggccg ccaccccg
 61 gacccctgag cgcaagagcc aagccgcag cgctgcgatg tggccacgc tgccgctgct
 121 ctgcgcggg gcctggctc tggagtcggc cgctctcggt gcccggaaac tgcgtggaa
 181 ctccttagag aagtttcaact tcaagtcatg gatgtctaag caccgttaaga cctacagtc
 241 ggaggagtag caccacaggc tgcagacgtt tgccagcaac tggaggaaga taaaacgcca
 301 caacaatggg aaccacatataaatttgc actgaaccaa ttttcagaca tgacgttgc
 361 tggaaaaaaa cacaagtatc tctggtcaga gcctcagaat tgctcagcca cccaaagtaa
 421 ctacccctcgatg ggtactggtc cttaccacc tccctggac tggggaaaaa aaggaaattt
 481 tgtctcacct gtggaaaaatc agggtgcgtt cggcagttgc tggactttctt ccaccactgg
 541 ggccctggag tctgcgatcg ccacatcgcaac cggaaagatg ctgtccttg cgaaacagca
 601 gctgggtggac tgcggccagg acttcaataa tcacggctgc caagggggtc tccccagcca
 661 gccttcgag tatatccctgt acaacaagg gatcatgggt gaagacaccc accccctacca
 721 gggcaaggat ggttacttgc agttccatggc tggaaaggcc atccggctttt tcaaggatgt
 781 agccaacatc acaatctatg acgaggaaaggc gatgggtggag gctgtggccc tctacaaccc
 841 tgtgagctt gcctttgggg tgacttcggg cttcatgtatg tataaaacgg gatctactc
 901 cagttttcc tggccataaaaa ctccagatataa agttaaaccat gcaacttgc ctgtgggta
 961 tggagaaaaaa aatgggatcc cttaactggat cgtggaaaaac tctttggggtc cccagggggg
 1021 aatgaacggg tacttcctca tgcggccgg aaagaacatg tggcccttg ctgcctgcgc
 1081 ctccctacccc atccctctgg tggagccgtt ggcagccgca ggcgcagactg gggagaagg
 1141 agagggaaacgg gcagccctggg cctgggtggaa aatccctgccc tggagggaaatg tgggggaga
 1201 tccactggga cccccaacat tctgcctca cctctgtgcc cggcctggaa acctacagac
 1261 aaggaggat tccaccatgtt gctccatggc gtctatgcac caaatgttac cagccatgtg
 1321 ctttagtgc cttcttaaca gactcaaacc acatggacca cggaaatatttcc ttctgtccag
 1381 aagggtactt ttccacatata agagctccatgg gggactgtttt ttctgttattt gctgttcaat
 1441 aaacatttgc tggccatccccc cccagatggaa gatgtgttgc cctggaaaaaa aaaa

(SEQ ID NO:2)

30

Figure 5: coding sequence of Homo sapiens CTSH transcript variant 2, mRNA (SEQ ID NO:3)

