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Heart and skeleton muscle specific nucleic acid, the production and use thereof

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(54) Title: HEART AND SKELETON MUSCLE SPECIFIC NUCLEIC ACID, THE PRODUCTION AND USE THEREOF (54) Bezeichnung: HERZ- UND SKELETTMUSKEL-SPEZIFISCHE NUKLEINSÄURE, IHRE HERSTELLUNG UND VERWENDUNG (57) Abstract The invention relates to a nucleic acid which is specific to the heart and skeleton muscle, to the production and use thereof as a diagnostic agent, a medicament and as a test for identifying functional interactors. (57) Zusammenfassung Die Erfindung betrifft eine Herz- und Skelettmuskel-spezifische Nukleinsäure, ihre Herstellung und Verwendung als Diagnostikum, Arzneimittel und Test zur Identifizierung funktioneller Interaktoren.		



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Abstract

The invention relates to a myocardium- and skeletal muscle-specific nucleic acid, its preparation and use as diagnostic aid, medicinal product and test for identifying functional interactors.



5 **Myocardium- and skeletal muscle-specific nucleic acid,
 its preparation and use**

 The invention relates to a nucleic acid which
is expressed in human myocardium and skeletal muscle,
to its preparation and use as diagnostic aid, medicinal
10 product and test for identifying functional
interactors.

 The heart is a muscular hollow organ which has
the task of keeping the bloodstream in the vessels in
motion by alternating contraction (systole) and
15 relaxation (diastole) of atria and ventricles.

 The muscle of the heart, the myocardium, is
composed of specialized striped muscle cells between
which there is connective tissue. Each cell has a
central nucleus, is bounded by the plasma membrane, the
20 sarcolemma, and contains numerous contractile
myofibrils which are separated irregularly by
sarcoplasma. The contractile substance of the heart is
formed by long parallel myofibrils. Each myofibril is
divided into several identical structural and
25 functional units, the sarcomeres. The sarcomeres in
turn are composed of the thin filaments which mainly
consist of actin, tropomyosin and troponin, and the
thick filaments which mainly consist of myosin.

 The molecular mechanism of muscle contraction
30 is based on a cyclic attachment and detachment of the
globular myosin heads by the F actin filaments. On
electrical stimulation of the myocardium, Ca^{2+} is
released from the sarcoplasmic reticulum which
influences, through an allosteric reaction, the
35 troponin complex and tropomyosin, and thus opens the
way for contact of the actin filament with the myosin



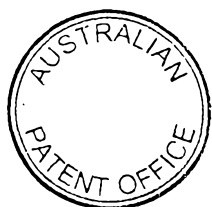
head. The attachment causes a conformational change in the myosin which thus pulls the actin filament along on itself. ATP is needed to reverse this conformational change and return to the start of a contraction cycle.

5 Short-term adjustment of the activity of the myocardium to the particular perfusion requirement, that is to say blood flow requirement, of the body is possible by nervous and hormonal regulation measures. It is thus possible to increase both the force of
10 contraction and the rate of contraction. Long-term overstrain results in physiological transformation processes in the myocardium, which are characterized mainly by an increase in myofibrils (myocyte hypertrophy).

15 If the myocardium is damaged, the originally physiological adaptation mechanisms often lead in the long term to pathophysiological states which develop into chronic cardiac insufficiency, that is to say cardiac weakness, and usually end with acute heart
20 failure. In cases of severe chronic insufficiency, the heart may no longer respond appropriately to changed output requirements, and even slight physical exertion leads to exhaustion and shortness of breath.

 Damage to the myocardium results from
25 ischaemia, that is to say depletion of blood, caused by coronary disease, bacterial or viral infections, toxins, metabolic abnormalities, autoimmune diseases or genetic defects. Therapeutic measures are currently aimed at strengthening the force of contraction and
30 controlling the compensatory neuronal and hormonal compensation mechanisms. Despite this treatment, the mortality from this disease remains high (35-50% in the first 5 years after diagnosis). Cardiac insufficiency is the main cause of death in the world. The only
35 causal therapy is a heart transplant.

 The molecular changes in chronic cardiac insufficiency are only inadequately known. In



particular, the genetic changes underlying cardiac insufficiency are substantially unknown. The question of why secondary damage by toxins or viruses leads to cardiac insufficiency in some people but not in others
5 also remains unanswered.

The present invention is thus based on the object of identifying and isolating genes which are at least partly responsible for, if not in fact the causes of, genetically related cardiac disorders.

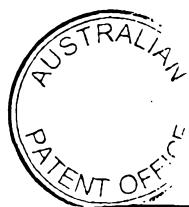
10 Surprisingly, a gene has now been found, in a human cardiac tissue cDNA bank, which is expressed more strongly in insufficient cardiac tissue than in healthy cardiac tissue and thus is causally connected with a genetically related cardiac insufficiency. A so-called
15 EST (expressed sequence tag) already exists for this gene, although it is faulty and no function at all can be assigned to it (Tanaka, T. et al. (1996) Genomics, 35, 231-235; EMBL AC:CO4498; clone 3NHC3467).

One aspect of the invention is therefore a
20 nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, preferably at least 10 nucleotides, in particular at least 15 nucleotides, especially at least
25 20 nucleotides, except a nucleic acid having the sequence:

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1  GCCAACACGC ANTCCGACGA CAGTGCAGCC ATGGTCATTG CAGAGATGCN TCAAAGTCAA
61 TGAGCACATC ACCAACGTAA ACGTCGAGTC CAACTTCATA ACGGGAAAGG GGATCCTGGC
121 CATCATGAGA GCTCTCCAGC ACAACACGGT GCTCACGGAG CTGCGTTTCC ATAACCAGAG
181 GCACATCATG GGCAGCCAGG TGGAAATGGA GATTGTCAAG CTNCTGAAGG AGAACACGAC
241 GCTNCTGAGG CTGGGNTACC ATTTTNAACT CCCAGGACC
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in which N denotes A, T, G or C.

The nucleic acid according to the invention was
30 isolated from a human cardiac tissue cDNA bank and sequenced. For this, firstly complete RNA was isolated by standard methods from a healthy and insufficient cardiac sample and transcribed with the aid of a



3'anchor primer mixture, for example a 5'-T₁₂ACN-3' primer, in which N denotes any deoxyribonucleotide, and reverse transcriptase into c-DNA. The cDNA was then amplified with a method based on the so-called differential display method of Liang and Pardee (Liang, P. & Pardee, A. (1992) *Science* 257, 967-970) under specific PCR conditions with the aid of a 3' primer, for example a T₁₂ACN primer, and of an arbitrarily selected 5'-decamer primer, for example a 5'-CCTTCTACCC-3' decamer primer. It was possible thereby to amplify a 321 base pair (bp)-long DNA fragment which is surprisingly present not in the healthy heart sample but distinctly in the insufficient heart sample. This was surprising because the conventional methods such as the differential display method or else subtractive cDNA gene banks are associated with the problem of redundancy, of under-representation and of false-positive clones. In particular, it is possible to identify the gene products of weakly expressed genes only under special conditions. It is therefore also not astonishing that the hit rate is generally very low (10-20%) and, for example in the differential display method, also depends on the chosen PCR conditions, the primer length or, for example in the production of subtractive banks, on the hybridization temperature. The complete gene was then isolated from a cDNA gene bank with the aid of the found DNA fragment and sequenced.

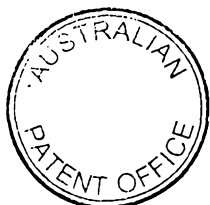
In every case it is necessary to find out by further methods whether the found cDNA can be assigned to an active and/or tissue-specific gene. Hence mRNAs from various human tissues were hybridized with the found DNA fragment in a so-called Northern blot, and the amount of bound m-RNA was determined, for example, via the radiolabelling of the DNA fragment. This experiment led to detection of the corresponding RNA in particular in striped muscle, that is to say myocardial



and skeletal muscle tissue, and very weakly in prostate tissue. In a further experiment comparing between healthy and insufficient cardiac tissues, increased expression was detected, for example expression of the
5 RNAs increased by about 35%, in insufficient tissue by comparison with healthy tissue. It was possible to demonstrate in particular that a relatively small RNA species preferentially shows increased expression in insufficient tissue by comparison with healthy tissue.
10 The increased expression of the relatively small RNA species is readily evident for example in the Northern blot in the form of a double band (see Fig. 5b).

Comparison of the derived polypeptide sequence with a protein database additionally revealed a certain
15 relationship (homology) with the protein tropomodulin (see Fig. 4). Tropomodulin is known to be a polypeptide which in chicken cardiomyocytes has an influence on the development of the myofibrils and the contractility of the cells (Gregorio et al. (1995) *Nature* 377, 83-86).
20 This protein binds on the one hand to tropomyosin, and on the other hand to the actin filaments, but is not itself regulated in its activity. The derived polypeptide according to the invention likewise has some of the structural features of tropomodulins, such
25 as, for example, a tropomyosin binding domain. In contrast to tropomodulin, the polypeptide according to the invention has additional structural features indicating regulation of the activity of the polypeptide by so-called tyrosine kinases (see Fig. 4).

30 The term "functional variant" therefore means for the purpose of the present invention polypeptides which are functionally related to the polypeptide according to the invention, that is to say can likewise be referred to as a regulable modulator of the
35 contractility of myocardial cells, are expressed in striped muscle, preferably in myocardial, skeletal muscle and/or prostate tissue, especially in myocardial



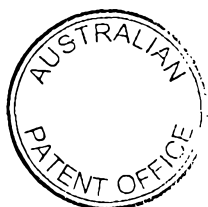
and/or skeletal muscle and, in particular, in myocardial cells, have structural features of tropomodulin, such as, for example, one or more tropomyosin binding domains, and/or whose activity can
5 be regulated by tyrosine kinases. Examples of functional variants are the corresponding polypeptides derived from other organisms than humans, preferably from non-human mammals such as, for example, monkeys.

