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(54) **1L1RL-1 as a cardiovascular disease marker**

1L1RL-1 als kardiovaskulärer Krankheitsmarker

1L1RL-1 en tant que marqueur de maladie cardiovasculaire

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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 molecules that can be used to evaluate the likelihood that a subject will benefit from treatment for 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 preventative 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.

**[0004]** This nucleic acid molecule is Interleukin 1 Receptor-Like 1 (IL1RL-1, also known as T1/ST2, ST2, and Fit-1, SEQ ID Nos: 1 an 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.

**[0005]** According to the invention, an *in vitro* method for evaluating the likelihood that a subject will benefit from treatment with an agent for reducing the risk of a cardiovascular condition, is provided.

**[0006]** In a first aspect, the invention provides an *in vitro* method for evaluating the likelihood that a subject will benefit from treatment with an agent selected from the group consisting of an anti-inflammatory agent, an antithrombotic agent, an anti-platelet agent, a fibrinolytic agent, a lipid reducing agent, a direct thrombin inhibitor, a glycoprotein IIb/IIIa receptor inhibitor, a monoclonal or polyclonal anti-cellular adhesion molecule antibody that inhibits the ability of white blood cells to attach to cellular adhesion molecules, a calcium channel blocker, a beta-adrenergic receptor blocker, a cyclooxygenase-2 inhibitor, and an angiotensin system inhibitor. The method comprises:

obtaining a first level of soluble IL1RL-1 protein in a sample from a subject;
comparing the level of the soluble IL1RL-1 protein to a predetermined value specific for the diagnosis of a cardiovascular condition selected from myocardial infarction and heart failure, wherein the predetermined value is a single cut-off value, and wherein a level of the soluble IL1RL-1 protein equal to or above the predetermined cut-off value is indicative of whether the subject will benefit from treatment with said agent.

**[0007]** Preferred features and embodiments of the invention are described in the attached dependent claims (claims 2-7).

**[0008]** In certain embodiments, the predetermined value specific for the diagnosis 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 myocardial infarction and 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-iM 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 T2/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 $ST_2$ protein and creatine kinase 1 day post myocardial infarction. *Log ST2=0.454(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. Alleyne et al., 2001, Applied Biochemistry and Biotechnology, Vol. 90. Pg. 97-105 discloses a method for evaluating if a subject suffering from myocardial infarction will benefit from treatment based on the evaluation of the levels of cytochrome-C in a sample.

**[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 disclosed characterized human sequences. 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 hydropathic 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 pyrolidone, 0.02% Bovine Serum Albumin, 2.5mM $NaH_2PO_4$[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. 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 GenGank 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.

**[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 or ordinary skill in the art.

**[0029]** Expression of any of the foregoing IL1RL-1 nucleic acids, 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 can also 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 recognised 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. 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 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 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. Thus, isolated means sufficiently pure to be used (i) to raised 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 may 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. Modification which create a polypeptide variant are typically made to the nucleic acid which encodes the polypeptide, and can include deletions, point mutation, 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 disclosed 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 labelled 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). 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 Productions, 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')2 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 invention can utilise F(ab')2, 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')2 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 (e.g. SEQ ID NO: 2), 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]** "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.

**[0058]** When the disorder is a cardiovascular condition selected from the group consisting of myocardial infarction 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.

**[0059]** Kits could be used to measure the levels of the nucleic acids of the invention, or expression products of the invention.

**[0060]** The invention embraces an *in vitro* method for evaluating the likelihood that a subject will benefit from treatment with an agent for reducing the risk of a cardiovascular condition. The agent is selected from the group consisting of an anti-inflammatory agent, an antithrombotic agent, an anti-platelet agent, a fibrinolytic agent, a lipid reducing agent, a direct thrombin inhibitor, a glycoprotein IIb/IIIa receptor inhibitor, a monoclonal or polyclonal anti-cellular adhesion molecule antibody that inhibits the ability of white blood cells to attach to cellular adhesion molecules, a calcium channel blocker, a beta-adrenergic receptor blocker, a cyclooxygenase-2 inhibitor, and an angiotensin system inhibitor. The method involves obtaining a first level of a soluble IL1RL-1 protein in a sample from a subject, and comparing the level of the soluble IL1RL-1 protein to a predetermined value specific for the diagnosis of a cardiovascular condition. The level of the IL1RL-1 molecule in comparison to the predetermined value is indicative of whether the subject will benefit from treatment with said agent. In certain embodiments, the predetermined value specific for the diagnosis 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 can be a condition selected from the group consisting of myocardial infarction and heart failure.

**[0061]** The predetermined value specific for the diagnosis of a cardiovascular condition is a 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]** As discussed above the invention provides a method for evaluating the likelihood that a subject will benefit from treatment with an agent for reducing risk of a future cardiovascular disorder. This method has important implications for patient treatment and also for clinical development of new therapeutics. Physicians select therapeutic regimens for patient treatment based upon the expected net benefits to the patient. The net benefit is derived from the risk to benefit ratio. The present invention permits selection of subjects who are more likely to benefit by intervention, thereby aiding the physician in selecting a therapeutic regimen. This might include using drugs with a higher risk profile where the likelihood of expected benefit has increased. Likewise, clinical investigators desire to select for clinical trials a population with a high likelihood of obtaining a net benefit. The present invention can help clinical investigators select such subjects. It is expected that clinical investigators now will use the present invention for determining entry criteria for clinical trials.

**[0064]** "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

**[0065]** fissures, ruptures, or ulcerates, and a mural thrombus forms leading to coronary artery occlusion.

**[0066]** 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 1L1RL-1 nucleic acid molecule, or an expression product thereof, are also important risk factors.

**[0067]** 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.

**[0068]** "Anti-inflammatory" agents include Alclofenac; Alclometasone Dipropionate; Algestone Acetonide; Alpha Amylase; Amcinafal; Amcinafide; Amfenac Sodium; Amiprilose Hydrochloride; Anakinra; Anirolac; Anitrazafen; Apazone; Balsalazide Disodium; Bendazac; Benoxaprofen; Benzydamine Hydrochloride; Bromelains; Broperamole; Budesonide; Carprofen; Cicloprofen; Cintazone; Cliprofen; Clobetasol Propionate; Clobetasone Butyrate; Clopirac; Cloticasone Pro-

pionate; Cormethasone Acetate; Cortodoxone; Deflazacort; Desonide; Desoximetasone; Dexamethasone Dipropionate; Diclofenac Potassium; Diclofenac Sodium; Diflorasone Diacetate; Diflumidone Sodium; Diflunisal; Difluprednate; Diftalone; Dimethyl Sulfoxide; Drocinonide; Endrysone; Enlimomab; Enolicam Sodium; Epirizole; Etodolac; Etofenamate; Felbinac; Fenamole; Fenbufen; Fenclofenac; Fenclorac; Fendosal; Fenpipalone; Fentiazac; Flazalone; Fluazacort; Flufenamic Acid; Flumizole; Flunisolide Acetate; Flunixin; Flunixin Meglumine; Fluocortin Butyl; Fluorometholone Acetate; Fluquazone; Flurbiprofen; Fluretofen; Fluticasone Propionate; Furaprofen; Furobufen; Halcinonide; Halobetasol Propionate; Halopredone Acetate; Ibufenac; Ibuprofen; Ibuprofen Aluminum; Ibuprofen Piconol; Ilonidap; Indomethacin; Indomethacin Sodium; Indoprofen; Indoxole; Intrazole; Isoflupredone Acetate; Isoxepac; Isoxicam; Ketoprofen; Lofemizole Hydrochloride; Lornoxicam; Loteprednol Etabonate; Meclofenamate Sodium; Meclofenamic Acid; Meclorisone Dibutyrate; Mefenamic Acid; Mesalamine; Meseclazone; Methylprednisolone Suleptanate; Momiflumate; Nabumetone; Naproxen; Naproxen Sodium; Naproxol; Nimazone; Olsalazine Sodium; Orgotein; Orpanoxin; Oxaprozin; Oxyphenbutazone; Paranyline Hydrochloride; Pentosan Polysulfate Sodium; Phenbutazone Sodium Glycerate; Pirfenidone; Piroxicam; Piroxicam Cinnamate; Piroxicam Olamine; Pirprofen; Prednazate; Prifelone; Prodolic Acid; Proquazone; Proxazole; Proxazole Citrate; Rimexolone; Romazarit; Salcolex; Salnacedin; Salsalate; Salycilates; Sanguinarium Chloride; Seclazone; Sermetacin; Sudoxicam; Sulindac; Suprofen; Talmetacin; Talniflumate; Talosalate; Tebufelone; Tenidap; Tenidap Sodium; Tenoxicam; Tesicam; Tesimide; Tetrydamine; Tiopinac; Tixocortol Pivalate; Tolmetin; Tolmetin Sodium; Triclonide; Triflumidate; Zidometacin; Glucocorticoids; and Zomepirac Sodium. One preferred anti-inflammatory agent is aspirin.

**[0069]** "Anti-thrombotic" and/or "fibrinolytic" agents include plasminogen (to plasmin via interactions of prekallikrein, kininogens, Factors XII, XIIIa, plasminogen proactivator, and tissue plasminogen activator[TPA]) Streptokinase; Urokinase: Anisoylated Plasminogen-Streptokinase Activator Complex; Pro-Urokinase; (Pro-UK); rTPA (alteplase or activase; "r" denotes recombinant); rPro-UK; Abbokinase; Eminase; Sreptase Anagrelide Hydrochloride; Bivalirudin; Dalteparin Sodium; Danaparoid Sodium; Dazoxiben Hydrochloride; Efegatran Sulfate; Enoxaparin Sodium; Ifetroban; Ifetroban Sodium; Tinzaparin Sodium; Retaplase; Trifenagrel; Warfarin; and Dextrans.

**[0070]** "Anti-platelet" agents include Clopridogrel; Sulfinpyrazone; Aspirin; Dipyridamole; Clofibrate; Pyridinol Carbamate; PGE; Glucagon; Antiserotonin drugs; Caffeine; Theophyllin Pentoxifyllin; Ticlopidine; and Anagrelide.

**[0071]** "Lipid reducing" agents include gemfibrozil, cholystyramine, colestipol, nicotinic acid, probucol lovastatin, fluvastatin, simvastatin, atorvastatin, pravastatin, and cirivastatin.

**[0072]** "Direct thrombin inhibitors" include hirudin, hirugen, hirulog, agatroban, PPACK, and thrombin aptamers.

**[0073]** "Glycoprotein IIb/IIIa receptor inhibitors" embraces both antibodies and non-antibodies, and include, but are not limited, to ReoPro (abcixamab), lamifiban, and tirofiban.