1 ccacgctcggt gcccgtcccc ccccgcgctc ccagttgacg ctctggggccg ccaccccg
 61 gacccctgag cgcaagagcc aagccgcag cgctgcgatg tggccacgc tgccgctgct
 121 ctgcgcggg gaactgtcg tgaactccctt agagaagttt cacttcaagt catggatgtc
 181 taaggccatgtt aagacccatcata gtaacggagga gtaccaccac aggctgcaga cttttggcc
 241 caactggagg aagataaaaccc cccacacaa tggaaaccac acatttaaaa tggactgtaa
 301 ccaattttca gacatgttgc ttgtggaaat aaaaacacaaat tatctctgggt cagagccctca
 361 gaattgtctca gccacaaaaa gtaacttaccc tgggggtactt ggtcccttacc cacccctcg
 421 ggactggccggg aaaaaggaa attttgtctc accttgcggaa aatcgggggtt cctggggcc
 481 ttgtgttgcact ttctccacca ctggggccctt ggagtctgcg atcccccatttcc caacccggaaa
 541 gatgtgttcc ttggccggaa acgcggctgtt ggacttgcgc caggacttca ataatcacgg
 601 ctgccaagggg ggtctccca gccaggctttt cgagtatatac ctgtacaaca aggggatcat
 661 gggtaagac accttccctt accaggggcaaa ggatgggtt tgcaagttcc aacctggaaa
 721 ggccatggc tttgtcaagg atgtacccaaat cttccatgttcatgacgggg aacgcgttgg
 781 ggaggctgtt gccccttaca accctgttgc ctttgcctt ggggttacttcc agggacttcat
 841 gatgtataga acggccatgtt acctccatgtt cccttccatgttcatgacgggg aacgcgttgg
 901 ccatgcgttgc ctggctgttgc ggtatggaga aaaaatggg atcccttactt gatgtgttgc
 961 aaacccctgg ggtcccttggggatggaa cgggtacttcc ctcatcgaccc gggggaaa
 1021 catgtgtggc ctggctgttgc ggccttccatgttcatgacgggg aacgcgttgg
 1081 cgcaggccggg aaggagggagg aacggggccggcc tggggcttgggg tggaaatcc
 1141 gcccctgggg aagttgtggg gagatccactt gggggccccc acattctgtcc ctcacccctcg
 1201 tggccctgggg gggaaacccatc acggccatgttcatgacgggg aacgcgttgg
 1261 gacgcaaaaga tcaaccaggc tggcccttgc tggcccttgc atggacttca aaccacatgg
 1321 accacaaata ttctttctgtt ccagaaggcc tttttccatgttcatgacgggg aacgcgttgg
 1381 tctttctgtt attcgctgtt caataaaat tggactgttgc cttcccttgggg tggggatgt
 1441 tggccatgggg aaaaaaaaaaaaaaaa

60 (SEQ ID NO:3)

Figure 6: coding sequence of mouse Cathepsin H (SEQ ID NO.4):

1 gggaccgggc agtgagcgcc gagatgtggg ctgcgcgtgcc gctgtgtgc gctggggcct
 61 ggctgcttag tactggggcc accgcggcgc tgaccgtgaa cgccatagaa aagtttcaact
 121 ttaagtcatg gatgaaaacag cataaaaaga cgtacagctc ggtggagtag aaccacacac
 181 tgcagatgtt tgccaacaac tggaggaaga ttcaagccca caaccagagg aaccacacat
 241 ttaaaaatggc attgaaccag ttttcagata tgagcttgc tgaataaaaa cacaattcc
 301 ttttgtcaga gcctcagaat tgctcagcca caaaaagtaa ctacctccga ggtacaggcc
 361 cttacccttc ctccatggac tggaggaaga aagaaaatgt ttttcgcca gtgataaaatc
 421 agggggcctg tggcagctgc tggactttctt ctaccacggg ggcccctagag tcagctgtgg
 481 ctattggccag tggaaaatgt ctgtctttgg ctgagcagca gctgggtggat tggcccaag
 541 cttcaacaa tcatggctgc aaaggaggc tccccagcca ggccctcgag tacatcttat
 601 acaacaaggg catcatggaa gaagacagct acccttacat aggcaaggat agttcatgca
 661 gattcaaccc caaaaaagct gttcattcg tcaagaatgt tgtcaacatc acactcaatg
 721 acgaggcgtgc aatggtttag gctgtggctc tataacaaccc tggacttttc gccttgagg
 781 tgactgaaga ttttttgat tataaaatgt ggctctactc cgttataatcc tggataaaa
 841 ctccagataaa agtaaaccat gcagttctgg cggttggcta tggagaacag aatggattac
 901 tctactggat tggaaaaaac tcttggggctt cccagtgggg ggagaatggg tacttcctca
 961 ttgaacgtgg gaagaacatg tggccctgg ctgtttgtgc ctccatccc attctcagg
 1021 tataagccac ggctgcacgg gcaactgattt ggcagaccaa gggaggaact ggtccttcga
 1081 tgagaatgcc accctggaga aaatttgtgtt gaaatccacc cagaggccct ctcactctg
 1141 agtctagacg cctaaagaca ggaaggacaa acttgaatag cgacaagccc acccacgtga
 1201 catcatcacc agaaaaatacgc ttggattgtt gttttttat gacccaaacc cacgtggacc
 1261 tagaategtc tcttttcca gctcttca tggactgggaa gctgtaatgg ttacctttc
 1321 tatgttgtgtt attcaataaaa cacacagtaa atacctcaaa aaaaaaaaaaa aa