In the wider sense, the term „functional
10 variant“ includes polypeptides which have a sequence homology, in particular a sequence identity, of about 70%, preferably about 80%, in particular about 90%, especially about 95%, with the polypeptide having the amino acid sequence shown in Fig. 4. These include, for
15 example, polypeptides encoded by a nucleic acid which is isolated from non-heart-specific tissue, for example skeletal muscle tissue, but which has, after expression in a heart-specific cell, the identified function(s). These furthermore include deletions of the polypeptide
20 in the region of about 1-60, preferably of about 1-30, in particular of about 1-15, especially of about 1-5, amino acids. For example, the first amino acid methionine can be absent with negligible alteration in the function of the polypeptide. These also include
25 fusion proteins which comprise the above-described polypeptides according to the invention, it being possible for the fusion proteins themselves to have the function of a regulable modulator of the contractility of myocardial cells, or to acquire the specific
30 function only after elimination of the fusion portion. They particularly include fusion proteins with a content of, in particular, non-heart-specific sequences of about 1-200, preferably about 1-150, in particular about 1-100, especially about 1-50, amino acids.
35 Examples of non-heart-specific peptide sequences are prokaryotic peptide sequences which may be derived, for example, from the galactosidase of E. coli.



The nucleic acid according to the invention is generally a DNA or RNA, preferably a DNA. Preferred for expression of the relevant gene is in general a double-stranded DNA and for use as probe is a single-stranded DNA. Particular preference is given to a double- or single-stranded DNA having a nucleic acid sequence as shown in Fig. 1, 2 or 3 and the parts thereof described in detail above, with the DNA region coding for the polypeptide being particularly preferred. This region starts with the nucleic acids "ATG" coding for methionine at position 89 to "TAG" coding for "amber" (stop) at position 1747.

The nucleic acid according to the invention can, for example, be chemically synthesized on the basis of the sequences disclosed in Figs. 1-3 or on the basis of the polypeptide sequence disclosed in Fig. 4 by use of the genetic code, for example by the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) *Chemical Reviews*, 90, 543-584, No. 4). Another possibility for obtaining the nucleic acid according to the invention is isolation from a suitable gene bank, for example from a heart-specific gene bank, using a suitable probe (see, for example, J. Sambrook et al., (1989), *Molecular Cloning. A Laboratory Manual* 2nd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Suitable as probe are, for example, single-stranded DNA fragments with a length of about 100-1000 nucleotides, preferably with a length of about 200-500 nucleotides, in particular with a length of about 300-400 nucleotides, whose sequence can be derived from the nucleic acid sequences shown in Figs. 1-3. One example of a probe is the DNA fragment of Example 1, which is 321 bp in size and corresponds to the underlined region in Fig. 1, using which the nucleic acid according to the invention has already been isolated successfully from human cardiac tissue (see Example 2).

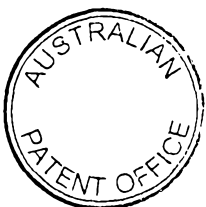


The nucleic acid according to the invention is normally present in a vector, preferably in an expression vector or vector effective for gene therapy. The vector effective for gene therapy preferably
5 contains heart-specific regulatory sequences such as, for example, the troponin C (cTNC) promoter (see, for example, Parmacek, M.S. et al. (1990) *J. Biol. Chem.* 265 (26) 15970-15976 and Parmacek, M.S. et al. (1992) *Mol. Cell Biol.* 12(5), 1967-1976), which is
10 functionally connected to the nucleic acid according to the invention.

The expression vectors may be prokaryotic or eukaryotic expression vectors. Examples of prokaryotic expression vectors for expression in *E. coli* are, for
15 example, the vectors pGEM or pUC derivatives, and of eukaryotic expression vectors for expression in *Saccharomyces cerevisiae* are, for example, the vectors p426Met25 or p426GAL1 (Mumberg et al. (1994) *Nucl. Acids Res.*, 22, 5767-5768) for expression in insect
20 cells are, for example, baculovirus vectors as disclosed in EP-B1 0 127 839 or EP-B1 0 549 721, and for expression in mammalian cells are, for example, the vectors Rc/CMV and Rc/RSV or SV40 vectors, which are all generally available.

The expression vectors generally also contain promoters suitable for the particular host cell, such as, for example, the trp promoter for expression in *E. coli* (see, for example, EP-B1 0 154 133), the ADH2 promoter for expression in yeasts (Russell et al.
25 (1983), *J. Biol. Chem.* 258, 2674-2682), the baculovirus polyhedrin promoter for expression in insect cells (see, for example, EP-B1 0 127 839) or the SV40 early promoter or LTR promoters, for example of MMTV (mouse mammary tumour virus; Lee et al. (1981) *Nature* 214,
30 228-232).

Examples of vectors effective for gene therapy are virus vectors, preferably adenovirus vectors, in



particular replication-deficient adenovirus vectors, or adeno-associated virus vectors, for example an adeno-associated virus vector which consists exclusively of two inverted terminal repeats (ITR).

5 An adenovirus vector and, in particular, a replication-deficient adenovirus vector are particularly preferred for the following reasons.

 The human adenovirus belongs to the class of double-stranded DNA viruses with a genome of about 36
10 kilobase pairs (kb). The viral DNA codes for about 2700 different gene products, a distinction being made between early ("early genes") and late ("late genes"). The "early genes" are divided into four transcriptional units E1 to E4. The late gene products code for the
15 capsid proteins. It is possible to distinguish immunologically at least 42 different adenoviruses and subgroups A to F, all of which are suitable for the present invention. A precondition for transcription of the viral genes is expression of the E1 region which
20 codes for a transactivator of adenoviral gene expression. This dependence of the expression of all subsequent viral genes on the E1 transactivator can be utilized to construct adenoviral vectors not capable of replication (see, for example, McGrory, W.J. et al.
25 (1988) *Viol.* 163, 614-617 and Gluzman, Y. et al. (1982) in "Eukaryotic Viral Vectors" (Gluzman, Y. ed.) 187 - 192, Cold Spring Harbor Press, Cold Spring Harbor, New York). In adenoviral vectors, especially of type 5 (for sequence, see Chroboczek, J. et al. (1992)
30 *Viol.* 186, 280-285) and especially of subgroup C, in general the E1 gene region is replaced by a foreign gene with its own promoter or by the nucleic acid construct according to the invention. Replacement of the E1 gene region which is a precondition for
35 expression of the downstream adenoviral genes results in an adenovirus not capable of replication. These



viruses are then able to replicate only in a cell line which replaces the missing E1 genes.

Replication-deficient adenoviruses are therefore generally formed by homologous recombination in the so-called 293 cell line (human embryonic kidney cell line) which has a copy of the E1 region stably integrated into the genome. For this purpose, the nucleic acid according to the invention is cloned into recombinant adenoviral plasmids under the control of its own promoter, for example the troponin C promoter mentioned above. Homologous recombination then takes place with an E1-deficient adenoviral genome such as, for example, dl327 or dell324 (adenovirus 5) in the 293 helper cell line. Where recombination is successful, viral plaques are harvested. The replication-deficient viruses produced in this way are employed in high titres (for example 10^9 to 10^{11} plaque forming units) for infecting the cell culture or for somatic gene therapy.

The exact site of insertion of the nucleic acid according to the invention into the adenoviral genome is in general not critical. It is, for example, also possible to clone the nucleic acid according to the invention in place of the deleted E3 gene (Karlsson, S. et al. (1986), *EMBO J.* 5, 2377 - 2385).

However, it is preferred for the E1 region or parts thereof, for example the E1A or E1B region (see, for example, WO 95/00655), to be replaced by the nucleic acid according to the invention, especially when the E3 region is also deleted.

However, the present invention is not confined to the adenoviral vector system; on the contrary, adeno-associated virus vectors are also particularly suitable in combination with the nucleic acid according to the invention for the following reasons.

The AAV virus belongs to the family of parvoviruses. These are distinguished by an



icosahedral, non-enveloped capsid which has a diameter of 18 to 30 nm and which contains a linear, single-stranded DNA of about 5 kb. For efficient replication of AAV, coinfection of the host cell with helper
5 viruses is necessary. Examples of suitable helpers are adenoviruses (Ad5 or Ad2), herpesviruses and vaccinia-viruses (Muzyczka, N. (1992) *Curr. Top. Microbiol. Immunol.* 158, 97-129). In the absence of a helper virus, AAV passes into a latency state where the virus
10 genome is able to integrate stably into the host cell genome. The property of AAV integrating into the host genome makes it particularly interesting as transduction vector for mammalian cells. Generally sufficient for the vector functions are the two
15 inverted terminal repeats (ITR: see, for example, WO 95/23867) which are about 145 bp long. They carry the signals necessary in "cis" for replication, packaging and integration into the host cell genome. For packaging into recombinant vector particles, a vector
20 plasmid which harbours the genes for non-structural proteins (rep proteins) and for structural proteins (cap proteins) is transfected into adenovirus-infected cells. After a few days, a cell-free lysate containing, besides the recombinant AAV particles, also
25 adenoviruses is prepared. The adenoviruses can advantageously be removed by heating at 56°C or by banding in a caesium chloride gradient. It is possible with this cotransfection method to achieve rAAV titres of 10^5 to 10^6 IE/ml. Contamination by wild-type viruses
30 is below the detection limit if the packaging plasmid and the vector plasmid have no overlapping sequences (Samulski, R.J. (1989) *J. Virol.* 63, 3822 - 3828).

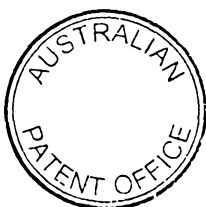
Transfer of the nucleic acid according to the invention into somatic cells can be effected by AAV
35 into resting, differentiated cells, which is particularly advantageous for gene therapy of the heart. The ability to integrate which has been



mentioned also ensures long-lasting gene expression in vivo, which in turn is particularly advantageous. A further advantage of AAV is that the virus is not pathogenic for humans and is relatively stable in vivo.

5 Cloning of the nucleic acid according to the invention into the AAV vector or parts thereof takes place by methods known to the skilled person, as described, for example, in WO 95/23867, in Chiorini, J.A. et al. (1995), *Human Gene Therapy* 6, 1531-1541 or Kotin, R.M.
10 (1994), *Human Gene Therapy* 5, 793-801.

