

(19)



(11)

**EP 1 532 269 B1**

(12)

**EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention  
of the grant of the patent:  
**23.11.2011 Bulletin 2011/47**

(51) Int Cl.:  
**C12Q 1/68 (2006.01) C07H 21/00 (2006.01)**

(21) Application number: **03728848.7**

(86) International application number:  
**PCT/US2003/014882**

(22) Date of filing: **09.05.2003**

(87) International publication number:  
**WO 2003/094856 (20.11.2003 Gazette 2003/47)**

(54) **1L1RL-1 AS A CARDIOVASCULAR DISEASE MARKER**

1L1RL-1 ALS MARKER FÜR KARDIOVASKULÄRE ERKRANKUNGEN

1L1RL-1 UTILISE COMME MARQUEUR DE MALADIES CARDIO-VASCULAIRES

(84) Designated Contracting States:  
**AT BE BG CH CY CZ DE DK EE ES FI FR GB GR  
HU IE IT LI LU MC NL PT RO SE SI SK TR**

(30) Priority: **09.05.2002 US 379173 P**

(43) Date of publication of application:  
**25.05.2005 Bulletin 2005/21**

(60) Divisional application:  
**10184644.2 / 2 336 359**

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(56) References cited:  
**WO-A-00/35473 WO-A-02/38794  
WO-A1-01/70817 WO-A1-01/70817  
US-B1- 6 288 218**

- **KUROIWA K ET AL: "CONSTRUCTION OF ELISA SYSTEM TO QUANTIFY HUMAN ST2 PROTEIN IN SERA OF PATIENTS" HYBRIDOMA, LIEBERT, NEW YORK, NY, US, vol. 19, no. 2, 2000, pages 151-159, XP008013066 ISSN: 0272-457X**
- **WEINBERG E.O. ET AL.: 'Identification of serum soluble ST2 receptor as a novel heart failure biomarker' CIRCULATION vol. 107, no. 5, February 2003, pages 721 - 726, XP002985431**

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**Description****Field of the Invention**

**[0001]** This invention relates to methods and compositions for the diagnosis of cardiovascular conditions. More specifically, the invention relates to isolated molecules that can be used to predict the outcome of cardiovascular conditions including myocardial infarction and heart failure.

**Background of the Invention**

**[0002]** Despite significant advances in therapy, cardiovascular disease remains the single most common cause of morbidity and mortality in the developed world. Thus, prevention and therapy of cardiovascular conditions such as myocardial infarction and stroke is an area of major public health importance. Currently, several risk factors for future cardiovascular disorders have been described and are in wide clinical use in the detection of subjects at high risk. Such screening tests include evaluations of total and HDL cholesterol levels. However, a large number of cardiovascular disorders occur in subjects with apparently low to moderate risk profiles, and ability to identify such patients is limited. Moreover, accumulating data suggests that the beneficial effects of certain preventive and therapeutic treatments for patients at risk for or known to have cardiovascular disorders differs in magnitude among different patient groups. At this time, however, data describing diagnostic tests to determine whether certain therapies can be expected to be more or less effective are lacking.

**Summary of the Invention**

**[0003]** This invention provides methods for the diagnosis of cardiovascular conditions. A gene was identified that is upregulated in cardiac cells when the cells are subjected to mechanically-induced deformation. This nucleic acid molecule encodes the Interleukin 1 Receptor-Like 1 (IL1RL-1, also known as T1/ST2, ST2, and Fit-1, SEQ ID NOs: 1 and 2 for the soluble form and SEQ ID NOs: 3 and 4 for the membrane form). The terms IL1RL-1, T1/ST2, ST2, and Fit-1, are used interchangeably hereinafter throughout the specification.

**[0004]** According to the invention, a method for predicting outcome of a cardiovascular condition is provided.

**[0005]** In a first aspect, the invention provides an *in vitro* method of predicting the risk of mortality and/or new or worsening congestive heart failure (CHF) for a subject with a cardiovascular condition selected from the group consisting of CHF and myocardial infarction (MI), the method comprising:

obtaining a level of soluble IL1RL-1 protein in a biological sample comprising serum from the subject; and  
comparing said level of soluble IL1RL-1 protein to a predetermined value specific for the cardiovascular condition;

wherein a raised level of soluble IL1RL-1 protein in comparison to the predetermined value is indicative of an increased risk of mortality within 30 days and/or new or worsening CHF within 30 days, and the soluble IL1RL-1 protein is an expression product of a nucleic acid sequence as set out in SEQ ID NO. 1.

**[0006]** In a second aspect, the invention provides an *in vivo* method for predicting the risk of mortality and/or new or worsening congestive heart failure (CHF) for a subject with a cardiovascular condition selected from the group consisting of CHF and myocardial infarction (MI), the method comprising:

obtaining a first level of soluble IL1RL-1 protein in a sample comprising serum from a subject;  
obtaining a second level of soluble IL1RL-1 protein in a subsequent sample comprising serum from the same subject;  
and  
comparing the change in the level of the IL1RL-1 protein to a predetermined value specific for the cardiovascular condition, wherein a change in the level of the IL1RL-1 protein exceeding the predetermined value is indicative of an increased risk of mortality within 30 days and/or new or worsening CHF within 30 days.

**[0007]** Preferred features and embodiments of the invention are described in the attached dependent claims (claims 2-8 and 10-23).

**[0008]** In certain embodiments, the predetermined value specific for the predicted outcome of a cardiovascular condition is a plurality of predetermined marker level ranges and said comparing step comprises determining in which of said predetermined marker level ranges said subjects level falls. The cardiovascular condition is selected from the group consisting of, myocardial infarction and congestive heart failure.

**[0009]** These and other objects of the invention will be described in further detail in connection with the detailed description of the invention.

**Brief Description of the Sequences**

[0010] SEQ ID NO:1 is the nucleotide sequence of the human IL1RL1 (Soluble) cDNA.

[0011] SEQ ID NO:2 is the predicted amino acid sequence of the translation product of the human IL1RL1 (Soluble) cDNA (SEQ ID NO:1).

[0012] SEQ ID NO:3 is the nucleotide sequence of the human IL1RL1 (Membrane) cDNA.

[0013] SEQ ID NO:4 is the predicted amino acid sequence of the translation product of the human IL1RL1 (Membrane) (SEQ ID NO:3).

[0014] SEQ ID NO: 5 is the nucleotide sequence of the rat Fit-1S cDNA.

[0015] SEQ ID NO:6 is the predicted amino acid sequence of the translation product of rat Fit-1S cDNA (SEQ ID NO:5).

[0016] SEQ ID NO:7 is the nucleotide sequence of the rat Fit-1M cDNA.

[0017] SEQ ID NO:8 is the predicted amino acid sequence of the translation product of the rat Fit-1M cDNA (SEQ ID NO:7).

**Brief Description of the Drawings**

[0018]

Figure 1 depicts by a Northern Blot the effects of 8% cyclic mechanical strain on the expression of Fit-1 in cultured cardiac myocytes over the course of time.

Figure 2 depicts by a Northern Blot the effects of 8% cyclic mechanical strain, angiotensin receptor blockade, angiotensin II, IL-1b, and phorbol ester, on the expression of IL1RL-1 in cultured cardiac myocytes over the course of time.

Figure 3 depicts by a Northern Blot the effects of 8% cyclic mechanical strain, hydrogen peroxide, and TIRON, on the expression of IL1RL-1 in cultured cardiac myocytes over the course of time.

Figure 4 depicts by a Northern Blot the effects of actinomycin D and cyclohexamide on the induction of IL1RL-1 expression during an 8% cyclic mechanical strain on cardiac myocytes over the course of time.

Figure 5 depicts by a Northern Blot the effects of 8% cyclic mechanical strain alone and in combination with IL-1b, and phorbol ester in the absence of strain, on the expression of IL1RL-1 in cultured cardiac myocytes over the course of time.

Figure 6 depicts by a Northern Blot the effects of an 8% cyclic mechanical strain on the expression of vacuolar ATPase in cultured cardiac myocytes over the course of time.

Figure 7 depicts a kit embodying features of the present invention.

Figure 8 depicts early (left) and late (right) time course of the mRNA induction of T1/ST2 by mechanical strain in cardiac myocytes. Maximal induction occurs at 3 hours, is sustained for 9 hours and declines by 15 hours. Top panels, T1/ST2 RNA; bottom panels, ethidium bromide. No str, no strain.

Figure 9 depicts mRNA induction of T1/ST2 by mechanical strain (8%), interleukin-1 (10 ng/ml) and phorbol ester (PMA, 200 nM) at 1 and 3 hours. PMA>strain>IL-1. Top panel, T1/ST2 mRNA, bottom panel, ethidium bromide.

Figure 10 shows that T1/ST2 may be a gene induced by NF-κB activation during IL-1/IL-receptor signaling in cardiac myocytes. IL-1 and strain induced T1/ST2 mRNA in the presence of infection with control adenovirus (left). With infection of IκB adenovirus (right), which decreases NF-κB DNA binding activity, the IL-1 induction of T1/ST2 was blocked. The strain induction of T1/ST2 was partially blocked by IκB adenovirus infection suggesting another pathway for induction of T1/ST2 by strain. Top panel, T1/ST2 mRNA; bottom panel, ethidium bromide.

Figure 11 shows expression of T1/ST2 protein following myocardial infarction in mice by immunohistochemistry at 1 day but not 3 days after infarction. 40X magnification.

Figure 12 shows in graphical form ST2 protein levels in the systemic circulation of human patients post myocardial infarction; a. ST2 protein was significantly increased on day 1 post myocardial infarction compared to day 14 and day 90; b. Linear regression analysis demonstrating a significant positive relationship ( $p < 0.001$ ) between circulating ST2 protein and creatine kinase 1 day post myocardial infarction.  $\text{Log ST2} = 0.454(\text{log CK}) - 1.07$ ; c. Quartile analysis of circulating ST2 protein levels day 1 post myocardial infarction and ejection fraction. Low ejection fraction is associated with high ST2 protein levels.

Figure 13 shows that elevated baseline levels of ST2 were indicative of higher mortality through 30 days of follow-up (log-rank,  $p = 0.0009$ ).

**Detailed Description of the Invention**

[0019] The invention involves the discovery of a number of genes that are upregulated in cardiac cells when the cells are subjected to a mechanically-induced strain deformation

**[0020]** "Upregulated," as used herein, refers to increased expression of a gene and/or its encoded polypeptide. "Increased expression" refers to increasing (i.e., to a detectable extent) replication, transcription, and/or translation of any of the disclosed nucleic acids (IL1RL-1, SEQ ID NOs:1, 3), since upregulation of any of these processes results in concentration/amount increase of the polypeptide encoded by the gene (nucleic acid). Conversely, "downregulation," or "decreased expression" as used herein, refers to decreased expression of a gene and/or its encoded polypeptide. The upregulation or downregulation of gene expression can be directly determined by detecting an increase or decrease, respectively, in the level of mRNA for the gene, or the level of protein expression of the gene-encoded polypeptide, using any suitable means known to the art, such as nucleic acid hybridization or antibody detection methods, respectively, and in comparison to controls.

**[0021]** A "cardiac cell", as used herein, refers to a cardiomyocyte.

**[0022]** A "molecule," as used herein, embraces both "nucleic acids" and "polypeptides."

**[0023]** "Expression," as used herein, refers to nucleic acid and/or polypeptide expression.

**[0024]** As used herein, a "subject" is a mammal or a non-human mammal. In all embodiments human nucleic acids, polypeptides, and human subjects are preferred. It is believed that the results obtained using the human and rat molecules described elsewhere herein are predictive of the results that may be obtained using other homologous sequences.

**[0025]** In general, homologs and alleles typically will share at least 80% nucleotide identity and/or at least 85% amino acid identity to the characterized human sequences of the invention. In further instances, homologs and alleles typically will share at least 90%, 95%, or even 99% nucleotide identity and/or at least 95%, 98%, or even 99% amino acid identity to the characterized human sequences, respectively. The homology can be calculated using various, publicly available software tools developed by NCBI (Bethesda, Maryland). Exemplary tools include the heuristic algorithm of Altschul SF, et al., (J Mol Biol, 1990, 215:403-410), also known as BLAST. Pairwise and ClustalW alignments (BLOSUM30 matrix setting) as well as Kyte-Doolittle hydrophobic analysis can be obtained using public (EMBL, Heidelberg, Germany) and commercial (e.g., the MacVector sequence analysis software from Oxford Molecular Group/Genetics Computer Group, Madison, WI, Accelrys, Inc., San Diego, CA). Watson-Crick complements of the foregoing nucleic acids also are embraced by the invention.

**[0026]** In screening for related genes, such as homologs and alleles of the sequences described elsewhere herein, a Southern blot may be performed using stringent conditions, together with a probe. The term "stringent conditions," as used herein, refers to parameters with which the art is familiar. With nucleic acids, hybridization conditions are said to be stringent typically under conditions of low ionic strength and a temperature just below the melting temperature ( $T_m$ ) of the DNA hybrid complex (typically, about 3°C below the  $T_m$  of the hybrid). Higher stringency makes for a more specific correlation between the probe sequence and the target. Stringent conditions used in the hybridization of nucleic acids are well known in the art and may be found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. An example of "high stringency conditions" is hybridization at 65°C in 6 x SSC. Another example of high stringency conditions is hybridization at 65°C in hybridization buffer that consists of 3.5 x SSC, 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% Bovine Serum Albumin, 2.5mM NaH<sub>2</sub>PO<sub>4</sub>[pH7], 0.5% SDS, 2mM EDTA. (SSC is 0.015M sodium chloride/0.15M sodium citrate, pH7; SDS is sodium dodecyl sulphate; and EDTA is ethylenediaminetetracetic acid). After hybridization, the membrane upon which the DNA is transferred is washed at 2 x SSC at room temperature and then at 0.1 x SSC/0.1 x SDS at temperatures up to 68°C. In a further example, an alternative to the use of an aqueous hybridization solution is the use of a formamide hybridization solution. Stringent hybridization conditions can thus be achieved using, for example, a 50% formamide solution and 42°C. There are other conditions, reagents, and so forth which can be used, and would result in a similar degree of stringency. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles of IL1RL-1 nucleic acids of the invention. The skilled artisan also is familiar with the methodology for screening cells and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing.

**[0027]** Given the teachings herein of full-length human and rat cDNA clones, other mammalian sequences such as (mouse, bovine, etc.) cDNAs corresponding to the related human and rat nucleic acids can be isolated from cDNA libraries using standard colony hybridization techniques, or can be identified using a homology search, for example, in GenBank using any of the algorithms described elsewhere herein or known in the art. For example, sequences with GenBank Accession numbers Y07519.1 and D13695.1 for the mouse IL1RL-1 homologs, can be used interchangeably with the homologous rat sequences of the invention, in all aspects of the invention without departing from the essence of the invention.

**[0028]** As used herein with respect to nucleic acids, the term "isolated" means: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An isolated nucleic acid is one which is readily manipulated by

recombinant DNA techniques well known in the art. Thus, a nucleotide sequence contained in a vector in which 5' and 3' restriction sites are known or for which polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated, but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need not be. For example, a nucleic acid that is isolated within a cloning or expression vector is not pure in that it may comprise only a tiny percentage of the material in the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulated by standard techniques known to those of ordinary skill in the art.

**[0029]** Expression of any of the foregoing IL1RL-1 nucleic acids of the present invention, including unique fragments of the foregoing, can be determined using different methodologies. A "unique fragment," as used herein, with respect to a nucleic acid is one that is a "signature" for the larger nucleic acid. For example, the unique fragment is long enough to assure that its precise sequence is not found in molecules within the human genome outside of the sequence for each nucleic acid defined above. Those of ordinary skill in the art may apply no more than routine procedures to determine if a fragment is unique within the human genome. Unique fragments, however, exclude fragments completely composed of nucleotide sequences previously published as of the filing date of this application.

**[0030]** Unique fragments can be used as probes in Southern and Northern blot assays to identify such nucleic acids, or can be used in amplification assays such as those employing PCR. As known to those skilled in the art, large probes such as 200, 250, 300 or more nucleotides are preferred for certain uses such as Southern and Northern blots, while smaller fragments will be preferred for other uses such as PCR. Unique fragments also can be used to produce fusion proteins for generating antibodies, or determining binding of the polypeptide fragments, or for generating immunoassay components. Likewise, unique fragments can be employed to produce nonfused fragments of, for example, the IL1RL-1 polypeptides, useful, for example, in the preparation of antibodies, immunoassays or therapeutic applications. Unique fragments further can be used as antisense molecules to inhibit the expression of the foregoing nucleic acids and polypeptides respectively.

