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(54) Title: DISEASE-ASSOCIATED PROTEIN

(57) Abstract: Disclosed are methods and compositions for early diagnosis, monitoring and treatment of retinal dystrophy, age-related macular degeneration, Bardet-Biedl syndrome, Bassen-kornzweig syndrome, best disease, chroidema, gyrate atrophy, congenital amurosis, retsun syndrome, stargardt disease and Usher syndrome. In particular, the invention relates to a protein, termed "2Rdcvl," that is differentially transcribed and expressed in subjects suffering from retinal dystrophies and the like, such as retinal dystrophy and age-related macular degeneration compared with non-sufferers, antibodies which recognize this protein, and methods for diagnosing such conditions.

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DISEASE-ASSOCIATED PROTEIN**FIELD OF THE INVENTION**

This invention relates to methods and compositions for detection and treatment of retinal degenerative diseases. In particular, the invention relates to a protein that protects against cone degeneration, nucleic acid molecules that encode such a protein, antibodies that recognize the protein, and methods for diagnosing retinal degenerative diseases.

BACKGROUND OF THE INVENTION

Photoreceptors are a specialized subset of retinal neurons, that are responsible for vision. Photoreceptors consist of rods and cones which are the photosensitive cells of the retina. Each rod and cone elaborates a specialized cilium, referred to as an outer segment, that houses the phototransduction machinery. The rods contain a specific light-absorbing visual pigment, rhodopsin. There are three classes of cones in humans, characterized by the expression of distinct visual pigments: the blue cone, green cone and red cone pigments. Each type of visual pigment protein is tuned to absorb light maximally at different wavelengths. The rod rhodopsin mediates scotopic vision (in dim light), whereas the cone pigments are responsible for photopic vision (in bright light). The red, blue and green pigments also form the basis of color vision in humans. The visual pigments in rods and cones respond to light and generate an action potential in the output cells, the rod bipolar neurons, which is then relayed by the retinal ganglion neurons to produce a visual stimulus in the visual cortex.

In humans, a number of diseases of the retina involve the progressive degeneration and eventual death of photoreceptors, leading inexorably to blindness. Degeneration of photoreceptors, such as by inherited retinal dystrophies (e.g., retinitis pigmentosa), age-related macular degeneration and other maculopathies, or retinal detachment, are all characterized by the progressive atrophy and loss of function of photoreceptor outer segments. In addition, death of photoreceptors or loss of photoreceptor function results in partial deafferentation of second order retinal neurons (rod bipolar cells and horizontal cells) in patients with retinal dystrophies, thereby decreasing the overall efficiency of the

propagation of the electrical signal generated by photoreceptors. Secondary glial and pigment epithelium changes secondary to photoreceptors degeneration result in vascular changes leading to ischemia and gliosis. Trophic factors that are capable of rescuing photoreceptors from cell death and/or restoring the function of dysfunctional (atrophic or dystrophic) photoreceptors may represent useful therapies for the treatment of such conditions.

The progression of these conditions points to a sequential loss of the two classes of photoreceptors: initially rods are lost as a direct result of a genetic or environmental or unknown lesion, resulting in night blindness and a reduction in visual field followed inevitably by loss of cones leading to total blindness. Thus, cones die indirectly since they do not express the primary lesion.

Not all of the genes associated with retinal dystrophy have yet been identified. Identification of such genes would make possible to both diagnose the disease and identify effective therapies.

SUMMARY OF THE INVENTION

The invention relates generally to a novel gene family, Rod-derived Cone Viability Factor (Rdcvf). In a first aspect, the invention provides an isolated polypeptide with an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4. Such a polypeptide, or fragments thereof, is found in the eye of sufferers of retinal dystrophies to a much lesser extent than in the eye of individuals without retinal dystrophy. Fragments of the isolated polypeptide with an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4 will comprise polypeptides comprising from about 5 to 10 amino acids, preferably from about 10 to about 20 amino acids, more preferably from about 20 to about 100 amino acids, and most preferably from about 20 to about 50 amino acids. In accordance with this aspect of the invention there are provided a novel polypeptide of mammalian origin, and in particular of mouse or human origin as well as biologically, diagnostically or therapeutically useful fragments, variants and derivatives thereof, variants and derivatives of the fragments, and analogs of the foregoing. Also within the scope of the present invention are polypeptides that are substantially similar to the

polypeptide with the amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4 e.g. an amino acid sequence as set forth in SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12 and SEQ ID NO: 14.

In a second aspect, the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3. Also within the scope of the present invention are nucleic acids that are substantially similar to the nucleic acid with the nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 e.g. nucleotide sequences as set forth in SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13. In a preferred embodiment, the invention provides an isolated nucleic acid molecule that encodes for a polypeptide selected from the group consisting of the polypeptides set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14, e.g. nucleotides 45-374 of SEQ ID NO:1, nucleotides 26-676 of SEQ ID NO:3, nucleotides 24-353 of SEQ ID NO:5, nucleotides 48-686 of SEQ ID NO:7, nucleotides 265-570 of SEQ ID NO:9, nucleotides 300-770 of SEQ ID NO: 11 or nucleotides 331-738 of SEQ ID NO:13. In a preferred embodiment, the isolated DNA takes the form of a vector molecule comprising the DNA as set forth in SEQ ID NO:1 or SEQ ID NO:3.

A third aspect of the present invention encompasses a method for the diagnosis of retinal dystrophy in a human which includes detecting the decreased transcription of messenger RNA transcribed from Rdcvf1 or Rdcvf2-encoding DNA in the eye from a mammalian organism, preferably a human, where such decreased transcription is diagnostic of the organisms' affliction with retinal dystrophy or pathological aging (ARMD). Another embodiment of the assay aspect of the invention provides a method for the diagnosis of retinal dystrophy in a mammalian organism, preferably a human, which requires measuring the amount of a Rdcvf1 or Rdcvf2 polypeptide or fragments thereof in the eye of a human suspected of suffering from a retinal dystrophy, where the presence of a decreased amount of the polypeptide or fragments thereof, relative to the amount of the polypeptide or fragments thereof in the eye of an individual not suffering from a retinal dystrophy, is diagnostic of the human's suffering from retinal dystrophy.

In accordance with another aspect of the invention there are provided anti-sense polynucleotides that regulate transcription of the Rdcvfl or Rdcvf2 gene; in another embodiment, double stranded RNA is provided that can regulate the transcription of the Rdcvfl or Rdcvf2 gene.

Another aspect of the invention provides a process for producing the aforementioned polypeptides, polypeptide fragments, variants and derivatives, fragments of the variants and derivatives, and analogs of the foregoing. In a preferred embodiment of this aspect of the invention there are provided methods for producing the aforementioned Rdcvfl polypeptides comprising culturing host cells having incorporated therein an expression vector containing an exogenously-derived Rdcvfl or Rdcvf2 - encoding polynucleotide under conditions sufficient for expression of Rdcvfl or Rdcvf2 polypeptides in the host and then recovering the expressed polypeptide.

In accordance with another aspect of the invention there are provided products, compositions, processes and methods that utilize the aforementioned polypeptides and polynucleotides for, *inter alia*, research, biological, clinical and therapeutic purposes.

In certain additional preferred aspects of the invention there are provided an antibody or a fragment thereof which specifically binds to a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8, i.e., Rdcvfl, or SEQ ID NO: 10, SEQ ID NO: 12 or SEQ ID NO: 14, i.e. Rdcvf2. In certain particularly preferred aspects in this regard, the antibodies are highly selective for mammalian, preferably mouse and in particular human Rdcvfl or Rdcvf2 polypeptides or portions of suchRdcvfl or Rdcvf2 polypeptides. In a related aspect, an antibody or fragment thereof is provided that binds to a fragment or portion of the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.

In another aspect, methods of treating a disease in a subject, where the disease is mediated by or associated with a change in Rdcvfl or Rdcvf2 gene expression e.g. a decrease in the presence of RDCVF1 or RDCVF2 polypeptide in the eye, by the administration of a therapeutically effective amount of a RDCVF1 or RDCVF2 protein as set out in SEQ ID NO:2 SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10,

SEQ ID NO:12 or SEQ ID NO:14 or a related protein or a fragment or portion thereof to the subject is provided. Also provided are methods for the diagnosis of a disease or condition associated with an decrease in Rdcvf1 or Rdcvf2 gene expression or decrease in the presence of RDCVF1 or RDCVF2 polypeptide in a subject, which comprises utilizing an antibody that binds to a polypeptide with the amino acid sequence set out in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14, or a fragment or portion thereof in an immunoassay.

In yet another aspect, the invention provides cells which can be propagated in vitro, preferably vertebrate cells, which are capable upon growth in culture of producing a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14 or fragments thereof, where the cells contain transcriptional control DNA sequences, other than mouse or human Rdcvf1 or Rdcvf2 transcriptional control sequences, where the transcriptional control sequences control transcription of DNA encoding a polypeptide with the amino acid sequence according to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14 or fragments thereof.

In a related aspect, the present invention provides a method for producing Rdcvf1 or Rdcvf2 polypeptides which comprises culturing a host cell having incorporated therein an expression vector containing an exogenously-derived Rdcvf1 or Rdcvf2 -encoding polynucleotide under conditions sufficient for expression of Rdcvf1 or Rdcvf2 polypeptides in the host cell, thereby causing the production of an expressed polypeptide, and recovering the expressed polypeptide.

In yet another aspect of the present invention there are provided assay methods and kits comprising the components necessary to detect abnormal, e.g. below-normal expression of Rdcvf1 or Rdcvf2 polynucleotides or polypeptides or fragments thereof in body tissue samples derived from a patient, such kits comprising e.g., antibodies that bind to Rdcvf1 or Rdcvf2 or oligonucleotide probes that hybridize with polynucleotides of the invention. In a preferred embodiment, such kits also comprise instructions detailing the procedures by which the kit components are to be used.

In another aspect, the invention is directed to an Rdcvfl or Rdcvf2 polypeptide for use in the treatment of a human or animal body. A related aspect is directed to the use of an Rdcvfl or Rdcvf2 polypeptide or fragment thereof, nucleotide encoding Rdcvfl or Rdcvf2 or a fragment thereof, or antibody that binds to Rdcvfl or Rdcvf2 or a fragment thereof in the manufacture of a medicament to treat a retinal dystrophy.

In another aspect, the invention provides a retinoprotective agent comprising a polypeptide selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14, and, optionally, a pharmaceutically acceptable carrier. In a related aspect the invention provides a pharmaceutical compositions comprising a Rdcvfl or Rdcvf2 polypeptide or fragment thereof, nucleotide encoding Rdcvfl or Rdcvf2 or a fragment thereof, for the treatment of a retinal dystrophy. In another related aspect, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of a polypeptide selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14 and a pharmaceutically acceptable carrier.

In a related aspect, the invention provides a method for the treatment of retinal dystrophy comprising administering a therapeutically effective amount of a polypeptide selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14, and pharmaceutically acceptable carrier, to a subject in need.

In another aspect, the invention is directed to methods for the identification of molecules that can bind to Rdcvfl or Rdcvf2 and/or modulate the activity of Rdcvfl or Rdcvf2 or molecules that can bind to nucleic acid sequences that modulate the transcription or translation of Rdcvfl or Rdcvf2. Such methods are disclosed in, e.g., U.S. Patent Nos. 5,541,070; 5,567,317; 5,593,853; 5,670,326; 5,679,582; 5,856,083; 5,858,657; 5,866,341; 5,876,946; 5,989,814; 6,010,861; 6,020,141; 6,030,779; and 6,043024, all of which are incorporated by reference herein in their entirety. Molecules identified by such methods also fall within the scope of the present invention.

In yet another aspect, the invention is directed to methods for the introduction of nucleic acids of the invention into one or more tissues of a subject in need of treatment

with the result that one or more proteins encoded by the nucleic acids are expressed and or secreted by cells within the tissue.

In another aspect the invention provides a method of providing photoreceptor cells for implantation wherein the photoreceptor cells are cultured together with RdCVF1 or RdCVF2.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: mouse Rdcvfl nucleotide sequence from the expression cloning and mouse RdCVF1 amino acid sequence.

Figure 2: mouse RdcvflL nucleotide sequence, and amino acid sequence.

Figure 3: human Rdcvfl and human Rdcvfl amino acids sequence.

Figure 4: human RdcvflL nucleotide sequence, human RdcvflL amino acids sequence.

Figure 5: mouse Rdcvfl2 nucleotide sequence and : mouse Rdcvfl2 amino acids.

Figure 6: mouse Rdcvfl2L nucleotide sequence and mouse Rdcvfl2L amino acid.

Figure 7: human Rdcvfl2 nucleotide sequence, and human Rdcvfl2 amino acids sequence.

Figure 8: depicts an amino acid alignments of the short forms of Rdcvfl : (SEQ ID No 2, 6, 10 and 14) and of the long forms of Rdcvfl : SEQ ID No4, 8, 12 and 14).

Figure 9 depicts the primers for GST-Rdcvfl.

Figure 10: Multiple Alignment of RDCVF1/RDCVF2.

Figure 11: Comparison of mouse and human RDCVF2

Figure 12: Multiple Alignment of mouse Rdcvfl2 with EST clones be552141, bi517442, bg707818 and bi603812.

Figure 13: Multiple Alignment of Rdcvfl with EST clones bg299078, ai716631, bg294111, be108041 and bg395178.

Figure 14: EST sequence bg299078 corrected to match Rdcvfl.

Figure 15: EST sequence bg294111 corrected to match RdcvflL.

Figure 16: Real-time RT-PCR analysis of the expression of rod arrestin (A) and RdCSF1 (B) in 5 weeks retina C57BL/6@N 5 weeks (green) and C3H/HE@N (red).

Figure 17: RT-PCR analysis showing that Rdcvfl2 is expressed in a rod-dependent manner and is expressed in another part of the CNS.

Figure 18: PCR analysis showing that RdCVF1 is expressed in a rod-dependent manner.

DETAILED DESCRIPTION OF THE INVENTION

All patent applications, patents and literature references cited herein are hereby incorporated by reference in their entirety.

In practicing the present invention, many conventional techniques in molecular biology, microbiology, and recombinant DNA are used. These techniques are well known and are explained in, for example, Current Protocols in Molecular Biology, Volumes I, II, and III, 1997 (F. M. Ausubel ed.); Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; DNA Cloning: A Practical Approach, Volumes I and II, 1985 (D. N. Glover ed.); Oligonucleotide Synthesis, 1984 (M. L. Gait ed.); Nucleic Acid Hybridization, 1985, (Hames and Higgins); Transcription and Translation, 1984 (Hames and Higgins eds.); Animal Cell Culture, 1986 (R. I. Freshney ed.); Immobilized Cells and Enzymes, 1986 (IRL Press); Perbal, 1984, A Practical Guide to Molecular Cloning; the series, Methods in Enzymology (Academic Press, Inc.); Gene Transfer Vectors for Mammalian Cells, 1987 (J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory); and Methods in Enzymology Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively).

As used herein, "differentially expressed gene" refers to (a) a gene containing at least one of the DNA sequences disclosed herein (e.g., as shown in Figure 1 and SEQ ID NO:1 or as shown in Figure 2 and SEQ ID NO:3); (b) any DNA sequence that encodes

the amino acid sequence encoded by the DNA sequences disclosed herein (e.g., as shown in Figure 1 and SEQ ID NO:2 or as shown in Figure 2 and SEQ ID NO:4); or (c) any DNA sequence that is substantially similar to the coding sequences disclosed herein.