Vectors effective for gene therapy can also be obtained by complexing the nucleic acid according to the invention with liposomes, because it is possible thereby to achieve a very high transfection efficiency,
15 in particular of myocardial cells (Felgner, P.L. et al. (1987), *Proc. Natl. Acad. Sci USA* 84, 7413-7417). In lipofection, small unilamellar vesicles of cationic lipids are prepared by ultrasound treatment of the liposome suspension. The DNA is bound ionically to the
20 surface of the liposomes, specifically in a ratio such that a positive net charge remains and the plasmid DNA is 100% complexed by the liposomes. Besides the lipid mixtures DOTMA (1,2-dioleoyloxypropyl-3-trimethylammonium bromide) and DOPE (dioleoylphosphatidyl-
25 ethanolamine) employed by Felgner et al. (1987, supra), numerous new lipid formulations have now been synthesized and tested for their efficiency in transfecting various cell lines (Behr, J.P. et al. (1989), *Proc. Natl. Acad. Sci. USA* 86, 6982-6986;
30 Felgner, J.H. et al. (1994) *J. Biol. Chem.* 269, 2550-2561; Gao, X. & Huang, L. (1991), *Biochim. Biophys. Acta* 1189, 195-203). Examples of the novel lipid formulations are DOTAP N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium ethyl
35 sulphate or DOGS (TRANSFECTAM; dioctadecylamidoglycylspermine). One example of the preparation of DNA-liposome complexes and successful use thereof in



For use of the nucleic acid according to the invention in gene therapy, it is also advantageous if the part of the nucleic acid which codes for the polypeptide contains one or more noncoding sequences, including intron sequences, preferably between the promoter and the start codon of the polypeptide, and/or a polyA sequence, in particular the naturally occurring polyA sequence or an SV40 virus polyA sequence, especially at the 3' end of the gene, because this makes it possible to stabilize the mRNA in the myocardial cell (Jackson, R.J. (1993) *Cell* 74, 9-14 and Palmiter, R.D. et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 478-482).

The present invention further relates to the polypeptide itself having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, preferably having at least 12 amino acids, in particular having at least 15 amino acids and especially having at least 164 amino acids, except a polypeptide having the sequence:

PTRNPTTVOPWSLORCIKVNEHITNVNVESNFITGKGILAIMRALQ

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HNTVLTFLRFHNORHIMGSOVEMEIVKLLKENTTLRLGYHFKLPG

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25 The polypeptide is prepared, for example, by
expression of the nucleic acid according to the
invention in a suitable expression system as described
above using methods generally known to the skilled
person. Examples of suitable host cells are the E. coli
30 strains DH5, HB101 or BL21, the yeast strain
Saccharomyces cerevisiae, the lepidopteran insect cell
line for example Spodoptera frugiperda, or the animal

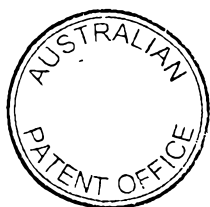


cells COS, Vero, 293 and HeLa, all of which are generally obtainable.

5 The said parts of the polypeptide can also be synthesized by classical synthesis (Merrifield technique). They are particularly suitable for obtaining antisera which can be used to screen suitable gene expression banks in order thus to obtain further functional variants of the polypeptide according to the invention.

10 The present invention therefore relates also to antibodies which react specifically with the polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, preferably
15 having at least 12 amino acids, in particular having at least 15 amino acids and especially having at least 164 amino acids, the abovementioned parts of the polypeptide either themselves being immunogenic or being able to be made immunogenic, or have their
20 immunogenicity increased, by coupling to suitable carriers such as, for example, bovine serum albumin.

The antibodies are either polyclonal or monoclonal. The preparation, to which the present invention also relates, takes place, for example, by
25 generally known methods, by immunizing a mammal, for example a rabbit, with the said polypeptide or the said parts thereof, where appropriate in the presence of, for example, Freund's adjuvant and/or aluminium hydroxide gels (see, for example, Diamond, B.A. et al.
30 (1981) *The New England Journal of Medicine*, 1344-1349). The polyclonal antibodies raised in the animal on the basis of an immunological response can then easily be isolated from the blood by generally known methods and purified, for example, by column chromatography. It was
35 thus possible, for example, to produce in rabbits a polyclonal antiserum against a polypeptide which had amino acids 1-90 according to the invention, as shown



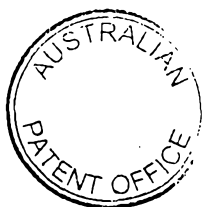
in Fig. 4, which was expressed as fusion protein in bacteria and purified by affinity chromatography. The antibodies according to the invention specifically recognized the corresponding protein of about 80 kD in
5 extracts of human heart tissue.

Monoclonal antibodies can be prepared, for example, by the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) *Nature*, 349, 293-299).

10 The present invention also relates to a medicinal product which contains a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof and the abovementioned parts thereof having at least 8
15 nucleotides, or a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof and the abovementioned parts thereof having at least 6 amino acids and, where appropriate, suitable additives or excipients, and to a process for producing
20 a medicinal product for treating cardiac disorders, in particular cardiac insufficiency, in which a said nucleic acid or a said polypeptide is formulated with a pharmaceutically acceptable carrier.

One example of the use of nucleic acid
25 fragments as therapeutic agent is the use of DNA fragments in the form of antisense oligonucleotides (Uhlmann, E. & Peyman, A. (1990) *Chemical Reviews*, 90, 543-584, No. 4).

A particularly suitable medicinal product for
30 use for human gene therapy is one which contains the said nucleic acid in naked form or in the form of one of the vectors effective for gene therapy which are described above, or in a form complexed with liposomes. The pharmaceutical carrier is, for example, a
35 physiological buffer solution, preferably with a pH of about 6.0-8.0, preferably of about 6.8-7.8, in particular of about 7.4 and/or an osmolarity of about

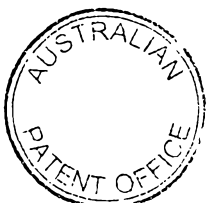


200-400 milliosmol/litre, preferably of about 290-310 milliosmol/litre. The pharmaceutical carrier may additionally contain suitable stabilizers such as, for example, nuclease inhibitors, preferably complexing agents such as EDTA and/or other excipients known to the skilled person.

The said nucleic acid is normally administered intravenously, for example with the aid of a catheter, where appropriate in the form of the virus vectors described in detail above or as liposome complexes. It is advantageous, for example, to infuse the nucleic acid according to the invention directly into the patient's coronary arteries (so-called percutaneous coronary gene transfer, PCGT), in particular in the form of recombinant adenovirus vectors or adeno-associated virus vectors. Administration with the aid of a balloon catheter is particularly preferred because it is possible thereby to confine the transfection not only to the heart but also to the injection site within the heart (see, for example, Feldman, L.J. et al. (1994) JACC 235A, 906-934).

It is also possible to administer the polypeptide itself intravenously or with the aid of a catheter or balloon catheter, where appropriate with suitable additives or excipients, such as, for example, physiological saline, stabilizers, proteinase inhibitors etc., in order to influence the function of the heart immediately and directly.

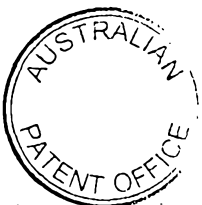
The present invention further relates to a diagnostic aid containing a nucleic acid, a polypeptide or antibody according to the present invention and, where appropriate, suitable additives or excipients and to a process for producing a diagnostic aid for diagnosing cardiac disorders, in particular cardiac insufficiency, in which a nucleic acid, a polypeptide or antibody according to the present invention is mixed with suitable additives or excipients.



It is possible, for example, according to the present invention to produce on the basis of the said nucleic acid a diagnostic aid based on the polymerase chain reaction (PCR diagnosis, for example as disclosed
5 in EP-0 200 362) or on a Northern blot as described in detail in Example 3 using the 321 bp DNA fragment according to the invention as probe. These tests are based on the specific hybridization of said nucleic acids with the complementary strand, normally of the
10 corresponding mRNA. The nucleic acid may also in this case be modified as described, for example, in EP 0 063 879. A DNA fragment, in particular the DNA fragment described in Example 1, is preferably labelled using suitable reagents, for example radioactively with
15 α -³²P-dCTP or non-radioactively with biotin, by generally known methods and incubated with isolated RNA, which has preferably been pre-bound to suitable membranes made of, for example, cellulose or nylon. It is additionally advantageous, before the hybridization
20 and binding to a membrane, for the isolated RNA to be fractionated according to size, for example by agarose gel electrophoresis. With the same amount of investigated RNA from each tissue sample, it is thus possible to determine the amount of mRNA specifically
25 labelled by the probe.

It is thus possible by using the diagnostic aid according to the invention also to measure a cardiac tissue sample in vitro specifically for the strength of expression of the corresponding gene in order to be
30 able to diagnose reliably possible cardiac insufficiency (see Example 1). A cDNA having a sequence as shown in Fig. 1 is particularly suitable for diagnosing a possible cardiac insufficiency (see Example 2).

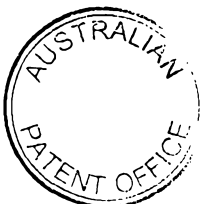
35 A further diagnostic aid contains the polypeptide according to the present invention or the immunogenic parts thereof described above in detail.



The polypeptide or the parts thereof, which are preferably bound to a solid phase, for example made of nitrocellulose or nylon, can, for example, be brought into contact in vitro with the body fluid to be
5 investigated, for example blood, in order to react for example with autoimmune antibodies. The antibody-peptide complex can then be detected for example by means of labelled antihuman IgG or antihuman IgM antibodies. The label is, for example, an enzyme such
10 as peroxidase which catalyses a colour reaction. The presence and the amount of autoimmune antibody present can thus be detected easily and rapidly by the colour reaction.

Another diagnostic aid contains the antibodies
15 according to the invention themselves. These antibodies can be used, for example, for investigating a cardiac tissue sample easily and quickly to find whether the relevant polypeptide is present in an increased amount, in order thus to obtain information about possible
20 cardiac insufficiency. In this case, the antibodies according to the invention are labelled for example with an enzyme, as already described above. The specific antibody-peptide complex can thus be detected easily and equally quickly by an enzymatic colour
25 reaction.