**[0031]** As will be recognized by those skilled in the art, the size of the unique fragment will depend upon its conservancy in the genetic code. Thus, some regions of SEQ ID NOs: 1, and 3, and complements will require longer segments to be unique while others will require only short segments, typically between 12 and 32 nucleotides long (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32 bases) or more, up to the entire length of each of the disclosed sequences. As mentioned above, this disclosure intends to embrace each and every fragment of each sequence, beginning at the first nucleotide, the second nucleotide and so on, up to 8 nucleotides short of the end, and ending anywhere from nucleotide number 8, 9, 10 and so on for each sequence, up to the very last nucleotide, (provided the sequence is unique as described above). For example, virtually any segment of the region of SEQ ID NO:1 beginning at nucleotide 1 and ending at nucleotide 1357, or SEQ ID NO:3 beginning at nucleotide 1 and ending at nucleotide 2058, or complements thereof, that is 20 or more nucleotides in length will be unique. Those skilled in the art are well versed in methods for selecting such sequences, typically on the basis of the ability of the unique fragment to selectively distinguish the sequence of interest from other sequences in the human genome of the fragment to those on known databases typically is all that is necessary, although *in vitro* confirmatory hybridization and sequencing analysis may be performed.

**[0032]** Expression vectors coding for proteins encoded by the nucleic acids corresponding to SEQ ID NOs: 1 and/or 3, fragments and variants thereof, and host cells containing those expression vectors, are disclosed. Virtually any cell, prokaryotic or eukaryotic, which can be transformed with heterologous DNA or RNA and which can be grown or maintained in culture, may be used in the practice of the invention. Examples include bacterial cells such as *Escherichia coli* and mammalian cells such as mouse, hamster, pig, goat, primate, etc. They may be of a wide variety of tissue types, including mast cells, fibroblasts, oocytes and lymphocytes, and they may be primary cells or cell lines. Specific examples include CHO cells and COS cells. Cell-free transcription systems also may be used in lieu of cells.

**[0033]** As used herein, a "vector" may be any of a number of nucleic acids into which a desired sequence may be inserted by restriction and ligation for transport between different genetic environments or for expression in a host cell. Vectors are typically composed of DNA although RNA vectors are also available. Vectors include, but are not limited to, plasmids, phagemids and virus genomes. A cloning vector is one which is able to replicate in a host cell, and which is further characterized by one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the new recombinant vector retains its ability to replicate in the host cell. In the case of plasmids, replication of the desired sequence may occur many times as the plasmid increases in copy number within the host bacterium or just a single time per host before the host reproduces by mitosis. In the case of phage, replication may occur actively during a lytic phase or passively during a lysogenic phase. An expression vector is one into which a desired DNA sequence may be inserted by restriction and ligation such that it is operably joined to regulatory sequences and may be expressed as an RNA transcript. Vectors may further contain one or more marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities

are detectable by standard assays known in the art (e.g.,  $\beta$ -galactosidase or alkaline phosphatase), and genes which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques (e.g., green fluorescent protein). Preferred vectors are those capable of autonomous replication and expression of the structural gene products present in the DNA segments to which they are operably joined.

**[0034]** As used herein, a coding sequence and regulatory sequences are said to be "operably joined" when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory sequences. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined if induction of a promoter in the 5' regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined to a coding sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

**[0035]** The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribed and 5' non-translated sequences involved with the initiation of transcription and translation respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Such 5' non-transcribed regulatory sequences will often include a promoter region which includes a promoter sequence for transcriptional control of the operably joined gene. Regulatory sequences may also include enhancer sequences or upstream activator sequences as desired. The vectors may optionally include 5' leader or signal sequences. The choice and design of an appropriate vector is within the ability and discretion of one of ordinary skill in the art.

**[0036]** Expression vectors containing all the necessary elements for expression are commercially available and known to those skilled in the art. See, e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989. Cells are genetically engineered by the introduction into the cells of heterologous DNA (RNA) encoding a polypeptide or fragment or variant thereof. That heterologous DNA (RNA) is placed under operable control of transcriptional elements to permit the expression of the heterologous DNA in the host cell.

**[0037]** Preferred systems for mRNA expression in mammalian cells are those such as pcDNA3.1 (available from Invitrogen, Carlsbad, CA) that contain a selectable marker such as a gene that confers G418 resistance (which facilitates the selection of stably transfected cell lines) and the human cytomegalovirus (CMV) enhancer-promoter sequences. Additionally, suitable for expression in primate or canine cell lines is the pCEP4 vector (Invitrogen, Carlsbad, CA), which contains an Epstein Barr virus (EBV) origin of replication, facilitating the maintenance of plasmid as a multicopy extra-chromosomal element. Still another preferred expression vector is an adenovirus, described by Stratford-Perricaudet, which is defective for E1 and E3 proteins (J. Clin. Invest. 90:626-630, 1992).

**[0038]** The above described SEQ ID NOs: 1 and/or 3, cDNA sequence-containing expression vectors, can be used to transfect host cells and cell lines, be these prokaryotic (e.g., *Escherichia coli*), or eukaryotic (e.g., CHO cells, COS cells, yeast expression systems and recombinant baculovirus expression in insect cells). Especially useful are mammalian cells such as mouse, hamster, pig, goat, primate, etc. They may be of a wide variety of tissue types, and include primary cells and cell lines. Specific examples include dendritic cells, U293 cells, peripheral blood leukocytes, bone marrow stem cells and embryonic stem cells.

**[0039]** The invention can utilise isolated polypeptides (including whole proteins and partial proteins), encoded by the foregoing nucleic acids (SEQ ID NOs: 1 and 3), and include the polypeptides of SEQ ID NOs: 2 and/or 4, and unique fragments thereof. Such polypeptides are useful, for example, alone or as part of fusion proteins to generate antibodies, as components of an immunoassay, etc. Polypeptides can be isolated from biological samples including tissue or cell homogenates, and can also be expressed recombinantly in a variety of prokaryotic and eukaryotic expression systems by constructing an expression vector appropriate to the expression system, introducing the expression vector into the expression system, and isolating the recombinantly expressed protein. Short polypeptides, including antigenic peptides (such as are presented by MHC molecules on the surface of a cell for immune recognition) also can be synthesized chemically using well-established methods of peptide synthesis.

**[0040]** As used herein with respect to polypeptides, the term "isolated" means separated from its native environment in sufficiently pure form so that it can be manipulated or used for any one of the purposes of the invention. Thus, isolated means sufficiently pure to be used (i) to raise and/or isolate antibodies, (ii) as a reagent in an assay, (iii) for sequencing, (iv) as a therapeutic, etc.

**[0041]** A unique fragment for each of the foregoing polypeptide, in general, has the features and characteristics of unique fragments as discussed above in connection with nucleic acids. As will be recognized by those skilled in the art, the size of the unique fragment will depend upon factors such as whether the fragment constitutes a portion of a conserved protein domain. Thus, some regions of a polypeptide will require longer segments to be unique while others will require only short segments, typically between 5 and 12 amino acids (e.g. 5, 6, 7, 8, 9, 10, 11 and 12 amino acids long or more, including each integer up to the full length of each polypeptide).

**[0042]** Unique fragments of a polypeptide preferably are those fragments which retain a distinct functional capability of the polypeptide. Functional capabilities which can be retained in a unique fragment of a polypeptide include interaction with antibodies, interaction with other polypeptides or fragments thereof, interaction with other molecules, etc. One important activity is the ability to act as a signature for identifying the polypeptide. Those skilled in the art are well versed in methods for selecting unique amino acid sequences, typically on the basis of the ability of the unique fragment to selectively distinguish the sequence of interest from non-family members. A comparison of the sequence of the fragment to those on known databases typically is all that is necessary.

**[0043]** Variants of the polypeptides described above can be useful. As used herein, a "variant" of a polypeptide is a polypeptide which contains one or more modifications to the primary amino acid sequence of a natural (e.g., "wild-type": a polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and 4) polypeptide. Modifications which create a polypeptide variant are typically made to the nucleic acid which encodes the polypeptide, and can include deletions, point mutations, truncations, amino acid substitutions and addition of amino acids or non-amino acid moieties to: (1) reduce or eliminate an activity of a polypeptide; (2) enhance a property of a polypeptide, such as protein stability in an expression system or the stability of protein-ligand binding; (3) provide a novel activity or property to a polypeptide, such as addition of an antigenic epitope or addition of a detectable moiety; or (4) to provide equivalent or better binding to a polypeptide receptor or other molecule. Alternatively, modifications can be made directly to the polypeptide, such as by cleavage, addition of a linker molecule, addition of a detectable moiety, such as biotin, addition of a fatty acid, and the like. Modifications also embrace fusion proteins comprising all or part of the polypeptide's amino acid sequence. One of skill in the art will be familiar with methods for predicting the effect on protein conformation of a change in protein sequence, and can thus "design" a variant polypeptide according to known methods. One example of such a method is described by Dahiyat and Mayo in Science 278:82-87, 1997, whereby proteins can be designed *de novo*. The method can be applied to a known protein to vary only a portion of the polypeptide sequence. By applying the computational methods of Dahiyat and Mayo, specific variants of any of the foregoing polypeptides can be proposed and tested to determine whether the variant retains a desired conformation.

**[0044]** Variants can include polypeptides which are modified specifically to alter a feature of the polypeptide unrelated to its physiological activity. For example, cysteine residues can be substituted or deleted to prevent unwanted disulfide linkages. Similarly, certain amino acids can be changed to enhance expression of a polypeptide by eliminating proteolysis by proteases in an expression system (e.g., dibasic amino acid residues in yeast expression systems in which KEX2 protease activity is present).

**[0045]** Mutations of a nucleic acid which encodes a polypeptide preferably preserve the amino acid reading frame of the coding sequence, and preferably do not create regions in the nucleic acid which are likely to hybridize to form secondary structures, such as hairpins or loops, which can be deleterious to expression of the variant polypeptide.

**[0046]** Mutations can be made by selecting an amino acid substitution, or by random mutagenesis of a selected site in a nucleic acid which encodes the polypeptide. Variant polypeptides are then expressed and tested for one or more activities to determine which mutation provides a variant polypeptide with the desired properties. Further mutations can be made to variants (or to non-variant polypeptides) which are silent as to the amino acid sequence of the polypeptide, but which provide preferred codons for translation in a particular host. The preferred codons for translation of a nucleic acid in, e.g., *Escherichia coli*, are well known to those of ordinary skill in the art. Still other mutations can be made to the noncoding sequences of a gene or cDNA clone to enhance expression of the polypeptide.

**[0047]** The skilled artisan will realize that conservative amino acid substitutions may be made in any of the foregoing polypeptides to provide functionally equivalent variants of the foregoing polypeptides, i.e., the variants retain the functional capabilities of each polypeptide. As used herein, a "conservative amino acid substitution" refers to an amino acid substitution which does not significantly alter the tertiary structure and/or activity of the polypeptide. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art, and include those that are found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

**[0048]** Thus functionally equivalent variants of polypeptides, i.e., variants of polypeptides which retain the function of the natural ("wild-type") polypeptides, are contemplated. Conservative amino acid substitutions in the amino acid sequence of polypeptides to produce functionally equivalent variants of each polypeptide typically are made by alteration of a nucleic acid encoding the polypeptide. Such substitutions can be made by a variety of methods known to one of ordinary skill in the art. For example, amino acid substitutions may be made by PCR-directed mutation, site-directed mutagenesis according to the method of Kunkel (Kunkel, Proc. Nat. Acad. Sci. U.S.A. 82: 488-492, 1985), or by chemical synthesis of a gene encoding a polypeptide. The activity of functionally equivalent fragments of polypeptides can be tested by cloning the gene encoding the altered polypeptide into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the altered polypeptide, and testing for a functional capability of the

polypeptides as disclosed herein.

**[0049]** A variety of methodologies well-known to the skilled artisan can be utilized to obtain isolated molecules. The polypeptide may be purified from cells which naturally produce the polypeptide by chromatographic means or immunological recognition. Alternatively, an expression vector may be introduced into cells to cause production of the polypeptide. In another method, mRNA transcripts may be microinjected or otherwise introduced into cells to cause production of the encoded polypeptide. Translation of mRNA in cell-free extracts such as the reticulocyte lysate system also may be used to produce polypeptides. Those skilled in the art also can readily follow known methods for isolating polypeptides. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography and immune-affinity chromatography.

**[0050]** The isolation of the described cDNAs also makes it possible for the artisan to diagnose a disorder characterized by an aberrant expression of any of the foregoing cDNAs. These methods involve determining expression of each of the identified nucleic acids, and/or polypeptides derived therefrom. In the former situation, such determinations can be carried out via any standard nucleic acid determination assay, including the polymerase chain reaction, or assaying with labeled hybridization probes as exemplified below. In the latter situation, such determination can be carried out via any standard immunological assay using, for example, antibodies which bind to the secreted protein.

**[0051]** The invention can also use isolated peptide binding agents which, for example, can be antibodies or fragments of antibodies ("binding polypeptides"), having the ability to selectively bind to any of the described polypeptides (e.g., SEQ ID NO: 2 or 4). Antibodies include polyclonal and monoclonal antibodies, prepared according to conventional methodology.

**[0052]** Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W.R. (1986) *The Experimental Foundations of Modern Immunology* Wiley & Sons, Inc., New York; Roitt, I. (1991) *Essential Immunology*, 7th Ed., Blackwell Scientific Publications, Oxford). The pFc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')<sub>2</sub> fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

**[0053]** Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

**[0054]** It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody. See, e.g., U.S. Patent Nos. 4,816,567; 5,225,539; 5,585,089; 5,693,762 and 5,859,205. Thus, for example, PCT International Publication Number WO 92/04381 teaches the production and use of humanized murine RSV antibodies in which at least a portion of the murine FR regions have been replaced by FR regions of human origin. Such antibodies, including fragments of intact antibodies with antigen-binding ability, are often referred to as "chimeric" antibodies.

**[0055]** Thus, as will be apparent to one of ordinary skill in the art, the present can utilise F(ab')<sub>2</sub>, Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric F(ab')<sub>2</sub> fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention can also use so-called single chain antibodies.

**[0056]** Thus, the invention can involve polypeptides of numerous size and type that bind specifically to polypeptides of the invention (e.g., SEQ ID NO: 2, or 4-its extracellular portions), and complexes of both the polypeptides and their binding partners. These polypeptides may be derived also from sources other than antibody technology. For example, such polypeptide binding agents can be provided by degenerate peptide libraries which can be readily prepared in solution, in immobilized form, as bacterial flagella peptide display libraries or as phage display libraries. Combinatorial



libraries also can be synthesized of peptides containing one or more amino acids. Libraries further can be synthesized of peptides and non-peptide synthetic moieties.

**[0057]** A method of diagnosing a disorder characterized by aberrant expression of a nucleic acid molecule, an expression product thereof, or a fragment of an expression product thereof, is described. The method involves contacting a biological sample isolated from a subject with an agent that specifically binds to the nucleic acid molecule, an expression product thereof, or a fragment of an expression product thereof, and determining the interaction between the agent and the nucleic acid molecule or the expression product as a determination of the disorder, wherein the nucleic acid molecule is a IL1RL-1 nucleic acid (SEQ ID NO.:1). In one embodiment, the disorder is myocardial infarction. In one embodiment, the disorder is heart failure.

**[0058]** In the case where the molecule is a nucleic acid molecule, such determinations can be carried out via any standard nucleic acid determination assay, including the polymerase chain reaction, or assaying with labeled hybridization probes as exemplified herein. In the case where the molecule is an expression product of the nucleic acid molecule, or a fragment of an expression product of the nucleic acid molecule, such determination can be carried out via any standard immunological assay using, for example, antibodies which bind to any of the polypeptide expression products.

**[0059]** "Aberrant expression" refers to decreased expression (underexpression) or increased expression (overexpression) of any of the foregoing IL1RL-1 molecules (nucleic acids and/or polypeptides) in comparison with a control (i.e., expression of the same molecule in a healthy or "normal" subject). A "healthy subject," as used herein, refers to a subject who is not at risk for developing a future cardiovascular condition (see earlier discussion and Harrison's Principles of Experimental Medicine, 13th Edition, McGraw-Hill, Inc., N.Y.- hereinafter "Harrison's"). Healthy subjects also do not otherwise exhibit symptoms of disease. In other words, such subjects, if examined by a medical professional, would be characterized as healthy and free of symptoms of a cardiovascular disorder or at risk of developing a cardiovascular disorder.

**[0060]** When the disorder is a cardiovascular condition selected from the group consisting of cardiac hypertrophy, myocardial infarction, stroke, arteriosclerosis, and heart failure, decreased expression of any of the foregoing molecules in comparison with a control (e.g., a healthy subject) is indicative of the presence of the disorder, or indicative of the risk for developing such disorder in the future.

**[0061]** The predetermined value specific for the cardiovascular condition can take a variety of forms. It can be single cut-off value, such as a median or mean. It can be established based upon comparative groups, such as where the risk in one defined group is double the risk in another defined group. It can be a range, for example, where the tested population is divided equally (or unequally) into groups, such as a low-risk group, a medium-risk group and a high-risk group, or into quadrants, the lowest quadrant being subjects with the lowest risk and the highest quadrant being subjects with the highest risk.

**[0062]** The predetermined value can depend upon the particular population selected. For example, an apparently healthy population (no detectable disease and no prior history of a cardiovascular disorder) will have a different 'normal' range of markers of systemic inflammation than will a smoking population or a population the members of which have had a prior cardiovascular disorder. Accordingly, the predetermined values selected may take into account the category in which the subject falls. Appropriate ranges and categories can be selected with no more than routine experimentation by those of ordinary skill in the art.