In its broadest sense, the term "substantially similar", when used herein with respect to a nucleotide sequence, means a nucleotide sequence corresponding to a reference nucleotide sequence, wherein the corresponding sequence encodes a polypeptide having substantially the same structure and function as the polypeptide encoded by the reference nucleotide sequence, e.g. where only changes in amino acids not affecting the polypeptide function occur. Desirably the substantially similar nucleotide sequence encodes the polypeptide encoded by the reference nucleotide sequence. The percentage of identity between the substantially similar nucleotide sequence and the reference nucleotide sequence desirably is at least 90%, more preferably at least 95%, still more preferably at least 99%. Sequence comparisons are carried out using a Smith-Waterman sequence alignment algorithm (see e.g. Waterman, M.S. *Introduction to Computational Biology: Maps, sequences and genomes*. Chapman & Hall. London: 1995. ISBN 0-412-99391-0, or at <http://www-hho.usc.edu/software/seqaln/index.html>). The localS program, version 1.16, is used with following parameters: match: 1, mismatch penalty: 0.33, open-gap penalty: 2, extended-gap penalty: 2. A nucleotide sequence "substantially similar" to reference nucleotide sequence hybridizes to the reference nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C, more desirably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C, more desirably still in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C, preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 50°C, more preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C, yet still encodes a functionally equivalent gene product.

The differentially expressed genes disclosed herein are expressed in eye tissue and in particular is produced in rod cells however, in a human afflicted with a retinal

dystrophy such as retinitis pigmentosa, age-related macular degeneration, Bardet-Biedel syndrome, Bassen-kornzweig syndrome, best disease, choroidema, gyrate atrophy, congenital amourosis, refsun syndrome, stargardt disease and Usher syndrome it is produced in decreases amounts relative to, i.e., to a lesser extent than in the corresponding tissues of humans who do not suffer from retinal dystrophy. Messenger RNA transcribed from the differentially expressed genes, and protein translated from such mRNA, is present in rod tissues and/or associated with such tissues in an amount at least about half, preferably at least about five times, more preferably at least amount ten times, most preferably at least about 100 times less than the levels of mRNA and protein found in corresponding tissues found in humans who do not suffer from a retinal dystrophy. Such decreases transcription of Rdcvfl or Rdcvf2 mRNA is referred to herein as "decreased transcription."

A "host cell," as used herein, refers to a prokaryotic or eukaryotic cell that contains heterologous DNA that has been introduced into the cell by any means, e.g., electroporation, calcium phosphate precipitation, microinjection, transformation, viral infection, and the like.

"Heterologous" as used herein means "of different natural origin" or represent a non-natural state. For example, if a host cell is transformed with a DNA or gene derived from another organism, particularly from another species, that gene is heterologous with respect to that host cell and also with respect to descendants of the host cell which carry that gene. Similarly, heterologous refers to a nucleotide sequence derived from and inserted into the same natural, original cell type, but which is present in a non-natural state, e.g. a different copy number, or under the control of different regulatory elements.

A vector molecule is a nucleic acid molecule into which heterologous nucleic acid may be inserted which can then be introduced into an appropriate host cell. Vectors preferably have one or more origin of replication, and one or more site into which the recombinant DNA can be inserted. Vectors often have convenient means by which cells with vectors can be selected from those without, e.g., they encode drug resistance genes. Common vectors include plasmids, viral genomes, and (primarily in yeast and bacteria) "artificial chromosomes."

"Plasmids" generally are designated herein by a lower case *p* preceded and/or followed by capital letters and/or numbers, in accordance with standard naming conventions that are familiar to those of skill in the art. Starting plasmids disclosed herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids by routine application of well known, published procedures. Many plasmids and other cloning and expression vectors that can be used in accordance with the present invention are well known and readily available to those of skill in the art. Moreover, those of skill readily may construct any number of other plasmids suitable for use in the invention. The properties, construction and use of such plasmids, as well as other vectors, in the present invention will be readily apparent to those of skill from the present disclosure.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated, even if subsequently reintroduced into the natural system. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

As used herein, the term "transcriptional control sequence" refers to DNA sequences, such as initiator sequences, enhancer sequences, and promoter sequences, which induce, repress, or otherwise control the transcription of protein encoding nucleic acid sequences to which they are operably linked.

As used herein, "Rdcvf1 transcriptional control sequences" or "Rdcvf2 transcriptional control sequences" are any of those transcriptional control sequences normally found associated with a mammalian Rdcvf1 or Rdcvf2 gene, preferably with the Rdcvf2 gene as found in the mouse or human genome.

As used herein, "non-human transcriptional control sequence" is any transcriptional control sequence not found in the human genome.

The term "polypeptide" is used interchangeably herein with the terms "polypeptides" and "protein(s)".

As used herein, a "chemical derivative" of a polypeptide of the invention is a polypeptide of the invention that contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed, for example, in Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, Pa. (1980).

As used herein, a "neuroprotective agent" is a compound that prevents or protects neuronal cells from degeneration. A "retinoprotective agent" is a compound that prevents or protects retinal cells from degeneration.

The invention includes nucleic acid molecules, preferably DNA molecules, such as (1) an isolated comprising a nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3, (2) isolated nucleic acid molecules that comprise nucleic acid sequences that hybridize under high stringency conditions to the isolated DNA as set forth in SEQ ID NO:1 or SEQ ID NO:3, and (3) nucleic acid sequences that hybridize to (1) or (2), above. Such hybridization conditions may be highly stringent or less highly stringent, as described above. In instances wherein the nucleic acid molecules are deoxyoligonucleotides ("oligos"), highly stringent conditions may refer, e.g., to washing in 6X SSC/0.05% sodium pyrophosphate at 37 °C. (for 14-base oligos), 48 °C. (for 17-base oligos), 55 °C. (for 20-base oligos), and 60 °C. (for 23-base oligos). Suitable ranges of such stringency conditions for nucleic acids of varying compositions are described in Krause and Aaronson (1991) Methods in Enzymology, 200:546-556 in addition to Maniatis et al., cited above.

These nucleic acid molecules may act as target gene antisense molecules, useful, for example, in target gene regulation and/or as antisense primers in amplification reactions of target gene nucleic acid sequences. Further, such sequences may be used as part of ribozyme and/or triple helix sequences, also useful for target gene regulation. Still

further, such molecules may be used as components of diagnostic methods whereby the presence of a RdCVF1 or RdCVF2 disease-causing allele, may be detected.

The invention also encompasses (a) vectors that contain any of the foregoing coding sequences (i.e., sense) and/or their complements (i.e., antisense); (b) expression vectors that contain any of the foregoing coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences; and (c) genetically engineered host cells that contain any of the foregoing coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell. As used herein, regulatory elements include but are not limited to inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression.

The invention includes fragments of any of the nucleic acid sequences disclosed herein. Fragments of the full length Rdcvfl or Rdcvf2 gene may be used as a hybridization probe for a cDNA library to isolate the full length gene and to isolate other genes which have a high sequence similarity to the Rdcvfl or Rdcvf2 gene and similar biological activity. Probes of this type preferably have at least about 30 bases and may contain, for example, from about 30 to about 50 bases, about 50 to about 100 bases, about 100 to about 200 bases, or more than 200 bases (e.g. 300). The probe may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete Rdcvfl or Rdcvf2 gene including regulatory and promoter regions, exons, and introns. An example of a screen comprises isolating the coding region of the Rdcvfl or Rdcvf2 gene by using the known DNA sequence to synthesize an oligonucleotide probe or random priming of the isolated sequence disclosed in Figure 1 to 8. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of human cDNA, genomic DNA to determine which individual clones of the library the probe hybridizes to.

In addition to the gene sequences described above, orthologs of such sequences, as may, for example be present in other species, may be identified and may be readily isolated, without undue experimentation, by molecular biological techniques well known in the art. Further, there may exist genes at other genetic loci within the genome that

encode proteins which have extensive homology (homologs) to one or more domains of such gene products. These genes may also be identified via similar techniques. Examples of orthologs or homologues are provided in Figures 8, 10, 11, 12 or 13.

For example, the isolated expressed gene sequence may be labeled and used to screen a cDNA library constructed from mRNA obtained from the organism of interest. Hybridization conditions will be of a lower stringency when the cDNA library was derived from an organism different from the type of organism from which the labeled sequence was derived. Alternatively, the labeled fragment may be used to screen a genomic library derived from the organism of interest, again, using appropriately low stringent conditions. Such low stringency conditions will be well known to those of skill in the art, and will vary predictably depending on the phylogeny of specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions see, for example, Sambrook et al. cited above.

Further, a previously unknown expressed gene-type sequence may be isolated by performing PCR using two degenerated oligonucleotide primer pools designed on the basis of amino acid sequences within the gene of interest. The template for the reaction may be cDNA obtained by reverse transcription of mRNA prepared from human or non-human cell lines or tissue known or suspected to express a homologue or splicing variants.

The PCR product may be subcloned and sequenced to ensure that the amplified sequences represent the sequences of a expressed gene-like nucleic acid sequence. The PCR fragment may then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment may be labeled and used to screen a bacteriophage cDNA library. Alternatively, the labeled fragment may be used to screen a genomic library.

PCR technology may also be utilized to isolate full length cDNA sequences. For example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source. A reverse transcription reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed"

with guanines using a standard terminal transferase reaction, the hybrid may be digested with RNAase H, and second strand synthesis may then be primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment may easily be isolated. For a review of cloning strategies which may be used, see e.g., Sambrook et al., 1989, *supra*.

In cases where the differentially expressed gene identified is the normal, or wild type, gene, this gene may be used to isolate mutant alleles of the gene. Such an isolation is preferable in processes and disorders which are known or suspected to have a genetic basis. Mutant alleles may be isolated from individuals either known or suspected to have a genotype which contributes to disease symptoms. Mutant alleles and mutant allele products may then be utilized in the diagnostic assay systems described below.

A cDNA of the mutant gene may be isolated, for example, by using RT-PCR, a technique which is well known to those of skill in the art. In this case, the first cDNA strand may be synthesized by hybridizing an oligo-dT oligonucleotide (or random hexamers) to mRNA isolated from tissue known or suspected to be expressed in an individual putatively carrying the mutant allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide that hybridizes specifically to the 5' end of the normal gene (or by any other means). Using these two primers, the product is then amplified via PCR, cloned into a suitable vector, and subjected to DNA sequence analysis through methods well known to those of skill in the art. By comparing the DNA sequence of the mutant gene to that of the normal gene, the mutation(s) responsible for the loss or alteration of function of the mutant gene product can be ascertained.

Alternatively, a genomic or cDNA library can be constructed and screened using DNA or RNA, from a tissue known to or suspected of expressing the gene of interest in an individual suspected of to carry the mutant allele. The normal gene or any suitable fragment thereof may then be labeled and used as a probe to identify the corresponding mutant allele in the library. The clone containing this gene may then be purified through methods routinely practiced in the art, and subjected to sequence analysis as described above.

Additionally, an expression library can be constructed utilizing DNA isolated from or cDNA synthesized from a tissue known to or suspected of expressing the gene of interest in an individual suspected of to carry the mutant allele. In this manner, gene products made by the putatively mutant tissue may be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against the normal gene product, as described, below. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where the mutation results in an expressed gene product with altered function (e.g., as a result of a missense mutation), a polyclonal set of antibodies are likely to cross-react with the mutant gene product. Library clones detected via their reaction with such labeled antibodies can be purified and subjected to sequence analysis as described above.

Differentially expressed gene products include those proteins encoded by nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:13, in particular, a polypeptide that is or includes the amino acid sequence set out in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14, or fragments thereof.

In addition, expressed gene products may include proteins that represent functionally equivalent gene products. Such an equivalent gene product may contain deletions, additions or substitutions of amino acid residues within the amino acid sequence encoded by the differentially expressed gene sequences described, above, but which result in a silent change, thus producing a functionally equivalent differentially expressed gene product (polymorphisms). Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved and on comparison with amino-acids sequence from other species.

For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and

glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Functionally equivalent," as utilized herein, may refer to a protein or polypeptide capable of exhibiting a substantially similar *in vivo* or *in vitro* activity as the endogenous differentially expressed gene products encoded by the differentially expressed gene sequences described above. "Functionally equivalent" may also refer to proteins or polypeptides capable of interacting with other cellular or extracellular molecules in a manner similar to the way in which the corresponding portion of the endogenous differentially expressed gene product would. For example, a "functionally equivalent" peptide would be able, in an immunoassay, to diminish the binding of an antibody to the corresponding peptide (i.e., the peptidic amino acid sequence of which was modified to achieve the "functionally equivalent" peptide) of the endogenous protein, or to the endogenous protein itself, where the antibody was raised against the corresponding peptide of the endogenous protein. An equimolar concentration of the functionally equivalent peptide will diminish the aforesaid binding of the corresponding peptide by at least about 5%, preferably between about 5% and 10%, more preferably between about 10% and 25%, even more preferably between about 25% and 50%, and most preferably between about 40% and 50%.

The differentially expressed gene products may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the differentially expressed gene polypeptides and peptides of the invention by expressing nucleic acid encoding differentially expressed gene sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing expressed gene protein coding sequences and appropriate transcriptional/translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Sambrook et al., 1989, *supra*, and Ausubel et al., 1989, *supra*. Alternatively, RNA or cDNA capable of encoding expressed gene protein sequences may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis",

1984, Gait, M. J. ed., IRL Press, Oxford, which is incorporated by reference herein in its entirety.

A variety of host-expression vector systems may be utilized to express the differentially expressed gene coding sequences of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the differentially expressed gene protein of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing differentially expressed gene protein coding sequences; yeast (e.g. *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the differentially expressed gene protein coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the differentially expressed gene protein coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid transformation vectors (e.g., Ti plasmid) containing differentially expressed gene protein coding sequences; or mammalian cell systems (e.g. COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothioneine promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter; Cytomegalovirus Early gene promoter).

Expression of RDCVF1 or RDCVF2 by a cell from an *Rdcvf1* or *Rdcvf2* gene that is native to a the cell can also be performed. Methods for such expression are detailed in, e.g., U.S. Patents 5,641,670; 5,733,761; 5,968,502; and 5,994,127, all of which are expressly incorporated by reference herein in their entirety. Cells that have been induced to express RDCVF1 or RDCVF2 by the methods of any of U.S. Patents 5,641,670; 5,733,761; 5,968,502; and 5,994,127 can be implanted into a desired tissue in a living animal in order to increase the local concentration of RDCVF1 or RDCVF2 in the tissue.

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the differentially expressed gene protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of antibodies or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the differentially expressed gene protein coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. PGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-asephagarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene protein can be released from the GST moiety by using these endopeptidases.

Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a chloramphenicol acetyl transferase ("cat") or luciferase transcription unit, downstream of restriction site or sites for introducing a candidate promoter fragment; i.e., a fragment that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the cat or luciferase gene engenders production of CAT or luciferase activity, which can be detected by standard CAT assays or luminometry. Vectors suitable to this end are well known and readily available. Three such vectors are pKK232-8, -pCM7 and pGL3 (Promega, E1751, Genebank Ass n° u47295). Thus, promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that readily may be obtained by the foregoing technique, using a reporter gene assay.

Among known bacterial promoters suitable for expression of polynucleotides and polypeptides in accordance with the present invention are the *E. coli* lacI and lacZ promoters, the T3 and T7 promoters, the T5 tac promoter, the lambda PR, PL promoters and the trp promoter. Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus ("RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is one of several insect systems that can be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The differentially expressed gene coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of differentially expressed gene coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (E.g., see Smith et al., 1983, *J. Virol.* 46: 584; Smith, U.S. Pat. No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the differentially expressed gene coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing differentially expressed gene protein in infected hosts. (e.g., See Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted differentially expressed gene coding sequences. These signals include the ATG initiation codon and adjacent sequences. In

cases where an entire differentially expressed gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the differentially expressed gene coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals (Kozack sequence) and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, *Methods in Enzymol.* 153:516-544).