The present invention also relates to a test for identifying functional interactors containing a nucleic acid according to the invention coding for a polypeptide having an amino acid sequence as shown in
30 Fig. 4 or a functional variant thereof and the abovementioned parts thereof having at least 8 nucleotides, a polypeptide having the amino acid sequence as shown in Fig. 4 or a functional variant thereof, and the abovementioned parts thereof having at
35 least 6 amino acids or the antibodies according to the invention and, where appropriate, suitable additives or excipients.



A suitable test for identifying functional interactors is, for example, the so-called two-hybrid system (Fields, S. & Sternglanz, R. (1994) *Trends in Genetics*, 10, 286-292).

5 In this test, a cell, for example a yeast cell, is transformed or transfected with one or more expression vectors which express a fusion protein which contains a polypeptide according to the present invention and a DNA binding domain of a known protein,
10 for example of Gal4 or LexA from *E. coli*, and/or expresses a fusion protein which contains an unknown polypeptide and a transcription activating domain, for example of Gal4, herpes virus VP16 or B42. The cell additionally contains a reporter gene, for example the
15 lacZ gene from *E. coli*, green fluorescence protein or the yeast amino acid biosynthesis genes His3 or Leu2, which is controlled by regulatory sequences, such as, for example, the LexA promoter/operator or by a so-called upstream activation sequence (UAS) of yeast. The
20 unknown polypeptide is encoded, for example, by a DNA fragment which is derived from a gene bank, for example from a human cardiac tissue-specific gene bank. Normally a cDNA gene bank is produced directly, using the expression vectors described, in yeast so that the
25 test can be carried out immediately thereafter.

 For example, a nucleic acid according to the present invention is cloned into a yeast expression vector in a functional unit with the nucleic acid coding for the LexA DNA binding domain, so that a
30 fusion protein consisting of the polypeptide according to the invention and the LexA DNA binding domain is expressed in the transformed yeast. In another yeast expression vector, cDNA fragments from a cDNA gene bank are cloned in a functional unit with the nucleic acid
35 coding for the Gal4 transcription activating domain, so that a fusion protein consisting of an unknown polypeptide and the Gal4 transcription activating



domain is expressed in the transformed yeast. The yeast which is transformed with the two expression vectors and is, for example, Leu2⁻ additionally contains a nucleic acid which codes for Leu2, and is controlled by
5 the LexA promoter/operator. In the event of a functional interaction between the polypeptide according to the invention and the unknown polypeptide, the Gal4 transcription activating domain binds via the LexA DNA binding domain to the LexA promoter/operator,
10 whereby the latter is activated and the Leu2 gene is expressed. The result of this is that the Leu2⁻ yeast is able to grow on minimal medium which contains no leucine.

On use of the lacZ or green fluorescence
15 protein reporter gene in place of an amino acid biosynthesis gene, activation of transcription can be detected by the formation of blue or green-fluorescing colonies. The blue or fluorescent coloration can also be quantified easily in a spectrophotometer, for
20 example at 585 nm in the case of a blue coloration.

Thus, it is possible to screen expression gene banks easily and quickly for polypeptides which interact with a polypeptide according to the present invention. It is then possible for the novel peptides
25 found to be isolated and further characterized.

Another possible use of the two-hybrid system is the influence on the interaction between a polypeptide according to the present invention and a known or unknown polypeptide by other substances such
30 as, for example, chemical compounds. Thus, it is also possible to find easily novel and valuable active substances which can be chemically synthesized and can be employed as therapeutic agent for treating a cardiac disorder. The present invention is therefore not
35 restricted to a method for finding polypeptide-like interactors, but also extends to a method for finding substances which are able to interact with the protein-



protein complex described above. Such polypeptide-like, as well as chemical interactors are therefore referred to as functional interactors for the purpose of the present invention.

5 The surprising advantage of the present invention is thus the possibility of using the subject-matters according to the invention for specific and reliable diagnosis and therapy of cardiac disorders, especially cardiac insufficiency. However, other
10 valuable therapeutic and diagnostic possibilities also emerge. For example, the functional interactors which can be easily identified using the described test methods are so advantageous because it is possible with their aid in the form of suitable medicinal products to
15 influence deliberately the activity of the polypeptide according to the invention in its natural environment in the myocardium and thus also the contractility of the myocardial cells, in particular since the activity of this polypeptide can be regulated as already
20 described in detail above.

 The following figures and examples are intended to illustrate the invention in detail without restricting it.

25 Description of the figures

 Fig. 1 shows a 1936 nucleotide-long heart-specific DNA sequence. The region which codes for the corresponding polypeptide is shown in bold. The DNA fragment from Example 1 is underlined.

30 Fig. 2 shows a 2080 nucleotide-long heart-specific DNA sequence which has an extension at the 5' end of the DNA sequence from Fig. 1. The region which codes for the corresponding polypeptide is once again shown in bold.

35 Fig. 3 shows a 2268 nucleotide-long heart-specific DNA sequence which has an extension at the 5' end of the DNA sequence from Fig. 1 or Fig. 2. The



region which codes for the corresponding polypeptide is likewise shown in bold.

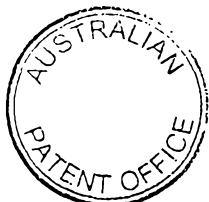
Fig. 4 shows a 552 amino acid-long polypeptide sequence encoded by one of the DNA sequences shown in Figs. 1-3. The regions homologous with human tropomodulin are shown in bold. The sequence motifs which indicate regulation of the polypeptide by tyrosine kinase signal transduction pathways are underlined.

Figs. 5a and 5b show Northern blots of mRNAs which correspond to the nucleic acid sequences shown in Figs. 1-3 for detecting expression in various human tissues (Fig. 5a) and for detecting expression in healthy and insufficient human cardiac tissue (Fig. 5b).

Examples

1. Isolation of a DNA fragment from human insufficient cardiac tissue

Complete RNA was initially isolated by standard methods (Chomczynski & Sacchi (1987), *Anal. Biochem*, 162 (1), 156-159) from a healthy and an insufficient cardiac tissue sample. The RNA was then treated with DNase in order to remove DNA contamination. An aliquot of this RNA (0.2 µg) was then incubated in a 20 µl reaction mix with 1 × RT buffer (Gibco Y00121), 10 mM DTT, 20 µM dNTP mix, 1 U/µl RNAsin (Promega N2511), 1 µM 3' anchor primer mixture of the 5'-T₁₂ACN-3' type, where N can be any deoxynucleotide, and 10 U/µl SuperScript RNase H⁻ reverser transcriptase at 37°C for 60 min and thus transcribed into cDNA. A cDNA aliquot was then subjected to a 20 µl PCR in 1 × PCR buffer (Perkin-Elmer) which, besides 1 µM 3' primer T₁₂AC and 1 µM 5'-decamer primer (5'-CCTTCTACCC-3'), contains 10 µCi of α-³²P-dCTP, 2 µM dNTP mix and 1 U of AmpliTaq (Perkin Elmer). The mixture was incubated firstly at 94°C for 1 min, then 40 cycles each of 30 s at 94°C,



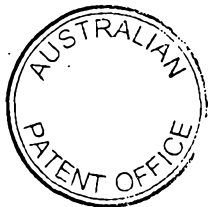
40°C for 2 min and 72°C for 30 s and finally at 72°C for 10 min. The resulting DNA fragment mixture was then fractionated on a 6% polyacrylamide gel and autoradiographed. A DNA fragment which is 321 bp in length and which is not present in the healthy heart sample but is distinctly present in the insufficient heart sample is thus prepared. This fragment was then cut out of the gel on the basis of the X-ray film and was reamplified by PCR under the conditions already described. The resulting fragment was then cloned into an appropriate vector, and the DNA sequence was determined. A fragment prepared in this way contains nucleotides 1627-1936 of the sequence according to Claim 1 and the 12 thymine nucleotides from the 3' anchor primer.

2. Isolation of heart-specific nucleic acids

A plaque hybridization was carried with a cDNA gene bank from cardiac tissue under standard conditions (see Sambrook, J., Frisch, E.F. & Maniatis, T. (1989) *Molecular Cloning, A Laboratory Manual*, ch. 8-10) using an α -³²P-dCTP-labelled DNA fragment from Example 1 which comprises the nucleotides from position 1627-1936 in Fig. 1. The cDNAs found were then isolated and sequenced. The sequences are shown in Figs. 1-3. It emerged from this that the cDNA having the sequence shown in Fig. 1 could be isolated with greater probability from insufficient cardiac tissue than the cDNA having the sequence shown in Fig. 2 or 3, which could be isolated with greater probability from healthy cardiac tissue.

3. Detection of the strength of expression of the heart-specific gene in various human tissues by means of Northern blots.

The DNA fragment 321 bp in length already described in Examples 1 and 2 and Fig. 1 was firstly

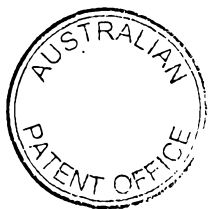


radiolabelled with α -³²P-dCTP by the random primer labelling method (Feinberg, A.P. & Vogelstein, B. (1983) Anal. Biochem., 132, 6). The RTS RadPrime DNA labelling system (GibcoBRL 10387-017) was used for this purpose. The hybridization of blots with poly A⁺ RNA from human tissues (see Figs. 5a and 5b) took place at 68°C for 1 hour in accordance with the manufacturer's instructions (Multiple Tissue Northern Blots I & II, Clontech Laboratories GmbH, Heidelberg, #7760-1, #7759-1) in ExpressHyb hybridization solution (Clontech #8015-1). The blots were then washed with 2 × SSC and 0.05% SDS for 30 minutes and thereafter with 0.1 × SSC and 0.1% SDS for 1 hour and autoradiographed. It emerged that the probe 321 bp in length hybridizes strongly with a polyA⁺ RNA of about 2400 bp strongly in cardiac tissue and skeletal muscle, very weakly in prostate tissue and not in leucocytes, large intestinal, small intestinal, ovarian, testicular, thymus, splenic, renal, hepatic, lung, placental and brain tissue (Fig. 5a).