**[0074]** "Calcium channel blockers" are a chemically diverse class of compounds having important therapeutic value in the control of a variety of diseases including several cardiovascular disorders, such as hypertension, angina, and cardiac arrhythmias (Fleckenstein, Cir, Res. v. 52, (suppl. 1), p.13-16 (1983); Fleckenstein, Experimental Facts and Therapeutic Prospects, John Wiley, New York (1983); McCall, D., Curr Pract Cardiol, v. 10, p. 1-11 (1985)). Calcium channel blockers are a heterogeneous group of drugs that prevent or slow the entry of calcium into cells by regulating cellular calcium channels. (Remington, The Science and Practice of Pharmacy, Nineteenth Edition, Mack Publishing Company, Eaton, PA, p.963 (1995)). Most of the currently available calcium channel blockers, and useful according to the present invention, belong to one of three major chemical groups of drugs, the dihydropyridines, such as nifedipine, the phenyl alkyl amines, such as verapamil, and the benzothiazepines, such as diltiazem. Other calcium channel blockers useful according to the invention, include, but are not limited to, amrinone, amlodipine, bencyclane, felodipine, fendiline, flunarizine, isradipine, nicardipine, nimodipine, perhexilene, gallopamil, tiapamil and tiapamil analogues (such as 1993RO-11-2933), phenytoin, barbiturates, and the peptides dynorphin, omega-conotoxin, and omega-agatoxin, and the like and/or pharmaceutically acceptable salts thereof.

**[0075]** "Beta-adrenergic receptor blocking agents" are a class of drugs that antagonize the cardiovascular effects of catecholamines in angina pectoris, hypertension, and cardiac arrhythmias. Beta-adrenergic receptor blockers include, but are not limited to, atenolol, acebutolol, alprenolol, befunolol, betaxolol, bunitrolol, carteolol, celiprolol, hedroxalol, indenolol, labetalol, levobunolol, mepindolol, methypranol, metindol, metoprolol, metrizoranolol, oxprenolol, pindolol, propranolol, practolol, practolol, sotalolnadolol, tiprenolol, tomalolol, timolol, bupranolol, penbutolol, trimepranol, 2-(3-(1,1-dimethylethyl)-amino-2-hydroxypropoxy)-3-pyridenecarbonitrilHCl, 1-butylamino-3-(2,5-dichlorophenoxy)-2-propanol, 1-isopropylamino-3-(4-(2-cyclopropylmethoxyethyl)phenoxy)-2-propanol, 3-isopropylamino-1-(7-methylindan-4-yloxy)-2-butanol, 2-(3-t-butylamino-2-hydroxy-propylthio)-4-(5-carbamoyl-2-thienyl)thiazol,7-(2-hydroxy-3-t-butylaminpropoxy)phthalide. The above-identified compounds can be used as isomeric mixtures, or in their respective levorotating or dextrorotating form.

**[0076]** Cyclooxygenase-2 (COX-2) is a recently identified form of a cyclooxygenase. "Cyclooxygenase" is an enzyme complex present in most tissues that produces various prostaglandins and thromboxanes from arachidonic acid. Nonsteroidal, anti-inflammatory drugs exert most of their anti-inflammatory, analgesic and antipyretic activity and inhibit hormone-induced uterine contractions and certain types of cancer growth through inhibition of the cyclooxygenase (also

known as prostaglandin G/H synthase and/or prostaglandin-endoperoxide synthase). Initially, only one form of cyclooxygenase was known, the "constitutive enzyme" or cyclooxygenase-1 (COX-1). It and was originally identified in bovine seminal vesicles.

**[0077]** Cyclooxygenase-2 (COX-2) has been cloned, sequenced and characterized initially from chicken, murine and human sources (see, e.g., U.S. Patent No. 5,543,297, issued August 6, 1996 to Cromlish et al., and assigned to Merck Frosst Canada, Inc., Kirkland, CA, entitled: "Human cyclooxygenase-2 cDNA and assays for evaluating cyclooxygenase-2 activity"). This enzyme is distinct from COX-1. COX-2 is rapidly and readily inducible by a number of agents including mitogens, endotoxin, hormones, cytokines and growth factors. As prostaglandins have both physiological and pathological roles, the constitutive enzyme, COX-1, is responsible, in large part, for endogenous basal release of prostaglandins and hence is important in their physiological functions such as the maintenance of gastrointestinal integrity and renal blood flow. By contrast, it is believed that the inducible form, COX-2, is mainly responsible for the pathological effects of prostaglandins where rapid induction of the enzyme would occur in response to such agents as inflammatory agents, hormones, growth factors, and cytokines. Therefore, it is believed that a selective inhibitor of COX-2 has similar anti-inflammatory, antipyretic and analgesic properties to a conventional non-steroidal anti-inflammatory drug, and in addition inhibits hormone-induced uterine contractions and also has potential anti-cancer effects, but with reduced side effects. In particular, such COX-2 inhibitors are believed to have a reduced potential for gastrointestinal toxicity, a reduced potential for renal side effects, a reduced effect on bleeding times and possibly a decreased potential to induce asthma attacks in aspirin-sensitive asthmatic subjects, and are therefore useful according to the present invention.

**[0078]** A number of selective "COX-2 inhibitors" are known in the art. These include, but are not limited to, COX-2 inhibitors described in U.S. Patent No. 5,474,995 "Phenyl heterocycles as COX-2 inhibitors"; U.S. Patent No. 5,521,213 "Diaryl bicyclic heterocycles as inhibitors of cyclooxygenase-2"; U.S. Patent No. 5,536,752 "Phenyl heterocycles as COX-2 inhibitors"; U.S. Patent No. 5,550,142 "Phenyl heterocycles as COX-2 inhibitors"; U.S. Patent No. 5,552,422 "Aryl substituted 5,5 fused aromatic nitrogen compounds as anti-inflammatory agents"; U.S. No. Patent 5,604,253 "N-Benzylindol-3-yl propanoic acid derivatives as cyclooxygenase inhibitors"; U.S. No. Patent 5,604,260 "5-Methanesulfonamido-1-indanones as an inhibitor of cyclooxygenase-2"; U.S. Patent No. 5,639,780 N-Benzyl indol-3-yl butanoic acid derivatives as cyclooxygenase inhibitors"; U.S. Patent No. 5,677,318 Diphenyl-l,2-3-thiadiazoles as anti-inflammatory agents"; U.S. Patent No. 5,691,374 "Diaryl-5-oxygenated-2-(5H)-furanones as COX-2 inhibitors"; U.S. Patent No. 5,698,584 "3,4-Diaryl-2-hydroxy-2,5-dihydrofurans as prodrugs to COX-2 inhibitors"; U.S. Patent No. 5,710,140 "Phenyl heterocycles as COX-2 inhibitors"; U.S. Patent No. 5,733,909 "Diphenyl stilbenes as prodrugs to COX-2 inhibitors"; U.S. Patent No. 5,789,413 "Alkylated styrenes as prodrugs to COX-2 inhibitors"; U.S. Patent No. 5,817,700 "Bisaryl cyclobutenes derivatives as cyclooxygenase inhibitors"; U.S. Patent No. 5,849,943 "Stilbene derivatives useful as cyclooxygenase-2 inhibitors"; U.S. Patent No. 5,861,419 "Substituted pyridines as selective cyclooxygenase-2 inhibitors"; U.S. Patent No. 5,922,742 "Pyridinyl-2-cyclopenten-1-ones as selective cyclooxygenase-2 inhibitors"; U.S. Patent No. 5,925,631 "Alkylated styrenes as prodrugs to COX-2 inhibitors"; all of which are commonly assigned to Merck Frosst Canada, Inc. (Kirkland, CA or Merck & Co., Inc. (Rahway, NJ). Additional COX-2 inhibitors are also described in U.S. Patent No. 5,643,933, assigned to G. D. Searle & Co. (Skokie, IL), entitled: "Substituted sulfonylphenylheterocycles as cyclooxygenase-2 and 5-lipoxygenase inhibitors."

**[0079]** A number of the above-identified COX-2 inhibitors are prodrugs of selective COX-2 inhibitors, and exert their action by conversion *in vivo* to the active and selective COX-2 inhibitors. The active and selective COX-2 inhibitors formed from the above-identified COX-2 inhibitor prodrugs are described in detail in WO 95/00501, published January 5, 1995, WO 95/18799, published July 13, 1995 and U.S. Patent No. 5,474,995, issued December 12, 1995. Given the teachings of U.S. Patent No. 5,543,297, entitled: "Human cyclooxygenase-2 cDNA and assays for evaluating cyclooxygenase-2 activity," a person of ordinary skill in the art would be able to determine whether an agent is a selective COX-2 inhibitor or a precursor of a COX-2 inhibitor, and therefore part of the present invention.

**[0080]** An "angiotensin system inhibitor" is an agent that interferes with the function, synthesis or catabolism of angiotensin II. These agents include, but are not limited to, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II antagonists, angiotensin II receptor antagonists, agents that activate the catabolism of angiotensin II, and agents that prevent the synthesis of angiotensin I from which angiotensin II is ultimately derived. The renin-angiotensin system is involved in the regulation of hemodynamics and water and electrolyte balance. Factors that lower blood volume, renal perfusion pressure, or the concentration of $Na^+$ in plasma tend to activate the system, while factors that increase these parameters tend to suppress its function.

**[0081]** Angiotensin I and angiotensin II are synthesized by the enzymatic renin-angiotensin pathway. The synthetic process is initiated when the enzyme renin acts on antiotensinogen, a pseudoglobulin in blood plasma, to produce the decapeptide angiotensin I. Angiotensin I is converted by angiotensin converting enzyme (ACE) to angiotensin II (angiotensin-[1-8] octapeptide). The latter is an active pressor substance which has been implicated as a causative agent in several forms of hypertension in various mammalian species, e.g., humans.

**[0082]** Angiotensin (renin-angiotensin) system inhibitors are compounds that act to interfere with the production of angiotensin II from angiotensinogen or angiotensin I or interfere with the activity of angiotensin II. Such inhibitors are

well known to those of ordinary skill in the art and include compounds that act to inhibit the enzymes involved in the ultimate production of angiotensin II, including renin and ACE. They also include compounds that interfere with the activity of angiotensin II, once produced. Examples of classes of such compounds include antibodies (e.g., to renin), amino acids and analogs thereof (including those conjugated to larger molecules), peptides (including peptide analogs of angiotensin and angiotensin I), pro-renin related analogs, etc. Among the most potent and useful renin-angiotensin system inhibitors are renin inhibitors, ACE inhibitors, and angiotensin II antagonists.