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host per se are routine skills in the art.

Generally, recombinant expression vectors will include origins of replication, a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, and a selectable marker to permit isolation of vector containing cells after exposure to the vector.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, etc.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the differentially expressed protein may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the differentially expressed protein. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the differentially expressed protein. These stable cell lines might be used as a way of cellular therapy directly or after encapsulation.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk, hgprt or aprt cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hygro, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147) genes.

An alternative fusion protein system allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, et al., 1991, Proc. Natl. Acad. Sci. USA 88: 8972-8976). In this system, the gene of interest is subcloned

into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

When used as a component in assay systems such as those described below, the differentially expressed protein may be labeled, either directly or indirectly, to facilitate detection of a complex formed between the differentially expressed protein and a test substance. Any of a variety of suitable labeling systems may be used including but not limited to radioisotopes such as ^{125}I ; enzyme labeling systems that generate a detectable calorimetric signal or light when exposed to substrate; and fluorescent labels.

Where recombinant DNA technology is used to produce the differentially expressed protein for such assay systems, it may be advantageous to engineer fusion proteins that can facilitate labeling, immobilization and/or detection.

Indirect labeling involves the use of a protein, such as a labeled antibody, which specifically binds to either a differentially expressed gene product. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression library.

In another embodiment, nucleic acids comprising a sequence encoding RDCVF1 or RDCVF2 protein or functional derivative thereof, are administered to promote cone function, by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. In this embodiment of the invention, the nucleic acid produces its encoded protein that mediates a therapeutic effect by promoting cone function.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

In a preferred aspect, the therapeutic comprises a Rdcvf1 or Rdcvf2 nucleic acid that is part of an expression vector that expresses a RDCVF1 or RDCVF2 protein or fragment or chimeric protein thereof in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the Rdcvf1 or Rdcvf2 coding region, said promoter

being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which the Rdcvfl or Rdcvf2 coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the Rdcvfl or Rdcvf2 nucleic acid (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by infection using a defective or attenuated retroviral or other viral vector (adenovirus, adeno-associated virus and lentivirus) (see, e.g., U.S. Pat. No. 4,980,286 and others mentioned *infra*), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see e.g., U.S. Patents 5,166,320; 5,728,399; 5,874,297; and 6,030,954, all of which are incorporated by reference herein in their entirety) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO

92/06180; WO 92/22635; WO92/20316; WO93/14188; and WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (see, e.g., U.S. Patents 5,413,923; 5,416,260; and 5,574,205; and Zijlstra et al., 1989, *Nature* 342:435-438).

In a specific embodiment, a viral vector that contains the Rdcvfl or Rdcvf2 nucleic acid is used. For example, a retroviral vector can be used (see, e.g., U.S. Patents 5,219,740; 5,604,090; and 5,834,182). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The Rdcvfl or Rdcvf2 nucleic acid to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Methods for conducting adenovirus-based gene therapy are described in, e.g., U.S. Patents 5,824,544; 5,868,040; 5,871,722; 5,880,102; 5,882,877; 5,885,808; 5,932,210; 5,981,225; 5,994,106; 5,994,132; 5,994,134; 6,001,557; and 6,033,8843, all of which are incorporated by reference herein in their entirety.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy. Adeno-associated viruses are especially attractive vehicles for delivering genes to the retina. Methods for producing and utilizing AAV are described, e.g., in U.S. Patents 5,173,414; 5,252,479; 5,552,311; 5,658,785; 5,763,416; 5,773,289; 5,843,742; 5,869,040; 5,942,496; and 5,948,675, all of which are incorporated by reference herein in their entirety.

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those

cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient directly or after encapsulation.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, epithelial cells are injected, e.g., subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the patient. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, a Rdcvfl or Rdcvf2 nucleic acid is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem-and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention. Such stem cells include but are not limited to hematopoietic stem cells (HSC), stem cells of epithelial tissues such as the skin and the lining of the gut, embryonic heart muscle cells, liver stem cells (see, e.g., WO 94/08598), and neural stem cells (Stemple and Anderson, 1992, Cell 71:973-985).

Epithelial stem cells (ESCs) or keratinocytes can be obtained from tissues such as the skin and the lining of the gut by known procedures (Rheinwald, 1980, Meth. Cell Bio. 21A:229). In stratified epithelial tissue such as the skin, renewal occurs by mitosis of stem cells within the germinal layer, the layer closest to the basal lamina. Stem cells within the lining of the gut provide for a rapid renewal rate of this tissue. ESCs or keratinocytes obtained from the skin or lining of the gut of a patient or donor can be grown in tissue culture (Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771). If the ESCs are provided by a donor, a method for suppression of host versus graft reactivity (e.g., irradiation, drug or antibody administration to promote moderate immunosuppression) can also be used. Retinal stem cells (Tropepe et al., 2000, Science, 287: 2032).

With respect to hematopoietic stem cells (HSC), any technique which provides for the isolation, propagation, and maintenance *in vitro* of HSC can be used in this embodiment of the invention. Techniques by which this may be accomplished include (a) the isolation and establishment of HSC cultures from bone marrow cells isolated from the future host, or a donor, or (b) the use of previously established long-term HSC cultures, which may be allogeneic or xenogeneic. Non-autologous HSC are used preferably in conjunction with a method of suppressing transplantation immune reactions of the future host/patient. In a particular embodiment of the present invention, human bone marrow cells can be obtained from the posterior iliac crest by needle aspiration (see, e.g., Kodo et al., 1984, J. Clin. Invest. 73:1377-1384). In a preferred embodiment of the present

invention, the HSCs can be made highly enriched or in substantially pure form. This enrichment can be accomplished before, during, or after long-term culturing, and can be done by any techniques known in the art. Long-term cultures of bone marrow cells can be established and maintained by using, for example, modified Dexter cell culture techniques (Dexter et al., 1977, J. Cell Physiol. 91:335) or Witlock-Witte culture techniques (Witlock and Witte, 1982, Proc. Natl. Acad. Sci. USA 79:3608-3612).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Described herein are methods for the production of antibodies capable of specifically recognizing one or more differentially expressed gene epitopes. Such antibodies may include, but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Such antibodies may be used, for example, in the detection of a fingerprint, target, gene in a biological sample, or, alternatively, as a method for the inhibition of abnormal target gene activity. Thus, such antibodies may be utilized as part of disease treatment methods, and/or may be used as part of diagnostic techniques whereby patients may be tested for abnormal levels of Rdcvf1 or Rdcvf2, or for the presence of abnormal forms of Rdcvf1 or Rdcvf2 by sampling the aqueous humor and/or vitreous by methods familiar to those of skill in the art (e.g. Forster, RK, Abbott, RL, Gelender, H. (1980) Management of infectious endophthalmitis Ophthalmology 87,313-319].

For the production of antibodies to a differentially expressed gene, various host animals may be immunized by injection with a differentially expressed protein, or a portion thereof or by DNA immunization. Such host animals may include but are not limited to rabbits, mice, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide,

surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals such as those described above, may be immunized by injection with differentially expressed gene product supplemented with adjuvants as also described above.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and Milstein, (1975, *Nature* 256:495-497; and U.S. Pat. No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, *Immunology Today* 4:72; Cole et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies And Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.*, 81:6851-6855; Neuberger et al., 1984, *Nature*, 312:604-608; Takeda et al., 1985, *Nature*, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable or hypervariable region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, 1988, *Science* 242:423-426; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-546) can be adapted to produce differentially expressed gene-single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Most preferably, techniques useful for the production of "humanized antibodies" can be adapted to produce antibodies to the polypeptides, fragments, derivatives, and functional equivalents disclosed herein. Such techniques are disclosed in U.S. Patent Nos. 5,932, 448; 5,693,762; 5,693,761; 5,585,089; 5,530,101; 5,910,771; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,545,580; 5,661,016; and 5,770,429, the disclosures of all of which are incorporated by reference herein in their entirety.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, *Science*, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Particularly preferred, for ease of detection, is the sandwich assay, of which a number of variations exist, all of which are intended to be encompassed by the present invention.

For example, in a typical forward assay, unlabeled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen binary complex. At this point, a second antibody, labeled with a reporter molecule capable of inducing a detectable signal, is then added and incubated, allowing time sufficient for the formation of a ternary complex of antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal, or may be quantitated by comparing with a

control sample containing known amounts of antigen. Variations on the forward assay include the simultaneous assay, in which both sample and antibody are added simultaneously to the bound antibody, or a reverse assay in which the labeled antibody and sample to be tested are first combined, incubated and added to the unlabeled surface bound antibody. These techniques are well known to those skilled in the art, and the possibility of minor variations will be readily apparent. As used herein, "sandwich assay" is intended to encompass all variations on the basic two-site technique. For the immunoassays of the present invention, the only limiting factor is that the unlabeled and the labeled antibodies be an RdCVF1- or RdCVF2-specific antibody.

The most commonly used reporter molecules in this type of assay are either enzymes, fluorophore- or radionuclide-containing molecules. In the case of an enzyme immunoassay an enzyme is conjugated to the second antibody, usually by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different ligation techniques exist, which are well-known to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, among others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine or toluidine are commonly used. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. A solution containing the appropriate substrate is then added to the tertiary complex of antibody-RdCVF1- or RdCVF2-labeled antibody. The substrate reacts with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an evaluation of the amount of Rdcvfl or Rdcvf2 which is present in the serum sample.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody absorbs the light energy, inducing a state of excitability in the molecule, followed by

emission of the light at a characteristic longer wavelength. The emission appears as a characteristic color visually detectable with a light microscope. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotopes, chemiluminescent or bioluminescent molecules may also be employed. It will be readily apparent to the skilled artisan how to vary the procedure to suit the required use.

This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of the gene encoding a polypeptide selected from the group consisting of the polypeptides set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14 which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered spatial or temporal expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents (SSCP), or by direct DNA sequencing (e.g., Myers et al., *Science* (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton et al., *Proc Natl Acad Sci USA* (1985) 85: 4397-4401). In another embodiment, an array of oligonucleotides probes comprising the Rdcvfl or Rdcvf2 nucleotide sequence or

fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M. Chec et al., *Science*, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to disease through detection of mutation in the Rdcvfl or Rdcvf2 gene by the methods described. In addition, such diseases may be diagnosed by methods comprising determining from a sample derived from a subject an abnormal Rdcvfl or Rdcvf2 expression. Expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as a polypeptide of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

- (a) a polynucleotide of the present invention, preferably the nucleotide sequence encoding a polypeptide selected from the group consisting of the polypeptides set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14, or a fragment thereof;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a polypeptide of the present invention, preferably the polypeptide of or a fragment thereof; or
- (d) an antibody to a polypeptide of the present invention, preferably to a polypeptide selected from the group consisting of the polypeptides set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, particularly retinitis pigmentosa, age -related macular degeneration, Bardet-Biedel syndrome, Bassen-kornzweig syndrome, best disease, choroidema, gyrate atrophy, congenital amurosis, refsun syndrome, stargardt disease and Usher syndrome. The nucleotide sequences of the present invention are also valuable for chromosome localization. The chromosomal location can be obtained by PCR on DNA prepared from a panel of hybrid cell lines (mouse-hamster). The chromosomal location of the human gene can be predicted from the location of the mouse gene by synthesis (McCarthy et al.(1997), Genome research, 7, 1153). The sequence is specifically targeted to, and can hybridize with, a particular location on an individual chromosome, including a mouse or a human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library, RetNet). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of Rdcvf1 or Rdcvf2, mimetics or agonists. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical

carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

The pharmaceutical compositions encompassed by the invention may be administered by any number of routes: e.g. Transscleral delivery of bioreactive protein to the choroid and retina Ambati et al. (2000) *Investigative Ophthalmology and Visual Science*, 41, 1186. The formulation of topical ophthalmic preparations, including ophthalmic solutions, suspensions and ointments is well known to those skilled in the art (see Remington's *Pharmaceutical Sciences*, 18th Edition, Chapter 86, pages 1581-1592 Mack Publishing Company, 1990). Other modes of administration are available, including intracameral injections (which may be made directly into the anterior chamber or directly into the vitreous chamber), subconjunctival injections and retrobulbar injections, and methods and means for producing ophthalmic preparations suitable for such modes of administration are also well known.

As used in this application, "extraocular" refers to the ocular surface and the (external) space between the eyeball and the eyelid. Examples of extraocular regions include the eyelid fornix or cul-de-sac, the conjunctival surface and the corneal surface. This location is external to all ocular tissue and an invasive procedure is not required to access this region. Examples of extraocular systems include inserts and "topically" applied drops, gels or ointments which may be used to deliver therapeutic material to these regions. Extraocular devices are generally easily removable, even by the patient.

The following patents disclose extraocular systems which are used to administer drugs to the extraocular regions. Higuchi et al. discloses in U.S. Pat. Nos. 3,981,303, 3,986,510 and 3,995,635, a biodegradable ocular insert which contains a drug. The insert can be made in different shapes for retention in the cul-de-sac of the eyeball, the extraocular space between the eyeball and the eyelid. Several common biocompatible polymers are disclosed as suitable for use in fabricating this device. These polymers include zinc alginate, poly (lactic acid), poly (vinyl alcohol), poly (anhydrides) and poly (glycolic acid). The patents also describe membrane coated devices with reduced permeation to the drug and hollow chambers holding the drug formulation.

Theeuwes, U.S. Pat. No. 4,217,898, discloses microporous reservoirs which are used for controlled drug delivery. These devices are placed extraocularly in the ocular cul-de-sac. Among the polymer systems of interest include poly (vinylchloride)-co-poly (vinyl acetate) copolymers. Kaufman discloses in U.S. Pat. Nos. 4,865,846 and 4,882,150 an ophthalmic drug delivery system which contains at least one bio-erodible material or ointment carrier for the conjunctival sac. The patent discloses polymer systems, such as, poly (lactide), poly (glycolide), poly (vinyl alcohol) and cross linked collagen, as suitable delivery systems.

In the presently described use of RDCVF1 or RDCVF2 protein product for the treatment of retinal disease or injury it is also advantageous that a topically applied ophthalmic formulation include an agent to promote the penetration or transport of the therapeutic agent into the eye. Such agents are known in the art. For example, Ke et al., U.S. Pat. No. 5,221,696 disclose the use of materials to enhance the penetration of ophthalmic preparations through the cornea.

Intraocular systems are those systems which are suitable for use in any tissue compartment within, between or around the tissue layers of the eye itself. These locations include subconjunctival (under the ocular mucous membrane adjacent to the eyeball), orbital (behind the eyeball), and intracameral (within the chambers of the eyeball itself). In contrast to extraocular systems, an invasive procedure consisting of injection or implantation is required to access these regions.

The following patents disclose intraocular devices. Wong, U.S. Pat. No. 4,853,224, discloses microencapsulated drugs for introduction into the chamber of the eye. Polymers which are used in this system include polyesters and polyethers. Lee, U.S. Pat. No. 4,863,457, discloses a biodegradable device which is surgically implanted intraocularly for the sustained release of therapeutic agents. The device is designed for surgical implantation under the conjunctiva (mucous membrane of the eyeball). Krezancaki, U.S. Pat. No. 4,188,373, discloses a pharmaceutical vehicle which gels at human body temperature. This vehicle is an aqueous suspension of the drug and gums or cellulose derived synthetic derivatives. Haslam et al. discloses in U.S. Pat. Nos. 4,474,751 and 4,474,752 a polymer-drug system which is

liquid at room temperature and gels at body temperature. Suitable polymers used in this system include polyoxyethylene and polyoxy propylene. Davis et al. disclose in U.S. Pat. No. 5,384,333 a biodegradable injectable drug delivery polymer which provides long term drug release. The drug composition is made up of a pharmaceutically active agent in a biodegradable polymer matrix, where the polymer matrix is a solid at temperatures in the range 20.degree. to 37.degree. C. and is flowable at temperatures in the range 38.degree. to 52.degree. C. The drug delivery polymer is not limited to the delivery of soluble or liquid drug formulations. For example, the polymer can be used as a matrix for stabilizing and retaining at the site of injection drug-containing microspheres, liposomes or other particulate-bound drugs.