Expression of the corresponding RNAs in healthy and insufficient cardiac tissue was also investigated. Complete RNA was isolated from various human cardiac tissue samples for this purpose (Chomczynski & Sacchi (1987), Anal. Biochem. 162, 156-159). Subsequently in each case 10 µg of RNA were fractionated using a 1% formaldehyde agarose gel and transferred by the capillary method to a charged nylon membrane (Zeta-Probe GT BioRad #162-0197). The membrane was briefly washed with 2 × SSC and then baked at 80°C for 30 minutes. The membranes were incubated with prehybridization solution (0.5 M Na₂HPO₄, pH 7.2; 7% SDS) at 65°C for at least 1 hour. The solution was then replaced by a fresh solution, and the radioactive, heat-denatured probe was added. The hybridization was carried out at 65°C for 15 hours. The membranes were then washed firstly with 40 mM Na₂HPO₄, pH 7.2; 5% SDS



at 65°C for 15 hours and then with 40 mM Na₂HPO₄, pH 7.2; 1% SDS at 65°C for 2 x 30 minutes, and subsequently autoradiographed. It emerged that various RNA species having a length from about 2200 to 2400 bp were fractionated in 1% agarose gels. These different species correspond well with the sizes of the three cDNAs found, including an average polyA tail 150 bp long (see Figs. 1-3). In particular, the smallest RNA species was more clearly detectable in diseased tissue than in healthy tissue. Quantification of the blot using a PhosphorImager and the ImageQuant software (Molecular Dynamics GmbH, Krefeld), taking into account a control hybridization with β 4-thymosin and actin, revealed an approximately 35% increased expression of the detected RNAs in insufficient cardiac tissue by comparison with healthy tissue.



SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT:
(A) NAME: MediGene Aktiengesellschaft
(B) STREET: Lochhamer Str. 11
(C) CITY: 82152 Martinsried
(D) COUNTRY: Germany
10 (F) POSTAL CODE: D-82152
(G) TELEPHONE: 089-89 56 32 0
(H) FAX: 089-89 56 32 20
- (ii) TITLE OF INVENTION: Myocardium- and skeletal
15 muscle-specific nucleic acid, its
preparation and use
- (iii) NUMBER OF SEQUENCES: 5
- 20 (iv) COMPUTER-READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Word Perfect 3.1

25

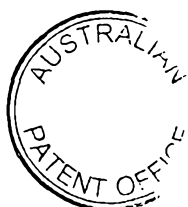
(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1936 base pairs
30 (B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35

(iii) HYPOTHETICAL: NO



(iv) ANTISENSE: YES

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: cardiac tissue

5 (xi) SEQUENCE DESCRIPTION SEQ ID NO: 1:

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TAAATACGAA	TCCATCGACG	AGGATGAACT	CCTCGCCTCC	CTGTCAGCCG	AGGAGCTGAA	180
GGAGCTAGAG	AGAGAGTTGG	AAGACATTGA	ACCTGACCGC	AACCTTCCCG	TGGGGCTAAG	240
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CTATTGGGAA	AAGGAGTCCC	AAAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGGAAA	360
GTTTGCAGAA	GACAAAGAGG	AAAGTGAAGA	AGAGCTTATC	TTTACTGAAA	GTAACAGTGA	420
GTTTCTGAG	GAAGTGATA	CAGAGGAGGA	GGAGGAGGAG	TCCCAGGAGG	AAGAGGAGGA	480
AGAAGACAGT	GACGAAGAGG	AAAGAACAAT	TGAAACTGCA	AAAGGGATTA	ATGGAAGTGT	540
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GAGCCGTCTT	CTGTCTCCTG	TGGCCACACT	TCCTCCTCCT	CCCCCTCCTC	CTCCTCCTCC	1380
CCCTCCTTCT	TCCCAAAGGC	TGCCACCACC	TCCTCCTCCT	CCCCCTCCTC	CACTCCCAGA	1440
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GGCATTACAA	AATGGACAAA	AAAAGAAAAA	AGGGAAAAAG	GTCAAGAAAC	AGCCAAACAG	1560
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TTCCCGACCT	TCTACTCCAC	AGAGATCAGC	TCATGAGAAAT	CTCATGGAAG	CAATTGCGGG	1680
AAGCAGCATA	AAACAGCTAA	AGCGGGTGGA	AGTTCCAGAA	GCCCTGCGAT	GGGAACATGA	1740
TCTTAGAAG	AGGATGCAGA	ACTGTTTCACT	GGTATTACAT	GAAATGCATT	GTGAGATGTT	1800
TCTAAATAAC	CTTCTTCAAT	TCAAAATGAT	CCCTGACTTT	AAAAATAATC	TCACCCATTA	1860
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TTCTTTTCTA	TGTCGT					1936



(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 2080 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(iii) HYPOTHETICAL: NO

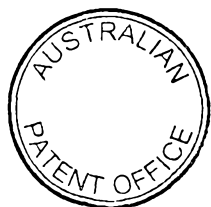
(iv) ANTISENSE: YES

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(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 2:



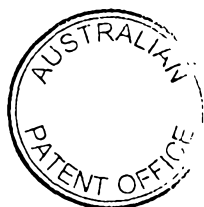
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CTATCTGTGG	ATGTGTTGGT	AACCTCCGAGT	TGTAATGAGT	TCATGAAATG	TGCTGTTATT	2040
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(2) INFORMATION FOR SEQ ID NO: 3:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2268 base pairs



(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

10

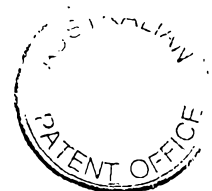
(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 3:

15

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GGAGCTAGAG	AGAGAGTTGG	AAGACATTGA	ACCTGACCGC	AACCTTCCCG	TGGGGCTAAG	240
GCAAAAGAGG	GTGACAGAGA	AAACCCCCAC	AGGGACATTC	AGCAGAGAGG	CACTGATGGC	300
CTATTGGGAA	AAGBAGTCCC	AAAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGGAAA	360
GTTTGCAGAA	BACAAAGAGG	AAAGTGAAGA	AGAGCTTATC	TTTACTGAAA	GTAACAGTGA	420
GTTTCTGAG	BAAJGTGATA	CAGAGGAGGA	GGAGGAGGAG	TCCCAGGAGG	AAGAGGAGGA	480
AGAASACAGT	BACBAGAGG	AAAGAACAAT	TGAAACTGCA	AAAGGGATTA	ATGGAACTGT	540
AAATTATGAT	ASTGTCAATT	CTGACAACTC	TAAGCCAAAG	ATATTTAAAA	GTCAAATAGA	600
GAACATAAAT	TTGACCAATG	GCAGCAATGG	GAGGAACACA	GAGTCCCCAG	CTGCCATTCA	660
CCCTTGTGGA	AATCCTACAG	TGATTGAGGA	CGCTTTGGAC	AAGATTAAAA	GCAATGACCC	720
TGACACCACA	GAAGTCAATT	TGAACAACAT	TGAGAACATC	ACAACACAGA	CCCTTACCCG	780
CTTTGCTGAA	JCCCTCAAGG	ACAACACTGT	GGTGAAGACG	TTCAGTCTGG	CCAACACGCA	840
TGCCGACGAC	ASTGCAGCCA	TGGCCATTGC	AGAGATGCTC	AAAGCCAATG	AGCACATCAC	900
CAACGTAAAC	GTCGAGTCCA	ACTTCATAAC	GGGAAAGGGG	ATCCTGGCCA	TCATGAGAGC	960
TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	GCGTTTCCAT	AACCAGAGGC	ACATCATGGG	1020
CAGCCAGGTG	GAAATGGAGA	TTGTCAAGCT	GCTGAAGGAG	AACACGACGC	TGCTGAGGCT	1080
GGGATACCAT	TTTGAACTCC	CAGGACCAAG	AATGAGCATG	ACGAGCATTT	TGACAAGAAA	1140
TATGGATAAA	CAGAGGCAAA	AACGTTTGCA	GGAGCAAAAA	CAGCAGGAGG	GATACGATGG	1200
AGGACCCAAAT	CTTAGGACCA	AAGTCTGGCA	AAGAGGAACA	CCTAGCTCTT	CACCTTATGT	1260
ATCTCCCAGG	CACTCACCCCT	GGTCATCCCC	AAAACCTCCC	AAAAAAGTCC	AGACTGTGAG	1320
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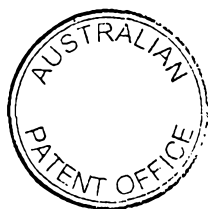
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TATTCTAAAG	GAAATAAAAA	ATTCTCTGAG	GTCAGTGCAA	GAGAAGAAAA	TGGAAGACAG	152C
TTCCCGACCT	TCTACCCAC	AGAGATCAGC	TCATGAGAAT	CTCATGGAAG	CAATTCGGGG	168C
AAGCAGCATA	AAACAGCTAA	AGCGGGTGGA	AGTTCAGAA	GCCCTGCGAT	GGGAACATGA	174C
TCTTTAGAAG	AGGATGCAGA	ACTGTTCACT	GGTATTACAT	GAAATGCATT	GTGAGATGTT	180C
TCTAAAATAC	CTTCTTCAAT	TCAAAATGAT	CCCTGACTTT	AAAAATAATC	TCACCCATTA	186C
ATTCCAAAGA	GAATCTTAAG	AAACAATCAG	CATGTTTCTT	CTGTAAATAT	GAAAATAAAT	192C
TTCTTTTTTA	TGTCGTGAGA	TTTGTATTGG	CAAGAAGCAG	TTAATTTAAA	GATGCTCTTC	198C
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TTGTAATCT	CAATAAATGT	GGATTGAAGT	TTTTTCCCTT	TTTTTAAAGC	CAAACATAA	210C
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TTTTTTCCCT	TTTTTTAAAA	AGCCAAACTA	ATATTTTTCT	GTGAGTTAAT	ACATCTGTCA	222C
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(2) INFORMATION FOR SEQ ID NO: 4:

- 5 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 552 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- 15 (iv) ANTISENSE: YES
- (vi) ORIGINAL SOURCE:
- (F) TISSUE TYPE: cardiac tissue
- 20 (xi) SEQUENCE DESCRIPTION SEQ ID NO: 4:



Met Ser Thr Phe Gly Tyr Arg Arg Gly Leu Ser Lys Tyr Glu Ser Ile
1 5 10 15
Asp Glu Asp Glu Leu Leu Ala Ser Leu Ser Ala Glu Glu Leu Lys Glu
20 25 30
Leu Glu Arg Glu Leu Glu Asp Ile Glu Pro Asp Arg Asn Leu Pro Val
35 40 45
Gly Leu Arg Gln Lys Ser Leu Thr Glu Lys Thr Pro Thr Gly Thr Phe
50 55 60