**[0083]** "Angiotensin II antagonists" are compounds which interfere with the activity of angiotensin II by binding to angiotensin II receptors and interfering with its activity. Angiotensin II antagonists are well known and include peptide compounds and non-peptide compounds. Most angiotensin II antagonists are slightly modified congeners in which agonist activity is attenuated by replacement of phenylalanine in position 8 with some other amino acid; stability can be enhanced by other replacements that slow degeneration in *vivo.* Examples of angiotensin II antagonists include: peptidic compounds (e.g., saralasin, [(San$^1$)(Val$^5$)(Ala$^8$)] angiotensin-(1-8) octapeptide and related analogs); N-substituted imidazole-2-one (U.S. Patent No. 5,087,634); imidazole acetate derivatives including 2-N-butyl-4-chloro-1-(2-chlorobenzile), imidazole-5-acetic acid (see Long et al., J. Pharmacol. Exp. Ther. 247(1), 1-7 (1988)); 4, 5, 6, 7-tetrahydro-1H-imidazo [4, 5-c] pyridine-6-carboxylic acid and analog derivatives (US Patent No. 4,816,463); N2-tetrazole beta-glucuronide analogs (US Patent No. 5,085,992); substituted pyrroles, pyrazoles, and tryazoles (US Patent No. 5,081,127); phenol and heterocyclic derivatives such as 1, 3-imidazoles (US Patent No. 5,073,566); imidazo-fused 7-member ring heterocycles (US Patent No. 5,064,825); peptides (e.g., US Patent No. 4,772,684); antibodies to angiotensin II (e.g., US Patent No. 4,302,386); and aralkyl imidazole compounds such as biphenyl-methyl substituted imidazoles (e.g., EP Number 253,310, January 20, 1988); ES8891 (N-morpholinoacetyl-(-1-naphthyl)-L-alanyl-(4, thiazolyl)-L-alanyl (35, 45)-4-amino-3-hydroxy-5-cyclo-hexapentanoyl-N-hexylamide, Sankyo Company, Ltd., Tokyo, Japan); SKF108566 (E-alpha-2-[2-butyl-1-(carboxyphenyl)methyl]1H-imidazole-5-yl[methylane]-2-thiophenepropanoic acid, Smith Kline Beecham Pharmaceuticals, PA); Losartan (DUP753/MK954, DuPont Merck Pharmaceutical Company); Remikirin (RO42-5892, F. Hoffman LaRoche AG); $A_2$ agonists (Marion Merrill Dow) and certain non-peptide heterocycles (G.D.Searle and Company).

**[0084]** "Angiotensin converting enzyme," (ACE), is an enzyme which catalyzes the conversion of angiotensin I to angiotensin II. ACE inhibitors include amino acids and derivatives thereof, peptides, including di- and tripeptides and antibodies to ACE which intervene in the renin-angiotensin system by inhibiting the activity of ACE thereby reducing or eliminating the formation of pressor substance angiotensin II. ACE inhibitors have been used medically to treat hypertension, congestive heart failure, myocardial infarction and renal disease. Classes of compounds known to be useful as ACE inhibitors include acylmercapto and mercaptoalkanoyl prolines such as captopril (US Patent No. 4,105,776) and zofenopril (US Patent Number 4,316,906), carboxyalkyl dipeptides such as enalapril (US Patent No. 4,374,829), lisinopril (US Patent No. 4,374,829), quinapril (US Patent No. 4,344,949), ramipril (US Patent No. 4,587,258), and perindopril (US Patent No. 4,508,729), carboxyalkyl dipeptide mimics such as cilazapril (US Patent No. 4,512,924) and benazapril (US Patent No. 4,410,520), phosphinylalkanoyl prolines such as fosinopril (US Patent No. 4,337,201) and trandolopril.

**[0085]** "Renin inhibitors" are compounds which interfere with the activity of renin. Renin inhibitors include amino acids and derivatives thereof, peptides and derivatives thereof, and antibodies to renin. Examples of renin inhibitors that are the subject of United States patents are as follows: urea derivatives ofpeptides (US Patent No. 5,116,835); amino acids connected by nonpeptide bonds (US Patent No. 5,114,937); di- and tri- peptide derivatives (US Patent No. 5,106,835); amino acids and derivatives thereof (US Patent Nos. 5,104,869 and 5,095,119); diol sulfonamides and sulfinyls (US Patent No. 5,098,924); modified peptides (US Patent No. 5,095,006); peptidyl beta-aminoacyl aminodiol carbamates (US Patent No. 5,089,471); pyrolimidazolones (US Patent No. 5,075,451); fluorine and chlorine statine or statone containing peptides (US Patent No. 5,066,643); peptidyl amino diols (US Patent Nos. 5,063,208 and 4,845,079); N-morpholino derivatives (US Patent No. 5,055,466); pepstatin derivatives (US Patent No. 4,980,283); N-heterocyclic alcohols (US Patent No. 4,885,292); monoclonal antibodies to renin (US Patent No. 4,780,401); and a variety of other peptides and analogs thereof (US Patent Nos. 5,071,837, 5,064,965, 5,063,207, 5,036,054, 5,036,053, 5,034,512, and 4,894,437).

**[0086]** Agents that bind to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules include polypeptide agents. Such polypeptides include polyclonal and monoclonal antibodies, prepared according to conventional methodology. Such antibodies already are known in the art and include anti-ICAM 1 antibodies as well as other such antibodies described above.

**[0087]** Anticoagulant agents include, but are not limited to, Ancrod; Anticoagulant Citrate Dextrose Solution; Anticoagulant Citrate Phosphate Dextrose Adenine Solution; Anticoagulant Citrate Phosphate Dextrose Solution; Anticoagulant Heparin Solution; Anticoagulant Sodium Citrate Solution; Ardeparin Sodium; Bivalirudin; Bromindione; Dalteparin Sodium; Desirudin; Dicumarol; Heparin Calcium; Heparin Sodium; Lyapolate Sodium; Nafamostat Mesylate; Phenprocoumon; Tinzaparin Sodium; and Warfarin Sodium.

**[0088]** Heparin may stabilize symptoms in evolving stroke, but anticoagulants are useless (and possibly dangerous) in acute completed stroke, and are contraindicated in hypertensives because of the increased possibility of hemorrhage into the brain or other organs. Although the timing is controversial, anticoagulants may be started to prevent recurrent

cardiogenic emboli. Clot lysing agents, including tissue plasminogen activator and streptokinase, are being evaluated for the very early treatment of acute stroke. Nimodipine has recently been shown to improve survival and clinical outcome after ischemic stroke.

**[0089]** Other than aspirin, ticlopidine is another antiplatelet agent that has been shown to be beneficial for stroke treatment. Endarterectomy may be indicated in patients with 70 to 99 percent narrowing of a symptomatic internal carotid artery. However, most authorities agree that carotid endarterectomy is not indicated in patients with TIAs that are referable to the basilar-vertebral system, in patients with significant deficits from prior strokes, or in patients in whom a stroke is evolving.

**[0090]** HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase is the microsomal enzyme that catalyzes the rate limiting reaction in cholesterol biosynthesis (HMG-CoA6Mevalonate). An HMG-CoA reductase inhibitor inhibits HMG-CoA reductase, and as a result inhibits the synthesis of cholesterol. A number of HMG-CoA reductase inhibitors has been used to treat subjects with hypercholesterolemia. More recently, HMG-CoA reductase inhibitors have been shown to be beneficial in the treatment of stroke (Endres M, et al., Proc Natl Acad Sci USA, 1998,95:8880-5).

**[0091]** HMG-CoA reductase inhibitors include, but are not limited to, simvastatin (U.S. Patent No. 4,444,784); lovastatin (U.S. Patent No. 4,231,938); pravastatin sodium (U.S. Patent No. 4,346,227); fluvastatin (U.S. Patent No. 4,739,073); atorvastatin (U.S. Patent No. 5,273,995); cerivastatin, and numerous others described in U.S. Patent No. 5,622,985; U.S. Patent No. 5,135,935; U.S. Patent No. 5,356,896; U.S. Patent No. 4,920,109; U.S. Patent No. 5,286,895; U.S. Patent No. 5,262,435; U.S. Patent No. 5,260,332; U.S. Patent No. 5,317,031; U.S. Patent No. 5,283,256; U.S. Patent No. 5,256,689; U.S. Patent No. 5,182,298; U.S. Patent No. 5,369,125; U.S. Patent No. 5,302,604; U.S. Patent No. 5,166,171; U.S. Patent No. 5,202,327; U.S. Patent No. 5,276,021; U.S. Patent No. 5,196,440, U.S. Patent No. 5,091,386; U.S. Patent No. 5,091,378; U.S. Patent No. 4,904,646; U.S. Patent No. 5,385,932; U.S. Patent No. 5,250,435; U.S. Patent No. 5,132,312; U.S. Patent No. 5,130,306; U.S. Patent No. 5,116,870; U.S. Patent No. 5,112,857; U.S. Patent No. 5,102,911; U.S. Patent No. 5,098,931; U.S. Patent No. 5,081,136; U.S. Patent No. 5,025,000; U.S. Patent No. 5,021,453; U.S. Patent No. 5,017,716; U.S. Patent No. 5,001,144; U.S. Patent No. 5,001,128; U.S. Patent No. 4,997,837; U.S. Patent No. 4,996,234; U.S. Patent No. 4,994,494; U.S. Patent No. 4,992,429; U.S. Patent No. 4,970,231; U.S. Patent No. 4,968,693; U.S. Patent No. 4,963,538; U.S. Patent No. 4,957,940; U.S. Patent No. 4,950,675; U.S. Patent No. 4,946,864; U.S. Patent No. 4,946,860; U.S. Patent No. 4,940,800; U.S. Patent No. 4,940,727; U.S. Patent No. 4,939,143; U.S. Patent No. 4,929,620; U.S. Patent No. 4,923,861; U.S. Patent No. 4,906,657; U.S. Patent No. 4,906,624; and U.S. Patent No. 4,897,402, the disclosures of which patents are incorporated herein by reference.

**[0092]** Nitric oxide (NO) has been recognized as a messenger molecule with many physiologic roles, in the cardiovascular, neurologic and immune systems (Griffith, TM et al., J Am Coll Cardiol, 1988, 12:797-806). It mediates blood vessel relaxation, neurotransmission and pathogen suppression. NO is produced from the guanidino nitrogen of L-arginine by NO Synthase (Moncada, S and Higgs, EA, Eur J Clin Invest, 1991, 21:361-374). Agents that upregulate endothelial cell Nitric Oxide Synthase include, but are not limited to, L-arginine, *rho* GTPase function inhibitors (see International Application WO 99/47153, the disclosure of which is incorporated herein by reference), and agents that disrupt actin cytoskeletal organization (see International Application WO 00/03746, the disclosure of which is incorporated hereby by reference).

**[0093]** 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**

**[0094]** 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.

**[0095]** **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 THREFOR, 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 ~ ± 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.

**[0096]** 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.

**[0097]** 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.

**[0098]** The electrodes for each dish were two arc-shaped $AgCl_2$ wire electrodes at the base of the inner surface of the dish, just above the deformable membrane. The electrodes were pre-made, 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.

**[0099]** **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.

**[0100]** 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 $NaHCO_3$, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 0.4 mM $KH_2PO_4$, 0.3 mM $Na_2HPO_4$, 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.