A particularly suitable vehicle for intraocular injection is sterile distilled water in which the RDCVF1 or RDCVF2 protein product is formulated as a sterile, isotonic solution, properly preserved. Yet another ophthalmic preparation may involve the formulation of the RDCVF1 or RDCVF2 protein product with an agent, such as injectable microspheres or liposomes, that provides for the slow or sustained release of the protein which may then be delivered as a depot injection. Other suitable means for the intraocular introduction of RDCVF1 or RDCVF2 protein product includes, implantable drug delivery devices or which contain the RDCVF1 or RDCVF2 protein product.

The ophthalmic preparations of the present invention, particularly topical preparations, may include other components, for example ophthalmically acceptable preservatives, tonicity agents, cosolvents, wetting agents, complexing agents, buffering agents, antimicrobials, antioxidants and surfactants, as are well known in the art. For example, suitable tonicity enhancing agents include alkali metal halides (preferably sodium or potassium chloride), mannitol, sorbitol and the like. Sufficient tonicity enhancing agent is advantageously added so that the formulation to be instilled into the eye is hypotonic or substantially isotonic. Suitable preservatives include, but are not limited to, benzalkonium chloride, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid and the like. Hydrogen peroxide may also be used as preservative. Suitable cosolvents include, but are not limited to, glycerin, propylene glycol and polyethylene glycol. Suitable complexing agents include caffeine,

polyvinylpyrrolidone, beta -cyclodextrin or hydroxypropyl- beta -cyclodextrin. Suitable surfactants or wetting agents include, but are not limited to, sorbitan esters, polysorbates such as polysorbate 80, tromethamine, lecithin, cholesterol, tyloxapol and the like. The buffers can be conventional buffers such as borate, citrate, phosphate, bicarbonate, or Tris-HCl.

The formulation components are present in concentrations that are acceptable to the extraocular or intraocular site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8. Additional formulation components may include materials which provide for the prolonged ocular residence of the extraocularly administered therapeutic agent so as to maximize the topical contact and promote absorption. Suitable materials include polymers or gel forming materials which provide for increased viscosity of the ophthalmic preparation. Chitosan is a particularly suitable material as an ocular release-rate controlling agent in sustained release liquid ophthalmic drug formulations (see U.S. Pat. No. 5,422,116, Yen, et. al.) The suitability of the formulations of the instant invention for controlled release (e.g., sustained and prolonged delivery) of an ophthalmic treating agent in the eye can be determined by various procedures known in the art, e.g., as described in Journal of Controlled Release, 6:367-373, 1987, as well as variations thereof.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethylcellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid

esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of Rdcvf1 or Rdcvf2, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate

concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example Rdcvf1 or Rdcvf2 or fragments thereof, antibodies to Rdcvf1, agonists, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc. Pharmaceutical formulations suitable for oral administration of proteins are described, e.g., in U.S.

Patents 5,008,114; 5,505,962; 5,641,515; 5,681,811; 5,700,486; 5,766,633; 5,792,451; 5,853,748; 5,972,387; 5,976,569; and 6,051,561.

The following Examples illustrate the present invention, without in any way limiting the scope thereof.

EXAMPLES

1. Expression cloning :

1) Total ARN purification from five weeks normal mouse retinas :

The cDNA library was constructed from retinas of Five weeks old C57BL/6@N mice generally according to the method of Glissin et al (1974), Biochemistry, 13, 2633-2637. Briefly, after killing, animals were enucleated and the eyes were first placed in Phosphate Buffer Saline (PBS) supplemented with 0,1% DiEthyl PyroCarbonate (DEPC). Neural retina was quickly dissected (the retinal pigmented epithelium was omitted in this tissue preparation). Quickly after each dissection, tissues were homogenised in fresh 6 M Guanidinium Chloride. Ten retinas were pooled in 2.4 ml GC in 4 ml sterile tubes, and the tissue was disrupted completely by strong homogenisation for 1 minute at room temperature.

Messenger RNA (mRNA) purification from five weeks normal mouse retina :
mRNA was isolated on oligo-dT coated porous beads (Oligotex, Qiagen) under stringent conditions according to the method of Kurabayashi et al, (1988). Nucleic Acids Res. Symposium series, 19, 61-64. Briefly, 100-150 µg total mouse retina RNA were mixed with 15 µl of oligo-dT beads in Binding Buffer [10 mM Tris pH 7.5 ; 0.3 M NaCl ; 0.1 M EDTA ; 0.5 % w/v Sodium Duodecyl Sulfate (SDS)] and incubated 6 minutes at 65°C in 0.5 l becher of water, then progressively cooled to room temperature for about 3-4 hours, then centrifuged at room temperature to recover the agarose beads. They were then washed twice by incubating 10 minutes in 0.4 ml of (0.1 M Tris pH 7.5 ; 0.1 M NaCl ; 1 mM EDTA ; 0.5 % w/v SDS). Bounded RNA (mRNA) were eluted in two steps with 50

μ l of 70°C-warmed RNase-free water, precipitated with 10 μ l Sodium Acetate pH 5.2 and 0.25 ml ethanol and incubated 12 hours at -70°C. mRNA were collected by centrifugation (1 hour at 15.000 rpm, followed by two washes with 70% ethanol) and resuspended in 20 μ l of RNase-free water. mRNA concentration was measured at 260 nm and the absence of rRNA contaminations was checked by gel electrophoresis under denaturating condition as above.

cDNA synthesis :

was carried out according to the method of Okayama and Berg,(1982),Mol Cell Biol., 2, 161-170. First strand synthesis was primed with 2.5 μ g of NotI adaptor oligonucleotide (5'TGTTACCAATCTGAAGTGGGAGCGGCCGACAA(T)₁₈ 3') and incubated for 2 hours with 50 units of modified Moloney Murine Leukaemia virus (M-MLV) reverse transcriptase (Superscript II, Life Technology) under the conditions recommended by the supplier. For second strand synthesis, reaction was incubated 4 hours at 14°C with 4 units of RNaseH and 100 units of DNA polymerase I in SS Buffer [40 mM Tris pH 7.2 ; 85 mM Potassium Chloride ; 4.4 mM Magnesium chloride ; 3 mM DTT ; 5 μ g/ml Bovin Serum Albumin (BSA)] in a final volume of 0.25 ml. EcoRI adapters (5'-OH AATTCCGGCACGAGG 3'-OH/3'-OH GCCGTGCTCC5'-PO₄) were ligated at both ends of double strand cDNA in 14 hours at 16°C using 40 units of T4 DNA ligase (Promega, Madison, USA) in a total volume of 20 μ l under the conditions recommended by the supplier. The products of this reaction are dscDNA that have an EcoRI half site in 5' and NotI half site in 3' that can be oriented in the cloning vector.

Ligation of dscDNA in pcDNA3 :

was performed according to Maniatis T.(1992), Molecular cloning: a laboratory manual, 2nd ed.,, using 10 μ g of pcDNA3 plasmid (Invitrogen) prepared by cutting with EcoRI and NotI (Promega, Madison, USA) under the conditions specified by the supplier.

Propagation of recombinant clones :

was performed generally according to the method of Birnboim et al (1979), Nucleic Acids Res., 7, 1513-1523. Briefly, In order to make pools of 100 primary clones, we slightly modified the XL1 Gold (Stratagene) transformation protocol (provided by Stratagene) as followed. After incubation in growth medium, the transformation reaction was brought to 20 % (vol./vol.) glycerol and 8 % (vol./vol.) horse serum albumin (HAS, Life Technologies). HAS and glycerol prevent death followed freezing thawing. A titration was done by plating on agar plates (100 µg/ml ampicillin) increasing volume of each transformation reaction to calculate the volume giving 100 colonies, while the bulk transformation reaction was stored at -80°C. Recombinant plasmids from the library were purified by 96 at once. In order to prepare 96 pools of 100 clones, the calculated volume corresponding to 100 clones was plated on agar and grown 20 hours at 37°C. The DNA was purified directly from colonies striped from agar plates. A stock of each culture at 23% glycerol was stored at -80°C. The DNA was purified using Qiawell ultra (Qiagen,) using a protocol recommended by the supplier. Typically, 10 µg of purified plasmid were obtained, the concentration of each preparation was measured using optical density at 260 nm. To sub-divide selected pools of 100 into pools of 10, 50 µl of a 1/250.000 dilution of the glycerol stock from the original pool was plated on an agar plate at 100 µg/ml ampicillin. After growth 16 hours at 37°C, individual 160 colonies were replicated on 16 agar plates (10 per plate) and grown 16 hours at 37°C. The 10 colonies from each plate were harvested and grown in liquid medium [Luria Broth (LB), 100 µg/ml ampicillin] for 3 hours at 37°C. A stock of these cultures at 30% glycerol was stored at -80°C. Plasmid DNA was prepared as before. To divide sub-pools of 10 into individual clones, 50 µl of a 1/250.000 dilution of the glycerol stock of the sub-pools of 10 was plated onto agar plate with 100 µg/ml ampicillin. After growth 16 hours at 37°C, 16 individual colonies were picked and grown 16 hours in 2 ml LB 100 µg/ml ampicillin. A stock of these cultures at 30% glycerol was stored at -80°C. Plasmid DNA was prepared as before.

Transient transfection in Cos-1 cells :

was performed using the method of Chen and Okayama, (1987). High-efficiency transformation of mammalian cells by plasmid DNA (Mol Cell Biol., 7, 2745-2752).

Chick embryo retinal cultures :

The protocol was adapted from Adler and Hatlcc [(1989) *Science*, 243, 391]. Chick embryonic retina (6 days *in ovo*) is dissociated and plated in monolayer culture. Under these culture conditions with the absence of differentiation signals, cones represent 60-80% of cells. We have produced polyclonal antibodies into rabbit against visinin (a chicken cone marker, Genbank accession number M84729) and verified that the proportion of cones in our culture is of 60-80%. The simple environment of our model (chemically defined medium, absence of cell to cell contacts) in addition to the ease and speed of the method make it a very appropriate system to study trophic factors involved in cone survival. Briefly, Retinas from embryos issued from a control isolate of progenitors, are dissected after six days of development *in ovo*, cells dissociated and plated at low density (10^5 cells/cm 2). During ten days cell viability (60-80% cones) was followed using LIVE/DEAD assay (Molecular probes, Eugene, USA) an assay that quantifies live and dead cells. The number of cells alive decreases down to 8% of initial cells number after seven days in culture in chemically defined medium. When performed in the presence of conditioned media from COS1 cells transfected with pools of clones from the library, live cells are counted after seven days *in vitro*.

Chicken progenitors (strain 657 red label) were maintained in a separated compartment for the purpose of this experiment in a hatching facility 25 km from the laboratory. Fertilized eggs obtained naturally were collected weekly, and maintained at 17°C (their biological zero) in the laboratory after hatching. Daily, 5 eggs are incubated for 24 hours at 20°C then 136 hours at 37°C with intermittent reversion of the inclination of the eggs in a humidified chamber. The day of the culture, eggs surface are washed with Mucocit-A then broken, the chicken embryos are transferred in PBS. The stage of development of the each embryo is verified be the 29th by visual comparison to Hamburger and Hamilton (1951), in (Essential Development Biology, Stern and Holland Ed.). Two of the embryos were chosen and enucleated, the eyes transferred in CO₂-independent medium (Life technologies). Retinas were dissected and transferred in Ringer buffer and washed twice. Retinas are cut in small pieces and treated 20 minutes at 37°C with a solution of trypsin

(0.25 % w/v). The reaction is stopped by addition of culture media (M199, Life Technologies) supplemented with 10 % inactivated FCS. The cell suspension is treated for few minutes in 25 µl of DNase I (1mg/ml, Sigma). The cell suspension is then washed twice in Chemical Defined Culture Media [CDCM, equal volumes of DMEM and M199 media (Life Technologies) and AB with Supplements (5µg/ml Insulin ; 5µg/ml Transforming ; 64 nM Progesterone ; 0.1mM Putrescine ; 5ng/ml Selenium ; 3mM Taurine ; 2.7 µM Cytidine 5'-diphosphoethanolamine ; 5.2 µM Cytidine 5'-diphosphocholine, 0.2 µg/ml Hydrocortisone ; 30 nM 3,3'-5-triiodo-L-thyronine ; 1 mM Sodium Pyruvate), 0.3 µM Prostaglandin D₂ ; 0.1 mg/ml linoleic acid] in order to remove the FCS. The concentration of cells stained with trypan blue is measured with Mallassez'cell and brought to two concentrations (5.6 and 1.12 10⁵ cells/ml) corresponding to the two plating densities (2 and 4 10⁵ cells/cm²).

Conditioned media from Cos-1 transfected cells are thaw on ice and 50 µl transferred to two 96 wells tissue culture treated black plate (Corning Costar) that have been coated a solution of 100 µg/ml Poly-L-lysine (Sigma) according the plan :

First round of screening :

1	1	1	1	2	C	2	2	2	3	3	P
C	3	3	4	4	4	C	4	5	5	5	5
6	C	6	6	6	7	7	C	7	7	8	8
8	8	C	9	9	9	9	10	C	10	10	10
11	11	11	C	11	12	12	12	12	C	13	13
13	13	14	14	C	14	14	15	15	15	C	15
16	16	16	16	17	C	17	17	17	18	18	C
18	18	19	19	19	19	P	20	20	20	C	20

Where numbers refer to n° of pools of 100 clones, C to conditioned media from Cos-1 cells transfected with the empty vector (pcDNA3) and P a positive control (conditioned media transfected with pcDNA-mouseGDNF).

Second and third round of screening :

x(y).01	x(y).01	x(y).01	x(y).01	x(y).02	C	x(y).02	x(y).02	x(y).02	x(y).03	x(y).03	P
C	x(y).03	x(y).03	x(y).04	x(y).04	x(y).04	C	x(y).04	x(y).05	x(y).05	x(y).05	x(y).05
x(y).06	C	x(y).06	x(y).06	x(y).06	x(y).07	x(y).07	C	x(y).07	x(y).07	x(y).08	x(y).08
x(y).08	x(y).08	C	x(y).09	x(y).09	x(y).09	x(y).09	x(y).10	C	x(y).10	x(y).10	x(y).10
x(y).11	x(y).11	x(y).11	C	x(y).11	x(y).12	x(y).12	x(y).12	x(y).12	C	x(y).13	x(y).13
x(y).13	x(y).13	x(y).14	x(y).14	C	x(y).14	x(y).14	x(y).15	x(y).15	x(y).15	C	x(y).15
x(y).16	x(y).16	x(y).16	x(y).16	x(y)	C	x(y)	x(y)	x(y)	C57	C57	C
C57	C57	C3H	C3H	C3H	C3H	P	0	0	0	C	0

Where x (second round) and y (third round) represent the n° of the pools selected in the first and second round respectively. 01 to 16 represent the sub-pools used. x(y) represents the parental pool from which the 16 pools derived. C is as is first round. P was modified as pCMVScript-CNTF in the second round and progressively to pcDNA-939.09.08 in the third round. 0 represents chick cone cells in CDM media only. C57 and C3H, conditioned media from explants of retinas prepared as described in (preparing conditioned media from explants of mouse retinas) from C57BL/6@N and C3H/He@N mouse retinas aged of 5 weeks respectively.