Ser Arg Glu Ala Leu Met Ala Tyr Trp Glu Lys Glu Ser Gln Lys Leu
65 70 75 80

Leu Glu Lys Glu Arg Leu Gly Glu Cys Gly Lys Val Ala Glu Asp Lys
85 90 95

Glu Glu Ser Glu Glu Glu Leu Ile Phe Thr Glu Ser Asn Ser Glu Val
100 105 110

Ser Glu Glu Val Tyr Thr Glu Glu Glu Glu Glu Ser Gln Glu Glu
115 120 125

Glu Glu Glu Glu Asp Ser Asp Glu Glu Glu Arg Thr Ile Glu Thr Ala
130 135 140

Lys Gly Ile Asn Gly Thr Val Asn Tyr Asp Ser Val Asn Ser Asp Asn
145 150 155 160

Ser Lys Pro Lys Ile Phe Lys Ser Gln Ile Glu Asn Ile Asn Leu Thr
165 170 175

Asn Gly Ser Asn Gly Arg Asn Thr Glu Ser Pro Ala Ala Ile His Pro
180 185 190

Cys Gly Asn Pro Thr Val Ile Glu Asp Ala Leu Asp Lys Ile Lys Ser
195 200 205

Asn Asp Pro Asp Thr Thr Glu Val Asn Leu Asn Asn Ile Glu Asn Ile
210 215 220

Thr Thr Gln Thr Leu Thr Arg Phe Ala Glu Ala Leu Lys Asp Asn Thr
225 230 235 240

Val Val Lys Thr Phe Ser Leu Ala Asn Thr His Ala Asp Asp Ser Ala
245 250 255

Ala Met Ala Ile Ala Glu Met Leu Lys Ala Asn Glu His Ile Thr Asn
260 265 270

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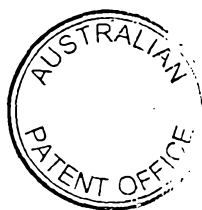
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305 310 315 320

Leu Leu Lys Glu Asn Thr Thr Leu Leu Arg Leu Gly Tyr His Phe Glu
325 330 335

Leu Pro Gly Pro Arg Met Ser Met Thr Ser Ile Leu Thr Arg Asn Met
340 345 350

Asp Lys Gln Arg Gln Lys Arg Leu Gln Glu Gln Lys Gln Gln Glu Gly
355 360 365

Tyr Asp Gly Gly Pro Asn Leu Arg Thr Lys Val Trp Gln Arg Gly Thr
370 375 380



Pro Ser Ser Ser Pro Tyr Val Ser Pro Arg His Ser Pro Trp Ser Ser
385 390 395 400

Pro Lys Leu Pro Lys Lys Val Gln Thr Val Arg Ser Arg Pro Leu Ser
405 410 415

Pro Val Ala Thr Leu Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro
420 425 430

Pro Ser Ser Gln Arg Leu Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro
435 440 445

Leu Pro Glu Lys Lys Leu Ile Thr Arg Asn Ile Ala Glu Val Ile Lys
450 455 460

Gln Gln Glu Ser Ala Gln Arg Ala Leu Gln Asn Gly Gln Lys Lys Lys
465 470 475 480

Lys Gly Lys Lys Val Lys Lys Gln Pro Asn Ser Ile Leu Lys Glu Ile
485 490 495

Lys Asn Ser Leu Arg Ser Val Gln Glu Lys Lys Met Glu Asp Ser Ser
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Ile Arg Gly Ser Ser Ile Lys Gln Leu Lys Arg Val Glu Val Pro Glu
530 535 540

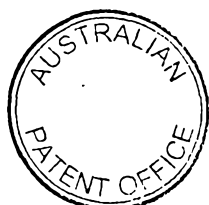
Ala Leu Arg Trp Glu His Asp Leu
545 550

(2) INFORMATION FOR SEQ ID NO: 5:

- 5 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 10 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: cDNA



(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

5 (vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 5

CCTTCTACCC

1:

10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 279 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

25 (vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 6:

30 GCCAACACGC ANTCCGACGA CAGTGCAGCC ATGGTCATTG CAGAGATGCN TCAAAGTCAA 60
TGAGCACATC ACCAACGTAA ACGTCGAGTC CAACTTCATA ACGGGAAAGG GGATCCTGGC 120
CATCATGAGA GCTCTCCAGC ACAACACGGT GCTCACGGAG CTGCGGTTTC ATAACCAGAG 180
GCACATCATG GGCAGCCAGG TGGAAATGGA GATTGTCAAG CTNCTGAAGG AGAACACGAC 240
GCTNCTGAGG CTGGGNTACC ATTTTNAACT CCCAGGACC 279

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 93 amino acids



THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Nucleic acid encoding for a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof having a sequence homology of at least about 70% of the said polypeptide, and parts thereof having at least 8 nucleotides, except a nucleic acid having the sequence:

1	GCCAACACGC	ANTCCGACGA	CAGTGCAGCC	ATGGTCATTG	CAGAGATGCN	TCAAAGTCAA
61	TGAGCACATC	ACCAACGTAA	ACGTCGAGTC	CAACTTCATA	ACGGGAAAGG	GGATCCTGGC
121	CATCATGAGA	GCTCTCCAGC	ACAACACGGT	GCTCACGGAG	CTGCGTTTCC	ATAACCAGAG
181	GCACATCATG	GGCAGCCAGG	TGGAAATGGA	GATTGTCAAG	CTNCTGAAGG	AGAACACGAC
241	GCTNCTGAGG	CTGGGNTACC	ATTTTNAACT	CCCAGGACC		

2. The nucleic acid according to claim 1, characterised in that the nucleic acid is a DNA or RNA, preferably DNA, in particular a double-stranded DNA.

3. The nucleic acid according to claim 1 or 2, characterised in that the nucleic acid contains a DNA having a nucleic acid sequence as shown in Figures 1, 2 or 3.

4. The nucleic acid according to any one of claims 1 to 3, characterised in that the nucleic acid is present in a vector, preferably in an expression vector.

5. The nucleic acid according to any one of claims 1 to 4, characterised in that the said nucleic acid is present in a vector when used for gene therapy.

6. The nucleic acid according to any one of claims 1 to 5, characterised in that the part of the nucleic acid which codes for the polypeptide contains one or more non-coding sequences and/or a polyA sequence.

7. A process for the preparation of a nucleic acid according to any one of claims 1 to 6, characterised in that the nucleic acid is chemically synthesised or isolated from a gene bank using a probe.



8. A polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof having a sequence homology of at least about 70% of the said polypeptide, and parts thereof having at least 6 amino acids, except a polypeptide having the sequence:

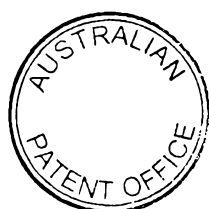
PTRNPTTVQPWSLQRCIKVNEHITNVNVESNFITGKGILAIMRALQ									
	10	20	30	40					
HNTVLTELRFHNQRHIMGSQVEMEIVKLLKENTTLLRLGYHFKLPG									
50	60	70	80	90					

9. The process for the preparation of a polypeptide according to claim 8, characterised in that a nucleic acid according to any one of claims 1 to 3 is expressed in a suitable host cell.

10. An antibody against a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof having a sequence homology of at least about 70% of the said polypeptide, and parts thereof having at least 6 amino acids.

11. A process for the preparation of an antibody according to claim 10, characterised in that a mammal is immunized with a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof having a sequence homology of at least about 70% of the said polypeptide, and parts thereof having at least 6 amino acids, and the resulting antibodies are isolated.

12. A medicinal product containing a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof having a sequence homology of at least about 70% of the said polypeptide, and parts thereof having at least 8 nucleotides, or a polypeptide having an amino sequence as shown in Figure 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, and, where appropriate, a pharmaceutically acceptable carrier/diluent or excipient.



13. A process for the preparation of a medicinal product as claimed in claim 12 wherein the said nucleic acid is formulated with a pharmaceutically acceptable carrier/diluent or excipient.

14. The use of a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof having a sequence homology of at least about 70% of the said polypeptide, and parts thereof having at least 8 nucleotides, or a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof, and parts thereof having at least 6 amino acids for the preparation of a medicament for the treatment of cardiac disorders.

15. A method of treatment of cardiac disorders including administering to a patient in need thereof, a therapeutically effective amount of a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof having a sequence homology of at least about 70% of the said polypeptide, and parts thereof having at least 8 nucleotides, or a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof, and parts thereof having at least 6 amino acids or an antibody according to claim 10 in combination with a pharmaceutically acceptable carrier/diluent or excipient.

16. A diagnostic aid containing a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof having a sequence homology of at least about 70% of the said polypeptide, and parts thereof having at least 8 nucleotides, a polypeptide having an amino acid sequence as shown in Figure 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, or an antibody according to claim 10 and, where appropriate, suitable additives or excipients.

17. A process for the preparation of a diagnostic aid as claimed in claim 16 for diagnosing cardiac disorders, wherein the said nucleic acid or antibody is mixed with a pharmaceutically acceptable carrier.



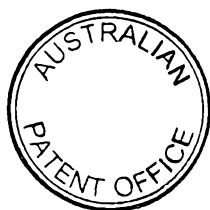
18. A test for identifying functional interactors containing a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, or a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, and, where appropriate, suitable additives or excipients.