**[0101]** 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.

**[0102]** **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.

**[0103]** **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 (Chomcyznski, et al., Anal. Biochem., 1987, 162:156-159). For Northern blotting, 15 $\mu g$ RNA was loaded on a 1.0% agarose-formaldehyde gel (2.0 mol/1), 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.

**[0104]** **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.

**[0105]** 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 ILIRL-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.

**[0106]** Figure 3 shows the effects of 8% mechanical strain, hydrogen peroxide ($H_2O_2$, 100 uM) 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 $H_2O_2$ in the absence of mechanical strain and blocked by TIRON, $H_2O_2$ 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.

**[0107]** 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.

**[0108]** 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

**[0109]** 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 $^{32}$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:

**[0110]** **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 FactorReviews. 1996;7:341-354; St. John SuttonMG, 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).

**[0111]** 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-α, 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-α 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-α serum levels are increased in patients with dilated cardiomyopathy (Ohtsuka T, et al. J Am Coll Cardiol. 2001;37:412-417).

**[0112]** **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.

**[0113]** **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).

**[0114]** **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).

**[0115]** 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.

**[0116]** 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.

**[0117]** **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).

**[0118]** 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:**

**[0119]** **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).

**[0120]** 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.

**[0121]** 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.

**[0122]** 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-κB. To investigate the role of NF-κB in interleukin-1$\beta$ and strain induction of T1/ST2 RNA, we overexpressed IκBα, which decreases NF-κB DNA binding activity. Cultured cardiac myocytes were infected with IκBα overexpression adenovirus vector or with $\beta$-galactosidase control vector and exposed for 4 hours to 8% cyclic mechanical strain or interleukin-1 (10nglml). RNA was analyzed by Northern blotting with $^{32}$P-labeled IL1RL-1 cDNA probe. Ectopic expression of IκBα 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-κB target gene through interleukin-1/interleukin-1 receptor signaling. In contrast, pathways in addition to NF-κB 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.

**[0123]** In addition to the above-noted results, we have shown that T1/ST2 is induced secondary to NF-κB activation by interleukin-1 and NF-κB 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**

**[0124]** **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 hemotoxylin and eosin.

**Patient studies and ELISA for ST2.**

**[0125]** *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).

**[0126]** *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.

**[0127]** Statistics. Each in vitro experiment shown was performed a minimum of three times. Values are means ± 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:**

**[0128]** **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.

**[0129] 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.

**[0130]** ***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±SEM, 3.8+0.4 ng/ml, $p<0.001$; range, 0.32 to 17.42 ng/ml) compared to day 14 (mean±SEM, 0.98±0.06 ng/ml; range, 0.25 to 3.42 ng/ml) and day 90 (mean±SEM, 0.79±0.07 ng/ml; range, 0.02 to 3.53 ng/ml; day 14 vs. day 90, P=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.

**[0131]** ***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.

**[0132] 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.

**[0133]** *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.

**[0134]** 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 | 5th Percentile | 95th 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/mm2)** | 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 | 5th Percentile | 95th 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/mm2)** | 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 |
| | *p value* | *<0.0001* | *0.1843* |
| | N | 161 | 139 |
| **Baseline ProANP (pmol/L)** | R | 0.35979 | -0.10967 |
| | *p value* | *<0.0001* | *0.1987* |
| | N | 161 | 139 |
| **Change in BNP* (pmol/L)** | R | -0.10184 | 0.21497 |
| | *p value* | *0.2329* | *0.0110* |
| | N | 139 | 139 |
| **Change in ProANP* (pmol/L)** | R | 0.05584 | 0.28847 |
| | *p value* | *0. 5138* | *0.0006* |
| | N | 139 | 139 |
| **Norepinephrine (pglml)** | *R* | *0.38535* | *-0.25253* |
| | *p value* | *<0. 0001* | *0.0032* |

(continued)

| Table 2. Relation of ST2 to Clinical Variables and Neurohormones: Spearman Correlations | | | |
|---|---|---|---|
| | | Baseline ST2 | Change in ST2 |
| | N | 156 | 134 |
| **Dopamine (pg/mL)** | R | 0.07879 | 0.22127 |
| | *p value* | *0.3283* | *0.0102* |
| | N | 156 | 134 |
| **Epinephrine (pg/mL)** | R | 0.08043 | -0.12110 |
| | *p value* | *0.3182* | *0.1634* |
| | N | 156 | 134 |
| **Angiotensin II (pg/mL)** | R | 0.00374 | -0.00725 |
| | *p value* | 0.9630 | 0.9335 |
| | N | 156 | 135 |
| **Adrenolutin (ng/mL)** | R | 0.00544 | -0.10422 |
| | *p value* | *0.9464* | *0.2308* |
| | N | 155 | 134 |
| **Creatinine (units)** | R | 0.16567 | 0.02513 |
| | *p value* | *0.0388* | *0.7724* |
| | N | 156 | 135 |
| **LV Ejection Fraction** | R | -0.08006 | 0.03651 |
| | *p value* | *0.3205* | *0.6742* |
| | N | 156 | 135 |
| **Age (years)** | R | -0.11768 | 0.19260 |
| | *p value* | *0.1447* | *0.0274* |
| | N | 155 | 134 |
| **Body Mass Index (units)** | R | 0.04561 | -0.05410 |
| | *p value* | 0.5731 | *0.5347* |
| | N | 155 | 134 |

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** | ***p-value*** |
| **Baseline ST2, per 0.1 ng/mL** | 1.114 | 0.961-1.300 | *0.1509* |
| **Baseline BNP, per 10 pmol/L** | 1.106 | 1.060-1.161 | *<0.0001* |
| **Baseline ProANP, per 10 pg/L** | 1.007 | 1.005-1.010 | *<0.0001* |
| **Change in ST2*, per change of 0.1 ng/mL** | 1.320 | 1.042-1.827 | *0.0482* |
| **Change in BNP*, per change of 10 pmol/L** | 1.033 | 0.966-1.110 | *0.3401* |
| **Change in ProANP*, per change of 10 pg/L** | 1.003 | 0.997-1.009 | *0.3413* |
| **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* |

(continued)

| Table 3. Univariate Predictors of Mortality and Transplantation (Endpoint) | | | |
|---|---|---|---|
| **Variable** | **Odds Ratio** | **95 % confidence interval** | ***p-value*** |
| **Etiology** | 1.543 | 0.744-3.336 | *0.2543* |
| **Body Mass Index, per 1kg/mm$^2$** | 0.972 | 0.919-1.021 | *0.2876* |

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** | *p* |
| **Baseline ST2 and Baseline BNP** | |
| Baseline BNP | *0.0003* |
| Baseline Dopamine | *0.0906* |
| Baseline ST2 | *0.6368* |
| **Baseline ST2 and Baseline ProANP** | |
| Baseline ProANP | *<0.0001* |
| Baseline Dopamine | *0.0944* |
| Baseline ST2 | *0.3306* |
| **Change in ST2* and Baseline BNP** | |
| Baseline BNP | *0.0001* |
| Change in ST2 | *0.0392* |
| **Change in ST2* and Baseline ProANP** | |
| Baseline ProANP | *<0.0001* |
| Change in ST2 | *0.0274* |

Baseline, values at trial enrollment; * Change, values at week 2 minus values at trial enrollment.

**EXAMPLE 3**

**Methods**

**[0135] 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 hemorrhage, severe renal insufficiency, and cardiogenic shock.

**[0136] 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 Chain. 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.

**[0137] 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 $25^{th}$ -$75^{th}$ percentiles. The association between baseline clinical characteristics and quartiles of ST2 were analyzed using the Kruskal-Wallis test for continuous variables and the $X^2$ 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

**[0138] 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 | 0% | 0% | 1.5% | 1.0% | 0.1 | 0.2 |
| Failure | | | | | | |
| Angina | 26% | 24% | 26% | 32% | 0.3 | 0.2 |
| Diabetes | 14% | 14% | 15% | 16% | 0.9 | 0.5 |
| Family history of | 73% | 73% | 73% | 73% | 0.2 | 0.08 |
| CAD | | | | | | |
| 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) | 71 ± 17 | 75 ± 17 | 72 ± 16 | 80 ± 17 | 0.001 | <0.0001 |

(continued)

| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | p trend | p Q4 vs Q1 |
|---|---|---|---|---|---|---|
| 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 | Spearmans 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)

**[0139] ST2 and Clinical Outcomes.** For the combined cohort of 810 patients, baseline ST2 was significantly associated 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 |

(continued)

| Outcome (30 days) | n | Median [25,75] | p value |
|---|---|---|---|
| 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

**[0140]** **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.

**[0141]** **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.

**[0142]** 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.

**[0143]** 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.

**[0144]** 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. Secondarily, 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, Shimpo 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.

**[0145]** 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.

**[0146]** Although ST2 maybe 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, Shimpo 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.

**[0147]** 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. $ST_2$ 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 $ST_2$ in acute myocardial infarction, since the levels of $ST_2$ predict outcome. Studies of the function of $ST_2$ in myocardial infarction are possible. In addition, identifying the ligand for the soluble and membrane $ST_2$ receptors could help further the understanding of the potentially competing roles of the membrane and soluble receptors.