**[0063]** "Myocardial infarction" is a focus of necrosis resulting from inadequate perfusion of the cardiac tissue. Myocardial infarction generally occurs with the abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by atherosclerosis. Generally, infarction occurs when an atherosclerotic plaque fissures, ruptures, or ulcerates, and a mural thrombus forms leading to coronary artery occlusion.

**[0064]** The diagnosis of myocardial infarction in a subject determines the need for treating the subject. A number of laboratory tests, well known in the art, are described, for example, in Harrison's. Generally, the tests may be divided into four main categories: (1) nonspecific indexes of tissue necrosis and inflammation, (2) electrocardiograms, (3) serum enzyme changes (e.g., creatine phosphokinase levels), and (4) cardiac imaging. A person of ordinary skill in the art could easily apply any of the foregoing tests to determine when a subject is at risk, is suffering, or has suffered, a myocardial infarction. In addition, increased levels of expression of a IL1RL-1 nucleic acid molecule, or an expression product thereof, are also important risk factors.

**[0065]** Heart failure is a clinical syndrome of diverse etiologies linked by the common denominator of impaired heart pumping and is characterized by the failure of the heart to pump blood commensurate with the requirements of the metabolizing tissues, or to do so only from an elevating filling pressure.

**[0066]** The invention will be more fully understood by reference to the following examples. These examples, however, are merely intended to illustrate the embodiments of the invention and are not to be construed to limit the scope of the invention.

Examples**EXAMPLE 1.****Experimental Protocols: Materials and Methods****Mechanical Strain Device**

**[0067]** Experiments of mechanically overloading cardiomyocytes have generally been performed by stretching cells with no control of the cardiac cycle, an approach that does not allow distinction between mechanical overload in contraction versus relaxation. In the present study, we designed and constructed a unique experimental system that allows precisely controlled mechanical strains as well as electrical pacing in cultured cardiomyocytes, to investigate, *inter alia*, how cardiomyocyte mechanotransduction is regulated by the cardiac cycle, and identify genes that are involved in such regulation.

**[0068] The Pacing-Strain Device.** The approach to mechanical stimulation used an apparatus that has multiple platens that contact the underside of silicone elastomer membranes to apply a spatially isotropic biaxial strain profile to the membrane (Schaffer JL, et al., J Orthop Res, 1993, 12:709-719; and U.S. Provisional Patent application filed on July 16, 1999 entitled "AN APPARATUS FOR STUDYING MYOCARDIAL MECHANICAL OVERLOAD HYPERTROPHY AND USES THEREFOR, by Richard T. Lee, and bearing Attorney Docket no. 100038.130 and express mail no. EL110243781US). Six individual 78mm membranes can be stretched at once with varying amplitudes of strain by controlling displacement of each platen with a stepper motor. Measured Green strains are accurate to  $\sim \pm 0.25\%$  at strains from 1-14% (Cheng GC, et al., Circ Res, 1997, 80:28-36; Brown TD, J Biomechanics, 2000, 33:3-14). Throughout this study, 8% biaxial strain was used.

**[0069]** To control the timing of mechanical strain relative to the cardiac cycle, the computer paced each dish electrically, and controlled: the phase between the mechanical strain and the electrical impulse, the electrical impulse duration, and the voltage of the impulse. In addition, the electrical impulses had alternating polarity to minimize electrochemical effects such as pH gradients at the electrodes. The two outputs were each connected to a single set of electrodes in each dish. The dishes were paced in parallel with a resistance of approximately 500 ohms per dish.

**[0070]** The positive and negative voltage sources were provided by two power supplies (6545A, Hewlett Packard Company, Palo Alto, CA). The control circuit was divided into two parts: a high voltage circuit and a low voltage or digital signal circuit. The high voltage circuit was a gate that switched the output based on the input signal. The low voltage circuit accepted two control signals from the computer and accepted the pulse width from a variable resistor, which controlled both the positive and negative voltage gates. The low voltage circuit allowed a voltage pulse between 0-120V DC amplitude and 2-37ms duration. Lights provided continuous monitoring of the pulses, and the timing of the circuits and calibration were validated by oscilloscope.

**[0071]** The electrodes for each dish were two arc-shaped  $\text{AgCl}_2$  wire electrodes at the base of the inner surface of the dish, just above the deformable membrane. The electrodes were premade, ethanol-sterilized, and placed into the dish just prior to each experiment to minimize potential toxicity from silver. Using this method no cellular death or detachment was observed in 24 hr experiments. Each arc was 120 degrees; we performed a two dimensional finite element analysis to estimate the uniformity of the potential field with this configuration. These calculations estimate a spatial variation in the potential field of {root mean square} = 29%. Thus, this system provides highly uniform biaxial mechanical strain, with a relatively small variation in the voltage field.

**[0072] Mechanical stimulation protocols.** We imposed strain only during first third of the cardiac cycle by electrical stimulation for strain imposed during the "systolic phase", and only during one third of the cardiac cycle in the relaxation phase for strain imposed during "diastolic phase," respectively. Conditions used in this study were: (1) control; (2) strain, no pacing; (3) pacing, no strain; (4) strain imposed during systolic phase; and (5) strain imposed during diastolic phase.

**[0073]** Neonatal rat ventricular myocytes (NRVM) from 1-day old Sprague-Dawley rats were isolated by previously described methods (Springhorn JP, and Claycomb WC., Biochem J, 1989; 258:73-78; Arstall MA, et al., J Mol Cell Cardiol, 1998, 30:1019-25). NRVM were plated on the coated membrane dish at a density of 2,000,000 cells/dish in DMEM containing 7% FCS and incubated 24 h. Approximate cell confluence was 85-90%. NRVM were then made quiescent by washing with 10 ml of Hanks' balanced salt solution (HBSS, 138 mM NaCl, 5.3 mM KCl, 4.0 mM  $\text{NaHCO}_3$ , 1.3 mM  $\text{CaCl}_2$ , 0.5 mM  $\text{MgCl}_2$ , 0.4 mM  $\text{MgSO}_4$ , 0.4 mM  $\text{KH}_2\text{PO}_4$ , 0.3 mM  $\text{Na}_2\text{HPO}_4$ , 5.6 mM glucose; Life Technologies, Inc., Rockville, MD) twice and incubating with 26 ml of DMEM containing 0.2% FCS for 48-72 hours.

**[0074]** In these cell culture conditions, cells beat at 40-60 beats/minute. At this rate, we have observed negligible competition when pacing at a rate of 70 beats/minute. We performed trial capture experiments; nine locations on each dish were sampled. Capture efficiency was similar at all locations, and maximal capture occurred at 60 V and above with 10 ms of pulse width. Therefore, a voltage of 70 V with 10 ms of impulse duration at a rate of 1.2 Hz (70 beats/minute) was selected. Under these conditions we did not observe partial cell detachment.

**[0075] Transcriptional Profiling.** The DNA microarray experiment was performed with rat neonatal cardiac myocytes cultured on fibronectin-coated membranes with serum-free medium for 48 hours. Cells were deformed with an 8% deformation imposed only during systole for a period of 30 minutes, and RNA was prepared after 6 hours of subsequent no strain conditions and no pacing conditions. This time point was based upon previous studies demonstrating that the gene tenascin (positive control for cardiomyocytes) is induced at this time period. The DNA microarray hybridization experiment was performed using the Affymatrix GeneChip RGU34A (Affymetrix, Inc., Santa Clara, CA). Data were analyzed using Affymatrix software.

**[0076] Northern Analyses.** The cDNA clones for differentially expressed genes were obtained by PCR using the GenBank sequences. Each clone was sequenced from both 5' and 3' ends to confirm identity. Positive elements in the DNA microarray were confirmed by Northern blot hybridization analysis in at least three independent experiments using three different sources of NRVMs. Total RNA was isolated by the guanidium thiocyanate and phenol chloroform method (Chomczynski, et al., Anal. Biochem., 1987, 162:156-159). For Northern blotting, 15 µg RNA was loaded on a 1.0% agarose-formaldehyde gel (2.0 mol/l), transferred to a nylon membrane (Amersham Pharmacia Biotech AB, Piscataway, NJ), and UV cross-linked with a UV Stratalinker (Stratagene, Inc., La Jolla, CA). Each probe was hybridized with ExpressHyb solution (Clontech Labs., Inc., Palo Alto, CA) at 68 °C for 1 hour. The membrane was washed with 2 x SSC, 0.05% SDS solution for 30 to 40 minutes and three times at room temperature and 0.1 x SSC, 0.1 % SDS solution with continuous shaking at 50 °C for 40 minutes. The membrane was exposed to film at -80 °C, and radiographs were scanned and analyzed with Optimas 5.0 software (Optimas Co./Media Cybernetics, Silver Springs, MD). Densitometric units were normalized to the ethidium-stained 28S ribosomal subunit on the membrane.

**[0077] Results.** Figure 1 shows the timecourse (early, left; late, right) of the induction of IL1RL-1 mRNA expression by 8% cyclic mechanical strain in neonatal cardiac myocytes in culture. Maximal induction occurs at 3 hours and is sustained for 15 hours.

**[0078]** Figure 2 shows the effects of 8% mechanical strain, angiotensin receptor blockade (ARB, CP-19116, 100 nM), angiotensin II (Ang II, 50 nM), interleukin-1β (IL-1β, 10 ng/ml), and phorbol ester (PMA, 200 nM) for 3 hours on the induction of IL1RL-1 mRNA expression in cultured neonatal rat cardiac myocytes. The induction of IL1RL-1 mRNA expression by strain was not blocked by angiotensin receptor blockade; furthermore, treatment with angiotensin II did not induce IL1RL-1 mRNA expression. Treatment with both IL-1β and PMA were associated with an induction of IL1RL-1 mRNA expression in the absence of mechanical strain.

**[0079]** Figure 3 shows the effects of 8% mechanical strain, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> 100 µM) and the antioxidant, TIRON (10 mM) on the induction of IL1RL-1 mRNA expression. Unlike the mRNA expression of the mechanically induced Tenascin-C gene which is induced by H<sub>2</sub>O<sub>2</sub> in the absence of mechanical strain and blocked by TIRON, H<sub>2</sub>O<sub>2</sub> does not induce IL1RL-1 in the absence of strain and blocks the strain-induced induction of IL1RL-1. TIRON slightly attenuated the mRNA expression of IL1RL-1 in the absence and presence of strain.

**[0080]** Figure 4 shows the effects of actinomycin D (5 µg/ml, left) and cyclohexamide (10 µg/ml, right) on the induction of IL1RL-1 mRNA expression by 8% mechanical strain. Actinomycin D and cyclohexamide were applied during mechanical strain. Actinomycin D blocked the induction of IL1RL-1 mRNA expression at both 2 and 4 hours suggesting that the induction of IL1RL-1 in response to strain is due to increased transcription of IL1RL-1. The protein synthesis inhibitor, cyclohexamide blocked the induction of IL1RL-1 mRNA expression in response to strain suggesting that new protein synthesis is required for the induction of IL1RL-1 mRNA expression.

**[0081]** Figure 5 shows the effects of 8% mechanical strain alone and in combination with interleukin-1β (IL-1β, 10 ng/ml), and phorbol ester in the absence of strain (PMA, 100 ng/ml) on IL1RL-1 mRNA expression in cultured neonatal cardiac myocytes. Both IL-1β and mechanical strain alone induced IL1RL-1 mRNA expression but the induction of IL1RL-1 by mechanical strain in the presence of IL-1β was not further increased suggesting that mechanical strain and IL-1β do not act in a synergistic or additive manner on the induction of IL1RL-1. The strongest induction of IL1RL-1 mRNA expression is seen with PMA. The rank order potency for the induction of IL1RL-1 mRNA expression is PMA>strain>IL-1β.

**[0082]** Figure 6 shows neonatal rat cardiac myocytes were exposed to 8% strain for 0, 1, 3, 6, 9 hours. Total RNA was isolated using a RNeasy kit. Five µg of total RNA were size-separated on 1% agarose-formaldehyde gel and transferred to nylon membrane. After crosslinking with UV light, membrane was hybridized with <sup>32</sup>P-labeled probe specific for V-ATPase B subunit. The membrane was then exposed to x-ray film for 3 hours at -80 °C with an intensifying screen.

## **EXAMPLE 2.**

### **Introduction:**

**[0083] Cytokines and Cardiac Injury.** Stress-activated cytokines participate in many forms of cardiac injury and pathophysiological conditions, the most characterized ones being tumor necrosis factor-α, interleukin-1 and interleukin-6. These molecules are not constitutively expressed in the normal heart but are rapidly induced during ischemia and reperfusion or upon hemodynamic overloading, suggesting that they play an important role in the initial myocardial

response to stress, injury or growth stimuli (Mann DL, Cytokine and Growth Factor Reviews. 1996;7:341-354; St. John Sutton MG, et al. Circulation. 2000;101:2981-2988). However, cytokines have also been shown to be stably expressed in pathologic myocardial conditions including ischemic heart disease and heart failure and are associated with a poor prognosis (Pulkki KJ, et al. Annals of Medicine. 1997; 29:339-343; Kubota T, et al Proc Natl Acad Sci. 1998;95:6930-6935; Aukrust P, et al. Am J Cardiol 1999;83:376-382; MacGowan GA, et al. Am J Cardiol 1997;79:1128-1132; Roig E, et al. Am J Cardiol 1998;688-690; Tsutamoto T, et al. J Am Coll Cardiol 1998;31:391-398; Prabhu SD, et al. Circulation. 2000; 101:2103-2109; Murray DR, et al. Annu Rev Immunol. 2000;18:451-494).

**[0084]** Interleukin-1 signaling through the interleukin-1 receptor is an early event in inflammatory cytokine signaling in many different systems (Trehu EG., Clin Cancer Res. 1996; 8:1341-51). In cardiac injury, interleukin-6 is produced by cardiac myocytes secondary to stimulation with interleukin-1, tumor necrosis factor- $\alpha$ , or lipopolysaccharide and has been detected in the post-ischemic lymph during reperfusion of ischemic myocardium (Gwechenberger M, et al. Circulation 1999;99:546-551). Recently recognized is the potential expression of counteracting anti-inflammatory cytokines in cardiac disease secondary to interleukin-1 signaling. Interleukin-4 and interleukin-10 can suppress the synthesis of tumor necrosis factor- $\alpha$  and enhance the release of soluble tumor necrosis factor receptors, which are ligand sinks for tumor necrosis factor (Joyce DA., 1994; Eur. J. Immunol. 11:2699-705). Interleukin-10 is increased in patients with heart failure (Yamaoka M, et al. Jpn Circ J. 1999;63:951-956) and interleukin-10 serum levels are increased when tumor necrosis factor- $\alpha$  serum levels are increased in patients with dilated cardiomyopathy (Ohtsuka T, et al. J Am Coll Cardiol. 2001;37:412-417).

**[0085] T1/ST2 (IL1RL-1): A Novel Mechanically Induced Receptor.** We have identified a novel potential stress-activated signaling pathway in the heart: regulation of the induction of an interleukin-1 family member gene, T1/ST2. Little is known of the induction, signaling and function of T1/ST2 in any cell type and T1/ST2 was shown in separate areas of investigation to have two seemingly unrelated functions. One of these is growth regulation and the other is immune modulation. Both compensatory hypertrophic growth and immune/inflammatory modulation are involved in the pathophysiology of cardiovascular diseases.

**[0086] Growth.** The T1/ST2 gene was first identified by its induction following serum stimulation of resting mouse 3T3 fibroblasts, suggesting that the T1/ST2 gene participates in growth regulation (Tominaga S., FEBS Letters 1989;258: 301-304). The same group later identified a longer transcript consisting of transmembrane and cytoplasmic domains homologous to the full-length interleukin-1 receptor (Yanagisawa K, et al. FEBS Letters. 1993;318:83-87).

**[0087] Immunity.** T1/ST2 is expressed on T helper-2, but not T helper-1, cells of the adaptive immune system, which produce interleukin-4, interleukin-5 and interleukin-10 (Yanagisawa KI, et al. J Biochem. 1997;121:95-103; Coyle AJ, et al. J Exp Med. 1999; 190:895-902). T helper-2 cells mediate beneficial responses to infection, but are detrimental in the development of allergy and asthma. There is a strong correlation between expression of T1/ST2 and interleukin-4 production on T helper-2 cells (Coyle AJ, et al. J Exp Med. 1999;190:895-902). T1/ST2 plays a *critical role in differentiation* to and activation of T helper-2 but not T helper-1 cells (O'Neill LAJ, et al. Immunology Today. 2000;21:206-209).

**[0088]** Inhibition of T1/ST2 signaling attenuated T helper 2-mediated induction of eosinophil inflammatory responses in lung and inhibited cytokine secretion from T helper-2 cells without modifying interferon-gamma secretion from T helper-1 cells (Coyle AJ, et al. J Exp Med. 1999; 190:895-902). These studies indicate that expression of T1/ST2 can alter the cytokine profile in favor of expression of interleukin-4, interleukin-5 and interleukin-10. Interleukin-10 has recently been shown to have anti-inflammatory effects in the setting of cardiac injury (Ohtsuka T, et al. J Am Coll Cardiol. 2001;37: 412-417). Similarly, the absence of T1/ST2 expression could result in a shift towards interferon-gamma expression, which may be deleterious following myocardial injury.