(SEQ ID NO:4)

Figure 7: Cathepsin H human protein sequence according to P09668 (SEQ ID NO.5):

30	10	20	30	40	50	60
	MWATLPLLCA	GAWLLGVPVC	GAAELSVNSL	EKFHFKSWMS	KHRKTYSTEE	YHHRLQTFAS
40	70	80	90	100	110	120
	NWRKINAHNN	GNHTFKMALN	QFSDMSSFAEI	KHKYLWSEPO	NCSATKSNYL	RGTGPYPPSV
40	130	140	150	160	170	180
	DWRKKGNFVS	PVKNQGACGS	CWTFSTTGAL	ESAIAIATGK	MLSLAEQQLV	DCAQDFNNHG
40	190	200	210	220	230	240
	CQGGGLPSQAF	EYILYNKGIM	GEDTYPYQGK	DGYCKFQPGK	AIGFVKDVAN	ITIYDEEAMV
40	250	260	270	280	290	300
	EAVALYNPVS	FAFEVTQDFM	MYRTGIYSST	SCHKTPDKVN	HAVLAVGYGE	KNGIPYWIVK
40	310	320	330			
	NSWGPQWGMN	GYFLIERGKN	MCGLAACASY	PIPLV		

(SEQ ID NO.5)

12/12

Figure 8: human prepro protein of isoform A (SEQ ID NO:6)

MWATLPLLCAGAWLLGVPVCGAAELCVNSLEKFHFKSWMSKHRKTYSTEEYHHLRLQTFAS
 NWRKINAHNNNGNHTFKMALNQFSDMSFAEIKHKYLWSEPNQCSATKSNYLRGTGPYPPSV
 DWRKKGNFVSPVKNQGACGSCWTFSTTGALESAIATGKMLSLAEQQLVDCAQDFNNHG
 CQGGGLPSQAFYEIILYNKGIMGEDTPYQGKDGYCKFQPGKAIGFVKDVAINTIYDEEAMV
 EAVALYNPVSFAFEVTQDFMMYRTGIYSSTSCHKTPDKVNHAFLAVGYGEKNGIPYWIVK
 NSWGPQWGMNGYFLIERGKNMCGLAACASYPPLV

10 (SEQ ID NO:6)

Figure 9: protein sequence of human precursor of isoform B (SEQ ID NO:7, translated from SEQ ID NO.3)

MWATLPLCAAELCVNSLEKFHFKSWMSKHRKTYSTEEYHHLRLQTFASNWRKINAHNNG
 NHTFKMALNQFSDMSFAEIKHKYLWSEPNQCSATKSNYLRGTGPYPPSVWRKKGNFV
 PVKNQGACGSCWTFSTTGALESAIATGKMLSLAEQQLVDCAQDFNNHGCGQGGLPSQA
 FEYIILYNKGIMGEDTPYQGKDGYCKFQPGKAIGFVKDVAINTIYDEEAMVEAVALYNP
 VSFAFEVTQDFMMYRTGIYSSTSCHKTPDKVNHAFLAVGYGEKNGIPYWIVKNSWPQW
 GMNGYFLIERGKNMCGLAACASYPPLV

20 (SEQ ID NO:7)

Figure 10: protein sequence of human precursor of isoform B according to NP_683880.1 (SEQ ID NO:8)