**[0148]** 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

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tcggccatca atcactagca c atg att ggc aaa tgg aga atg ggg ctt tgg       231
                        Met Ile Gly Lys Trp Arg Met Gly Leu Trp
                        1               5                   10
gct ttg gca att ctg aca gtt ccc atg tat ttc ata gtg aca gag ggc      279
Ala Leu Ala Ile Leu Thr Val Pro Met Tyr Phe Ile Val Thr Glu Gly
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aga aaa aca tcc tgg ggt cta gaa aac gag gct tta att gtc aga tgc      327
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ccc caa aga gga ggt gcg att aac cct gtg gaa tgg tat tat tca aat      375
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Thr Asn Glu Arg Ile Pro Thr Gln Lys Arg Asn Arg Ile Phe Val Ser
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Arg Asp Arg Leu Lys Phe Leu Pro Ala Lys Val Glu Asp Ser Gly Ile
75                  80                  85                  90
tat acg tgt gtt atc aga agc cct gaa tcg att aag acc gga tct ttg      519
Tyr Thr Cys Val Ile Arg Ser Pro Glu Ser Ile Lys Thr Gly Ser Leu
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aat gtc acc ata tat aaa aga cca cca aac tgc aaa atc cct gat tac      567
Asn Val Thr Ile Tyr Lys Arg Pro Pro Asn Cys Lys Ile Pro Asp Tyr
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atg atg tac tcg aca gta gat gga tca gat aaa aat tcc aag ata aca      615
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tgt cca aca att gcc ttg tat aat tgg aca gcg cct gtt cag tgg ttt      663
Cys Pro Thr Ile Ala Leu Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe
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Lys Asn Cys Lys Ala Leu Gln Gly Pro Arg Phe Arg Ala His Met Ser
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tat ttg ttc att gac aaa gtg agt cat gtt gat gaa ggt gac tac aca      759
Tyr Leu Phe Ile Asp Lys Val Ser His Val Asp Glu Gly Asp Tyr Thr
                175                 180                 185
tgt cga ttc act cac acg gag aac gga acc aat tac att gtg act gcc      807
Cys Arg Phe Thr His Thr Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala
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acc aga tca ttc aca gtt gaa gaa aaa ggc ttc tct aca ttt cca gta      855
Thr Arg Ser Phe Thr Val Glu Glu Lys Gly Phe Ser Thr Phe Pro Val
        205                 210                 215
att aca aac cct cca cac aac tac aca gtg gaa gtg gaa ata gga aaa      903
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    220                 225                 230
aca gca aac att gcc tgc tca gct tgc ttt ggc aca gcc tct cag ttc      951
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235                 240                 245                 250
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Val Ala Val Leu Trp Gln Ile Asn Lys Thr Arg Ile Gly Ser Phe Gly
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aaa gca aga att caa gaa gag aaa ggc cca aat aaa agt tcc agc aat     1047
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Gly Met Ile Cys Leu Thr Ser Leu Leu Arg Ile Thr Gly Val Thr Asp
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aag gac ttc tcc ctg aaa tat gac tgt gtg gcc atg aac cat cac gga     1143
Lys Asp Phe Ser Leu Lys Tyr Asp Cys Val Ala Met Asn His His Gly
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Val Ile Arg His Pro Val Arg Leu Arg Arg Lys Gln Pro Ser Lys Glu
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Cys Leu Ser Gln Ile Ala
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gggattagtt ggccagtttc ctcaaatgtg tttttcatgt tgagaaagct ccttaaatct   1362
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Ile Asn Pro Val Glu Trp Tyr Tyr Ser Asn Thr Asn Glu Arg Ile Pro
    50                  55                  60
Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
65                  70                  75                  80
Leu Pro Ala Lys Val Glu Asp Ser Gly Ile Tyr Thr Cys Val Ile Arg
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Ser Pro Glu Ser Ile Lys Thr Gly Ser Leu Asn Val Thr Ile Tyr Lys
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Arg Pro Pro Asn Cys Lys Ile Pro Asp Tyr Met Met Tyr Ser Thr Val
        115                 120                 125
Asp Gly Ser Asp Lys Asn Ser Lys Ile Thr Cys Pro Thr Ile Ala Leu
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Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
145                 150                 155                 160
Gln Gly Pro Arg Phe Arg Ala His Met Ser Tyr Leu Phe Ile Asp Lys
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Val Ser His Val Asp Glu Gly Asp Tyr Thr Cys Arg Phe Thr His Thr
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Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
        195                 200                 205
Glu Glu Lys Gly Phe Ser Thr Phe Pro Val Ile Thr Asn Pro Pro His
    210                 215                 220
Asn Tyr Thr Val Glu Val Glu Ile Gly Lys Thr Ala Asn Ile Ala Cys
225                 230                 235                 240
Ser Ala Cys Phe Gly Thr Ala Ser Gln Phe Val Ala Val Leu Trp Gln
                245                 250                 255
Ile Asn Lys Thr Arg Ile Gly Ser Phe Gly Lys Ala Arg Ile Gln Glu
            260                 265                 270
Glu Lys Gly Pro Asn Lys Ser Ser Ser Asn Gly Met Ile Cys Leu Thr

        275                 280                 285
Ser Leu Leu Arg Ile Thr Gly Val Thr Asp Lys Asp Phe Ser Leu Lys
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Tyr Asp Cys Val Ala Met Asn His His Gly Val Ile Arg His Pro Val
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acatcagaca gcacacatca accgcctagt ggactcaccg ttaccttcct gtgccattgc    240
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Gly Leu Trp Ala Leu Ala Ile Leu Thr Val Pro Met Tyr Phe Ile Val
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aca gag ggc aga aaa aca tcc tgg ggt cta gaa aac gag gct tta att      391
Thr Glu Gly Arg Lys Thr Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile
    25                  30                  35
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Val Arg Cys Pro Gln Arg Gly Gly Ala Ile Asn Pro Val Glu Trp Tyr
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Tyr Ser Asn Thr Asn Glu Arg Ile Pro Thr Gln Lys Arg Asn Arg Ile
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ttc gtc tca aga gat cgt ctg aag ttt cta cca gcc aaa gtg gaa gac      535
Phe Val Ser Arg Asp Arg Leu Lys Phe Leu Pro Ala Lys Val Glu Asp
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Ser Gly Ile Tyr Thr Cys Val Ile Arg Ser Pro Glu Ser Ile Lys Thr
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Gly Ser Leu Asn Val Thr Ile Tyr Lys Arg Pro Pro Asn Cys Lys Ile
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cct gat tac atg atg tac tcg aca gta gat gga tca gat aaa aat tcc      679
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Gln Trp Phe Lys Asn Cys Lys Ala Leu Gln Gly Pro Arg Phe Arg Ala
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cac atg tcc tat ttg ttc att gac aaa gtg agt cat gtt gat gaa ggt      823
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        170                 175                 180
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Asp Tyr Thr Cys Arg Phe Thr His Thr Glu Asn Gly Thr Asn Tyr Ile
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Ile Gly Lys Thr Ala Asn Ile Ala Cys Ser Ala Cys Phe Gly Thr Ala
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tct ttt ggc aaa gca aga att caa gaa gag aaa ggc cca aat aaa agt     1111
Ser Phe Gly Lys Ala Arg Ile Gln Glu Glu Lys Gly Pro Asn Lys Ser
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cat cac gga gtg ata agg cac ccc gta aga ctg aga agg aaa caa cca     1255
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            315                 320                 325
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gag gtt gct ctg ttc tgg aga gat ata atg gca cct tac aaa acc cag     1399
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360                 365                 370                 375
aat gat gga aag ctc tat gat gct tac atc att tac cct cgg gtc ttc     1447
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cgg ggc agc gca gca ggg acc ggc tct gtg gag tac ttt gtt cac tac     1495
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act ctg ccc gac gtt ctc gaa aat aaa tgt ggc tac aag ttg tgc att     1543
Thr Leu Pro Asp Val Leu Glu Asn Lys Cys Gly Tyr Lys Leu Cys Ile
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tac ggg aga gac ctg ctg cct ggg caa gat gcg gcc act gtg gtg gaa     1591
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440                 445                 450                 455
atg atg cac agc aaa gag ttt gcc tat gag cag gag atc gcc ctg cac     1687
Met Met His Ser Lys Glu Phe Ala Tyr Glu Gln Glu Ile Ala Leu His
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agc gcc ctc atc cag aac aac tcc aag gtg att ctg att gaa atg gag     1735
Ser Ala Leu Ile Gln Asn Asn Ser Lys Val Ile Leu Ile Glu Met Glu
            475                 480                 485
cct atg ggt gag gca agc cga ctg cag ctt ggg gat ctg caa gat tct     1783
Pro Met Gly Glu Ala Ser Arg Leu Gln Leu Gly Asp Leu Gln Asp Ser
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ctc cag cat ctt gtg aaa atg cag ggg acc atc aag tgg agg gaa gac     1831
Leu Gln His Leu Val Lys Met Gln Gly Thr Ile Lys Trp Arg Glu Asp
    505                 510                 515
cac gtg gcc gac aaa cag tct cta agc tcc aaa ttc tgg aag cat gtg     1879
His Val Ala Asp Lys Gln Ser Leu Ser Ser Lys Phe Trp Lys His Val
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gcc gct ccg ttg agt ggc aag gtg tgc ttg gac ctg aaa cac ttt tga     1975
Ala Ala Pro Leu Ser Gly Lys Val Cys Leu Asp Leu Lys His Phe
            555                 560                 565
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    50                  55                  60
Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
65                  70                  75                  80
Leu Pro Ala Lys Val Glu Asp Ser Gly Ile Tyr Thr Cys Val Ile Arg
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Ser Pro Glu Ser Ile Lys Thr Gly Ser Leu Asn Val Thr Ile Tyr Lys
            100                 105                 110
Arg Pro Pro Asn Cys Lys Ile Pro Asp Tyr Met Met Tyr Ser Thr Val
        115                 120                 125
Asp Gly Ser Asp Lys Asn Ser Lys Ile Thr Cys Pro Thr Ile Ala Leu
    130                 135                 140
Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
145                 150                 155                 160
Gln Gly Pro Arg Phe Arg Ala His Met Ser Tyr Leu Phe Ile Asp Lys
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Val Ser His Val Asp Glu Gly Asp Tyr Thr Cys Arg Phe Thr His Thr
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Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
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Glu Glu Lys Gly Phe Ser Thr Phe Pro Val Ile Thr Asn Pro Pro His
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Asn Tyr Thr Val Glu Val Glu Ile Gly Lys Thr Ala Asn Ile Ala Cys
225                 230                 235                 240
Ser Ala Cys Phe Gly Thr Ala Ser Gln Phe Val Ala Val Leu Trp Gln
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Tyr Asp Cys Val Ala Met Asn His His Gly Val Ile Arg His Pro Val
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Arg Leu Arg Arg Lys Gln Pro Ile Asp His Gln Ser Thr Tyr Tyr Ile
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Val Ala Gly Cys Ser Leu Leu Leu Met Phe Ile Asn Val Leu Val Ile
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Val Leu Lys Val Phe Trp Ile Glu Val Ala Leu Phe Trp Arg Asp Ile
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Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu Pro Gly Gln
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Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser Arg Arg Gln
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            500                 505                 510
Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln Ser Leu Ser
        515                 520                 525
Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val Pro Lys Arg
    530                 535                 540
Pro Pro Lys Met Ala Ser Val Ala Ala Pro Leu Ser Gly Lys Val Cys
545                 550                 555                 560
Leu Asp Leu Lys His Phe
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## Claims

**1.** An *in vitro* method for:

evaluating the likelihood that a subject will benefit from treatment with an agent selected from the group consisting of an anti-inflammatory agent, an antithrombotic agent, an anti-platelet agent, a fibrinolytic agent, a lipid reducing agent, a direct thrombin inhibitor, a glycoprotein IIb/IIIa receptor inhibitor, a monoclonal or polyclonal anti-cellular adhesion molecule antibody that inhibits the ability of white blood cells to attach to cellular adhesion molecules, a calcium channel blocker, a beta-adrenergic receptor blocker, a cyclooxygenase-2 inhibitor, and a renin-angiotensin system inhibitor, wherein the method comprises:

obtaining a first level of soluble IL1RL-1 protein in a sample from a subject;
comparing the level of the soluble IL1RL-1 protein to a predetermined value specific for the diagnosis of a cardiovascular condition selected from myocardial infarction and heart failure, wherein the predetermined value is a single cut-off value, and wherein a level of the soluble IL1RL-1 protein equal to or above the predetermined cut-off value is indicative of whether the subject will benefit from treatment with said agent.

**2.** A method as claimed in claim 1, wherein the soluble IL1RL-1 protein is an expression product of a nucleic acid sequence as set out in SEQ ID NO: 1, or comprises the amino acid sequence set out in SEQ ID NO: 2.