DATED this 22nd day of November 2001

MEDIGENE AKTIENGESELLSCHAFT

WATERMARK PATENT & TRADEMARK ATTORNEYS
290 BURWOOD ROAD
HAWTHORN VICTORIA 3122
AUSTRALIA

Case: P16586AU00 KJS/ALJ/PXT



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Fig. 1

	CAGCCTGCCA	CTTGCCTCCC	TGCCTGCTTC	TGGCTGCCTT
	GAATGCCTGG	TCCTTCAAGC	TCCTTCTGGG	TCTGACAAAG
5	CAGGGACCAT	GTCTACCTTT	GGCTACCGAA	GAGGACTCAG
	TAAATACGAA	TCCATCGACG	AGGATGAACT	CCTCGCCTCC
	CTGTCAGCCG	AGGAGCTGAA	GGAGCTAGAG	AGAGAGTTGG
	AAGACATTGA	ACCTGACCGC	AACCTTCCCC	TGGGGCTAAG
	GCAAAAGAGC	CTGACAGAGA	AAACCCCCAC	AGGGACATTC
10	AGCAGAGAGG	CACTGATGGC	CTATTGGGAA	AAGGAGTCCC
	AAAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGGAAA
	GGTTGCAGAA	GACAAAGAGG	AAAGTGAAGA	AGAGCTTATC
	TTTACTGAAA	GTAACAGTGA	GGTTTCTGAG	GAAGTGTATA
	CAGAGGAGGA	GGAGGAGGAG	TCCCAGGAGG	AAGAGGAGGA
15	AGAAGACAGT	GACGAAGAGG	AAAGAACAAT	TGAAACTGCA
	AAAGGGATTA	ATGGAAGTGT	AAATTATGAT	AGTGTCAATT
	CTGACAATC	TAAGCCAAAG	ATATTTAAAA	GTCAAATAGA
	GAACATAAAT	TTGACCAATG	GCAGCAATGG	GAGGAACACA
	GAGTCCCCAG	CTGCCATTCA	CCCTTGTGGA	AATCCTACAG
20	TGATTGAGGA	CGCTTTGGAC	AAGATTAAAA	GCAATGACCC
	TGACACCACA	GAAGTCAATT	TGAACAACAT	TGAGAACATC
	ACAACACAGA	CCCTTACCCG	CTTTGCTGAA	GCCCTCAAGG
	ACAACACTGT	GGTGAAGACG	TTCACTCTGG	CCAACACGCA
	TGCCGACGAC	AGTGCAGCCA	TGGCCATTGC	AGAGATGCTC
25	AAAGCCAATG	AGCACATCAC	CAACGTAAAC	GTCGAGTCCA
	ACTTCATAAC	GGGAAAGGGG	ATCCTGGCCA	TCATGAGAGC
	TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	GCGTTTCCAT

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	AACCAGAGGC	ACATCATGGG	CAGCCAGGTG	GAAATGGAGA
	TTGTCAAGCT	GCTGAAGGAG	AACACGACGC	TGCTGAGGCT
	GGGATACCAT	TTTGA ACTCC	CAGGACCAAG	AATGAGCATG
	ACGAGCATTT	TGACAAGAAA	TATGGATAAA	CAGAGGCAAA
5	AACGTTTGCA	GGAGCAAAAA	CAGCAGGAGG	GATACGATGG
	AGGACCCAAT	CTTAGGACCA	AAGTCTGGCA	AAGAGGAACA
	CCTAGCTCTT	CACCTTATGT	ATCTCCCAGG	CACTCACCCCT
	GGTCATCCCC	AAA ACTCCCC	AAAAAAGTCC	AGACTGTGAG
	GAGCCGTCCT	CTGTCTCCTG	TGGCCACACT	TCCTCCTCCT
10	CCCCCTCCTC	CTCCTCCTCC	CCCTCCTTCT	TCCCAAAGGC
	TGCCACCACC	TCCTCCTCCT	CCCCCTCCTC	CACTCCCAGA
	GAAAAAGCTC	ATTACCAGAA	ACATTGCAGA	AGTCATCAAA
	CAACAGGAGA	GTGCCCAACG	GGCATTACAA	AATGGACAAA
	AAAAGAAAAA	AGGGAAAAAG	GTCAAGAAAC	AGCCAAACAG
15	TATTCTAAAG	GAAATAAAAA	ATTCTCTGAG	GTCAGTGCAA
	GAGAAGAAAA	TGGAAGACAG	<u>TTCCCGACCT</u>	<u>TCTACCCAC</u>
	<u>AGAGATCAGC</u>	<u>TCATGAGAAT</u>	<u>CTCATGGAAG</u>	<u>CAATTCGGGG</u>
	<u>AAGCAGCATA</u>	<u>AAACAGCTAA</u>	<u>AGCGGGTGGA</u>	<u>AGTTCCAGAA</u>
	<u>GCCCTGCGAT</u>	<u>GGGAACATGA</u>	<u>TCTTTAGAAG</u>	<u>AGGATGCAGA</u>
20	<u>ACTGTT CAGT</u>	<u>GGTATTACAT</u>	<u>GAAATGCATT</u>	<u>GTGAGATGTT</u>
	<u>TCTAAAATAC</u>	<u>CTTCTTCAAT</u>	<u>TCAAAATGAT</u>	<u>CCCTGACTTT</u>
	<u>AAAATAATC</u>	<u>TCACCCATTA</u>	<u>ATTCCAAAGA</u>	<u>GAATCTTAAG</u>
	<u>AAACAATCAG</u>	<u>CATGTTTCTT</u>	<u>CTGTAAATAT</u>	<u>GAAAATAAAT</u>
	<u>TTCTTTTTTA</u>	<u>TGTCGT</u> -poly(A)-Schwanz		

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Fig. 2

	CAGCCTGCCA	CTTGCCTCCC	TGCCTGCTTC	TGGCTGCCTT
	GAATGCCTGG	TCCTTCAAGC	TCCTTCTGGG	TCTGACAAAG
5	CAGGGACCAT	GTCTACCTTT	GGCTACCGAA	GAGGACTCAG
	TAAATACGAA	TCCATCGACG	AGGATGAACT	CCTCGCCTCC
	CTGTCAGCCG	AGGAGCTGAA	GGAGCTAGAG	AGAGAGTTGG
	AAGACATTGA	ACCTGACCGC	AACCTTCCCG	TGGGGCTAAG
	GCAAAAGAGC	CTGACAGAGA	AAACCCCCAC	AGGGACATTC
10	AGCAGAGAGG	CACTGATGGC	CTATTGGGAA	AAGGAGTCCC
	AAAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGGAAA
	GGTTGCAGAA	GACAAAGAGG	AAAGTGAAGA	AGAGCTTATC
	TTTACTGAAA	GTAACAGTGA	GGTTTCTGAG	GAAGTGTATA
	CAGAGGAGGA	GGAGGAGGAG	TCCCAGGAGG	AAGAGGAGGA
15	AGAAGACAGT	GACGAAGAGG	AAAGAACAAT	TGAAACTGCA
	AAAGGGATTA	ATGGAACTGT	AAATTATGAT	AGTGTCAATT
	CTGACAACCTC	TAAGCCAAAG	ATATTTAAAA	GTCAAATAGA
	GAACATAAAT	TTGACCAATG	GCAGCAATGG	GAGGAACACA
	GAGTCCCCAG	CTGCCATTCA	CCCTTGTTGA	AATCCTACAG
20	TGATTGAGGA	CGCTTTGGAC	AAGATTAAAA	GCAATGACCC
	TGACACCACA	GAAGTCAATT	TGAACAACAT	TGAGAACATC
	ACAACACAGA	CCCTTACCCG	CTTTGCTGAA	GCCCTCAAGG
	ACAACACTGT	GGTGAAGACG	TTCAGTCTGG	CCAACACGCA
	TGCCGACGAC	AGTGCAGCCA	TGGCCATTGC	AGAGATGCTC
25	AAAGCCAATG	AGCACATCAC	CAACGTAAAC	GTCGAGTCCA
	ACTTCATAAC	GGGAAAGGGG	ATCCTGGCCA	TCATGAGAGC
	TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	GCGTTTCCAT
	AACCAGAGGC	ACATCATGGG	CAGCCAGGTG	GAAATGGAGA

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	TTGTCAAGCT	GCTGAAGGAG	AACACGACGC	TGCTGAGGCT
	GGGATACCAT	TTTGAACTCC	CAGGACCAAG	AATGAGCATG
	ACGAGCATTT	TGACAAGAAA	TATGGATAAA	CAGAGGCAAA
	AACGTTTGCA	GGAGCAAAAA	CAGCAGGAGG	GATACGATGG
5	AGGACCCAAT	CTTAGGACCA	AAGTCTGGCA	AAGAGGAACA
	CCTAGCTCTT	CACCTTATGT	ATCTCCCAGG	CACTCACCCCT
	GGTCATCCCC	AAAAC TCCCC	AAAAAAGTCC	AGACTGTGAG
	GAGCCGTCCT	CTGTCTCCTG	TGGCCACACT	TCCTCCTCCT
	CCCCCTCCTC	CTCCTCCTCC	CCCTCCTTCT	TCCCAAAGGC
10	TGCCACCACC	TCCTCCTCCT	CCCCCTCCTC	CACTCCCAGA
	GAAAAAGCTC	ATTACCAGAA	ACATTGCAGA	AGTCATCAAA
	CAACAGGAGA	GTGCCCAACG	GGCATTACAA	AATGGACAAA
	AAAAGAAAAA	AGGGAAAAAG	GTCAAGAAAC	AGCCAAACAG
	TATTCTAAAG	GAAATAAAAA	ATTCTCTGAG	GTCAGTGCAA
15	GAGAAGAAAA	TGGAAGACAG	TTCCCGACCT	TCTACCCAC
	AGAGATCAGC	TCATGAGAAT	CTCATGGAAG	CAATTCGGGG
	AAGCAGCATA	AAACAGCTAA	AGCGGGTGGA	AGTTCCAGAA
	GCCCTGCGAT	GGGAACATGA	TCTTTAGAAG	AGGATGCAGA
	ACTGTTTCAGT	GGTATTACAT	GAAATGCATT	GTGAGATGTT
20	TCTAAAATAC	CTTCTTCAAT	TCAAAATGAT	CCCTGACTTT
	AAAATAATC	TCACCCATTA	ATTCCAAAGA	GAATCTTAAG
	AAACAATCAG	CATGTTTCTT	CTGTAAATAT	GAAAATAAAT
	TTCTTTTTTA	TGTCGTGAGA	TTTGTATTGG	CAAGAAGCAG
	TTAATTTAAA	GATGCTCTTC	CTATCTGTGG	ATGTGTTGGT
25	AACTCCGAGT	TGTAATGAGT	TCATGAAATG	TGCTGTTATT
	TTTGTAATCT	CAATAAATGT	GGATTGAAGT	TTTTTCCCTT