**[0089]** Taken together, the involvement of T1/ST2 in growth responses and immune function coupled with the clinical recognition of the role of cytokines in the inflammatory response to ischemia/reperfusion are suggestive that T1/ST2 activation is a growth- or stress-activated signaling pathway that contributes to myocardial growth and remodeling.

**[0090] Phenotype of T1/ST2 Null Mice.** (Townsend MJ, et al. J Exp Med. 2000;191:1069-1075). The absence of T1/ST2 in T1/ST2 null mice does not compromise their basal immune function in the absence of immune challenge. However, T1/ST2 null mice have an impaired ability to generate IL-4, IL-5, and IL-10, but not IFN- $\gamma$  (a Th1 cytokine) and to generate a T helper-2 inflammatory response during eosinophilic infiltration in the lung (a Th2 response).

**[0091]** We have begun to study the induction of T1/ST2 in cardiac myocytes and its involvement in survival/death signaling within the context of the myocyte signaling pathways. Preliminary studies presented below show that T1/ST2 is induced in cardiac myocytes in response to interleukin-1 and mechanical strain and that the induction of T1/ST2 by interleukin-1 may be dependent on NF- $\kappa$ B activation. T1/ST2 mRNA is also induced in human adult vascular smooth muscle cells in response to interleukin-1. T1/ST2 protein is expressed in the mouse heart early after myocardial ischemia in vivo as well as in human aorta tissue from patients with unstable plaque.

## Results:

**[0092] IN VITRO STUDIES.** The following studies demonstrate the induction of T1/ST2 by mechanical strain and

interleukin-1, possibly through activation of NF- $\kappa$ B. Both transcripts of T1/ST2 (that is, IL1RL-1S-soluble- and IL1RL-1M -membrane-) are induced by strain in cardiac myocytes although the more abundant transcript was the soluble isoform. T1/ST2 mRNA is induced by mechanical strain in cultured neonatal cardiac myocytes (Figure 8).

[0093] T1/ST2 mRNA is induced by mechanical strain in cultured neonatal cardiac myocytes. Neonatal rat ventricular myocytes were isolated by collagenase digestion, plated on fibronectin-coated silicone membrane dishes at a density of 3.5 million cells/dish in 13 ml media as previously described (Yamamoto K, et al. J Biol Chem. 1999;274:21840-21846). This technique yields cultures with  $\geq 95\%$  myocytes. Mechanical deformation was applied using a device that provides uniform biaxial cyclic strain as previously described (Yamamoto K, et al. J Biol Chem. 1999;274:21840-21846). RNA was extracted (Qiagen) and Northern blotting was performed using as a probe a  $^{32}$ P-labelled 600bp PCR fragment specific to rat T1/ST2. Maximal induction occurs at 3 hours, is sustained for 9 hours and declines by 15 hours.

[0094] Both interleukin-1 $\beta$  and mechanical strain each induce T1/ST2 RNA in cardiac myocytes (Figure 9). Shown is the induction of T1/ST2 by interleukin-1 and strain. We also found that the induction of T1/ST2 by mechanical strain in the presence of interleukin-1 $\beta$  was not further increased suggesting that interleukin-1 does not sensitize myocytes to the effects of mechanical strain (or vice versa) on the induction of T1/ST2. The 1 hour time point was included in the event that induction by strain is saturated at 3 hours and therefore masks an additive effect of interleukin-1 $\beta$ . Shown in the two right lanes are the effects of phorbol ester (PMA) at 1 and 3 hours. The rank order potency for the induction of T1/ST2 mRNA expression is PMA>strain> interleukin-1 $\beta$ . Since interleukin-1 $\beta$  signals through NF- $\kappa$ B and PMA through PKC these results suggest that NF- $\kappa$ B and PKC activation both participate in the induction of T1/ST2.

[0095] T1/ST2 may be a NF- $\kappa$ B target gene in cardiac myocytes through interleukin-1/interleukin-1 receptor signaling (Figure 10). Previously reported by us (Yamamoto K, et al. J Biol Chem. 1999;274:21840-21846), mechanical strain of cardiac myocytes activates NF- $\kappa$ B. To investigate the role of NF- $\kappa$ B in interleukin-1 $\beta$  and strain induction of T1/ST2 RNA, we overexpressed I $\kappa$ B $\alpha$ , which decreases NF- $\kappa$ B DNA binding activity. Cultured cardiac myocytes were infected with I $\kappa$ B $\alpha$  overexpression adenovirus vector or with  $\beta$ -galactosidase control vector and exposed for 4 hours to 8% cyclic mechanical strain or interleukin-1 (10ng/ml). RNA was analyzed by Northern blotting with  $^{32}$ P-labeled IL1RL-1 cDNA probe. Ectopic expression of I $\kappa$ B $\alpha$  blocked interleukin-1 $\beta$  induction of T1/ST2-1 mRNA and partially blocked strain induction of T1/ST2 mRNA expression when compared with T1/ST2 induction in cells treated with the  $\beta$ -galactosidase control vector. These results suggest that T1/ST2 is an early, NF- $\kappa$ B target gene through interleukin-1/interleukin-1 receptor signaling. In contrast, pathways in addition to NF- $\kappa$ B activation may be involved in the induction of T1/ST2 RNA by mechanical strain. T1/ST2 mRNA is also induced by interleukin-1 but not PMA or tumor necrosis factor (TNF) in human adult vascular smooth muscle cells.

[0096] In addition to the above-noted results, we have shown that T1/ST2 is induced secondary to NF- $\kappa$ B activation by interleukin-1 and NF- $\kappa$ B is linked to cardiac myocyte survival. Further in vitro studies are performed to confirm that T1/ST2 activation is linked to cell growth and survival.

## IN VIVO STUDIES.

### Materials and Methods

[0097] **Experimental myocardial infarction in mice.** Experimental procedures on mice were approved by the Harvard Medical School Standing Committee on Animals. Experimental myocardial infarction was created in mice by coronary artery ligation as previously described (13). Hearts were harvested from mice 1 and 3 days after coronary artery ligation followed by perfusion fixation of the heart with Z-Fix (Anatech LTD). Hearts were then immersion fixed in Z-Fix overnight at 4°C. After dehydration in graded ethanol solutions, hearts were placed in Histo-Clear (National Diagnostics) and paraffin-embedded. Five micron tissue sections were deparaffinized, rehydrated, incubated with 3% hydrogen peroxide, rinsed in water followed by phosphate buffered saline. Sections were blocked, incubated in 1:50 anti-mouse ST2 primary antibody (Morwell Diagnostics) and 1:100 anti-rat HRP conjugated secondary antibody (Vector Laboratories). Slides were counterstained with hematoxylin and eosin.

### **Patient studies and ELISA for ST2.**

[0098] HEART study The Healing and Early Afterload Reducing Therapy (HEART) study was a randomized, double-blind, placebo-controlled trial that enrolled 352 patients with acute myocardial infarction (MI) from 36 centers in the United States and Canada. Men and women over the age of 21 years who had experienced an MI within 24 hours were eligible. Inclusion and exclusion criteria, and details of the trial design have been previously described (Pfeffer M.A., et al., Circulation, 1997, 95:2643-2651; Greaves S.C., et al., Am. J. Cardiol, 1997, 80:442-448; Solomon S.D., et al., Ann. Intern. Med., 2001, 134:451-458; Aikawa Y., et al., Am. Heart J, 2001, 141:234-242). Serial blood samples from days 1, 14, and 90 after myocardial infarction from 69 randomly chosen patients in the HEART trial were available for this study. Soluble T1/ST2 was assayed with a double monoclonal sandwich ELISA assay that has been previously described

(Kuroiwa K., et al., Hybridoma, 2000, 19:151-159). The assay is commercially available (MBL International, Watertown, MA).

**[0099] PRAISE study** The Prospective Randomized Amlodipine Survival Trial (PRAISE) study was a prospective large-scale study of amlodipine in patients with heart failure due to coronary artery disease. The results of this trial were null for a benefit of Amlodipine in severe heart failure. Blood samples were drawn at the beginning of this study before therapy and then twice more during the study. Soluble T1/ST2 was assayed as described above. One of the key current blood tests for heart failure is brain natriuretic peptide (BNP). We examined whether T1/ST2 levels in heart failure patients were altered and whether T1/ST2 levels correlated with BNP levels in these patients.

**[0100] Statistics.** Each in vitro experiment shown was performed a minimum of three times. Values are means  $\pm$  SEM. Data were analyzed by one-way ANOVA, or ANOVA for repeated measures, with post hoc Bonferroni multiple comparison analyses where appropriate. Linear regression was performed on serum values with log transformed values due to non-normal parameter distributions. P values  $<0.05$  were considered statistically significant.

## Results:

**[0101] In vivo Expression of T1/ST2 Protein in Myocardial Infarction in Mice.** To evaluate expression of T1/ST2 in injured myocardium, mice were subjected to experimental myocardial infarction through coronary artery ligation. Figure 11 shows protein expression of T1/ST2 using immunohistochemistry in mouse hearts 1 and 3 days post myocardial infarction. Positive staining was seen 1 day post myocardial infarction (post-MI) in all regions of the left ventricle, normal, infarct and border zones, but not at 3 days post myocardial infarction. No staining for T1/ST2 was observed in 1 and 3 day sham-operated controls. These results suggest that T1/ST2 protein is expressed in response to acute injury during the early phase of post-infarction remodeling before the migration of macrophages into the infarct and border zones seen at 3 days. The monoclonal antibody used for these studies does not distinguish between soluble and membrane forms of T1/ST2.

**[0102] Soluble T1/ST2 is increased in the systemic circulation of patients one day after myocardial infarction.** Since soluble T1/ST2 is highly induced in cardiac myocytes, and T1/ST2 protein is highly expressed in mouse myocardium following experimental myocardial infarction, we hypothesized that soluble T1/ST2 is increased in the systemic circulation of patients following myocardial infarction.

**[0103] Methods and Results:** Using a double monoclonal sandwich ELISA assay, we assayed blood samples from 69 participants of the HEART Study on the day of myocardial infarction (day 1), as well as day 14 and day 90 after infarction. As shown in Figure 12a, systemic T1/ST2 protein was significantly increased one day after myocardial infarction (mean  $\pm$  SEM,  $3.8 \pm 0.4$  ng/ml,  $p < 0.001$ ; range, 0.32 to 17.42 ng/ml) compared to day 14 (mean  $\pm$  SEM,  $0.98 \pm 0.06$  ng/ml; range, 0.25 to 3.42 ng/ml) and day 90 (mean  $\pm$  SEM,  $0.79 \pm 0.07$  ng/ml; range, 0.02 to 3.53 ng/ml; day 14 vs. day 90,  $P = \text{NS}$ ). Mean values at day 90 were similar to published mean values for healthy controls (Kuroiwa K., et al., Hybridoma, 2000, 19:151-159). Systemic T1/ST2 protein levels correlated positively with peak creatine kinase levels ( $r = 0.41$ ,  $p < 0.001$ ), shown in Figure 12b. High systemic ST2 protein levels were also associated with low ejection fraction one day after myocardial infarction as shown in quartile analysis ( $p = 0.03$ ) in Figure 12c.

**[0104] Conclusions:** These results suggest a coordinated regulation between the extent of myocardial injury and synthesis and secretion of soluble T1/ST2 into the systemic circulation in the clinical setting of myocardial infarction.

**[0105] Soluble T1/ST2 is increased in the systemic circulation of patients with severe chronic heart failure.** This study tested the hypothesis that soluble T1/ST2 levels in the serum of patients with severe chronic heart failure are associated with levels of BNP, ProANP and norepinephrine, neurohormones that are increased in heart failure.

**[0106] Methods and Results:** Serum samples, clinical variables and neurohormone levels from the neurohormone substudy of the Prospective Randomized Amlodipine Survival Evaluation 2 study (PRAISE-2) heart failure trial (New York Heart Association functional class III or IV, end point: mortality or transplantation) were used. The PRAISE-2 study was a multi-center, randomized, double blinded, parallel group, placebo-controlled study to evaluate the effects of amlodipine 10 mg/day on survival in patients with congestive heart failure of a non-ischemic etiology. The trial consisted of - patients recruited from 240 sites in the United States and Canada. The neurohormone substudy consisted of 181 patients recruited from 26 centers participating in the main study. Both the main PRAISE-2 and the neurohormonal substudy were approved by the institutional review boards of the participating institutions. Patients were eligible if they were at least 18 years of age, had heart failure of a non-ischemic etiology, symptoms at rest or upon minimal exertion (New York Heart Association functional class III or IV) and a left ventricular ejection fraction lower than 30%. All patients were on treatment with ACE inhibitors and digoxin for at least 3 months. Patients were excluded if they had a recent or remote history of angina.

**[0107] Assays for T1/ST2, Neurohormones and Measurement of Oxidative Stress.** Blood samples were evaluated at baseline and 2 weeks (Table 1). Soluble T1/ST2 was measured with a sandwich double monoclonal antibody ELISA method (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan) according to the manufacturer's instruction. In brief, serum samples or standards were incubated in the microwells coated with anti-human T1/ST2 antibody. After

washing, peroxidase-conjugated anti-human T1/ST2 antibody was added into the microwell and incubated. After another washing, the peroxidase substrate was added and the optical density at 450 nm was determined. Circulating catecholamines (norepinephrine, epinephrine, dopamine), angiotensin II, natriuretic peptides (pro-atrial natriuretic peptide (Pro-ANP), brain natriuretic peptide (BNP)) and indices of oxidative stress (malondialdehyde, adrenolutin) were measured as previously described (Dhalla KS, et al., Mol Cell Biochem, 1989;87:85-92; Moe GW, et al., Am Heart J, 2000;139:587-95). T1/ST2 serum measurements were performed on samples from 162 patients obtained at trial enrollment and from 135 of the same patients obtained 2 weeks after trial enrollment. Baseline T1/ST2 levels correlated with baseline BNP levels ( $r=0.3511$ ,  $p<0.0001$ ), baseline ProANP levels ( $r=0.3598$ ,  $p<0.0001$ ) and baseline norepinephrine levels ( $r=0.3854$ ,  $p<0.0001$ ) (Table 2). The change in T1/ST2 (T1/ST2 levels at 2 weeks minus T1/ST2 levels at trial enrollment) was significant as a univariate predictor of mortality or transplantation ( $p=0.048$ ) as was baseline BNP ( $p<0.0001$ ) and baseline ProANP ( $p<0.0001$ ) (Table 3). In multivariate models including BNP and ProANP, the change in T1/ST2 remained significant as an independent predictor of mortality or transplantation independent of BNP and ProANP (Table 4).

Table 1. Baseline Characteristics

## A. All Patients

	N	Median	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
Baseline ST2 (ng/mL)	161	0.24	0.16	0.70
Baseline BNP (pmol/L)	162	56.0	3.70	264.30
Baseline ProANP (pg/L)	162	1778.50	531.00	5615.00
Norepinephrine (pg/mL)	158	401.58	165.90	1096.00
Dopamine (pg/mL)	158	39.06	4.22	398.40
Epinephrine (pg/mL)	158	54.92	11.64	139.90
Angiotensin II (pg/mL)	157	22.60	7.00	67.30
Adrenolutin (ng/mL)	156	22.84	4.31	369.31
Creatinine (mmol/L)	158	1.10	0.80	1.90
Age (years)	157	59.9	32.5	78.2
Body Mass Index (kg/mm <sup>2</sup> )	157	27.6	20.4	39.7
LV Ejection Fraction	158	22.0	11.0	30.0

## B. Patients With Blood Samples at Baseline and Week 2

	N	Median	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
Baseline ST2 (ng/mL)	135	0.24	0.15	0.81
Baseline BNP (pmol/L)	135	54.90	3.30	264.30
Baseline ProANP (pg/L)	135	1788.00	488.00	4788.00
Norepinephrine (pg/mL)	130	395.05	171.70	1118.00
Dopamine (pg/mL)	130	64.02	4.32	405.50
Epinephrine (pg/mL)	130	56.07	12.24	134.80
Angiotensin II (pg/mL)	131	21.70	7.00	58.30
Adrenolutin (ng/mL)	130	24.41	4.43	369.31
Creatinine (mmol/L)	135	1.10	0.80	2.00
Age (years)	134	60.5	34.4	78.2
Body Mass Index (kg/mm <sup>2</sup> )	134	27.4	20.5	39.7
LV Ejection Fraction	135	22.0	11.0	30.0

Table 2. Relation of ST2 to Clinical Variables and Neurohormones: Spearman Correlations

		Baseline ST2	Change in ST2
Baseline BNP (pmol/L)	R	0.3511	-0.11327
	<i>p</i> value	<0.0001	0.1843
	N	161	139
Baseline ProANP (pmol/L)	R	0.35979	-0.10967

(continued)

**Table 2. Relation of ST2 to Clinical Variables and Neurohormones: Spearman Correlations**

		Baseline ST2	Change in ST2
	<i>p value</i>	<i>&lt;0. 0001</i>	<i>0.1987</i>
	N	161	139
	<b>Change in BNP* (pmol/L)</b>	R	-0.10184
	<i>p value</i>	<i>0.2329</i>	<i>0.0110</i>
	N	139	139
	<b>Change in ProANP* (pmol/L)</b>	R	0.05584
	<i>p value</i>	<i>0.5138</i>	<i>0. 0006</i>
	N	139	139
	<b>Norepinephrine (pg/ml)</b>	R	<i>0.38535</i>
	<i>p value</i>	<i>&lt;0. 0001</i>	<i>0.0032</i>
	N	156	134
	<b>Dopamine (pg/mL)</b>	R	0.07879
	<i>p value</i>	<i>0.3283</i>	<i>0.0102</i>
	N	156	134
	<b>Epinephrine (pg/mL)</b>	R	0.08043
	<i>p value</i>	<i>0.3182</i>	<i>0.1634</i>
	N	156	134
	<b>Angiotensin II (pg/mL)</b>	R	0.00374
	<i>p value</i>	<i>0.9630</i>	<i>0.9335</i>
	N	156	135
	<b>Adrenolutin (ng/mL)</b>	R	0.00544
	<i>p value</i>	<i>0.9464</i>	<i>0.2308</i>
	N	155	134
	<b>Creatinine (units)</b>	R	0.16567
	<i>p value</i>	<i>0.0388</i>	<i>0.7724</i>
	N	156	135
	<b>LV Ejection Fraction</b>	R	-0.08006
	<i>p value</i>	<i>0.3205</i>	<i>0.6742</i>
	N	156	135
	<b>Age (years)</b>	R	-0.11768
	<i>p value</i>	<i>0.1447</i>	<i>0.0274</i>
	N	155	134
	<b>Body Mass Index (units)</b>	R	0.04561
	<i>p value</i>	<i>0.5731</i>	<i>0.5347</i>
	N	155	134

[0108] R, Spearman correlation coefficient; N, sample number. Baseline, values at trial enrollment; \* Change, values at week 2 minus values at trial enrollment.