**3.** The method of claim 1, wherein the beta-adrenergic receptor blocker is selected from the group consisting of: atenolol, acebutolol, alprenolol, betunolol, betaxolol, bunitrolol, carteolol, celiprolol, hedroxalol, indenolol, labetalol, levobunolol, mepindolol, methypranol, metindol, metoprolol, metrizoranolol, oxprenolol, pindolol, propranolol, practolol, sotalolnadolol, tiprenolol, tomalolol, timolol, bupranolol, penbutolol, trimepranol, 2-(3-(1,1-dimethylethyl)-amino-2-hydroxypropoxy)-3-pyridenecarbonitril hydrochloride, 1-butylamino-3-(2,5-dichlorophenoxy)-2-propanol, 1-isopropylamino-3-(4-(2-cyclopropylmethoxyethyl)phenoxy)-2-propanol, 3-isopropylamino-1-(7-methylindan-4-yloxy)-2-butanol, and 2-(3-t-butylamino-2-hydroxy-propylthio)-4-(5-carbamoyl-2-thienyl)thiazol,7-(2-hydroxy-3-t-butylaminpropoxy) phthalide.

**4.** The method of claim 1, wherein the renin-angiotensin system inhibitor is selected from the group consisting of: an angiotensin-converting enzyme (ACE) inhibitor, an angiotensin II receptor antagonist, an agent that activates the catabolism of angiotensin II, and an agent that prevents the synthesis of angiotensin I.

5. The method of claim 1 or 2, wherein the sample is blood or serum.

6. The method of claim 1 or 2, wherein the subject is free of symptoms calling for treatment with the agent.

7. The method of claim 1 or 2, wherein the subject is human.

**Patentansprüche**

1. In-vitro-Verfahren zum:

Feststellen der Wahrscheinlichkeit, dass ein Individuum von einer Behandlung mit einem Mittel profitiert, das aus der aus einem entzündungshemmenden Mittel, einem antithrombotischen Mittel, einem Thrombozytenaggregationshemmer, einem Fibrinolytikum, einem lipidreduzierenden Mittel, einem direkten Thrombininhibitor, einem Glykoprotein-IIb/IIIa-Rezeptorinhibitor, einem monoklonalen oder polyklonalen Antikörper gegen zelluläre Adhäsionsmoleküle, der die Fähigkeit weißer Blutkörperchen hemmt, sich an zelluläre Adhäsionsmoleküle anzulagern, einem Calciumkanalblocker, einem Beta-Adrenozeptorblocker, einem Cyclooxygenase-2-Inhibitor und einem Inhibitor des Renin-Angiotensin-Systems bestehenden Gruppe ausgewählt ist, wobei das Verfahren Folgendes umfasst:

Ermitteln eines ersten Spiegels an löslichem IL1RL-1-Protein in einer Probe von einem Individuum;
Vergleichen des Spiegels an löslichem IL1RL-1-Protein mit einem vorher festgelegten Wert, der für die Diagnose eines Herz-Kreislauf-Leidens spezifisch ist, das aus der aus Myokardinfarkt und Herzversagen bestehenden Gruppe ausgewählt ist, wobei es sich bei dem vorher festgelegten Wert um einen einzelnen Ausschlussgrenzwert handelt und wobei ein Spiegel an löslichem IL1RL-1-Protein größer gleich dem vorher festgelegten Ausschlussgrenzwert anzeigt, ob das Individuum von der Behandlung mit dem Mittel profitieren wird.

2. Verfahren nach Anspruch 1, wobei es sich bei dem löslichen IL1RL-1-Protein um ein Expressionsprodukt einer Nukleinsäuresequenz gemäß SEQ ID NO: 1 handelt oder es die Aminosäuresequenz gemäß SEQ ID NO: 2 umfasst.

3. Verfahren nach Anspruch 1, wobei der Beta-Adrenozeptorblocker ausgewählt ist aus der Gruppe bestehend aus: Atenolol, Acebutolol, Alprenolol, Befunolol, Betaxolol, Bunitrolol, Carteolol, Celiprolol, Hedroxalol, Indenolol, Labetalol, Levobunolol, Mepindolol, Metipranol, Metindol, Metoprolol, Metuzoranolol, Oxprenolol, Pindolol, Propranolol, Practolol, Sotalolnadolol, Tiprenolol, Tomalolol, Timolol, Bupranolol, Penbutolol, Trimepranol, 2-(3-(1,1-Dimethylethyl)-amino-2-hydroxypropoxy)-3-pyridencarbonitrilhydrochlorid, 1-Butylamino-3-(2,5-dichlorphenoxy)-2-propanol, 1-Isopropylamino-3-(4-(2-cyclopropylmethoxyethyl)phenoxy)-2-propanol, 3-Isopropylamino-1-(7-methylindan-4-yloxy)-2-butanol und 2-(3-t-Butylamino-2-hydroxypropylthio)-4-(5-carbamoyl-2-thienyl)thiazol, 7-(2-Hydroxy-3-t-butylaminpropoxy)phthalid.

4. Verfahren nach Anspruch 1, wobei der Inhibitor des Renin-Angiotensin-Systems ausgewählt ist aus der Gruppe bestehend aus: einem Inhibitor des Angiotensin-konvertierenden Enzyms (ACE), einem Angiotensin-II-Rezeptor-Antagonisten, einem Mittel, das den Abbau von Angiotensin II aktiviert, und einem Mittel, das die Synthese von Angiotensin I verhindert.

5. Verfahren nach Anspruch 1 oder 2, wobei es sich bei der Probe um Blut oder Serum handelt.

6. Verfahren nach Anspruch 1 oder 2, wobei das Individuum frei von Symptomen ist, die eine Behandlung mit dem Mittel erfordern.

7. Verfahren nach Anspruch 1 oder 2, wobei es sich bei dem Individuum um einen Menschen handelt.

**Revendications**

1. Procédé *in vitro* pour :

évaluer la probabilité pour un sujet de bénéficier d'un traitement avec un agent sélectionné dans le groupe

comprenant : un agent anti-inflammatoire, un agent antithrombotique, un agent antithrombocyte, un agent fibrinolytique, un agent lipo-réducteur, un inhibiteur de thrombine direct, un inhibiteur de récepteur de glycoprotéine IIb/IIIa, un anticorps moléculaire anti-adhérence cellulaire monoclonal ou polyclonal qui inhibe l'aptitude des globules blancs à se fixer aux molécules d'adhérence cellulaire, un inhibiteur calcique, un inhibiteur de récepteur bêta-adrénergénique, un inhibiteur de cyclooxygénase-2, et un inhibiteur de système rénine-angiotensine, le procédé comprenant :

l'obtention d'un premier niveau de protéine IL1RL-1 soluble dans un échantillon prélevé chez un sujet ;
la comparaison du niveau de la protéine IL1RL-1 soluble à une valeur prédéterminée spécifique pour le diagnostic d'une affection cardiovasculaire sélectionnée entre infarctus du myocarde et insuffisance cardiaque, la valeur prédéterminée étant une seule valeur limite, et un niveau de la protéine IL1RL-1 soluble égal ou supérieur à la valeur limite prédéterminée indiquant si le sujet bénéficiera d'un traitement avec ledit agent.

**2.** Procédé selon la revendication 1, dans lequel la protéine IL1RL-1 soluble est un produit de l'expression d'une séquence d'acides nucléiques définie dans SEQ ID NO: 1, ou comprend la séquence d'acides aminés définie dans SEQ ID NO: 2.

**3.** Procédé selon la revendication 1, dans lequel l'inhibiteur de récepteur bêta-adrénergénique est sélectionné dans le groupe comprenant : aténolol, acébutolol, alprénolol, béfunolol, bétaxolol, bunitrolol, cartéolol, céliprolol, hédroxalol, indénolol, labétalol, lévobunolol, mépindolol, méthypranol, métindol, métoprolol, métrizoranolol, oxprénolol, pindolol, propranolol, practolol, sotalolnadolol, tiprénolol, tomalolol, timolol, bupranolol, penbutolol, tumépranol, 2-(3-(1,1-diméthylethyl)-amino-2-hydroxypropoxy)-3-pyridènecarbonitlil hydrochloride, 1-butylamino-3-(2,5-dichlorophénoxy)-2-propanol, 1-isopropylamino-3-(4-(2-cyclopropylméthoxyéthyl)phénoxy)-2-propanol, 3-isopropylamino-1-(7-méthylindan-4-yloxy)-2-butanol, et 2-(3-t-butylamino-2-hydroxy-propylthio)-4-(5-carbamoyl-2-thienyl)thiazol,7-(2-hydroxy-3-t-but ylaminpropoxy) phthalide.

**4.** Procédé selon la revendication 1, dans lequel l'inhibiteur de système rénine-angiotensine est sélectionné dans le groupe comprenant : un inhibiteur de l'enzyme de conversion de l'angiotensine (ECA), un antagoniste de récepteur d'angiotensine II, un agent qui active le catabolisme de l'angiotensine II, et un agent qui empêche la synthèse de l'angiotensine I.

**5.** Procédé selon la revendication 1 ou 2, dans lequel l'échantillon est du sang ou du sérum.

**6.** Procédé selon la revendication 1 ou 2, dans lequel le sujet est exempt de symptômes nécessitant un traitement avec l'agent.