50 µl of cell suspensions corresponding to two densities (2 and 4 10⁵ cells/cm²) were added to the well of the two 96 wells plates filled conditioned media with a 8 channel motorized pipette (Biohit) in order to minimized experimental errors. Cells were incubated for 7 days at 37°C in 5% CO₂.

Preparing conditioned media from explants of mouse retinas :

The second and third round screening included positive controls adapted from Mohand-Saïd et al. (1998). Five weeks aged mouse 5C57BL/6@N (wild type) and C3H/He@N (*rdl*) were sacrificed and enucleated. Two retinas were dissected and incubated in 24 hours at 37°C in 5% CO₂ in 1.5 ml of CDM in 12 wells plates. Conditioned media were

recovered and concentrated by a factor 40 by ultra-filtration on Vivaspin (Sartorius, cutting point 10 kDa). Conditioned media were frizzed in liquid nitrogen and stored in aliquots at -20°C before used. The day of use, conditioned media were thawed on ice, diluted 10 times in CDM and sterilized by filtration on 0.22 µm filter (Acrodisk 13, Gelman Sciences).

Functional assay, Live/Dead assay :

The functional assay is based on the number of chicken retinal cells alive after 7 days incubation *in vitro*. We used Live/Dead assay kit (Molecular probes, Eugene, USA) that is based on the use of two fluorogenic dyes (Calcein AM and Ethidium dimer) that stain live and dead cells respectively. A cell that is alive processes a metabolic activity (here an esterase activity) that converts the substrate (Calcein AM) in its fluorescent product emitting at 520 nm. The membrane permeability of a dead cell is altered and permits the DNA staining of the nucleus by Ethidium dimmer emitting at 635 nm. A cell is alive : emitting at 520 nm after excitation at 485 nm, or dead : emitting at 635 nm after excitation at 520 nm. Using epifluorescence microscopy, the two types of fluorescent cells can be visualized separately. After 7 days *in vitro*, cells were incubated for 30 minutes at room temperature in the dark with 2.7 µM Calcein-AM and 0,3 mM Ethidium dimer.

Image acquisition :

Briefly, image acquisition consisted of autofocussing each well, automatic cell counting in two fluorescence's followed by processing of the raw data using specialist software e.g. Metamorph (Universal Imaging Corporation, West Chester, USA) to obtain digitalised pictures of each well of the plate. We used a inverted microscope (Nikon TE 200) equipped with a mercury epifluorescent lamp with two excitation filters 485 and 520 nm, two emission filters 520 and 635 nm, an object (x10), a computer driven motorized platine (Multicontrol 2000, Martzauzer and a CCD camera (Cohu).

To record the plate, it is positioned on the motorised platine, and the focus done manually on the first well and this plane is recorded (z origin). The threshold of the image dead and

live is set from the first well. The centre of the first well is adjusted using white light by aligning manually the bottom of the first well to the bottom of the image on the computer monitor, then by aligning the extreme right of the first well to the right of the image on the computer screen and to record the two positions. The centre of the first well is calculated and gives the position of the centre of each well of the plate. We have noticed in the development process that there is a slight higher density of cells at the edge of the well and excluded the edge from the acquisitions. It is important that the image from each well be centred perfectly in order to avoid any misleading results. When set-up, the first scan of the plate executes a recording of dead cells. The dead cells density is the less variable under these conditions. The application executes an auto focusing by taking images at different focal planes and choosing the brightest one, the right focus. This z position is stored and the platine executes programmed movements in the x and y axes taking a total of 4 images that when reconstituted in one image represents 2/3 of the surface of the well. A pile of images from the focus planes is stored for control. The platine executes an auto focusing and four acquisitions for each well of the plate starting by wells A1 to A12, then B12 to B1, C1 to C12 etc... At the end, the platine moves outside the plate in order to overexpose the last well (H1). The scanning of dead cells takes 30 minutes. The second scan (live cells) is executed after switching the filter. This second scan is using the recorded z positions of each well from the dead scan. Four images are taken from each well as for dead cells. At the end of the second scan (22 minutes) dead and live reconstituted images are stored in a file that is named automatically with the date of the day. Cells numbers (dead and live) are counted automatically with pre-set morphometric parameters (average) and displayed on the computer monitor in order to check if the experiment is correct. It is important to check on a daily basis that the number of cells alive is not too high. We have observed that if plated at too important density, the chicken retinal cells survived longer most likely by producing their own survival factor. We screened cells in the absence of this effect. Before scanning the second plate (same experiment with twice density of cells plated), we add an α at the end of the name of the log file from the first plate. Images of each experiment were stored on CD-rom. We have generated a library of more than 250 CD-rom.

Cells counting and selection of the pools :

The cells numbers (live and dead) were counted using images of each experiment stored on CD-rom. The log file from an experiment was first loaded on a computer (counting off-line) and opened using Metamorph software. In a first step, the images corresponding to the 14 wells C (conditioned media from Cos-1 cells transfected with the empty vector) are opened. After adjusting the threshold of the image, the command Integrated Morphometry Analysis is used to measure the distribution of the areas between 10 and 250 of the object (number of live cells for each total area) for these control wells. The distribution follows a Gaussian curve with the maximal number of objects corresponding to an isolated cell. This standard value (SV) is then used to calculate the value of the area above which an object will be counted as two cells (standard object cut, SOC) through our empirical function: SOC = 29/20.74 SV. The SOC value of each individual plate is used to count the number of live cells of the plate. These numbers are then transferred into a excel table.

For the first round of screening, the value were plotted for each pool as fold difference (increase or decrease live cells numbers) versus the average of the 14 control wells + the standard deviation. In order to redress the variations coming from the position difference within the plate, we calculated the average of the fold difference individually between the 80 wells corresponding to positions where the pools are tested and the 14 wells control for 200 independent plates. In average, the differences observed are due only to position difference and cells numbers were corrected with this coefficient. In order to discriminate in more stringent way the pools to be selected, the live cells counts were plotted as fold difference versus as control all values not exceeding 1.3 but exceeding 0.4. In this manner, all pools were fold difference versus the 14 wells corresponding to empty vector are in the interval of 0.4 to 1.3 are considered as having no effect and used as control. After correction, the fold difference versus control of the two plates was multiplied and the result sorted by decreasing fold difference. The pools on the top of that list were further checked by visual inspection of the graph corresponding to the 20 pools of the experiment (both plates) and of the images of live and dead cells to avoid to screen misleading pools.

For the second and third round, the plates screening for sub-pools include additional control. We prepared conditioned media from retina explants of 5 weeks of age. We selected experiments where positive effects were recorded for retina explants from C57BL/6@N and dismissed the others. The results were plotted as fold difference versus the 14 control wells, no recalculation of the control was made. The fold difference versus control of the two plates was multiplied and the results were sorted by decreasing fold difference for the 16 sub-pools.

Isolated cDNA were sequenced using T7 primer (5' GTAATACGACTCACTATAGGGC 3') on a capillary sequencer (CEQ2000, Beckman Coulter). DNA sequence was compared to databases using Basic Local Alignment Search Tool (BLAST).

Identification of Rdcvfl2 and human homologues:

Using the Rdcvfl sequence (the nucleotide sequence encoding the polypeptides set forth in SEQ ID NO:2 or SEQ ID NO:4) and BLAST, homologous murine and human polypeptides were identified (Figure 8). EST clones with homology to mouse RdCVF2 (GenBank Accession No: bc016199) were identified: GenBank Accession Nos: be552141, bi517442, bg707818, bi603812, ai433287, be088414, bg297383, bg297304 (see also Figure 12). EST clones with homology to mouse Rdcvfl (SEQ ID NO:1) were identified: GenBank Accession Nos: bg299078, ai716631, bg294111, be108041, bg395178 (see also Figure 13).

Real-time RT-PCR analysis of Rdcvfl expression :

The retinal expression of Rdcvfl by mouse C57BL/6@N and C3H/He@N aged as well as congenic C3H (+/+ and rd/rd) of 5 weeks is studied using real-time RT-PCR on a lightcycler (Roche) with sybergreen PCR kit (Roche). cDNAs are produced by priming with a random hexamer oligonucleotide (pdN6, Amersham), M-MLV reverse transcriptase (superscript II, Life Technologies) and total RNA from mouse retina prepared as in 1). cDNAs are normalized using a ubiquitous messenger Glucose-6-Phosphate DeHydrogenase (G6PDH). 0.2 μ l of first strand cDNAs synthesis (an equivalent of 10 ng of total RNA) is amplified with 2 μ M of the oligonucleotides of SEQ ID NO:24 and SEQ ID NO:25 in triple in a total volume of 25 μ l using the following

program : 30 seconds at 95°C, and 35 cycles of a sequence (1 second at 95°C, 18 seconds at 55°C, 10 seconds at 72°C). The analysis (Figure 16) shows that Rdcvfl expression decreases after rod degeneration in the rd1 mouse (C3H/He@N). Rdcvfl was also shown to be directly expressed by photoreceptors by real-time RT-PCR using RNA prepared from outer layer of the retina by vibratome sectionning.

Products were checked by agarose gel electrophoresis. Similar results are obtained with another pair of Rdcvfl specific primers. As a positive control, the expression of rod arrestin (Ass n° M24086) is monitored in the same conditions with primers (5' CTATTACGTCAAGCCTGTAGCC 3' and 5' CATCCTCATCTTCTTCCCTTC 3'). Confirmation that Rdcvfl is the cone protective factor can be obtained by adding a suitable amount of Rdcvfl to a retinal explant of 5 week old rd1 mouse (C3H/He@N). What is a suitable amount can be arrived at by some initial titration experiments. Compare with appropriate controls after 7 days cone survival will be increased.

RT-PCR Analysis of Rdcvf2

RT-PCR for Rdcvf2 expression was carried out using primers 5' GCCAGCGTTTCTGCCTTTAC 3' and 5' AAGCCCTGCCTGCTCTAACATC 3'. The analysis shows that RdCVF2 is expressed in a rod-dependent manner and that Rdcvf2 expression is not restricted to the retina, but that also other neuronal cells express Rdcvf2. (Figure 17), whereas the expression of Rdcvfl seems to be restricted to retinal cells.

Live/Death Assays of Rdcvfl or Rdcvf2

COS-1 cells are transfected with a suitable expression vector carrying the Rdcvfl or Rdcvf2 under control of a inducible promoter. Control cells are transfected with the empty vector. The cells are incubated for a suitable period of time upon induction of Rdcvfl or Rdcvf2 expression. Subsequently, the number of surviving cone cells incubated with conditioned media from COS-1 cells transfected with Rdcvfl or Rdcvf2 and the number of surviving control cells are counted according to the above described method. The cells expressing Rdcvfl or Rdcvf2 show a significantly higher amount of surviving cells.

Rod specific factor

Real-time RT-PCR analysis, carried out under standard conditions, of the expression of rod arrestin (control) and Rdcvfl in 5 weeks retinal explants from C57BL/6@N 5 weeks and C3H/HE@N, using the primers:

SEQ ID NO: 24 : 5' TCTATGTGTCCCAGGACCCTACAG 3'

SEQ ID NO: 25 : 5' TTTATGCACAAGTAGTACCAGGACAG 3'

demonstrates that RdCVF1 is expressed only in the presence of rods (Rod-derivedCVF1).

Production of polyclonal antibodies :

Polyclonal antibodies are prepared by injecting a Gluthation-S-Transfcerase (GST) purified fusion protein (GST-Rdcvfl) as well as from mouse RdCVF1 peptide sequence amino acids 11 to 32 from SEQ ID N02 (Ab n°2) and peptide sequence amino acids 79 to 96 from SEQ ID N02 (Ab n°3) into rabbit. The fusion construct pGST-Rdcvfl is prepared by amplification with oligonucleotides of SEQ ID NO:26 and SEQ ID NO:27 using pcDNA-Rdcvfl as template under standard conditions. The Open Reading Frame (ORF) of Rdcvfl is cloned in frame into pGex2TK (Pharmacia) between the BamHI and EcoRI restriction sites, and transformed into E. coli [BL21 (DE3) pLysS, Promega] by standard procedure. A single colony is grown in 3 litres of LB liquid media with 100 µg/ml ampicillin at 30°C and protein production is induced by addition of 1 µg/ml isopropylthio-β-D-galactoside (IPTG) and continued for 5 hours at 30°C. Cells are harvested, lysed by sonication and purified onto glutathione sepharose under standard protocol. The fusion protein is eluted with 10 mM reduced glutathione at room temperature. The Eluted protein is dialyzed into PBS before injection to rabbits. Protein purity is monitored by polyacrylamide gel electrophoresis. Two rabbits are immunized by intradermic injection at 80 sites of 100 µg of purified GST-Rdcvfl. Serum is harvested after 8 weeks.

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The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A pharmaceutical composition comprising a therapeutically effective amount of a polypeptide selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14 or a therapeutically useful fragment thereof and a pharmaceutically acceptable carrier.
2. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:13 and a pharmaceutically acceptable carrier.
3. Use of a polypeptide selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14 or a fragment thereof in the manufacture of a medicament for the treatment of retinal dystrophy.
4. Use of a nucleotide encoding a polypeptide selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14 or a fragment thereof in the manufacture of a medicament for the treatment of retinal dystrophy.
5. Use of an antibody that binds a polypeptide selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14 or a fragment thereof in the manufacture of a medicament for the treatment of retinal dystrophy.
6. The use according to any one of claims 3 to 5 wherein said treatment is retinitis pigmentosa, age-related macular degeneration, Bardet-Biedel syndrome, Basson-Kornzweig syndrome, best disease, choroidema, gyrate atrophy, congenital amourosis, retsun syndrome, stargardt disease or Usher syndrome.

7. A method for the treatment of retinal dystrophy comprising administering a therapeutically effective amount of a polypeptide selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or 5 SEQ ID NO:14, and a pharmaceutically acceptable carrier, to a subject in need.

8. A method according to claim 7 for the treatment of retinitis pigmentosa, age-related macular degeneration, Bardet-Biedel syndrome, Bassen-Kornzweig syndrome, best disease, chorioidema, gyrate atrophy, congenital amourosis, retsun syndrome, stargardt 10 disease or Usher syndrome.

9. A method for the diagnosis of retinal dystrophy in a human which comprises:

detecting the decreased transcription of messenger RNA transcribed corresponding 15 to a RDCVF1 or RDCVF2 cDNA as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:13, in the eye of a human, wherein said decreased transcription is diagnostic of said human's suffering from retinal dystrophy.

20 10. A method for the diagnosis of retinal dystrophy in a human which comprises:

measuring the amount of an RDCVF1 or RDCVF2 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14, or fragments thereof, in rod 25 cells of a human, wherein the presence of a decreased amount of said polypeptide or fragments thereof, relative to the amount of said polypeptide or fragments thereof in normal eye tissue, is diagnostic of said human's suffering from retinal dystrophy.

11. The method according to claim 10, wherein said detecting step comprises 30 contacting said tissue with an antibody which specifically binds to an RDCVF1 or RDCVF2 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2,

SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14, or a fragment thereof and detecting specific binding of said antibody with a polypeptide in said tissue, wherein detection of specific binding to a polypeptide indicates the presence of a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14, or a fragment thereof.

12. An isolated polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2, or a biologically useful fragment thereof or a substantially similar 10 polypeptide with at least 90% identity.