**Table 3. Univariate Predictors of Mortality and Transplantation (Endpoint)**

Variable	Odds Ratio	95 % confidence interval	<i>p-value</i>
Baseline ST2, per 0.1 ng/mL	1.114	0.961-1.300	<i>0.1509</i>
Baseline BNP, per 10 pmol/L	1.106	1.060-1.161	<i>&lt;0.0001</i>
Baseline ProANP, per 10 pg/L	1.007	1.005-1.010	<i>&lt;0.0001</i>
Change in ST2*, per change of 0.1 ng/mL	1.320	1.042-1.827	<i>0.0482</i>
Change in BNP*, per change of 10 pmol/L	1.033	0.966-1.110	<i>0.3401</i>
Change in ProANP*, per change of 10 pg/L	1.003	0.997-1.009	<i>0.3413</i>



(continued)

**Table 3. Univariate Predictors of Mortality and Transplantation (Endpoint)**

Variable	Odds Ratio	95 % confidence interval	<i>p</i> -value
Norepinephrine, per 1 pg/mL	1.001	1.000-1.002	0.0562
Dopamine, per 10 pg/mL	1.029	1.006-1.059	0.0433
Epinephrine, per 1 pg/mL	0.999	0.995-1.001	0.6645
Angiotensin II, per 1 pg/mL	0.997	0.977-1.017	0.7921
Adrenolutin, per 10 ng/mL	0.985	0.943-1.017	0.4167
Creatinine, per 1 mmol/L	2.487	0.997-6.417	0.0526
LV Ejection Fraction	0.952	0.897-1.007	0.0906
Race	1.947	0.946-4.192	0.0776
Gender	1.225	0.576-2.728	0.6061
Age	1.435	1.099-1.914	0.0104
Etiology	1.543	0.744-3.336	0.2543
Body Mass Index, per 1 kg/mm <sup>2</sup>	0.972	0.919-1.021	0.2876

[0109] Baseline, values at trial enrollment.; \* Change, values at week 2 minus values at trial enrollment.

**Table 4. Multivariate Predictors of Mortality and Transplantation (Endpoint): Predictive Value of ST2**

Variables	<i>p</i>
<b>Baseline ST2 and Baseline BNP</b>	
Baseline BNP	0.0003
Baseline Dopamine	0.0906
Baseline ST2	0.6368
<b>Baseline ST2 and Baseline ProANP</b>	
Baseline ProANP	<0.0001
Baseline Dopamine	0.0944
Baseline ST2	0.3306
<b>Change in ST2* and Baseline BNP</b>	
Baseline BNP	0.0001
Change in ST2	0.0392
<b>Change in ST2* and Baseline ProANP</b>	
Baseline ProANP	<0.0001
Change in ST2	0.0274

[0110] Baseline, values at trial enrollment.; \* Change, values at week 2 minus values at trial enrollment.

### EXAMPLE 3

#### Methods

[0111] **Study populations.** The Thrombolysis in Myocardial Infarction (TIMI) 14 trial was a randomized, open-label, dose-ranging study of combination reperfusion therapy for patients with ST-segment elevation MI conducted between March 1997 and July 1998. Specifically, this study was an angiographic trial comparing 4 different thrombolytic combinations: abciximab alone, alteplase alone, abciximab with reduced dose of alteplase, and abciximab with reduced dose of streptokinase (Antman EM et al., Circulation, 1999; 99:2720-32; Antman EM et al., Eur Heart J, 2000; 21:1944-53). The ENTIRE-TIMI 23 trial was an open-label, dose-ranging, multicenter study conducted between February 2000 and September 2001 to evaluate enoxaparin as adjunctive antithrombin therapy with various forms of pharmacological reperfusion, including full-dose tenecteplase and half-dose tenecteplase plus abciximab (Antman EM et al., Circulation. 2002;105:1642-9). In both studies, patients were eligible for inclusion if they had a qualifying episode of ischemic discomfort of at least 30 min within 6 hr (ENTIRE) or 12 hr (TIMI 14), and exhibited at least 0.1 mV ST-segment elevation in 2 contiguous precordial electrocardiographic leads. Exclusion criteria for both trials included increased risk of hem-

orrhage, severe renal insufficiency, and cardiogenic shock.

**[0112] Laboratory analyses.** Serum samples collected at baseline, 1, 3, 12, and 24 hr after enrollment in TIMI 14 were evaluated. Serum samples from the ENTIRE trial were available only at baseline. Serum was isolated within 60 min of sample collection and stored at -20 °C or colder until shipped to the TIMI Biomarker Core Lab (Boston, MA), where samples were maintained at -70°C. Soluble ST2 was measured with a sandwich double monoclonal antibody ELISA method (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Serum samples or standards were incubated in microwells coated with anti-human ST2 antibody. After washing, peroxidase-conjugated anti-human ST2 antibody was added into the microwell and incubated. After washing again, the peroxidase substrate was added and the optical density at 450 nm was determined. High sensitivity C-reactive protein (hs-CRP, Dade-Behring Inc, Deerfield, IL), creatine kinase MB isoenzyme (CK-MB), B-type natriuretic peptide (SHIONORIA BNP, Shionogi, Osaka, Japan). and cardiac troponin I (ACS:180, Bayer Diagnostics, Tarrytown, NY) were measured using previously described methods (Morrow DA et al., J Am Coll Cardiol. 1998;31:1460-5; Morrow DA et al., Clin Chem. 2000;46:453-460). Creatine kinase isoenzyme levels were measured locally at the site on admission, at 3 hours, and at 6 to 8 hour intervals for the first 24 hours. Due to sample availability, BNP levels were measured in samples from ENTIRE-TIMI 23, but not TIMI 14.

**[0113] Statistical analysis.** Patients were divided into quartiles on the basis of their ST2 serum levels at the time of enrollment into the studies. ST2 levels are described using the median and 25<sup>th</sup>-75<sup>th</sup> percentiles. The association between baseline clinical characteristics and quartiles of ST2 were analyzed using the Kruskal-Wallis test for continuous variables and the  $\chi^2$  test for categorical variables. Correlations between ST2 and other continuous baseline variables were studied with a non-parametric (Spearman's) correlation coefficient. For evaluation of association with clinical outcomes, ST2 was compared between patients who met a study end point and those who did not using the Wilcoxon rank-sum test. Multivariable analysis of the association of ST2 with outcomes was performed using logistic regression including terms for established predictors of mortality in ST-elevation myocardial infarction (STEMI) (Morrow, DA et al., Circulation 2000; Oct 24; 102(17):2031-7). Except where stated, results presented are for the combined TIMI 14 and ENTIRE-TIMI 23 study population.

## Results

**[0114] Baseline ST2 and Clinical Variables.** Most baseline clinical characteristics, including gender, age, weight, and extent of coronary artery disease did not correlate with baseline ST2 levels (Table 5). Few patients in this population had either a prior history or presented with clinical evidence of heart failure. Interestingly, heart rate correlated positively with ST2 levels ( $p < 0.0001$ ) and systolic blood pressure showed a modest correlation with ST2 levels ( $p = 0.05$ ), consistent with the theory that ST2 is secreted by cardiac myocytes under biomechanical stress. The biomarkers cardiac troponin I, BNP, and CRP—which have all been shown to predict outcome after myocardial infarction (de Lemos JA et al., N Engl J Med 2001; 345:1014-21; Antman EM et al, N Engl J Med 1996; 335:1342-9; Morrow DA et al., J Am Coll Cardiol 1998; 31:1460-5) were correlated with ST2 by quartile analysis and cardiac troponin I and CRP were statistically significant. When these biomarkers were evaluated as continuous variables, quantitatively weak correlations were observed (Table 6).

**Table 5. Baseline Clinical Characteristics According to Quartiles of ST2 (ng/mL)**

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p trend	p Q4 vs Q1
Range, ng/mL	0.085 - 0.179	0.180 - 0.235	0.236 - 0.346	0.347 - 6.88		
n	204	202	202	202		
Time CP to randomization (hrs)	2.8 ± 1.6	3.1 ± 1.5	3.2 ± 1.4	4.0 ± 1.9	<0.0001	<0.0001
Age (years)	58 ± 10	58 ± 10	58 ± 11	58 ± 10	0.9	1.0
Male	74%	77%	85%	81%	0.03	0.09
White	88%	89%	90%	88%	0.9	1.0
Past Medical History						
Hypertension	25%	24%	36%	33%	0.02	0.09
Congestive Heart Failure	0%	0%	1.5%	1.0%	0.1	0.2
Angina	26%	24%	26%	32%	0.3	0.2
Diabetes	14%	14%	15%	16%	0.9	0.5

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(continued)

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p trend	p Q4 vs Q1
Family history of CAD	73%	73%	73%	73%	0.2	0.08
Hypercholesterolemia	22%	21%	21%	29%	0.2	0.1
Smoking status:						
Current smoker	57%	48%	49%	48%	0.2	0.06
Physical findings						
Weight kg	83 ± 16	81 ± 15	82 ± 14	83 ± 15	0.4	0.8
Systolic BP (mm Hg)	139 ± 21	138 ± 22	141 ± 23	143 ± 22	0.1	0.05
HR (BPM)	7 ± 17	75 ± 17	72 ± 16	80 ± 17	0.001	<0.0001
Killip Class II-IV	2.0 %	1.5%	3.6%	4.5%	0.3	0.2
Diagnostic Testing						
cTnI > 0.1 ng/ml*	61%	69%	77%	84%	0.001	<0.0001
BNP > 80 pg/ml*	1.8%	5.4%	7.2%	14.4%	0.003	0.001
CRP > 1.5 ng/ml	2.1%	8.8%	8.1%	11.4%	0.006	<0.0001
Creatinine mg/dL	1.0 ± 0.21	1.0 ± 0.20	1.0 ± 0.25	1.1 ± 0.28	0.1	0.03
Extent CAD (50% stenosis)					0.3	0.2
1 vessel	48%	55%	45%	50%		
2 vessel	38%	28%	34%	30%		
3 vessel	15%	18%	20%	20%		
EF (%)**	58 ± 15	58 ± 15	57 ± 15	57 ± 15	1.0	0.9
CP = Chest Pain; HR = Heart Rate; cTnI = Cardiac Troponin I; BNP = B type Natriuretic Peptide; CRP = C Reactive Protein; CAD = Coronary Artery Disease; EF = Ejection Fraction *Measured in the ENTIRE-TIMI 23 population only; N=448 except **(N = 469)						

**Table 6. Correlation between ST2 and Continuous Variables**

Variable	Spearman's rho	P value
Time CP to randomization	0.29	<0.0001
Age	-0.003	0.9
Weight (kg)	0.01	0.8
CKMB peak	0.08	0.02
cTnI*	0.26	<0.0001
CRP	0.10	0.007
BNP*	0.068	0.15
Creatinine	0.09	0.01
LVEF**	-0.005	0.9
CP = Chest Pain; CKMB = MB isoenzyme of creatine kinase; cTnI = Cardiac Troponin I; BNP = B type Natriuretic Peptide; CRP = C Reactive Protein; CAD = Coronary Artery Disease; EF = Ejection Fraction. *Measured in the ENTIRE-TIMI 23 population only; N=448 except **(N=469)		

**[0115] ST2 and Clinical Outcomes.** For the combined cohort of 810 patients, baseline ST2 was significantly asso-

ciated with clinical outcomes at 30 days (Table 7). Specifically, levels of ST2 were significantly higher at presentation among patients who subsequently died ( $p=0.0001$ ), or developed new or worsening CHF ( $p=0.009$ ), by 30 days after enrollment. Dichotomized at the median, elevated baseline levels of ST2 were indicative of higher mortality through 30 days of follow-up (log-rank,  $p = 0.0009$ , Figure 13). Moreover, in an analysis by quartiles of ST2, the risk of both death ( $p=0.001$ ) and the composite of death or CHF ( $p=0.001$ ) increased in a graded, stepwise fashion with higher levels of ST2. This association between ST2 and clinical events was homogeneous between the two individual trials (TIMI 14 and ENTIRE-TIMI 23).

**Table 7. Association between Baseline ST-2 Concentration (ng/ml) and Outcomes**

Outcome (30 days)	n	Median [25,75]	p value
Dead	28	0.379 [0.267,0.611]	0.0001
Alive	782	0.233 [0.178, 0.340]	
MI	29	0.213 [0.171,0.259]	0.11
No MI	781	0.237 [0.181,0.348]	
CHF	21	0.287 [0.237, 0.470]	0.009
No CHF	789	0.233 [0.178, 0.345]	
Death/CHF	47	0.317 [0.246,0.590]	<0.0001
No Death/CHF	763	0.231 [0.177,0.339]	
MI= Myocardial Infarction; CHF = Congestive Heart Failure			

**[0116] Evolution of ST2 serum levels.** Baseline ST2 levels analyzed by quartile were significantly correlated with the time to randomization (Tables 5 and 6). ST2 levels were anticipated to increase in the first day following coronary occlusion and return to normal over the next 14 days (6). Among the TIMI 14 patients, analysis of serial measurements of serum ST2 in 228 patients revealed an increase with time, with most patients reaching a peak ST2 level at 12 hours, although, a few patients had ST2 serum levels that continued to increase past this time point.

**[0117] Multivariate analysis.** After controlling for established clinical predictors in STEMI including age, heart rate, systolic blood pressure, location of myocardial infarction, Killip class, and time from onset of chest pain, increasing levels of ST2 remained an independent predictor of death at 30 days (OR 1.77; 95% CI 1.01 - 3.12,  $p=0.047$ ). This association was no longer significant when BNP was added to the clinical model (assessment was limited to ENTIRE). The predictive capacity of ST2 ascertained at later time points (3 and 12 hours in TIMI 14) was also evaluated; revealing a stronger association between ST2 and mortality risk.

**[0118]** Serum soluble T1/ST2, therefore is a novel biomarker for severe heart failure that parallels neurohormonal activation. In patients with severe chronic NYHA Class III-IV heart failure, the change in T1/ST2 levels is an independent predictor of the endpoint of mortality or transplantation.

**[0119]** In this study, we explored the potential role of serum measurement of a recently-identified receptor of the interleukin-1 family in acute myocardial infarction. The soluble form of this receptor is rapidly secreted by cardiac myocytes when the cells are biomechanically overloaded; this suggests that the receptor may play a role in conditions where the myocardium is rapidly overloaded, such as in myocardial infarction. To explore this, we measured serum ST2 levels at the time of presentation in a cohort of patients with acute myocardial infarction. The results demonstrate that ST2 levels at the time of presentation in these patients are associated with in-hospital and 30-day mortality. Furthermore, multivariate analysis indicated that ST2 level is independently associated with outcome after controlling for important clinical factors.

**[0120]** Thus, the significance of these data is twofold. Foremost, these data suggest that the interleukin receptor family, which participates in host defense and differentiation of T cells (Sims JE. IL-1 and IL-18 receptors, and their extended family. *Curr Opin Immunol.* 2002;14:117-22), may participate in early events in acute myocardial infarction. These data implicate this receptor as a potential novel target for modifying prognosis in patients with myocardial infarction. Secondly, ST2 represents a novel biomarker that offers prognostic information in patients with acute myocardial infarction; thus, extending upon our prior work demonstrating an association between ST2 and mortality among patients with non-ischemic congestive heart failure (Weinberg EO, Shimp M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation.* 2003;107:721-6), another condition of myocardial overload.