**7.** Procédé selon la revendication 1 ou 2, dans lequel le sujet est humain.

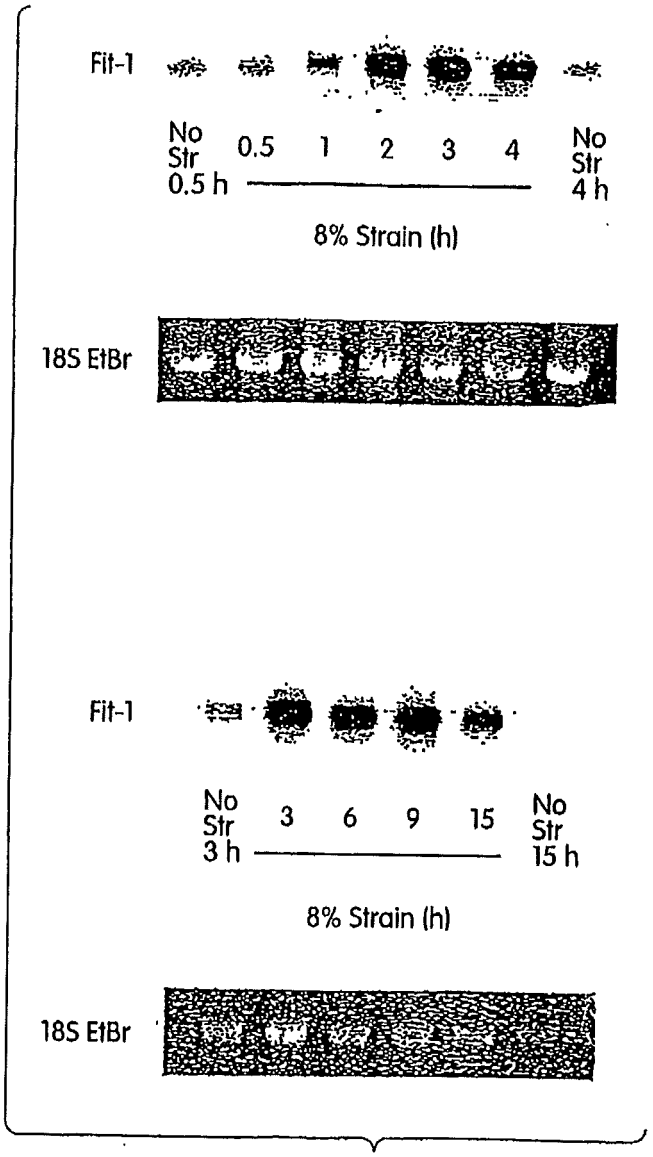
Fit-1
No Str 0.5 h
0.5
1
2
3
4
No Str 4 h
8% Strain (h)
18S EtBr
Fit-1
No Str 3 h
3
6
9
15
No Str 15 h
8% Strain (h)
18S EtBr

Fig. 1

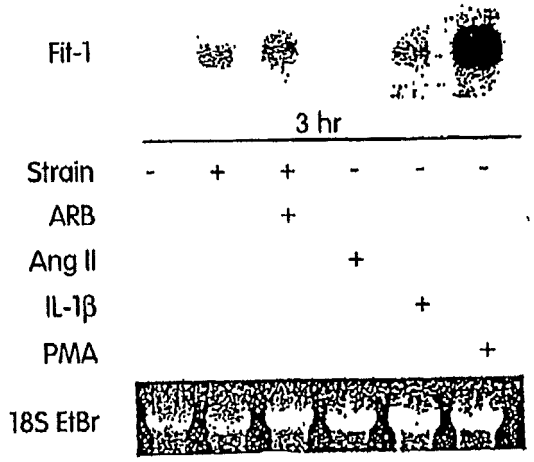
Fit-1
3 hr
Strain - + + - - -
ARB +
Ang II +
IL-1β +
PMA +
18S EtBr

Fig. 2

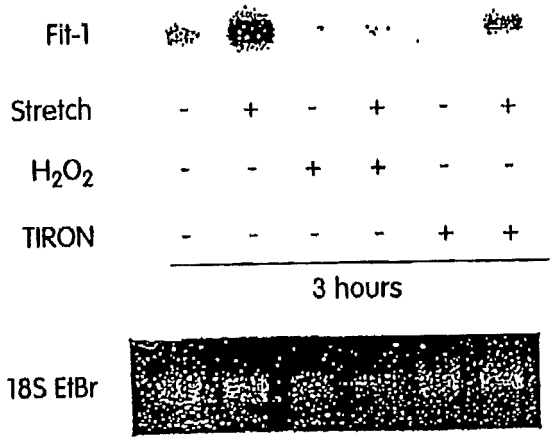
Fit-1
Stretch - + - + - +
$H_2O_2$ - - + + - -
TIRON - - - - + +
3 hours
18S EtBr

Fig. 3

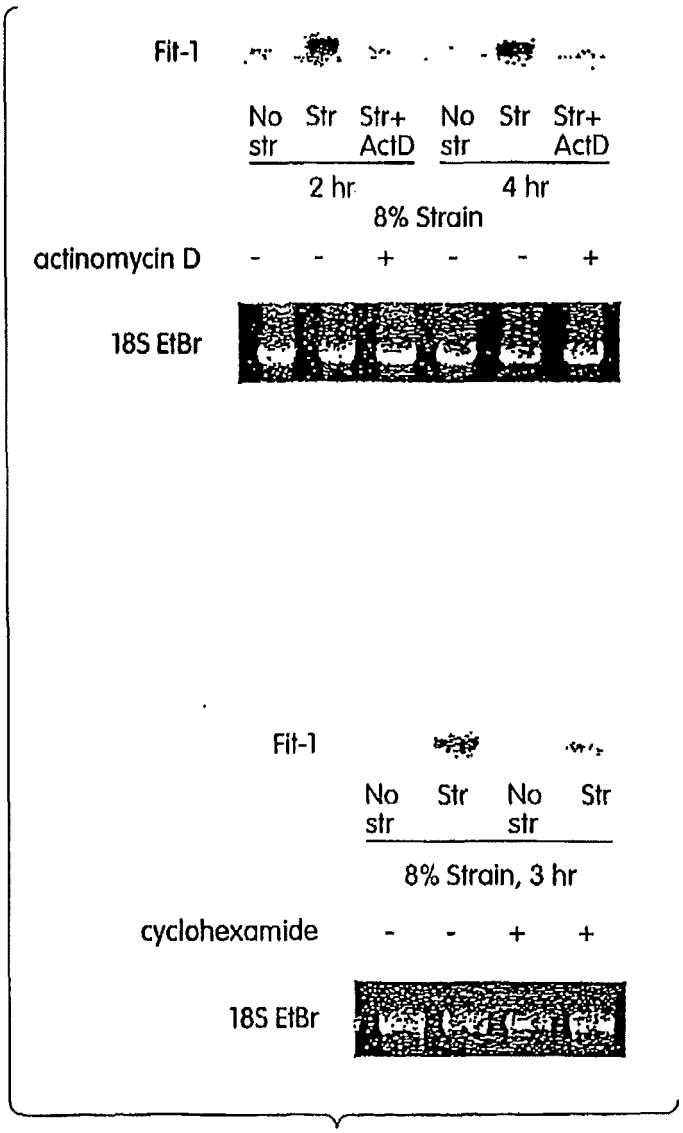
Fit-1
No str
Str
Str+ ActD
No str
Str
Str+ ActD
2 hr
4 hr
8% Strain
actinomycin D
- - + - - +
18S EtBr
Fit-1
No str
Str
No str
Str
8% Strain, 3 hr
cyclohexamide
- - + +
18S EtBr

Fig. 4

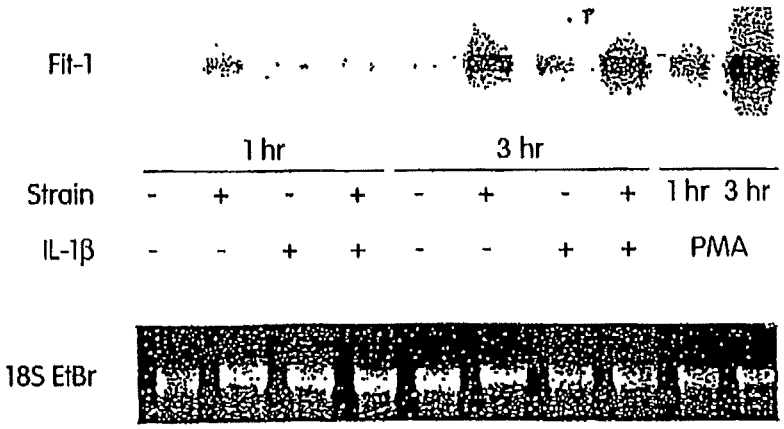
Flt-1
1 hr
3 hr
Strain - + - + - + - + 1 hr 3 hr
IL-1β - - + + - - + + PMA
18S EtBr

Fig. 5

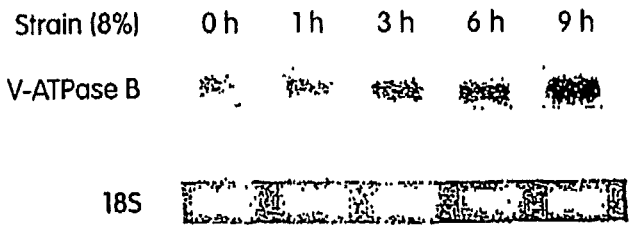
Strain (8%) 0 h 1 h 3 h 6 h 9 h
V-ATPase B
18S

Fig. 6

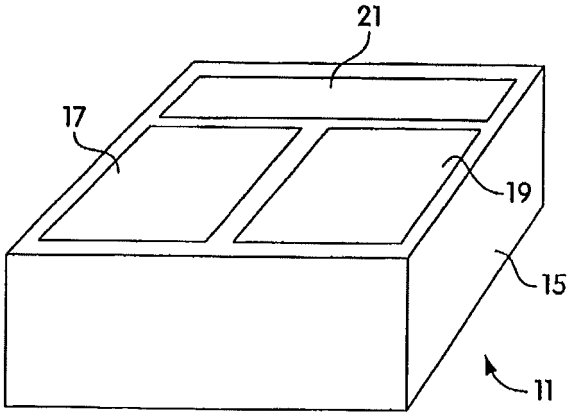


Fig. 7

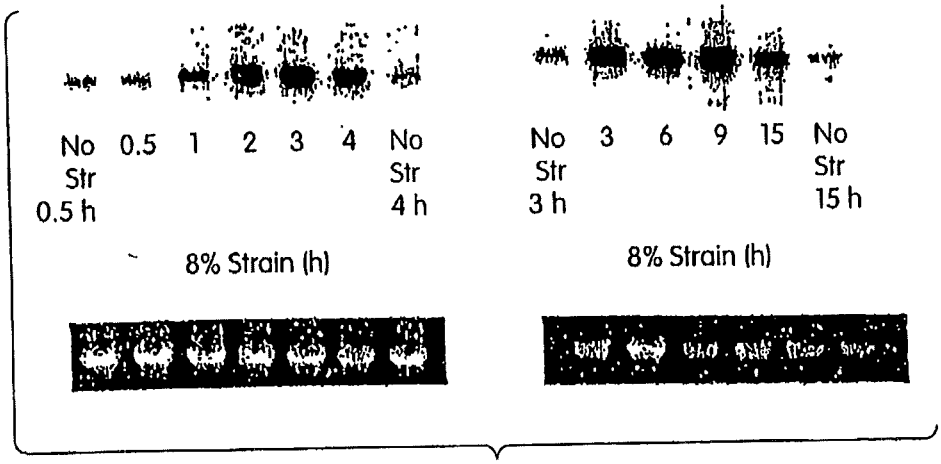
No Str 0.5 h
0.5
1
2
3
4
No Str 4 h
8% Strain (h)
No Str 3 h
3
6
9
15
No Str 15 h
8% Strain (h)

Fig. 8

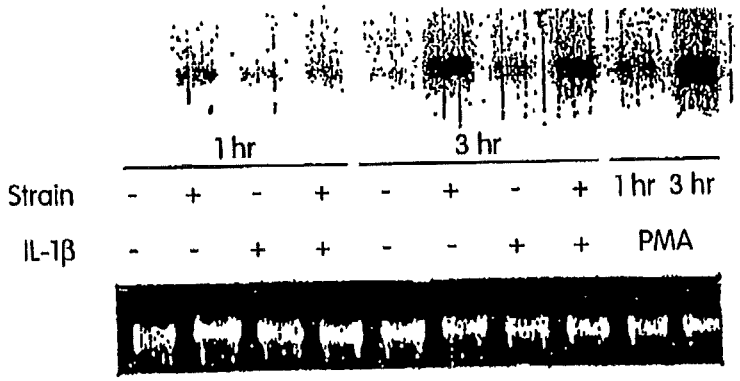
1 hr
3 hr
Strain
- + - + - + - + 1 hr 3 hr
IL-1β
- - + + - - + + PMA