13. An isolated polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:4, or a substantially similar polypeptide with at least 99% identity.

15 14. A method for producing RDCVF1 or RDCVF2 polypeptides according to claims 12 or 13 comprising:

16 culturing a host cell having incorporated therein an expression vector containing an exogenously-derived polynucleotide encoding a polypeptide according to claim 12 or claim 13 under conditions sufficient for expression of said polypeptides in the host cell, 20 thereby causing the production of an expressed polypeptide; and recovering the polypeptide produced by said cell.

17. A vector comprising a nucleic acid sequence encoding an RDCVF1 or RDCVF2 polypeptide comprising an amino acid sequence according to claim 12 or claim 25 13 operably linked to a transcription control sequence for use in gene therapy.

18. The vector according to claim 15 wherein the vector is derived from an adenovirus or an adeno-associated virus.

30 19. A purified antibody or a fragment thereof which specifically binds to a polypeptide that comprises the amino acid according to claim 12 or claim 13.

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18. An antibody fragment according to claim 17 which is an Fab or F(ab')₂ fragment.

5 19. An antibody according to claim 17 which is a polyclonal antibody.

20. An antibody according to claim 17 which is a monoclonal antibody.

10 21. A kit for the diagnosis of retinal dystrophy in a human comprising a means for collecting a sample and an antibody according to claim 17.

DATED this 11th day of November, 2005

15 **Novartis AG AND Universite Louis Pasteur**

By DAVIES COLLISON CAVE

Patent Attorneys for the Applicants

20

Figure 1:**Mouse RdCVF1 cDNA: SEQ ID NO:1**

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1 ATCGGATCCCTCTCTGGTCCCCAGCTCCCTGCATACTGCTACCCATGGCA 50
51 TCTCTTCTCTGGACCCATCTGATCAGGAACAACAGGACCAAGGATGAA 100
101 ACGGGAGACAGAGGAGAGCTGAGCCGAGGGTAGGTTAGAGAATCGCTGGGTG 150
151 TCGTGTCTTCGGCGGGCGCCCTGCCCCAGTGCAGGGCTTTGCCCGA 200
201 GTCCCTAAAGACCTTCCTCGCGGCCCTACTGACGAGTTCTACGTGCTGCG 250
251 GGCAGCACAGCTGGCCCTGGCTATGTTGTCAGGACCCCTACAGAGGAGC 300
301 AACAGGACCTCTTCCTCAGGGACATGCGTAAAAATGGCTTCCCTGCCG 350
351 TTCCATGATGAACTGAGGAGGTGAGGCCAGGGAAAGACCAGGGAGGCT 400
401 TCTCGAGAGGGCATTTCCCTGGAGGTTACTGTCGTTACTTGTG 450
451 CATAAAGAGGTATTCCTC 468

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Polypeptide : ATG to TGA: SEQ ID NO:2

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1 MASLFSGRIL IRNNSDQDEV ETEAELSRLR ENRLVLLFFG AGACPOCQAF
51 APVLKDFFVR LTDEFYVLRA AQLALVIVSQ DPTEEQQDLF LRDMPEKWL
101 LPFHDELRR

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Figure 2:**Mouse RdCVF1L cDNA: SEQ ID NO:3**

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1 CCCAGCTCC TTGCATACTG CTACCATGGC ATCTCTCTTC TCTGGACGCA
51 TCTTGTACAG GACAAACAGC GACCAAGGATG AACTGGAGAC AGAGGCAAGAG
101 CTGAGCCGTA GGTTAGAGAA TCGCTGTGTT TTGCTGTCT TCGGCCCGGG
151 CGCTGTCCC CAGTGCCAGG CCTTGTCCCC AGTCTCTAAA GACTCTTCG
201 TGCGGCTCAC TGACGAGTT TACCTGCTGG GGGCAGCACAA GCTGGCCCTG
251 GTCATATGTTT CCAAGGACCC TACAGAGGAG CAACAGGACC TCTTCCCTAG
301 GGACATGCT GAAAATGGC TCTTCCCTGCC GTTCCATGAT GAACTGAGGA
351 CGGACCTCGG CGGCCAGGTT TCTCTCCCTC AACTGCCAGC GGTTGTGTTA
401 CTTAAAGCTTG GTGGGGAGCT GCTGACAGC GACGCCAGG AGGAGATCCA
451 GCGTCTGGGA CCCGCTGCT TTGCCAACTG GCAGGAGGCC CGAGAGCTCC
501 TGACCCAGGTT TTCTCTGCAA CGGGAGGATT TGATGAGGCC TGCGCCGCC
551 AGCATCAGG AGCCCTCTCGG CGGTGCGAAG TACCGAGTAG ACCGGATGT
601 CGGCGGGAGC GGGCGAAAC GGCGCAGTC TGTTGAACCC CAGGGGAGC
651 CGGGTACAGG GGCGAGCTC TGTTGACTCC CACGGTACCA CTGGGGACCG
701 GAGCTCTGGT GACACCAAGG TACCGGTGCA CGACCGAGGT TGATGACCT
751 CCCGAAGGAA CGGG

```

Polypeptide : ATG to TGA: SEQ ID NO:4

```

1 MASLFSGRIL IRNNSDQDEV ETEAELSRLR ENRLVLLFFG AGACPOCQAF
51 APVLKDFFVR LTDEFYVLRA AQLALVIVSQ DPTEEQQDLF LRDMPEKWL
101 LPFHDELRRR LGRQFSVRQL PAVVVLKPGG DVLTSDATEE IQLGPACFA
151 NWQEEAELLRSFLQPEDLD EPARRSITEP LRRKRYVRDR DVGGSGAKRR
201 DSGEPQGDAG TRARLW

```

Figure 3:**Human RdCVF1 cDNA: SEQ ID NO:5**

```

1  CCCAGCACCC AACCCAGGTT ACCATGGCCT CCCTTCTTC TGCCGGCATC
51  CTGATCCGCA ACAATAGCGA CCAGGACAG CTGGATACCG AGGCTGAGGT
101 CAGTCGCAGG CTGGAGAACC GGCTGGTCT GCTGTTCTT GTGCTGGGG
151 CTGTCCACA GTGCCAGGCC TTCCGAGCCA TCCTCAAGGA CTCTTCTCGT
201 CGGCTCACAG ATGAGTTCTA TGTAATGCCG CGGGCTCACG TGCCCTGCT
251 GTACGTGTCC CAGGACTCCA CGGAGGAGCA GCAGGACCTG TTCTCAAGG
301 ACATGCCAAA GAAATGGCTT TTCCIGCCCT TTGAGGATCA TCTGAGGAGC
351 TGA

```

Polypeptide : ATG to TGA: SEQ ID NO:6

```

1  MASLFSGRIL IRNNSDQDEL DTEAEVSRL ENRLVLLFFG AGACPQCQAF
51  VPILKDFFVR LTDEFYVLRA AQLALVYVSQ DSTEEQQDLF LKDMPKKKWLF
101 LPFEDDLRRD LGQFQFSVERL PAVVVLKEDG DVLTRDGADE IQLIGTACFA

```

Figure 4:**Human RdCVF1L cDNA: SEQ ID NO:7**

```

1  CCGGGGACCA CACGCCGCGC TGTCGCCAGC ACCAACCCA GGTATTACATG
51  GCCTCCCTGT TCTCTGGCCG CATCTGATC CGCACACAATA CGGACCCAGGA
101 CGAGCTGGAT ACGGAGGCTG AGGTCAGTCG CAGGTGGAG AACCGGCTGG
151 TGCTGCTGTT CTTTGGTCT GGGGCTTGTG CACAGTGCCA GGCTTCTCGT
201 CCCATCCCTCA AGGACTTTCT CGTCCGGCTC ACAGATGAGT TCTATGTA
251 GCGGGCGGCT CAGGTGGCCC TGIGTGACGT GTCCCAGGAC TCAACGGAGG
301 AGCAGCAGGA CCTGTTCCTC AAGGACATGC CAAAGAAATG GCTTTTCTG
351 CCCCTTGAGG ATGATCTGAG GAGGGACCTC GGCGCCCAAGT TCTCAGTGGA
401 GCGCCTGCGG CGGGTCGTGG TGCTCAAGCC GGACGGGGAC GTGCTCACTC
451 CGCACGGCGC CGACGAGATC CAGCGCTGG GCACCGCCTG CTTCGCAAC
501 TGGCAGGAGG CGGCCGAGGT GCTGGACCCG AACCTCCAGG TGCCAGAGGA
551 CCTGGAGGAC CAGGAGGCC CGAGGCTCAC CGAGTGCTG CGGGGCCACA
601 AGTACCGCGT GAAAAGGCG CGCGGAGGCG CGCGGACCC CGGGGGAGGG
651 GGTGGGGAGG AGGGCGGGGC CGGGGGCTG TTCTGA

```

Polypeptide : ATG to TGA: SEQ ID NO:8

```

1  MASLFSGRIL IRNNSDQDEL DTEAEVSRL ENRLVLLFFG AGACPQCQAF
51  VPILKDFFVR LTDEFYVLRA AQLALVYVSQ DSTEEQQDLF LKDMPKKKWLF
101 LPFEDDLRRD LGQFQFSVERL PAVVVLKEDG DVLTRDGADE IQLIGTACFA
151 NWQEEAAEVLD RNQQLPEDLE DQEPRSLITEC LRRHKYRVEK AARGGRDPPG
201 GGGEEGGAGG LF

```

Figure 5:**Mouse RdCVF2 cDNA: SEQ ID NO:9**

```

1  ATAAATAGA GGGTGGGAGA GCTTGTGGC GGGGCTCTGC TTTTGGTGC
51  GGGGCACCCA GCTGTGATCG CTGCTGTCCC ACCTTCTGGA GTGGCCACTG

101 TGCTCTCTCC TCCCTTCGGC TCAAGGTGAG CTGTTCCAGC AGAAGGGGG
151 GCTGAGAGGC GCCTAGTGTG GCGGGAGGCT CAGTGTCACTC TCCAGCTAA
201 CAGGTGGCG TCAAGCCCCAG GCCTCGTCTC TCCACTGTGT CCTCTTCAGC
251 CGGAGCTGTG GCGGATGGTGC GACGTGCTGG GCGGGCGGGG CCTGGTGACC
301 CGGGAGGGCA CGCTGCTGGA GCGCCGAGGTG GCGCTGCAGA ACAAGGTGGT
351 AGCTTGTAC TTGCGGCGG GCCGGTGCTC GCCCAGCCGC GACTTCACGC
401 CGCTCTCTCG CGACTTCTAC ACGGAGCTGG TGAGGGAGGC CGGGCGGCC
451 GCTCCCTTGG AGGTGGTTT CTGTCGGCA GACGGCAGTG CGGAGGAGAT
501 GTTGGACTTC ATGCAGGAGC TGCACTGGCTC CTGGCTGGCA TTGCCCCCTCC
551 ACGACCCCTA CGGGCAGTGA GTGGGGACCC AGGGGTCATG GGGCTGGCGC

```

Polypeptide : ATG to TGA: SEQ ID NO:10

```

1  MVDVLGGRRL VTRREGTVVVA EVALQNKVVA LYFAAGRCSP SRDFTPLLCD
51  FYTELVSEAR RPAPFEVVVFV SADGSAEEML DFMRELHGSW LalPFHDPR
101 Q

```

Figure 6:**Mouse RdCVF2L cDNA: SEQ ID NO:11**

```

1  TTGACTCTGG TGGGTAGAGA GGGTTTGCAA CGCAGGATAA AATAGAGGCT
51  GGGAGAGGTT GATGGCGTGG CTCTGCTTT TGTTGCGGGG CACCAAGCTGT
101 CATCGCTCT GTCGCACTT CTGGAGTGGC CACTGTGTC TCTCTCCCT
151 TCGGCTCAAG GTGAGCTGTT CCAGCAGAAG GCGGGGCTGA GAGGGGCCCTA
201 GTGCTGCGG AGGCTCAGTG TCATCTTCCA GCTAACAGGT GCGCGTGCAG
251 CCCAGGGCTC GTCTCTCCAC TGTTGCTCTC TCAACCCGAG CTCTGCGGGA
301 TGTTGACCT GTCTGGCGGG CGGGCGCTGG TGACCCGGGA GGGCACGGTG
351 GTGGAGGGCCG AGGTGGCGCT GCAAGAACAG GTGGTAGCTT TGTAATTG
401 GGGGGCCGG TGCTGGCCA CGCCGGACTT CACCCGGCTG CTCTGCCACT
451 TCTACACCGA GCTGGTGAGC GAGGGCGCCG GGGCGGCTCC CTTCGAGGTG
501 GTTTTCGTG CGGCAGACGG CAGTGGCGAG GAGATGTTGG ACTTCATGCG
551 CGAGCTGCAC GGCTCTGCG TGCGATTGCG CTTCACCGAC CCCTACCGGC
601 ATGAACTGAA GAAGAGGTAC GAAATCACCG CCATCCCCAA GCTGCGTGC
651 ATCAAGCAGA ACGGAGCTGT CATCACCAAC AAAGGGCGGA AGCAGATCCG
701 AGAGGGCGGG CTAGCTTCTG TTCAAGACTG GTGGAAGCA CGCCGATGTT
751 TCCAAAACCT CTGGGGGTGA CGAGGGCAGT TGCTGGAAGT TCAGGGCAAC
801 TATCTTCAAA AAGGGCTTAG CTGGTTCCCT TCTCTGCTGA GGAATGTCAT
851 TGAGAGTCA CCATGCTGTG ACAGAGACCA TAAACTGTC AGGAAGAAC
901 TACGTCTGCC CCCTGTGGGT CCTAGAGCTC CGTTGAATGT TTATTCTTA
951 CACCTTTCTC CACGGGTGCC TAGGATCCAG GACACATCAG CCACGGAGTTA
1001 ACAGAACTCT ATGCAAGATG CTCTTCTCA CAGGAAATT TTGATGATTTA
1051 TTGACCTATG GAGGTGATAC ATTTCTGAT GACATTGTTG TGATGCTTG
1101 GTAAACGTAT TTATTAATCG GGTTTGTAGA CTGTCGTAATT TAATAAACCA
1151 AACTCACAC TTG

```

Polypeptide : ATG to TGA: SEQ ID NO:12