**[0121]** Although not excluded, it is unlikely that the relationship of ST2 and outcome after myocardial infarction is simply a reflection of the association of chronic elevations in inflammatory markers like CRP and risk of myocardial

infarction. ST2, like BNP, may be synthesized by cardiac myocytes themselves and data from patients without apparent ischemic disease suggests that ST2 predicts prognosis in the absence of coronary artery disease. Furthermore, preliminary data suggest that ST2 levels in outpatients with stable coronary artery disease are unrelated to CRP levels. While our data support the complementary value of ST2 for risk assessment when added to a robust clinical model (REF TIMI Risk Score), ST2 did not contribute additional information to BNP in the smaller data set limited to ENTIRE-TIMI 23. There may also be prognostic value of ST2 in conjunction with other available biomarkers.

**[0122]** Although ST2 may be secreted by mechanically-overloaded cardiac myocytes, many cells can secrete ST2. It is therefore possible that elevations in serum ST2 are not completely specific for acute myocardial infarction. In addition to non-ischemic heart failure (Weinberg EO, Shimp M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation*. 2003;107:721-6), patients with asthma (Oshikawa K, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, Ohno S, Tominaga SI, Sugiyama Y. Elevated soluble ST2-protein levels in sera of patients with asthma with an acute exacerbation. *Am J Respir Crit Care Med*. 2001;164:277-81) or autoimmune diseases like systemic lupus erythematosus (Kuroiwa K, Arai T, Okazaki H, Minota S, Tominaga S. Identification of human ST2 protein in the sera of patients with autoimmune diseases. *Biochem Biophys Res Commun*. 2001;284:1104-8) may also have increased serum ST2 levels. Therefore, the usefulness of ST2 measurement in the initial diagnosis of acute myocardial infarction in such subjects is not unequivocal.

**[0123]** However, ST2 remains a possible target for therapy in patients with MI. These data demonstrate how genomic technology can reveal a new potential pathophysiological pathway in a common disease. ST2 was initially identified through studies of the interleukin-1 family, but its role in myocardial disease was only recently suggested by genomic studies with DNA microarrays. Studies with DNA microarrays allow identification of potential new disease pathways, but this is only an initial step in understanding the role of the pathway. The above data supports the role for ST2 in acute myocardial infarction, since the levels of ST2 predict outcome. Studies of the function of ST2 in myocardial infarction are possible. In addition, identifying the ligand for the soluble and membrane ST2 receptors could help further the understanding of the potentially competing roles of the membrane and soluble receptors.

**[0124]** The results described establish that the T1/ST2 is secreted during a heart attack and/or heart failure, and can be easily measured, thereby supporting the asserted utilities of the invention.

#### SEQUENCE LISTING

##### **[0125]**

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<223> Fit-1M

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## Claims

1. An *in vitro* method of predicting the risk of mortality and/or new or worsening congestive heart failure (CHF) for a subject with a cardiovascular condition selected from the group consisting of CHF and myocardial infarction (MI), the method comprising:

obtaining a level of soluble IL1RL-1 protein in a biological sample comprising serum from the subject; and comparing said level of soluble IL1RL-1 protein to a predetermined value specific for the cardiovascular condition;

wherein a raised level of soluble IL1RL-1 protein in comparison to the predetermined value is indicative of an increased risk of mortality within 30 days and/or new or worsening CHF within 30 days, and the soluble IL1RL-1 protein is an expression product of a nucleic acid sequence as set out in SEQ ID NO. 1.

2. The method of claim 1, wherein the level of soluble IL1RL-1 protein is determined within 12 hours of an episode of ischemic discomfort or 1, 3, 12 or 24 hours thereafter.

3. The method of claim 2, wherein the level of soluble IL1RL-1 protein is determined within 6 hours of an episode of ischemic discomfort or 1, 3, 12, or 24 hours thereafter.

4. The method of claim 1 comprising the steps of:

(a) contacting a biological sample comprising serum from a subject with an antibody or antigen-binding fragment thereof that specifically binds to soluble IL1RL-1 protein; and  
 (b) measuring the amount of bound antibody and determining therefrom if the level of said soluble IL1RL-1 protein is raised in comparison to the predetermined level.

5. The method of claim 4, wherein the antibody or antigen-binding fragment thereof is labelled with a detectable label.

6. The method of claim 1 or 4, further comprising measuring at least one other biomarker.

7. The method of any of claims 1 to 3 and 6, wherein the predetermined value specific for the cardiovascular condition is a plurality of predetermined value ranges and said comparing step comprises determining in which of said predetermined value ranges said subject's level falls.

8. The method of claim 1, wherein the subject has received or is receiving treatment for a cardiovascular condition.

9. An *in vitro* method for predicting the risk of mortality and/or new or worsening congestive heart failure (CHF) for a subject with a cardiovascular condition selected from the group consisting of CHF and myocardial infarction (MI), the method comprising:

obtaining a first level of soluble IL1RL-1 protein in a sample comprising serum from a subject;  
obtaining a second level of soluble IL1RL-1 protein in a subsequent sample comprising serum from the same subject; and  
comparing the change in the level of the IL1RL-1 protein to a predetermined value specific for the cardiovascular condition, wherein a change in the level of the IL1RL-1 protein exceeding the predetermined value is indicative of an increased risk of mortality within 30 days and/or new or worsening CHF within 30 days.

10. The method of claim 9, wherein the first level is obtained within 24 hours of an MI.

11. The method of claim 8 or 9, wherein the second level is obtained about 2 weeks after the first.

12. The method of claim 9, wherein a change in the level of soluble IL1RL-1 protein exceeding the predetermined value is indicative of an increased risk of a requirement for cardiac transplantation.

13. The method of claim 9, further comprising measuring at least one other biomarker in the subject.

14. The method of claims 6 or 13, wherein the other biomarker is a circulating catecholamine, angiotensin II, creatinine, creatinine kinase MB isoenzyme (CK-MB), C-reactive protein (CRP), troponin, a natriuretic peptide or an index of oxidative stress.

15. The method of claim 1, 4, or 9, further comprising measuring troponin and brain natriuretic peptide (BNP) in the subject.

16. The method of claim 14 or 15, wherein the troponin is cardiac troponin I.

17. The method of claim 14, wherein the circulating catecholamine is norepinephrine, dopamine or epinephrine.

18. The method of claim 14, wherein the natriuretic peptide is brain natriuretic peptide (BNP) or pro-atrial natriuretic peptide (ProANP).

19. The method of claim 18, wherein the natriuretic peptide is brain natriuretic peptide (BNP).

20. The method of claim 14, wherein the index of oxidative stress is adrenolutin or malondialdehyde.

21. The method of claim 1, 4, or 19, wherein the biological sample is a blood or serum sample.

22. The method of claim 9, wherein the subject has received or is receiving treatment for a cardiovascular condition.

23. The method of any of claims 1 to 22, wherein the soluble IL1RL-1 protein has a sequence of SEQ ID NO.2.

## Patentansprüche

1. *In-vitro*-Verfahren zur Vorhersage des Risikos der Mortalität und/oder eines neuen oder verschlimmernden kongestiven Herzversagens (CHF) für ein Subjekt mit einem kardiovaskulären Leiden, das aus der Gruppe ausgewählt ist, die aus CHF und Myokardinfarkt (MI) besteht, wobei das Verfahren Folgendes umfasst:

Erhalten eines Gehalts an löslichem IL1RL-1 Protein in einer biologischen Probe, die Serum des Subjekts

umfasst; und

Vergleichen von genanntem Gehalt an löslichem IL1RL-1 Protein mit einem Vorbestimmten Wert, der für das kardiovaskuläre Leiden spezifisch ist;

- 5 worin ein erhöhter Gehalt an löslichem IL1RL-1 Protein im Vergleich zum Vorbestimmten Wert ein erhöhtes Risiko der Mortalität innerhalb von 30 Tagen und/oder eines neuen oder verschlimmernden CHF innerhalb von 30 Tagen anzeigt und das lösliche IL1RL-1 Protein ein Expressionsprodukt einer Nukleinsäuresequenz, wie in SEQ.-ID-NR. 1 dargelegt, ist.
- 10 2. Verfahren nach Anspruch 1, worin der Gehalt an löslichem IL1RL-1 Protein innerhalb von 12 Stunden eines Vorfalls von ischämischen Beschwerden oder 1, 3, 12 oder 24 Stunden danach bestimmt wird.
3. Verfahren nach Anspruch 2, worin der Gehalt an löslichem IL1RL-1 Protein innerhalb von 6 Stunden eines Vorfalls von ischämischen Beschwerden oder 1, 3, 12 oder 24 Stunden danach bestimmt wird.
- 15 4. Verfahren nach Anspruch 1, das folgende Schritte umfasst:
  - (a) Inkontaktbringen einer biologischen Probe, die Serum eines Subjekts umfasst, mit einem Antikörper oder Antigenbindenden Fragment davon, der/das spezifisch zum löslichen IL1RL-1 Protein bindet; und
  - 20 (b) Messen der Menge von gebundenem Antikörper und Bestimmen davon, ob der Gehalt an genanntem löslichem IL1RL-1 Protein im Vergleich zum Vorbestimmten Gehalt erhöht ist.
5. Verfahren nach Anspruch 4, worin der Antikörper oder das Antigen-bindende Fragment davon mit einer detektierbaren Markierung markiert ist.
- 25 6. Verfahren nach Anspruch 1 oder 4, das weiter das Messen von mindestens einem anderen Biomarker umfasst.
7. Verfahren nach einem der Ansprüche 1 bis 3 und 6, worin der Vorbestimmte Wert, der für das kardiovaskuläre Leiden spezifisch ist, eine Vielzahl von Vorbestimmten Wertebereichen ist und genannter Vergleichsschritt das Bestimmen davon umfasst, in welchem der genannten Vorbestimmten Wertebereiche der genannte Gehalt des Subjekts fällt.
- 30 8. Verfahren nach Anspruch 1, worin das Subjekt eine Behandlung für ein kardiovaskuläres Leiden erhalten hat oder erhält.
- 35 9. *In-vitro*-Verfahren zur Vorhersage des Risikos der Mortalität und/oder eines neuen oder verschlimmernden kongestiven Herzversagens (CHF) für ein Subjekt mit einem kardiovaskulären Leiden, das aus der Gruppe ausgewählt ist, die aus CHF und Myokardinfarkt (MI) besteht, wobei das Verfahren Folgendes umfasst:
  - 40 Erhalten eines ersten Gehalts an löslichem IL1RL-1 Protein in einer Probe, die Serum eines Subjekts umfasst; Erhalten eines zweiten Gehalts an löslichem IL1RL-1 Protein in einer nachfolgenden Probe, die Serum desselben Subjekts umfasst; und
  - 45 Vergleichen der Änderung des Gehalts des IL1RL-1 Proteins mit einem Vorbestimmten Wert, der für das kardiovaskuläre Leiden spezifisch ist, worin eine Änderung des Gehalts des IL1RL-1 Proteins, die größer als der Vorbestimmte Wert ist, ein erhöhtes Risiko der Mortalität innerhalb von 30 Tagen und/oder eines neuen oder verschlimmernden CHF innerhalb von 30 Tagen anzeigt.
10. Verfahren nach Anspruch 9, worin der erste Gehalt innerhalb von 24 Stunden eines MI erhalten wird.
- 50 11. Verfahren nach Anspruch 8 oder 9, worin der zweite Gehalt etwa 2 Wochen nach dem ersten erhalten wird.
12. Verfahren nach Anspruch 9, worin eine Änderung des Gehalts an löslichem IL1RL-1 Protein, die größer als der Vorbestimmte Wert ist, ein erhöhtes Risiko des Erfordernisses für eine Herztransplantation anzeigt.
- 55 13. Verfahren nach Anspruch 9, das weiter das Messen von mindestens einem anderen Biomarker im Subjekt umfasst.
14. Verfahren nach den Ansprüchen 6 oder 13, worin der andere Biomarker ein zirkulierendes Catecholamin, Angiotensin II, Creatinin, Creatinin-Kinase-MB-Isoenzym (CK-MB), C-reaktives Protein (CRP), Troponin, ein natriuretisches



Peptid oder ein Indikator für oxidativen Stress ist.

15. Verfahren nach Anspruch 1, 4 oder 9, das weiter das Messen von Troponin und B-Typ natriuretischem Peptid (BNP) bei dem Subjekt umfasst.

16. Verfahren nach Anspruch 14 oder 15, worin das Troponin kardiales Troponin I ist.

17. Verfahren nach Anspruch 14, worin das zirkulierende Catecholamin Norepinephrin, Dopamin oder Epinephrin ist.

18. Verfahren nach Anspruch 14, worin das natriuretische Peptid das B-Typ natriuretische Peptid (BNP) oder proatriale natriuretische Peptid (ProANP) ist.

19. Verfahren nach Anspruch 18, worin das natriuretische Peptid das B-Typ natriuretische Peptid (BNP) ist.

20. Verfahren nach Anspruch 14, worin der Indikator für oxidativen Stress Adrenolutin oder Malondialdehyd ist.

21. Verfahren nach Anspruch 1, 4 oder 19, worin die biologische Probe eine Blut- oder Serumprobe ist.

22. Verfahren nach Anspruch 9, worin das Subjekt eine Behandlung für ein kardiovaskuläres Leiden erhalten hat oder erhält.

23. Verfahren nach einem der Ansprüche 1 bis 22, worin das lösliche IL1RL-1 Protein eine Sequenz von SEQ.-ID-NR. 2 hat.

## Revendications

1. Procédé in vitro de prédiction du risque de mortalité et/ou d'une nouvelle insuffisance cardiaque congestive (CHF) ou d'une insuffisance cardiaque congestive qui se dégrade pour un sujet avec une affection cardiovasculaire sélectionnée parmi le groupe constitué de la CHF et d'un infarctus du myocarde (MI), le procédé comprenant :

l'obtention d'un niveau de protéine IL1RL-1 soluble dans un échantillon biologique comprenant du sérum provenant du sujet ;

la comparaison dudit niveau de protéine IL1RL-1 soluble à une valeur prédéterminée spécifique pour l'affection cardiovasculaire ;

dans lequel un niveau élevé de protéine IL1RL-1 soluble comparé à la valeur prédéterminée est indicateur d'un risque accru de mortalité dans les 30 jours et/ou d'une nouvelle CHF ou d'une CHF qui se dégrade dans les 30 jours, et la protéine IL1RL-1 soluble est un produit d'expression d'une séquence d'acide nucléique telle qu'établie dans SEQ ID N° 1.

2. Procédé selon la revendication 1, dans lequel le niveau de protéine IL1RL-1 soluble est déterminé dans les 12 heures suivant un épisode de gêne ischémique ou 1, 3, 12 ou 24 heures après celui-ci.

3. Procédé selon la revendication 2, dans lequel le niveau de protéine IL1RL-1 soluble est déterminé dans les 6 heures suivant un épisode de gêne ischémique ou 1, 3, 12 ou 24 heures après celui-ci.

4. Procédé selon la revendication 1, comprenant les étapes consistant à :

(a) mettre en contact un échantillon biologique comprenant du sérum provenant d'un sujet avec un anticorps ou fragment de liaison à l'antigène de celui-ci qui se fixe spécifiquement à la protéine IL1RL-1 soluble ; et

(b) mesurer la quantité d'anticorps fixé et déterminer à partir de celle-ci si le niveau de ladite protéine IL1RL-1 soluble est élevé comparé au niveau prédéterminé.

5. Procédé selon la revendication 4, dans lequel l'anticorps ou fragment de liaison à l'antigène de celui-ci est marqué avec un traceur détectable.