30 1 mwatplllca aelcvnslek fhfkswmskh rktysteeyh hrlqtfasnw rkinahnng
 61 61 htftkmalnqf sdmsfaeikh kylwsepnc satksnylrg tgpyppsvdw rkkgnfvspv
 121 121 knqgacgscw tfsttgales aiaiatgkml slaeqqlvdc aqdfnnhgcq gglpsqafey
 181 181 ilynkgimge dtypyqgkdg yckfqpgkai gfvkdvanit iydeeamvea valynpvsfa
 241 241 fevtqdfmmy rtgiysstsc hktptdkvnha vlavgygekn gipywivkns wgpqwgmnny
 301 301 fliergknmc glaacsyplv

(SEQ ID NO:8)

40 1 mwatplllca gawllgvpc gaaelcvnsl ekhfkfswms khrktystee yhhrlqtfas
 61 61 nwrkinahnn gnhtfkmaln qfsdmsfaei khkylwsepq ncsatksnyl rgtgpypssv
 121 121 dwrkgnfvs pvknqgacgs cwtftgal esaiaiatgk mlsaeqqlv dcaqdfnnhg
 181 181 cqggglpsqaf eyilynkgim gedtpyqgk dgycckfqpgk aigfvkdvan itiydeeamv
 241 241 eavalynpvs fafevtqdfm myrtgiysst schktpdkvn havlavgyge kngipywivk
 301 301 nswgpqwgmn gyfliergkn mcglaacsy pipl

50 (SEQ ID NO:9)

INTERNATIONAL SEARCH REPORT

International application No PCT/EP2010/062525

A. CLASSIFICATION OF SUBJECT MATTER
 INV. G01N33/68 G01N33/573 C12Q1/37
 ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/241074 A1 (WOOLF C. [US] ET AL.) 26 October 2006 (2006-10-26) * abstract paragraphs [0004] – [0010], [0037] – [0038], [0048] – [0049], [0053], [0093], [0125] – [0138], [0167] – [0174] figure 3 claims 1-8 ----- US 2007/015271 A1 (ROSEN C.A. [US] ET AL.) 18 January 2007 (2007-01-18) * abstract paragraphs [0457], [0801] – [0817], [0883] – [0902], [0932] examples 8,12,13,14,15,19,20,46,47 claims 1-32 SEQ ID NO:219 (nucleic acid) and SEQ ID NO: 3119 identify cathepsin H. ----- -/-/	1-17
X		1-17

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search 9 November 2010	Date of mailing of the international search report 08/12/2010
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	Authorized officer Giry, Murielle

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/062525

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/260697 A1 (WANG K.K.-W. [US] ET AL.) 24 November 2005 (2005-11-24) * abstract paragraphs [0010] - [0011], [0099], [0104], [0120] - [0121], [0138] - [0139], [0148], [0242] - [0244] claims 1,92 -----	1-17
A	US 2003/212003 A1 (LUBBERT H. [DE] ET AL.) 13 November 2003 (2003-11-13) * abstract paragraphs [0064] - [0091] examples 1-4 claims 1-10 -----	1-17
A	US 2003/144234 A1 (BUXTON F.P. [US] ET AL.) 31 July 2003 (2003-07-31) * abstract claims 1-37 -----	1-17
A,P	WO 2009/118137 A1 (SANOFI - AVENTIS [FR]) 1 October 2009 (2009-10-01) * abstract claims 1-58 -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2010/062525

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 2006241074	A1 26-10-2006	NONE		
US 2007015271	A1 18-01-2007	NONE		
US 2005260697	A1 24-11-2005	US 2009317805 A1		24-12-2009
US 2003212003	A1 13-11-2003	NONE		
US 2003144234	A1 31-07-2003	US 2007129366 A1		07-06-2007
WO 2009118137	A1 01-10-2009	AR 071034 A1 AU 2009228611 A1 EP 2105742 A1		19-05-2010 01-10-2009 30-09-2009