```

1  MVDVLGGRRL VTRREGTVVVA EVALQNKVVA LYFAAGRCSP SRDFTPLLCD

```

51 FYTELVSEAR RPAPFEVVVFV SADGSAEEML DFMRELHGSW LALPFHDPR
 101 HELKKRYEIT AIPKLVVVIKQ NGAVITNKGR KQIRERGLAC FQNNVEAADV
 151 FQNFSG

Figure 7:

Human RdCVF2 cDNA: SEQ ID NO:13

1 CTGTGGCCGG CGCCGAGTTG GGGGAGGGTG CAGAGACCTG AGGGCTTGAG
 51 GTTGCCCTGGC TGCCCGCGCT CCCAGAGCGG GGTGCCGCGC TGTGCCAG
 101 GTATCTGGGG TCTCTGGTGT CTGAGTGTCT CATTGTCGGC GCGAACACAA
 151 TTGGCTCCAGC CACAGGGAG GCCTGGCCAA GGTTGGGGCG CATCTAGGGC
 201 AGGTCTTGAG AGGTCCAGGG CCCGGTGTG CGGACAGAGG CGGGGCACCG
 251 CGGCCTCGC CGCCGCTCC CGCGAGGTGA TCATCCCTCT GCAGGTGTCC
 301 TCGGGCTCGG GTGCTGGCC ATGGTTGACAA TTCTGGCCGA
 351 CGGGCACCTG GTGACCTGTA AGGGGGGAC CGTGGAGGCC GAGGGGGCGC
 401 TGCAAGAACAA GTGTGGGCA CTGACTTCG CGGGCGCCCG GTGGCGCCG
 451 ACAGCCGACT TCACCCCGCT GCTCTGGCAG TTCTTATACGG CGCTGGTGC
 501 CGAGGCCCGG CGGCCCGCGC CCTTCGAAGT GGTCCTTCGTG TCAGCCGACG
 551 GCAGCTGCCA GGAGATGCTG GACITCATGC GCGAGCTGCA TGCGCCTGG
 601 CTGGGCTCGC CCTTCACAGA CCCCTACCGG CAACGGAGTC TCGCTCTGTT
 651 GCCAAGCTG GAGTGCAGTG CGGTGATCT AGCTCACTGC AACCTTTC
 701 TCCTGGGTC AAGTGATTCT CTAGCTTCTAG CCTCTTGAGC ATCTGGGACT
 751 ACAGCCGACT CTGTGAATTA CGTGGGGAA AGATATTGAA GAGGAGITGG
 801 ACACTCCGAG ATGTGAGCTG TTCTCCCGGC GCACCATCGG TGTCCTGCAT
 851 TCTGCGAGTC TGTGCTCATT AACAAATGTGC TGTCGACCATG TGACTCAGCA
 901 ATCCCTGCTGC TGGGTATATA CCCGAAAGAA AGGAAAAGGA AGCCAGTATA
 951 TTGAAGAGGT ATCTGCACCC CCACTTAT TGCAACACTG TTCAACACAG
 1001 CCAAGATTG GAAGCAACCT AAGTGTCCAT CAACAGATGA ATGGATAAAG
 1051 AAAACGCTGT ACATATACAC AATGGAGTAC TCCTCAGCCA TTAAAAAAAT
 1101 GAGATTCTGT CATTGGAAT AATATAGATG GAAAAGGAGG CCCTTATGTG
 1151 AAGTGAATAA AGCCAGGAC AGAAAAGACAA ACATCACATG TTCTCACITA
 1201 TTGTGGGAT CTAATGATCA AACAAATGGA ACTCTTGAC ATAGAGACTA
 1251 GAAGGTTGT TACCAAGAACG TGGAAGGAA ATGCGGTG GGAGGAAGGT
 1301 GGGAAATGTT AATAGGTACA AAAAATACA AAGAATAAAT AAGACCTAAT
 1351 ATTGATAGC ACAACAGITG GACTACTGTC AATAATCATT TAATITGTACA
 1401 TTAAAAATA ACTATAATG CATTGTTGT AACACAAAAG ATAATGCTT
 1451 GAGGAGAAA AAAAAAAA AA

Polypeptide : ATG to TGA : SEQ ID NO:14

1 MVDILGERHL VTCKGATVEA EAALQNKVVA LYFAAARCAP SRDFTPLLCD
 51 FYTALVAEAR RPAPFEVVVFV SADGSCQEML DFMRELHGSW LALPFHDPR
 101 QRSLALLPRL ECGSVILAHG NLCLLGSDDS LALAS

Figure 8:

Pileup short sequences :

1	50
MRdCVF1 MASLFSGRIL IRNNNSDQDEV ETEAELSRRRL ENRLVLLFFG AGACPQCQAF	
hRdCVF1 MASLFSGRIL IRNNSDQDEL DTEAEVSRRL ENRLVLLFFG AGACPQCQAF	
MRdCVF2 MVDVLGGRRL VTRREG...T VVEAEVA..L QNKVVALYFA AGRCSPSRDF	
hRdCVF2 MVDILGERHL VTCKG...A TVEAEAA..L QNKVVALYFA AARCAPSRDF	

	51	100
mRdCVF1	APVLIKDFVRLA LTDEFYVLRA AQLALVYVVSQ DPTEEQQDLF LRDMPEKWL	
hRdCVF1	VPILKDFVRLA LTDEFYVLRA AQLALVYVVSQ DPTEEQQDLF LKDMPEKWL	
mRdCVF2	TPLLCDFYTE LVSE..ARRP APFEVVFVSA DGSAEEMLDF MRELGWLA	
hRdCVF2	TPLLCDFYTA LVAE..ARRP APFEVVFVSA DGSCQEMLDF MRELGWLA	
	101	143
mRdCVF1	LPFHDELRR	
hRdCVF1	LPFEDDLRR	
mRdCVF2	LPFHDPYRQ	
hRdCVF2	LPFHDPYRQR SLALLPRLEC SGVILAHCNL CLLGSSDSLA LAS	

Pileup long sequences :

	1	50
mRdCVF1L	MASLFSGRL IRNNSDQDEV ETEAELSRRRL ENRLVLLFFG AGACFQCQAF	
hRdCVF1L	MASLFSGRL IRNNSDQDEL DTEAEVSRRRL ENRLVLLFFG AGACFQCQAF	
mRdCVF2L	MVDVLGGRRL VTRREG....T VVEAEVA..L QNKVVALYFA AGRCSPSRDF	
hRdCVF2	MVDILGERHL VTCKG....A TVEAEAA..L QNKVVALYFA AARCAPSRRDF	
	51	100
mRdCVF1L	APVLKDFVRLA LTDEFYVLRA AQLALVYVVSQ DPTEEQQDLF LRDMPEKWL	
hRdCVF1L	VPILKDFVRLA LTDEFYVLRA AQLALVYVVSQ DPTEEQQDLF LKDMPEKWL	
mRdCVF2L	TPLLCDFYTE LVSE..ARRP APFEVVFVSA DGSAEEMLDF MRELGWLA	
hRdCVF2	TPLLCDFYTA LVAE..ARRP APFEVVFVSA DGSCQEMLDF MRELGWLA	
	101	150
mRdCVF1L	LPFHDELRRD ...LGRQFSV RQLPAVVVLK PGDVLTSDA TEEIQRIGPA	
hRdCVF1L	LPFEDDLRRD ...LGRQFSV ERLPAVVVLK PDGDVLTTRDG ADEIQRIGTA	
mRdCVF2L	LPFHDPYRHE ...LKKRYEI TAIPKLVVIK QNGAVITNKG RKQIRERGLA	
hRdCVF2	LPFHDPYRQR SLALLPRLEC SGV...ILAH CNLCLLGSSD SLALAS	
	151	200
mRdCVF1L	CFANWQEAEE LLDRSFLQPE DLDEPARRSI TEPLRRRKYR VDRDVGGSGA	
hRdCVF1L	CFANWQEAEE VLDRNFQLPE DLDEQEPRLS TECLRRHKYR VEKAARGG..	
mRdCVF2L	CFQNWVEAAD VFQNFSG	
hRdCVF2		
	201	219
mRdCVF1L	KRDSGEPOQ DAGTRAELW	
hRdCVF1L	..RDPGGGGG EGGAGGLF	
mRdCVF2L		
hRdCVF2		

Figure 9:

SEQ ID NO: 26: 5' cggatccATGGCATCTCTCTCTGGACGC 3'

SEQ ID NO: 27: 5' ggaatTCACCTCCTCAGTTCATCATGGAA 3'

Figure 10:

RdCVF1	1	50
MASLFSGRIL IRNNNSDQDEV ETEAELSRLI ENRLVLLFFG AGACPQQCQAF		
RdCVF2	MVDVLGGRRL V....TREGT VVEAEVA..L QNKVVVALYFA AGRCSPSRDF	
RdCVF1	51	100
RdCVF2	APVLIKDFVRL LTDEFYVLRA AQLALVYVSQ DPTEEQQDLF LRDMPEKWL	
RdCVF1	101	100
RdCVF2	LPFHDELRR LPFHDPYRQ	

Figure 11:

bc016199	mouse RdCVF2	
bg707818	human RdCVF2	
bc016199	1	50
bg707818	MVDVLGGRRL VTRREGTVVVA EVALONKVVA LYFAAGRCSP SRDFTPLLCD	
bc016199	51	100
bg707818	FYTELVSEAR RPAPFEVVVFV SADGSAEEML DFMRELHGSW LALPFHDPR	
bc016199	101	120
bg707818	Q* QRSLALLPRL ECSVILAH*	

Figure 12:

Name: mRdCVF2	mouse sequence bc016199
Name: be552141	homo sapiens
Name: bi517442	homo sapiens
Name: bg707818	homo sapiens
Name: bi603812	homo sapiens
mRdCVF2	ATGGT GGACGTGCTG
be552141	TGTCCTCGGG TCTCAGGTGG CTGCGTGTCT GCGCCATGGT TGACATTCTG
bi517442	TGTCCTCGGG TCTCAGGTGG CTGCGTGTCT GCGCCATGGT TGACATTCTG
bg707818	TGTCCTCGGG TCTCAGGTGG CTGCGTGTCT GCGCCATGGT TGACATTCTG
bi603812	TGTCCTCGGG TCTCAGGTGG CTGCGTGTCT GCGCCATGGT TGACATTCTG
mRdCVF2	GGCGGGCGGC GCCTGGTGAC CGCGGAGGGC ACGGTGGTGG AGGCCGAGGT
be552141	GGCGAGCGGC ACCTGGTGAC CTGTAAGGGC GCGACGGTGG AGGCCGAGGC
bi517442	GGCGAGCGGC ACCTGGTGAC CTGTAAGGGC GCGACGGTGG AGGCCGAGGC
bg707818	GGCGAGCGGC ACCTGGTGAC CTGTAAGGGC GCGACGGTGG AGGCCGAGGC
bi603812	GGCGAGCGGC ACCTGGTGAC CTGTAAGGGC GCGACGGTGG AGGCCGAGGC
mRdCVF2	GGCGCTGCAG AACRAGGTGG TAGCTTTGTA CTTCGGCGCG GGGCGGTGCT
be552141	GGCGCTGCAG AACAAAGGTGG TGGCACTGTA CTTCGGCGCG GCGCGGTGCG
bi517442	GGCGCTGCAG AACAAAGGTGG TGGCACTGTA CTTCGGCGCG GCGCGGTGCG
bg707818	GGCGCTGCAG AACAAAGGTGG TGGCACTGTA CTTCGGCGCG GCGCGGTGCG
bi603812	GGCGCTGCAG AACAAAGGTGG TGGCACTGTA CTTCGGCGCG GCGCGGTGCG

```

mRdCVF2 CGCCCGAGCG CGACTTCACG CGCTGCTCT GCGACTTCTA CACGGAGCTG
be552141 CGCCGAGCG CGACTTCACG CGCTGCTCT GCGACTTCTA TACGGCGCTG
bi517442 CGCCGAGCG CGA.TTCACG CGCTGCTCT GCGACTTCTA TACGGCGCTG
bg707818 CGCCGAGCG CGACTTCACG CGCTGCTCT GCGACTTCTA TACGGCGCTG
bi603812 CGCCGAGCG CGACTTCACG CGCTGCTCT GCGACTTCTA TACGGCGCTG

mRdCVF2 GTGAGCGAGG CGCGGCGGCC CGCTCCCTTC GAGGTGGTTT TCGTGTCCGC
be552141 GTGGCCGAGG CGCGGCGGCC CGCGCCCTTC GAAGTGGTCT TCGTGTCCGC
bi517442 GTGGCCGAGG ...CGGGGG CGCGCCCTTC GAAGTGGTCT TCGTGTCCGC
bg707818 GTGGCCGAGG CGCGGCGGCC CGCGCCCTTC GAAGTGGTCT TCGTGTCCGC
bi603812 GTGGCCGAGG CGCGGCGGCC CGCGCCCTTC GAAGTGGTCT TCGTGTCCGC

mRdCVF2 AGACGGCAGT GCGGAGGAGA TGT.TGGACT TCATGCGCGA GCTGCACCGC
be552141 CGACGGCAGC TCCCAAGGAGA TGC.TGGACT TCATGCGCGA GCTGCATGGC
bi517442 CGACGGCAGC TCCCAAGGAGA TGC.TGGACT TCATGCGCGA GCTG.ATGGC
bg707818 CGACGGCAGC TGCCAGGAGA TGC.TGGACT TCATGCGCGA GCTGCATGGC
bi603812 CGACGGCAGC TGCCAGGAGA TGC.TGGACT TCATGCGCGA GCTGCATGGC

mRdCVF2 TCCTGGC.TG GCATTGCCCT TCCACGACCC CTACCGGCAG TGA
be552141 GCCTGGC.TG GCGCTGCCCT TCCACGACCC CTACCGGCAC CATTGCTGTG
bi517442 GCCTGGC.TG GCGCTG.CCT TCCACGACCC CTACCGGCAC AGCCGGAGCC
bg707818 GCCTGGC.TG GCGCTGCCCT TCCACGAAAC CTACCGGCAA CGGAGTCTCG
bi603812 GCCTGGCTTG GCGCTGCCCT TCCACGACCC CTACCGGCAA CGGAGTCTCG

```

Figure 13:

pileup

```

GapWeight: 5
GapLengthWeight: 1

Name: RdCVF1
Name: bg299078
Name: ai716631
Name: bg294111
Name: be108041
Name: bg395178 Rev

      1                               50
RdCVF1 ----- ----- ----- ----- ----- ----- -----
bg299078 ----- ----- ----- ----- ----- ----- -----
ai716631 ----- ----- ----- ----- ----- ----- -----
bg294111 ----- ----- ----- ----- ----- ----- -----
be108041 ACAGGGTCAA CCTTGGCTAC ACAGGGACTC TGAGGACAGC ATGGNTACA
bg395178 ----- ----- ----- ----- ----- ----- -----
                                         51                               100
RdCVF1 ----- ----- ----- ----- ATGGC ATCTCTCTTC TCTGGACGCA
bg299078 CCCGAGCTCC TTGCATACTG CTACCATGGC ATCTCTCTTC TCTGGACGCA
ai716631 TAAGACGGAT CCCGACTAC GTAGTCGGGA ATCGGCACG AGGGGCGGCA
bg294111 ---CAGCTCC TTGCATACTG CTACCATGGC ATCTCTCTTC TCTGGACGCA
be108041 AGAAACCTTC TCTCAAAACC AAAAAGGCC TGCGAGTACT AGTGCACCTG
bg395178 TCCCCAGCAC CCAACCCAGG TTACCATGGC CTCCCTGTT TCTGGCCCA