-poly(A) -Schwanz

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Fig. 3

	CAGCCTGCCA	CTTGCCTCCC	TGCCTGCTTC	TGGCTGCCTT
	GAATGCCTGG	TCCTTCAAGC	TCCTTCTGGG	TCTGACAAAG
5	CAGGGACCAT	GTCTACCTTT	GGCTACCGAA	GAGGACTCAG
	TAAATACGAA	TCCATCGACG	AGGATGAACT	CCTCGCCTCC
	CTGTCAGCCG	AGGAGCTGAA	GGAGCTAGAG	AGAGAGTTGG
	AAGACATTGA	ACCTGACCGC	AACCTTCCCG	TGGGGCTAAG
	GCAAAAGAGC	CTGACAGAGA	AAACCCCCAC	AGGGACATTC
10	AGCAGAGAGG	CACTGATGGC	CTATTGGGAA	AAGGAGTCCC
	AAAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGGAAA
	GGTTGCAGAA	GACAAAGAGG	AAAGTGAAGA	AGAGCTTATC
	TTTACTGAAA	GTAACAGTGA	GGTTTCTGAG	GAAGTGTATA
	CAGAGGAGGA	GGAGGAGGAG	TCCCAGGAGG	AAGAGGAGGA
15	AGAAGACAGT	GACGAAGAGG	AAAGAACAAT	TGAAACTGCA
	AAAGGGATTA	ATGGAAGTGT	AAATTATGAT	AGTGTCAATT
	CTGACAACTC	TAAGCCAAAG	ATATTTAAAA	GTCAAATAGA
	GAACATAAAT	TTGACCAATG	GCAGCAATGG	GAGGAACACA
	GAGTCCCCAG	CTGCCATTCA	CCCTTGTGGA	AATCCTACAG
20	TGATTGAGGA	CGCTTTGGAC	AAGATTAAAA	GCAATGACCC
	TGACACCACA	GAAGTCAATT	TGAACAACAT	TGAGAACATC
	ACAACACAGA	CCCTTACCCG	CTTTGCTGAA	GCCCTCAAGG
	ACAACACTGT	GGTGAAGACG	TTCAGTCTGG	CCAACACGCA
	TGCCGACGAC	AGTGCAGCCA	TGGCCATTGC	AGAGATGCTC
25	AAAGCCAATG	AGCACATCAC	CAACGTAAAC	GTCGAGTCCA
	ACTTCATAAC	GGGAAAGGGG	ATCCTGGCCA	TCATGAGAGC
	TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	GCGTTTCCAT
	AACCAGAGGC	ACATCATGGG	CAGCCAGGTG	GAAATGGAGA

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	TTGTCAAGCT	GCTGAAGGAG	AACACGACGC	TGCTGAGGCT
	GGGATACCAT	TTTGAACTCC	CAGGACCAAG	AATGAGCATG
	ACGAGCATTT	TGACAAGAAA	TATGGATAAA	CAGAGGCAAA
	AACGTTTGCA	GGAGCAAAAA	CAGCAGGAGG	GATACGATGG
5	AGGACCCAAT	CTTAGGACCA	AAGTCTGGCA	AAGAGGAACA
	CCTAGCTCTT	CACCTTATGT	ATCTCCCAGG	CACTCACCTT
	GGTCATCCCC	AAAACCTCCCC	AAAAAAGTCC	AGACTGTGAG
	GAGCCGTCCT	CTGTCTCCTG	TGGCCACACT	TCCTCCTCCT
	CCCCCTCCTC	CTCCTCCTCC	CCCTCCTTCT	TCCCAAAGGC
10	TGCCACCACC	TCCTCCTCCT	CCCCCTCCTC	CACTCCCAGA
	GAAAAAGCTC	ATTACCAGAA	ACATTGCAGA	AGTCATCAAA
	CAACAGGAGA	GTGCCCCAACG	GGCATTACAA	AATGGACAAA
	AAAAGAAAAA	AGGGAAAAAG	GTCAAGAAAC	AGCCAAACAG
	TATTCTAAAG	GAAATAAAAA	ATTCTCTGAG	GTCAGTGCAA
15	GAGAAGAAAA	TGGAAGACAG	TTCCCGACCT	TCTACCCAC
	AGAGATCAGC	TCATGAGAAT	CTCATGGAAG	CAATTCGGGG
	AAGCAGCATA	AAACAGCTAA	AGCGGGTGGA	AGTTCCAGAA
	GCCCTGCGAT	GGGAACATGA	TCTTTAGAAG	AGGATGCAGA
	ACTGTTTCAGT	GGTATTACAT	GAAATGCATT	GTGAGATGTT
20	TCTAAAATAC	CTTCTTCAAT	TCAAAATGAT	CCCTGACTTT
	AAAATAATC	TCACCCATTA	ATTCCAAAGA	GAATCTTAAG
	AAACAATCAG	CATGTTTCTT	CTGTAAATAT	GAAAATAAAT
	TTCTTTTTTA	TGTCGTGAGA	TTTGTATTGG	CAAGAAGCAG
	TTAATTTAAA	GATGCTCTTC	CTATCTGTGG	ATGTGTTGGT
25	AACTCCGAGT	TGTAATGAGT	TCATGAAATG	TGCTGTTATT
	TTTGTAATCT	CAATAAATGT	GGATTGAAGT	TTTTTCCCTT
	TTTTTAAAGC	CAAATAATA	TTTTTCTGTG	ACTTGATACA
	TCTGTCAGAT	TTTTGTAATC	TCGATAAATG	TGTATTGAAG

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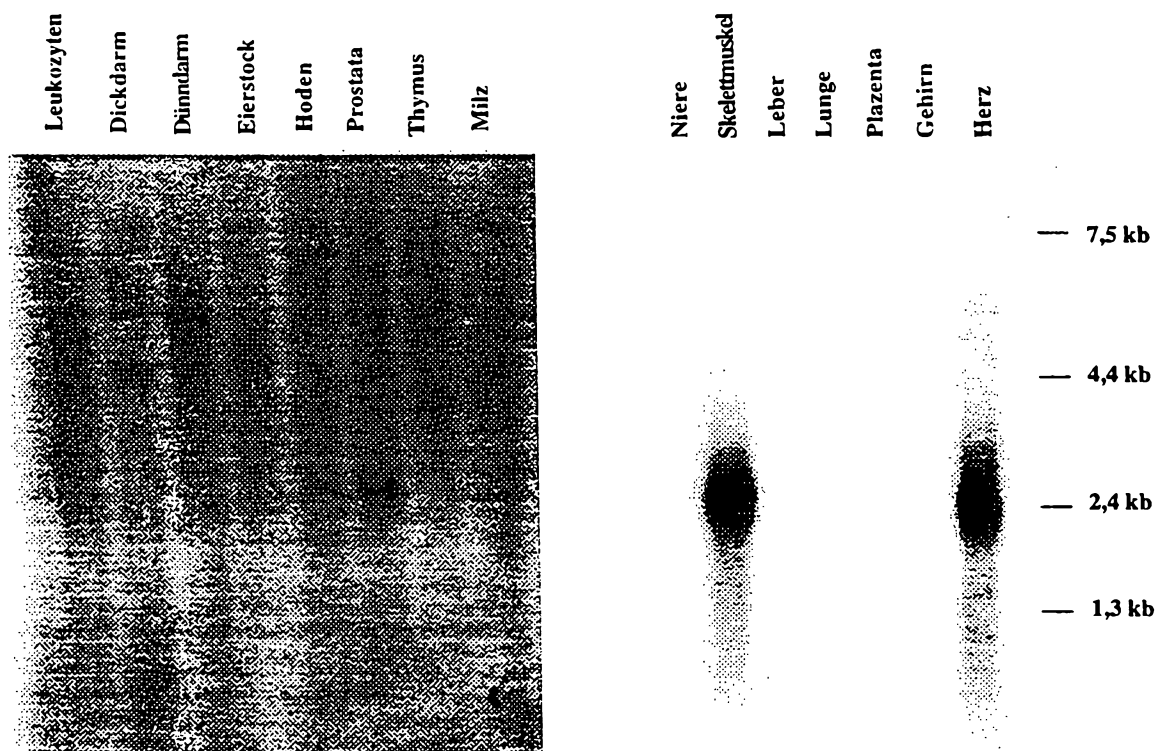
TTTTTCCCT	TTTTTAAAA	AGCCAACTA	ATATTTTCT
GTGAGTTAAT	ACATCTGTCAG	GTGTGTATGT	AACATTACTG
GACATTAAAA	AAAATTATTAC	ATTCTC-poly (A)	-Schwanz

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Fig. 4

	MSTFGYRRGL	SKYESIDED	LLASLSAEEL	KELERELEDI
5	EPDRNLPVGL	RQKSLTEKTP	TGTF SREALM	AYWEKESQKL
	LEKERLGECG	KVAEDKEESE	EELIFTESNS	EVSEEVYTEE
	EEEESEQEEE	EEDSDEEERT	IETAKGINGT	VNYDSVNSDN
	SKPKIFKSQI	ENINLTNGSN	GRNTESPAAI	HPCGNPTVIE
	DALDKIKSND	PDTTEVNLNN	IENITTQTLT	RFAEALKDNT
10	VVKTFSLANT	HADDSAAMAI	AEMLKANEHI	TNVNVESNFI
	TGKGILAIMR	ALQHNTVLTE	LRFHNQRHIM	GSQVEMEIVK
	LLKENTTLLR	LGYHFELPGP	RMSMTSILTR	NMDKQRQKRL
	QEQKQQEGYD	GGPNLRTKVV	QRGTPSSSPY	VSPRHSPWSS
	<u>PKLPKKVQTV</u>	RSRPLSPVAT	LPPPPPPPPP	PPPSSQRLPP
15	<u>PPPPPPPPPLP</u>	<u>EKKLITR</u> NIA	EVIKQQESAQ	RALQNGQKKK
	KGKKVKKQPN	SILKEIKNSL	RSVQEKKMED	SSRPSTPQRS
	AHENLMEAIR	GSSIKQLKRV	EVPEALRWEH	DL.

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Fig. 5a Nachweis der Expression in menschlichen Geweben

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Fig. 5b Nachweis der Expression in gesundem und insuffizientem menschlichem Herzgewebe