Fig. 9

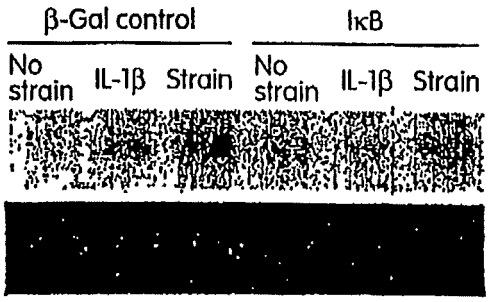
β-Gal control
IκB
No strain
IL-1β
Strain
No strain
IL-1β
Strain

Fig. 10

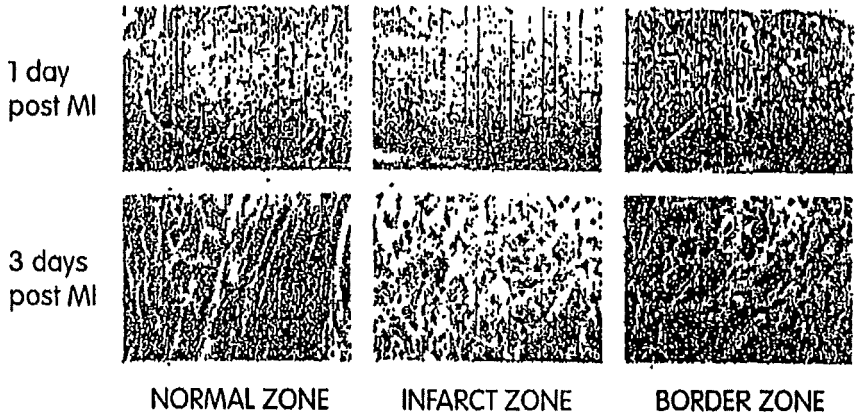
1 day post MI
3 days post MI
NORMAL ZONE
INFARCT ZONE
BORDER ZONE

Fig. 11

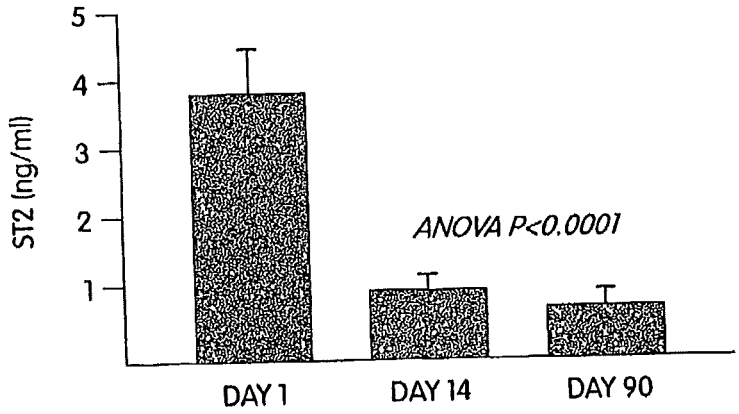


Fig. 12A

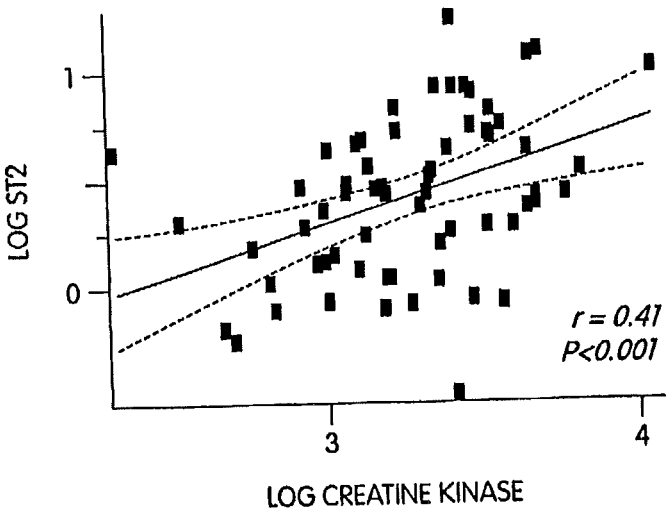


Fig. 12B

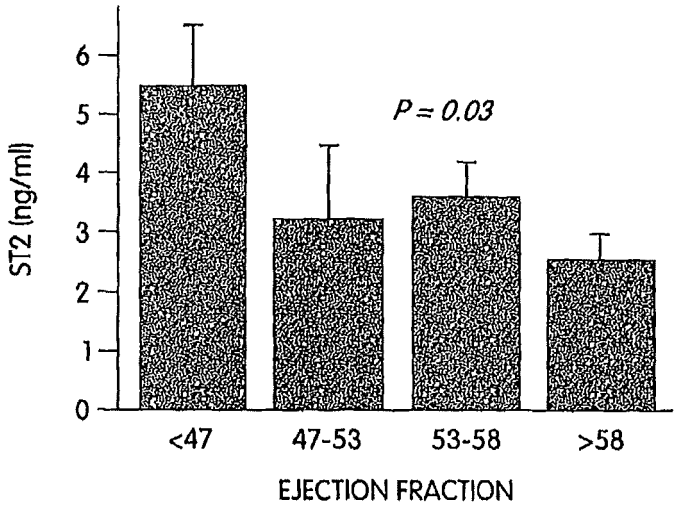


Fig. 12C

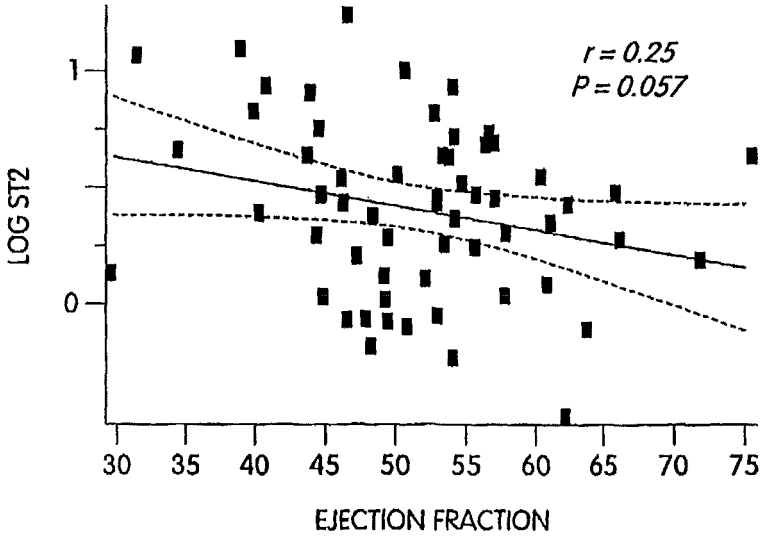


Fig. 12D

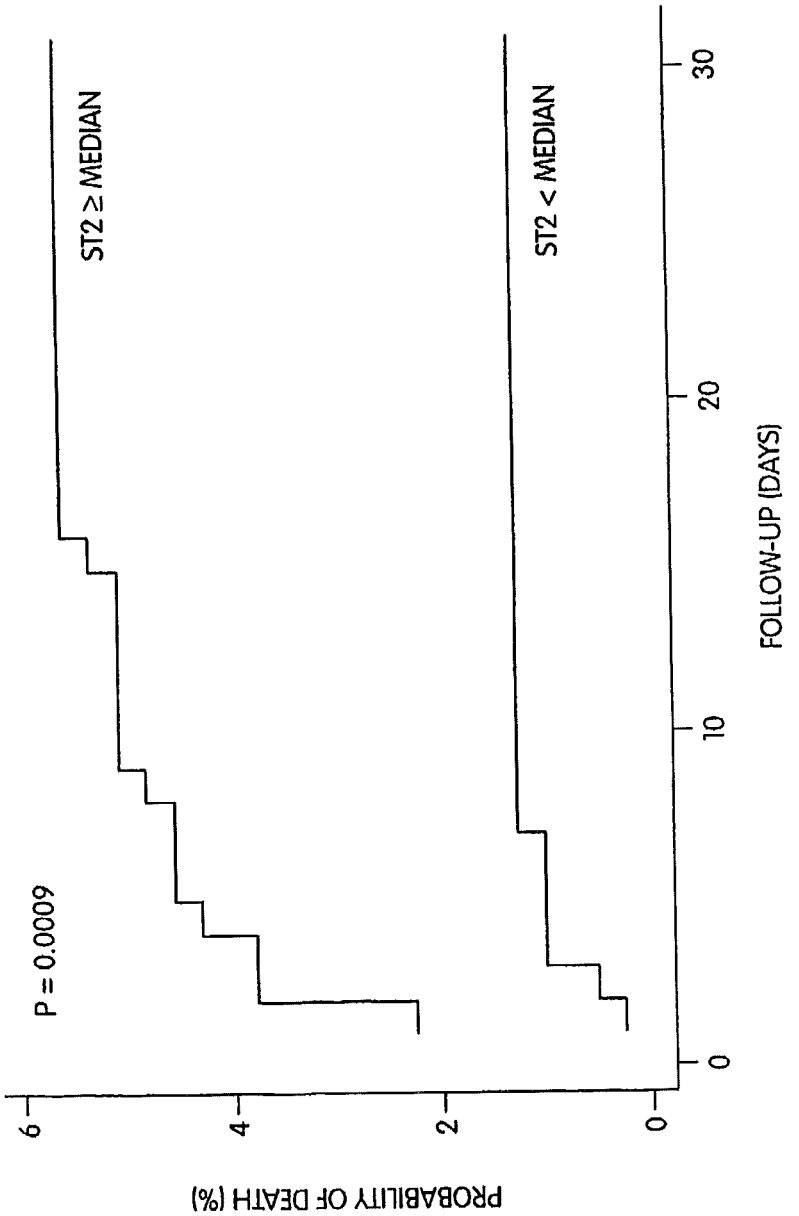
PROBABILITY OF DEATH (%)
0
2
4
6
0
10
20
30
FOLLOW-UP (DAYS)
ST2 ≥ MEDIAN
ST2 < MEDIAN
P = 0.0009

Fig. 13

## REFERENCES CITED IN THE DESCRIPTION

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摘要(译)

本发明涉及用于诊断和治疗心血管疾病的方法和组合物。更具体地，本发明涉及可用于诊断和/或治疗心血管疾病的分离的分子，包括心脏肥大，心肌梗塞，中风，动脉硬化和心力衰竭。