6. Procédé selon la revendication 1 ou 4, comprenant en outre la mesure d'au moins un autre biomarqueur.

7. Procédé selon l'une quelconque des revendications 1 à 3 et 6, dans lequel la valeur prédéterminée spécifique pour l'affection cardiovasculaire est une pluralité de plages de valeur prédéterminées et ladite étape de comparaison comprend le fait de déterminer dans laquelle desdites plages de valeur prédéterminées le niveau dudit sujet tombe.
- 5 8. Procédé selon la revendication 1, dans lequel le sujet a reçu ou reçoit un traitement pour une affection cardiovasculaire.
9. Procédé in vitro de prédiction du risque de mortalité et/ou d'une nouvelle insuffisance cardiaque congestive (CHF) ou d'une insuffisance cardiaque congestive qui se dégrade pour un sujet avec une affection cardiovasculaire sélectionnée parmi le groupe constitué de la CHF et d'un infarctus du myocarde (MI), le procédé comprenant :  
10 l'obtention d'un premier niveau de protéine IL1RL-1 soluble dans un échantillon comprenant du sérum provenant d'un sujet ;  
l'obtention d'un second niveau de protéine IL1RL-1 soluble dans un échantillon subséquent comprenant du  
15 sérum provenant du même sujet ; et  
la comparaison du changement de niveau de protéine IL1RL-1 à une valeur prédéterminée spécifique pour l'affection cardiovasculaire, dans lequel un changement de niveau de protéine IL1RL-1 dépassant la valeur prédéterminée est indicateur d'un risque accru de mortalité dans les 30 jours et/ou d'une nouvelle CHF ou d'une CHF qui se dégrade dans les 30 jours.  
20 10. Procédé selon la revendication 9, dans lequel le premier niveau est obtenu dans les 24 heures suivant un MI.  
11. Procédé selon la revendication 8 ou 9, dans lequel le second niveau est obtenu environ 2 semaines après le premier.  
12. Procédé selon la revendication 9, dans lequel un changement de niveau de protéine IL1RL-1 soluble dépassant la  
25 valeur prédéterminée est indicateur d'un risque accru d'un besoin de transplantation cardiaque.  
13. Procédé selon la revendication 9, comprenant en outre la mesure d'au moins un autre biomarqueur chez le sujet.  
14. Procédé selon la revendication 6 ou 13, dans lequel l'autre biomarqueur est une catécholamine en circulation, angiotensine II, créatinine, isoenzyme de créatinine kinase MB (CK-MB), protéine C réactive (CRP), troponine, un  
30 peptide natriurétique ou un indice de stress oxydatif.  
15. Procédé selon la revendication 1, 4 ou 9, comprenant en outre la mesure de la troponine et du peptide cérébral natriurétique (BNP) chez le sujet.  
35 16. Procédé selon la revendication 14 ou 15, dans lequel la troponine est la troponine cardiaque I.  
17. Procédé selon la revendication 14, dans lequel la catécholamine en circulation est la norépinéphrine, la dopamine ou l'épinéphrine.  
40 18. Procédé selon la revendication 14, dans lequel le peptide natriurétique est le peptide cérébral natriurétique (BNP) ou le peptide natriurétique pro-atrial (ProANP).  
19. Procédé selon la revendication 18, dans lequel le peptide natriurétique est le peptide cérébral natriurétique (BNP).  
20. Procédé selon la revendication 14, dans lequel l'indice de stress oxydatif est l'adrénolutine ou le malondialdéhyde.  
21. Procédé selon la revendication 1, 4 ou 19, dans lequel l'échantillon biologique est un échantillon sanguin ou sérique.  
50 22. Procédé selon la revendication 9, dans lequel le sujet a reçu ou reçoit un traitement pour une affection cardiovasculaire.  
23. Procédé selon l'une quelconque des revendications 1 à 22, dans lequel la protéine IL1RL-1 soluble possède une  
55 séquence de SEQ ID N° 2.

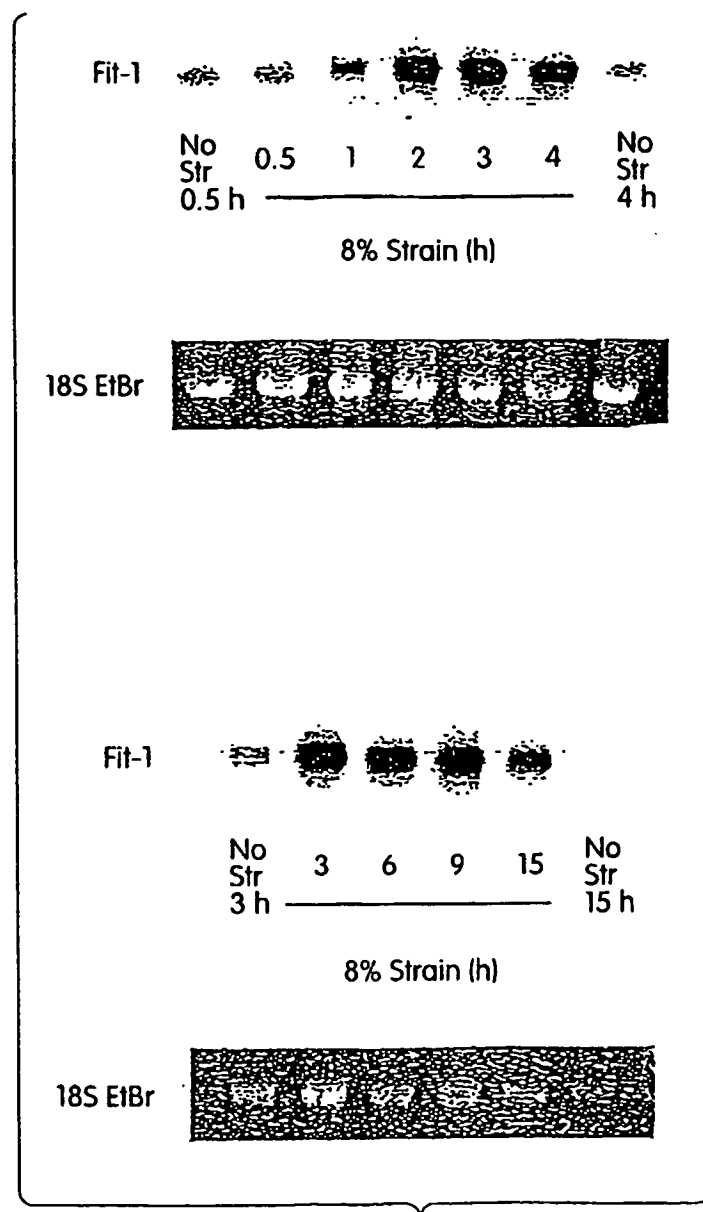


Fig. 1

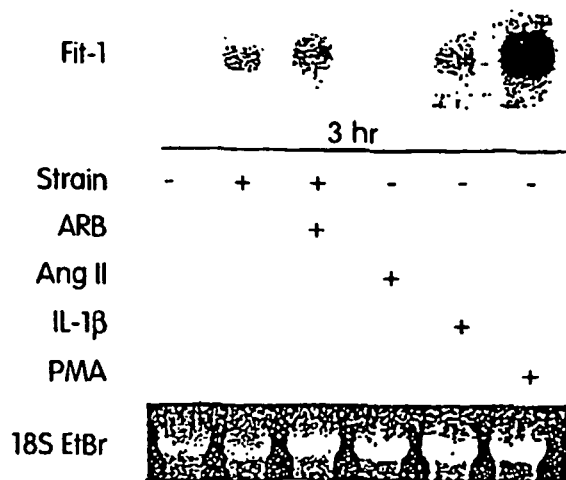


Fig. 2

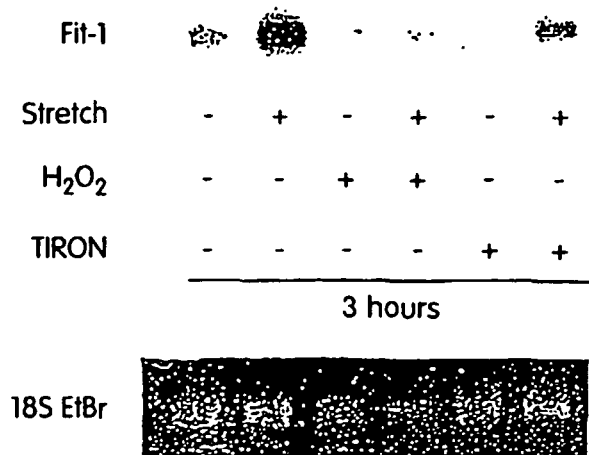


Fig. 3

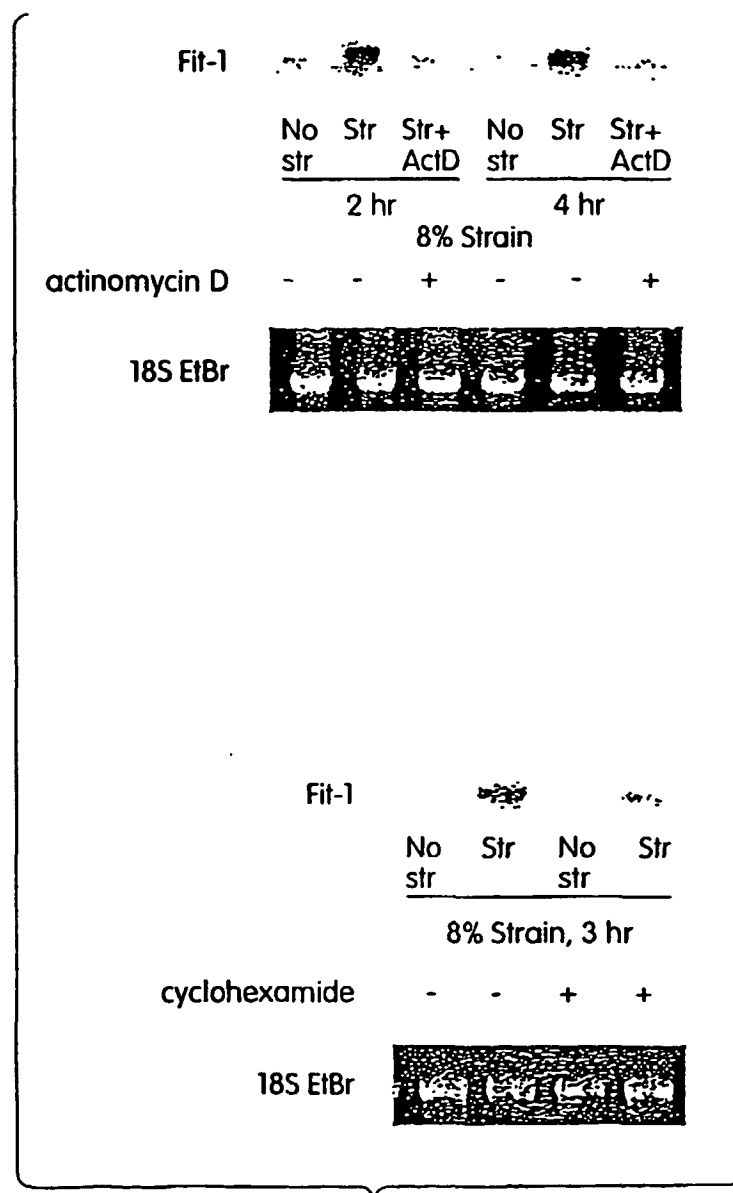


Fig. 4

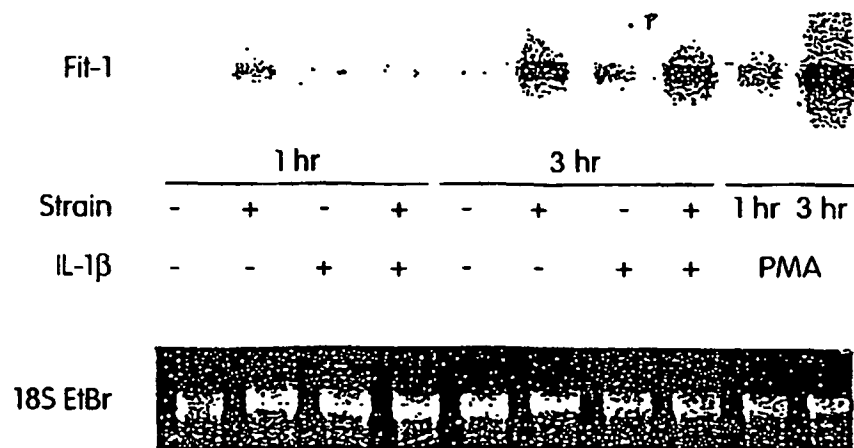


Fig. 5

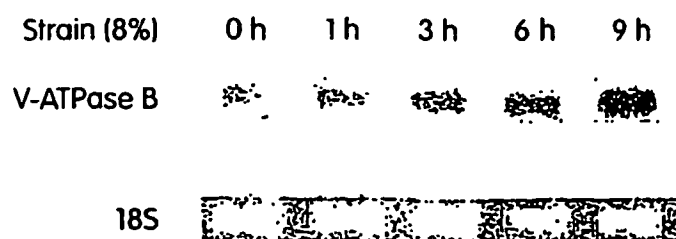


Fig. 6

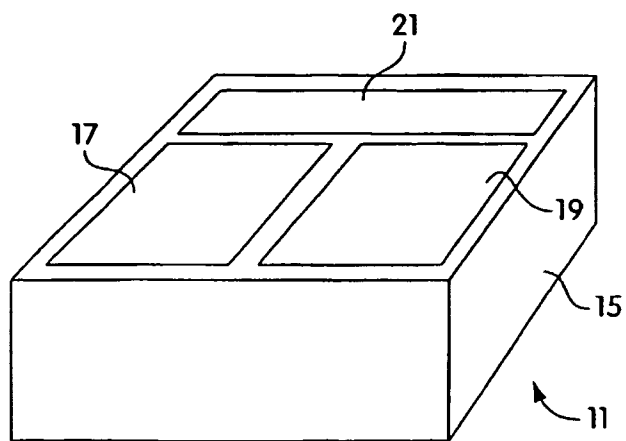


Fig. 7

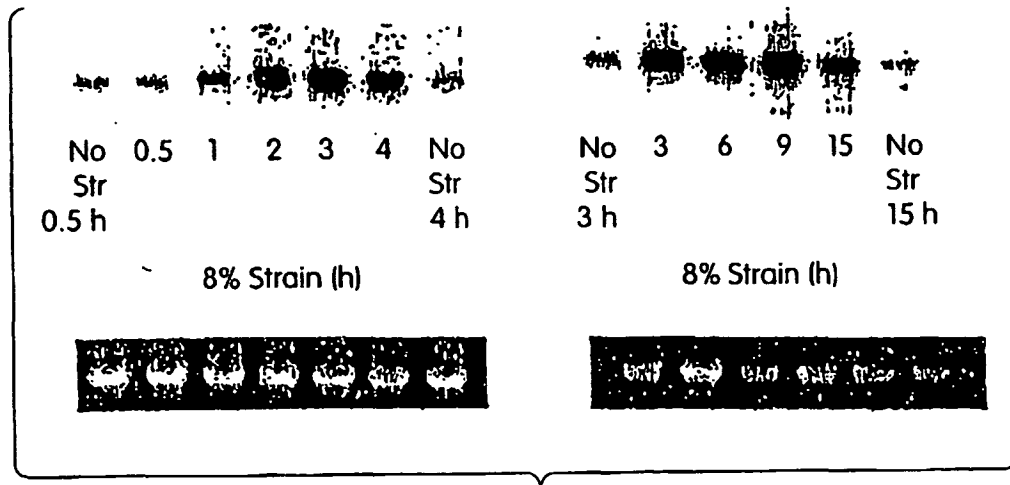


Fig. 8

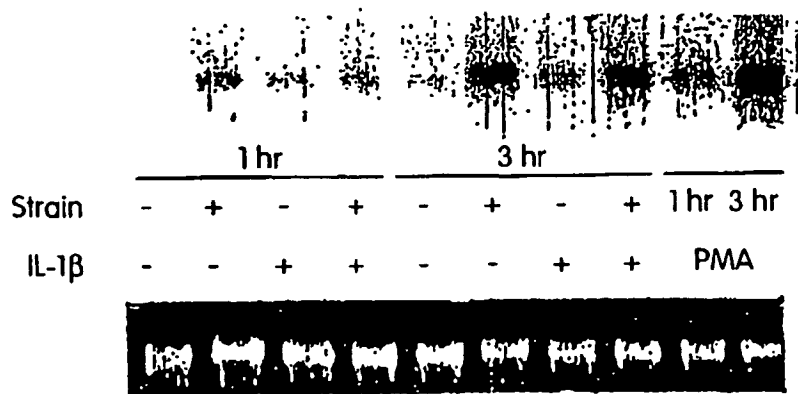


Fig. 9



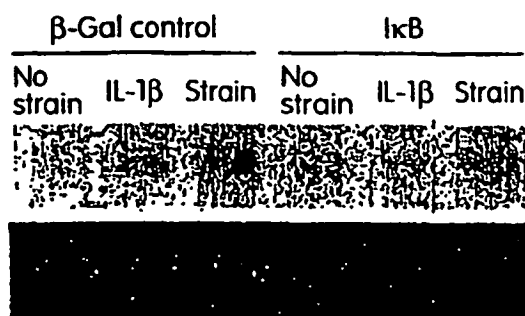


Fig. 10

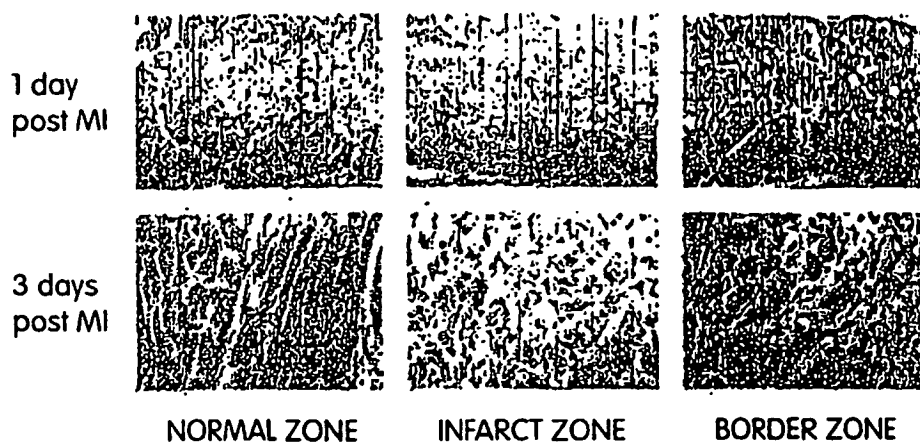


Fig. 11

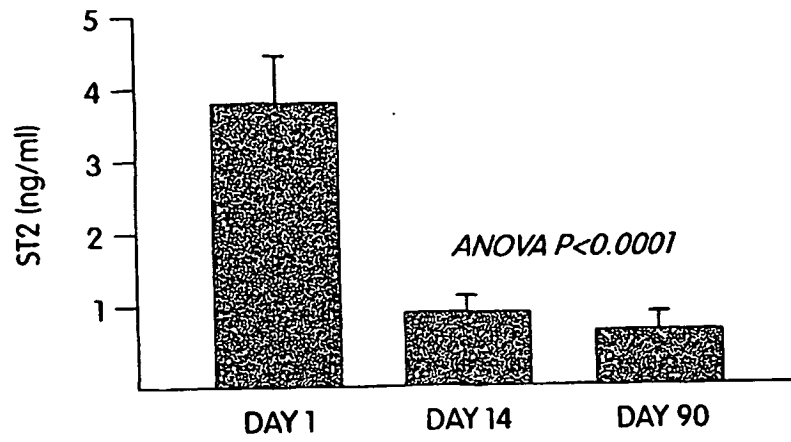


Fig. 12A

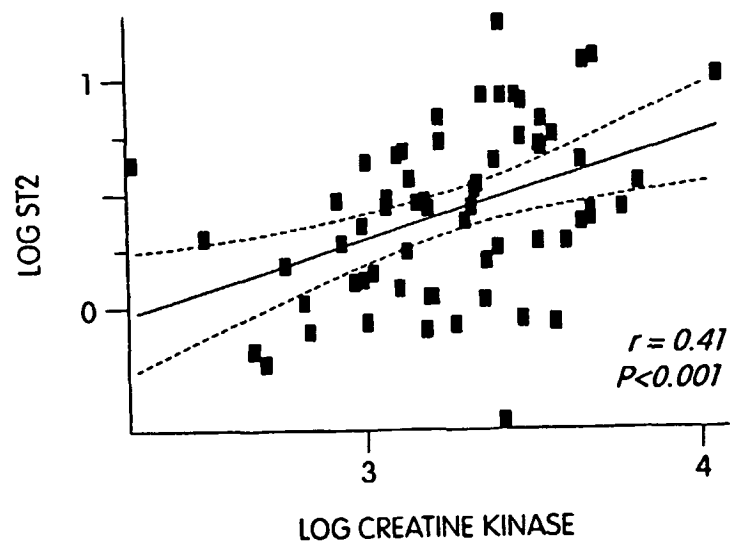


Fig. 12B

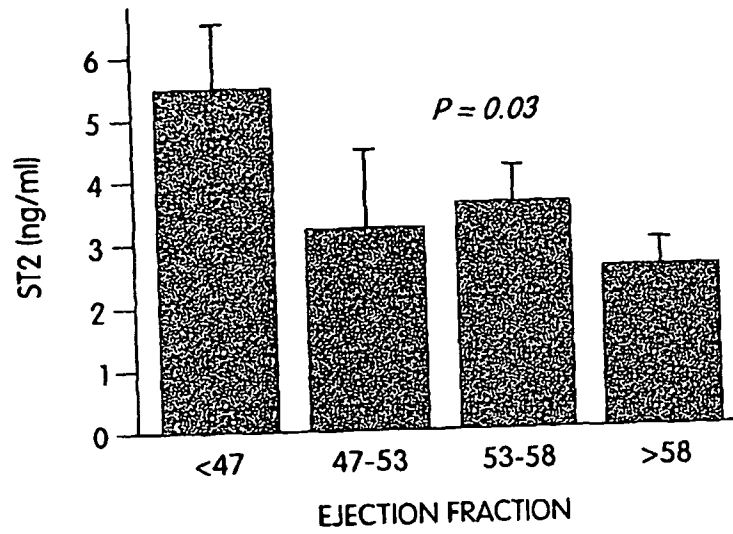


Fig. 12C

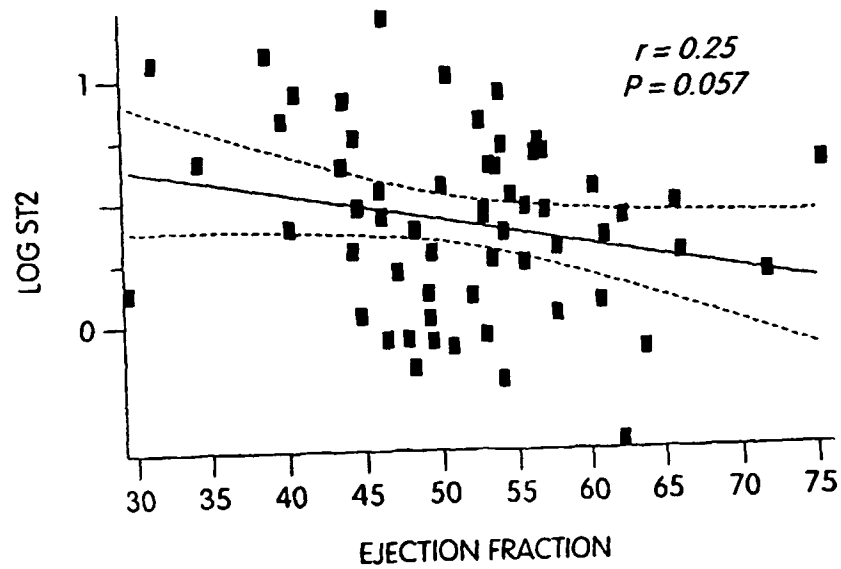


Fig. 12D

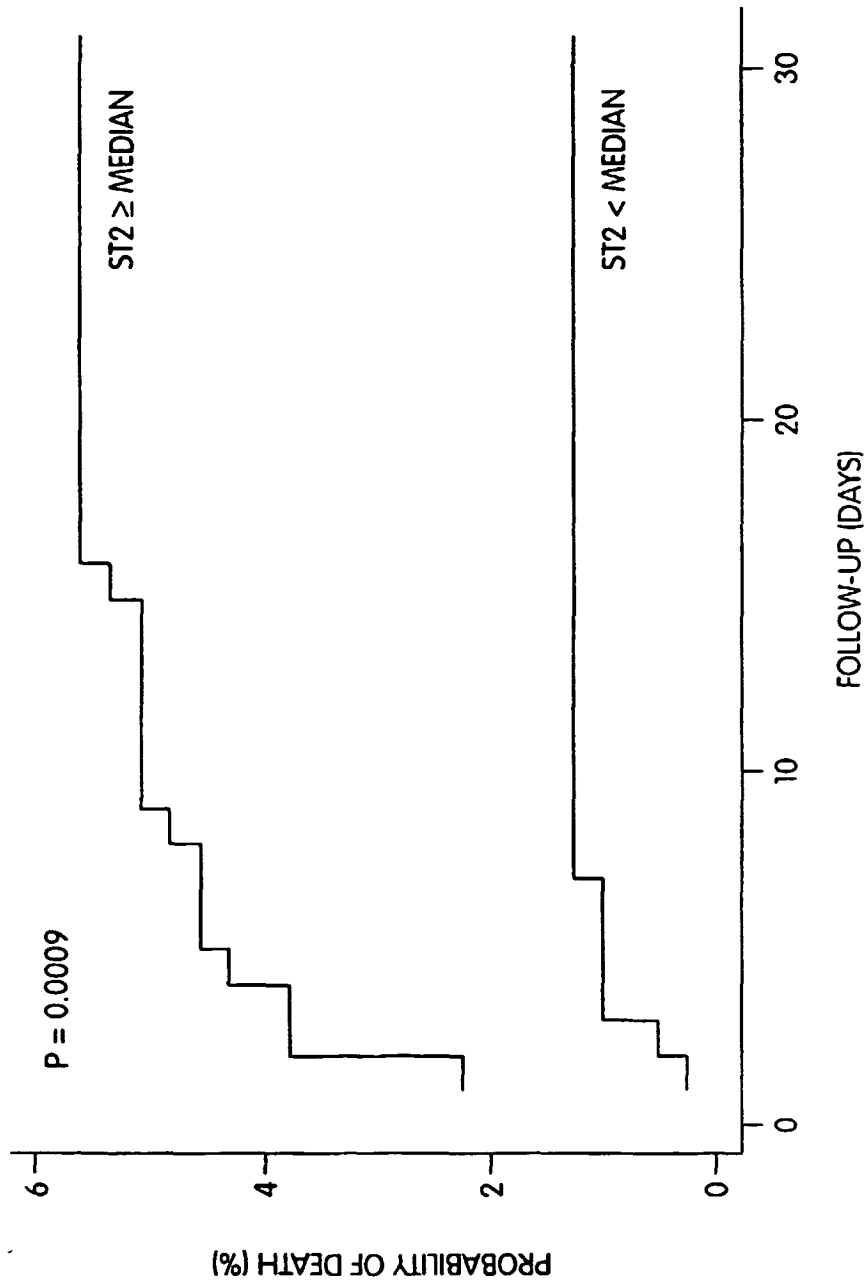


Fig. 13

## REFERENCES CITED IN THE DESCRIPTION

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## Patent documents cited in the description

- US 4816567 A [0054]
- US 5225539 A [0054]
- US 5585089 A [0054]
- US 5693762 A [0054]
- US 5859205 A [0054]
- WO 9204381 A [0054]
- US 60379173 B [0125]

## Non-patent literature cited in the description

- **ALTSCHUL SF et al.** *J Mol Biol*, 1990, vol. 215, 403-410 [0025]
- Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 [0026] [0047]
- Current Protocols in Molecular Biology. John Wiley & Sons, Inc, [0026] [0047]
- *J. Clin. Invest.*, 1992, vol. 90, 626-630 [0037]
- **DAHIYAT ; MAYO.** *Science*, 1997, vol. 278, 82-87 [0043]
- **KUNKEL.** *Proc. Nat. Acad. Sci. U.S.A.*, 1985, vol. 82, 488-492 [0048]
- **CLARK, W.R.** The Experimental Foundations of Modern Immunology. Wiley & Sons, Inc, 1986 [0052]
- **ROITT, I.** Essential Immunology. Blackwell Scientific Publications, 1991 [0052]
- Harrison's Principles of Experimental Medicine. McGraw-Hill, Inc, [0059]
- **SCHAFFER JL et al.** *J Orthop Res*, 1993, vol. 12, 709-719 [0068]
- **CHENG GC et al.** *Circ Res*, 1997, vol. 80, 28-36 [0068]
- **BROWN TD.** *J Biomechanics*, 2000, vol. 33, 3-14 [0068]
- **SPRINGHOM JP ; CLAYCOMB WC.** *Biochem J*, 1989, vol. 258, 73-78 [0073]
- **ARSTALL MA et al.** *J Mol Cell Cardiol*, 1998, vol. 30, 1019-25 [0073]
- **CHOMCZYNSKI et al.** *Anal. Biochem.*, 1987, vol. 162, 156-159 [0076]
- **MANN DL.** *Cytokine and Growth Factor Reviews*, 1996, vol. 7, 341-354 [0083]
- **ST. JOHN SUTTON MG et al.** *Circulation*, 2000, vol. 101, 2981-2988 [0083]
- **PULKKI KJ et al.** *Annals of Medicine*. 1997, vol. 29, 339-343 [0083]
- **KUBOTA T et al.** *Proc Natl Acad Sci.*, 1998, vol. 95, 6930-6935 [0083]
- **AUKRUST P et al.** *Am J Cardiol*, 1999, vol. 83, 376-382 [0083]
- **MACGOWAN GA et al.** *Am J Cardiol*, 1997, vol. 79, 1128-1132 [0083]
- **ROIG E et al.** *Am J Cardiol*, 1998, 688-690 [0083]
- **TSUTAMOTO T et al.** *JAm Coll Cardiol*, 1998, vol. 31, 391-398 [0083]
- **PRABHU SD et al.** *Circulation*, 2000, vol. 101, 2103-2109 [0083]
- **MURRAY DR et al.** *Annu Rev Immunol.*, 2000, vol. 18, 451-494 [0083]
- **TREHU EG.** *Clin Cancer Res.*, 1996, vol. 8, 1341-51 [0084]
- **GWECHENBERGER M et al.** *Circulation*, 1999, vol. 99, 546-551 [0084]
- **JOYCE DA.** *Eur. J. Immunol.*, 1994, vol. 11, 2699-705 [0084]
- **YAMAOKA M et al.** *Jpn Circ J.*, 1999, vol. 63, 951-956 [0084]
- **OHTSUKA T et al.** *J Am Coll Cardiol.*, 2001, vol. 37, 412-417 [0084] [0088]
- **TOMINAGA S.** *FEBS Letters*, 1989, vol. 258, 301-304 [0086]
- **YANAGISAWA K et al.** *FEBS Letters.*, 1993, vol. 318, 83-87 [0086]
- **YANAGISAWA KI et al.** *J Biochem.*, 1997, vol. 121, 95-103 [0087]
- **COYLE AJ et al.** *J Exp Med.*, 1999, vol. 190, 895-902 [0087] [0088]
- **O'NEILL LAJ et al.** *Immunology Today*, 2000, vol. 21, 206-209 [0087]
- **TOWNSEND MJ et al.** *J Exp Med.*, 2000, vol. 191, 1069-1075 [0090]
- **YAMAMOTO K et al.** *J Biol Chem.*, 1999, vol. 274, 21840-21846 [0093] [0095]
- **PFEFFER M.A. et al.** *Circulation*, 1997, vol. 95, 2643-2651 [0098]
- **GREAVES S.C. et al.** *Am. J. Cardiol*, 1997, vol. 80, 442-448 [0098]
- **SOLOMON S.D. et al.** *Ann. Intern. Med.*, 2001, vol. 134, 451-458 [0098]
- **AIKAWA Y. et al.** *Am. Heart J*, 2001, vol. 141, 234-242 [0098]
- **KUROIWA K. et al.** *Hybridoma*, 2000, vol. 19, 151-159 [0098] [0103]

- **DHALLA KS et al.** *Mol Cell Biochem*, 1989, vol. 87, 85-92 [0107]
- **MOE GW et al.** *Am Heart J*, 2000, vol. 139, 587-95 [0107]
- **ANTMAN EM et al.** *Circulation*, 1999, vol. 99, 2720-32 [0111]
- **ANTMAN EM et al.** *Eur Heart J*, 2000, vol. 21, 1944-53 [0111]
- **ANTMAN EM et al.** *Circulation*, 2002, vol. 105, 1642-9 [0111]
- **MORROW DA et al.** *J Am Coll Cardiol.*, 1998, vol. 31, 1460-5 [0112]
- **MORROW DA et al.** *Clin Chem.*, 2000, vol. 46, 453-460 [0112]
- **MORROW, DA et al.** *Circulation*, 24 October 2000, vol. 102 (17), 2031-7 [0113]
- **DE LEMOS JA et al.** *N Engl J Med*, 2001, vol. 345, 1014-21 [0114]
- **ANTMAN EM et al.** *N Engl J Med*, 1996, vol. 335, 1342-9 [0114]
- **MORROW DA et al.** *J Am Coll Cardiol*, 1998, vol. 31, 1460-5 [0114]
- **SIMS JE.** IL-1 and IL-18 receptors, and their extended family. *Curr Opin Immunol.*, 2002, vol. 14, 117-22 [0120]
- **WEINBERG EO ; SHIMPO M ; HURWITZ S ; TOMINAGA S ; ROULEAU JL ; LEE RT.** Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation*, 2003, vol. 107, 721-6 [0120] [0122]
- **OSHIKAWA K ; KUROIWA K ; TAGO K ; IWAHANA H ; YANAGISAWA K ; OHNO S ; TOMINAGA SI ; SUGIYAMA Y.** Elevated soluble ST2-protein levels in sera of patients with asthma with an acute exacerbation. *Am J Respir Crit Care Med.*, 2001, vol. 164, 277-81 [0122]
- **KUROIWA K ; ARAI T ; OKAZAKI H ; MINOTA S ; TOMINAGA S.** Identification of human ST2 protein in the sera of patients with autoimmune diseases. *Biochem Biophys Res Commun*, 2001, vol. 284, 1104-8 [0122]

专利名称(译)	111rl-1作为心血管疾病的标志物		
公开(公告)号	<a href="#">EP1532269B1</a>	公开(公告)日	2011-11-23
申请号	EP2003728848	申请日	2003-05-09
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申请(专利权)人(译)	布里格姆妇女医院，INC.		
当前申请(专利权)人(译)	布里格姆妇女医院，INC.		
[标]发明人	LEE RICHARD T		
发明人	LEE, RICHARD, T.		
IPC分类号	C12Q1/68 C07H21/00 G01N33/50 A61K45/00 A61P9/00 A61P9/10 C12N15/09 G01N33/15 G01N33/53 G01N33/566 G01N33/68		
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代理机构(译)	TIMOTHY JOHN SIMON，跳		
优先权	60/379173 2002-05-09 US		
其他公开文献	EP1532269A2 EP1532269A4		
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