      101                               150
RdCVF1 TCTTGATCAG GACAACAGC GACCAGGATG AAGTGGACAC AGAGGCAGAG
bg299078 TCTTGATCAG GACAACAGC GACCAGGATG AAGTGGAGAC AGAGGCAGAG

```

ai716631	TCTTGATCG	GAACAACAGC	GACCAGGTG	AACTGGAGAC	AGAGGCAGAG	
bg294111	TCTTGATCG	GAACAACAGC	GACCAGGTG	AACTGGAGAC	AGAGGCAGAG	
be108041	GGAGGCGAG	GAACAACAGC	GACCAGGTG	AACTGGAGAC	AGAGGCAGAG	
bg395178	TCTTGATCG	CAACAATAGC	GACCAGGACG	AGCTGGATAC	GGAGGCTGAG	
	151				200	
RdCVF1	CTGAGCCGTA	GGTTAGAGAA	TCGTCCTGGT	TTGCTGTTCT	TCGGCGCCGG	
bg299078	CTGAGCCGTA	GGTTAGAGAA	TCGTCCTGGT	TTGCTGTTCT	TCGGCGCCGG	
ai716631	CTGAGCCGCG	GGTTAGAGAA	TCGTCCTGGT	CTACTGTTCT	TCGGTGTCTGG	
bg294111	CTGAGCCGTA	GGTTAGAGAA	TCGTCCTGGT	<u>TCGTCCTGGT</u>	TCGGCGCCGG	
be108041	CTGAGCCGCC	GGTTAGAGAA	TCGTCCTGGT	CTACTGTTCT	TCGGTGTCTGG	
bg395178	GTCAGTCGCA	GGCTGGAGAA	CCGGCTGGTG	CTGCTGTTCT	TTGGTGTCTGG	
	201				250	
RdCVF1	CGCCCTGTCCC	CAGTGCCAGG	CCCTTGCCCC	AGTCCTCAAA	GACTTCCTCG	
bg299078	CGCCCTGTCCC	CAGTGCCAGG	<u>CC</u> .TTGCC	AGTCCTCAAA	GACTTCCTCG	
ai716631	CGCCCTGTCCC	CAGTGCCAGG	CCCTTGCCCC	AGTCCTCAAA	GACTTCCTCG	
bg294111	CGCCCTGTCCC	CAGTGCCAGG	<u>CC</u> .TTGCC	AGTCCTCAAA	GACTTCCTCG	
be108041	CGCCCTGTCCC	CAGTGCCAGG	CCCTTGCCCC	AGTCCTCAAA	GACTTCCTCG	
bg395178	CGCCCTGTCCC	CAGTGCCAGG	CCCTTGCCCC	CATCCTCAAG	GACTTCCTCG	
	251				300	
RdCVF1	TGCCGCTCAC	TGACCGAGTT	TACGTGCTGC	GGGCAGCAC	GCTGGCCCTG	
bg299078	TGCCGCTCAC	TGACCGAGTT	TACGTGCTGC	GGGCAGCAC	GCTGGCCCTG	
ai716631	TGCCGCTCAC	TGATGAGTT	TACGTGCTAC	GGGCAGCAC	GCTGGCCCTG	
bg294111	TGCCGCTCAC	TGACCGAGTT	TACGTGCTGC	GGGCAGCAC	GCTGGCCCTG	
be108041	TGCCGCTCAC	TGATGAGTT	TACGTGCTAC	GGGCAGCAC	GCTGGCCCTG	
bg395178	TGCCGCTCAC	AGATGAGTT	TATGTACTGC	GGGGGGCTCA	GCTGGCCCTG	
	301				350	
RdCVF1	GTCTATGTGT	CCCAAGGACCC	TACAGAGGG	CAACAGGACC	TCTTCCTCAG	
bg299078	GTCTATGTGT	CCCAAGGACCC	TACAGAGGG	CAACAGGACC	TCTTCCTCAG	
ai716631	GTCTATGTGT	CCCAAGGACCC	TACAGAGGG	CAACAGGACC	TCTTCCTCAG	
bg294111	GTCTATGTGT	CCCAAGGACCC	TACAGAGGG	CAACAGGACC	TCTTCCTCAG	
be108041	GTCTATGTGT	CCCAAGGACCC	TACAGAGGG	CAACAGGACC	TCTTCCTCAG	
bg395178	GTGTACGTGT	CCCAAGGACTC	CACGGAGGAG	CAGCAGGACC	TGTTCCTCAA	
	351				400	
RdCVF1	GGACATGCT	AAAAAAATGGC	TCTTCCTGCG	GTTCGA.TGA	TGAACGTGAGG	
bg299078	GGACATGCT	AAAAAAATGGC	TCTTCCTGCG	GTCCCCATGGA	TGAACGTGAGG	
ai716631	GGACATGCT	AAAAAGTGGC	TCTTCCTGCG	GTTCGA.TGA	TGAACCTGAGG	
bg294111	GGACATGCT	AAAAAAATGGC	TCTTCCTGCG	GTTCGA.TGA	TGAACGTGAGG	
be108041	GGACATGCT	AAAAAGTGGC	TCTTCCTGCG	GTTCGA.TGA	TGAACCTGAGG	
bg395178	GGACATGCTA	AAAAAAATGGC	TTTTCCTGCG	CTTTGA.GGA	TGATCTGAGG	
	401				450	
RdCVF1	AGGTGA-----	-----	-----	-----	-----	
bg299078	AGGTGAGGCC	CCAGGGAAAGA	CCAGGGAGGG	CTTCCTGGAG	AAGGCATTTC	
ai716631	AGAGACCTCG	GGGCCAGTT	CTCCGT-----	-----	-----	
bg294111	AGGGACCTCG	GGGCCAGTT	CTCTGTCCGT	CAACTGCCAG	CGTTTGTGGT	
be108041	AGTAATAAAA	ATTAGAGGTT	GTGGCTCAA	AAAAAAA	AAAA-----	
bg395178	AGGGACCTCG	GGGCCAGTT	CTCAGTGGAG	CGCTGCGCG	CGTCGTGGT	
	451				500	
RdCVF1	-----	-----	-----	-----	-----	
bg299078	CCTGGAGGTT	TACTGTCTG	GTACTACTTG	TGCACTAAAG	AGGTATTCC	
ai716631	-----	-----	-----	-----	-----	
bg294111	ACCTAAGCT	GGTGGGGACG	TGCTGACAAG	CGACGCCACG	GAGGAGATCC	
be108041	-----	-----	-----	-----	-----	
bg395178	GCTCAAGCG	GACGGGGACG	TGCTCACTCG	CGACGGCGCC	GACGAGATCC	

501	550
RdCVF1	-----
bg299078	CCACACCAAC CACAGGCAC AACACACAC AAGAGGTGTC CCATCCGCTC
ai716631	-----
bg294111	AGCGTCTGGG ACCCGCCTGC TTGCCAATC GGCAGGAGGC CGCAGAGCTC
be108041	-----
bg395178	ACCGCCTGGG CACCGCCTGC TTGCCAATC GGCAGGAGGC CGCCGAGGTG
551	600
RdCVF1	-----
bg299078	TTCATCACA GCCCACTGAC GCCAGACAGC ATCGCAGC TCACGGCTCA
ai716631	-----
bg294111	CTGGACCGCA GCTTCCTGCA ACCGGAGGAT TTGGATGAGC CTGGCGGGCG
be108041	-----
bg395178	CTGGACCGCA ACTTCAGCT GCCACAGGAC CTGGAGGACC AGGAGCCACG
601	650
RdCVF1	-----
bg299078	GAAAAACACA GGTAGTCCTCA CAGGGCTGCC ATCCCTAAATAC TGGCCACCT
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bg294111	CAGCATCACC GAGCCTCTGC GCGTCGCAA GTACCGACTA GACCGGGATG
be108041	-----
bg395178	GAGCCTCACC GAGTGCCTGC GCCGCCACAA GTACCGCGTG GAAAAGGGCG
651	700
RdCVF1	-----
bg299078	GAGCACAAAGA CGCATGGCTA CAAGCCTCAA GGCTAGAAATC TAAAACCAAG
ai716631	-----
bg294111	TCGGCGGGAG CGGGCGAAA CGGGCGACT CTGGTGAACC CCAGGGGGAC
be108041	-----
bg395178	CGCGAGGCG CGCGACCCCG GGGAGGGCT CGGGACGGAG GCCGGGGCCC
701	750
RdCVF1	-----
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ai716631	-----
bg294111	GCGGGTACAA GGGCGGAGCT CTGGTGACTC CCAGGGTAGG AGTGGGGACC
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751	800
RdCVF1	-----
bg299078	-----
ai716631	-----
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be108041	-----
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801	850
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ai716631	-----
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be108041	-----
bg395178	AGGGCGAAGA CCTGACCCAA AGCACAAACCA CGGGGACAC TAAACGACTC
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RdCVF1	-----
bg299078	-----
ai716631	-----
bg294111	-----

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be108041 -----
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RdCVF1 -----
bg299078 -----
ai716631 -----
bg294111 -----
be108041 -----
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Figure 14:

BG299078

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TGGACGCATCTGATCAGGAACAAACAGGCCACCAGGATGAAGTGGAGACAGAGGCAGAGCT
61 -----+-----+-----+-----+-----+-----+-----+-----+ 120
ACCTGGCTAGAACTAGTCCTCTTGCTCGCTGGCTACTTCACCTCTGTCCTCCGCTCGA

GAGCCGTTAGGTTAGAGAAATCGTCTGGTGTGTTCTCGGCCGCCGCCGCT 180
121 -----+-----+-----+-----+-----+-----+-----+-----+ 180
CTCGGCATCCAAATCTCTTAGCAGACCCAAACGACAAGAACAGCGGCCGCCGAGACAGGGGT
+T
GTGCCAGGCCCTGGCCAGTCCTCAAAGACTTCTCTGTCGGCTCACTGACGAGTTCTAC
181 -----+-----+-----+-----+-----+-----+-----+-----+ 240
CACGGTCCGGAACGGGTCAAGGAGTTCTGAAAGAACACGCCGAGTCAGTGCTCAAGAGATG

GTGCTGCCGGCAGCACAGCTGGCCCTGGCTATGTGTCCTGGACCCCTACAGAGGAGCAA
241 -----+-----+-----+-----+-----+-----+-----+-----+ 300
CAAGACGCCCTCTGTCGACGGGACCAAGATAACAGGTTCTGGATGTCCTCTGTT
removed
CAGGACCTCTCCTCAGGGACATGCGTCAAAGGGCTCTCTGCGCTCCACTGATGA
301 -----+-----+-----+-----+-----+-----+-----+-----+ 360
GTCCTGGAGAAGGGTCCCTGACGGGACTTTTACCGAGAACGGACGGCAGGGTACTACT
ACTGAGGGAGGTGAGGCCCGAGGGAAAGACCAAGGGAGGGCTCTGGAGAACGGCTTTCCCT
361 -----+-----+-----+-----+-----+-----+-----+-----+ 420
TGACTCTCCCACTCGGGGTCCTCTGTCCTCCGAGAACGGCTCTCCGTAAGAGGAAAGGA
GGAGGTTACTGCTGGTACTACTTGTGCACTAAAGAGGTATTCTCCACACCAACCAAC
421 -----+-----+-----+-----+-----+-----+-----+-----+ 480
CCTCCAAATGACAGGACCATGATGAAACACGTGATTTCTCCATAAGGAGGTGTTGGTG
AGGGACAAACACACAAAGGGTCTCCCATCGCTCTTCCATCACAGGCCACTGACGCC
481 -----+-----+-----+-----+-----+-----+-----+-----+ 540
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AGACAGCATCGGACGCTCACGGCTCAGAAAACACAGGTAGTCTCACAGGCCCTGCCATC
541 -----+-----+-----+-----+-----+-----+-----+-----+ 600
TCTGTCGTAACGCTGCGAGTGCCGAGTCCTTGTGTCATCAGAGTGTCGGACGGTAG
CTAATACTGCCACCCCTGAGCACAAAGAGCGATGCCATCAAGGCCAAGGCTAGAACTAA
601 -----+-----+-----+-----+-----+-----+-----+-----+ 660
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 661 TTGGTGCTCCACCCCTGGCATCGGGGTGAGGGGCCCTCGCG 702

Figure 15:

BG294111

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 61 GTTGTGCGTGGTCTACTTCACCTCTGTCTCGTCTCGACTCGGATCCAATCTCTTACG
 +T
 121 TCTGGGTGCTGTTCTTCGGCGCCGGCCGCTGTCCCGTAGGCTTGCCCCAGTCC 180
 121 AGACCACACGACAAGAAGCCGGCGCGGACAGGGTCACGGTCCGGAACGGGGTCAGG
 131 TCAAAAGACTTCCTCGTGGGCTCACTGACGAGTTCTACGTGCTGCGGGCAGCACAGCTGG 240
 131 AGTTTCTGAAGAAGGACCCCGAGTGACTGCTCAAGATGCAACGCCCCGTGTCGACCC
 241 CCTGGTCTATGTGTCAGGACCCCTACAGAGGAGAACAGGACCTTCCCTCAGGACA 300
 241 GGGACCAAGATAACAGGGTCTGGGATGTCCTCGTGTCTGGAGAACGGAGTCCCTGT
 301 TGCCCTGAAAATGGCTTCCCTGGCTTCCATGATGAACTGAGGAGGGACCTCGGGCGCC 360
 301 ACGGACTTTTACCGAGAACGGACGGCAAGGTACTACTTGACTCCTCCCTGGAGGCCCGGG
 361 AGTTCTCTCGCTCACTGCCAGCGTTGCTACITAAGCCTGGTGGGACGTGCTGA 420
 361 TCAAGAGACAGGCAGTTGACGGTCCCAACACCATGAATTGGACCAACCCCTGCACGACT
 421 CAAGCGACGCCACGGAGGAGATCCAGCGTCTGGACCCGCTGCTTGCCAACCTGGCAGG 480
 421 GTTCGCTGCGGTGCTCTCTAGGTGCGAGACCTGGCGGAGAACGGTTGACCGTCC
 481 AGGCCGAGACTCTGGACCGCAGCTTCTGCAACCGGAGGATTTGGATGAGCCTGCGC 540
 481 TCCGGCGTCTGAGGACCTGGCGTCGAAGGACGTTGGCCCTCTAAACCTACTCGGACGG
 541 GGGCGACGATCACCGAGCCTCTGCGCCGTCGCAAGTACCGAGTAGACCGGGATGTCGGC 600
 541 CCGCGTGTAGTGGCTCGAGACGCGGAGCGGTCATGGCTCATCTGGCCCTACAGCCGC
 601 CGAGCGGGCGAACGGCGGACTCTGGTGAACCCAGGGGACGCGGGTACAAGGGCGG 660
 601 CCTCGCCCCGCTTGGCGCTGAGACCACCTGGGTCCCTGCGCCCATGTTCCCGCC
 661 AGCTCTGGTGAATCCCAGGGTAGGAGTGGGACCGGAGCTGGTGAACACAAAGTACCG 720
 661 TCGAGACCACTGAGGGTCCATCCCTACCCCTGGCTCGAGACCACCTGGTTTCATGGC
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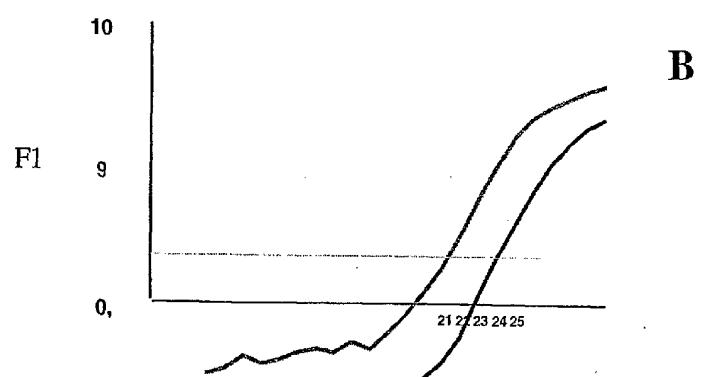
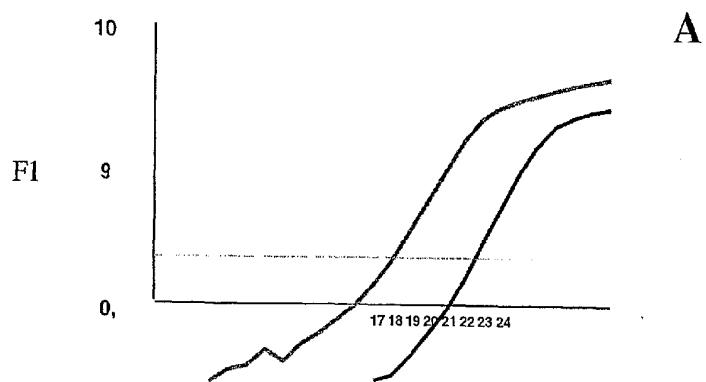


Figure 17:

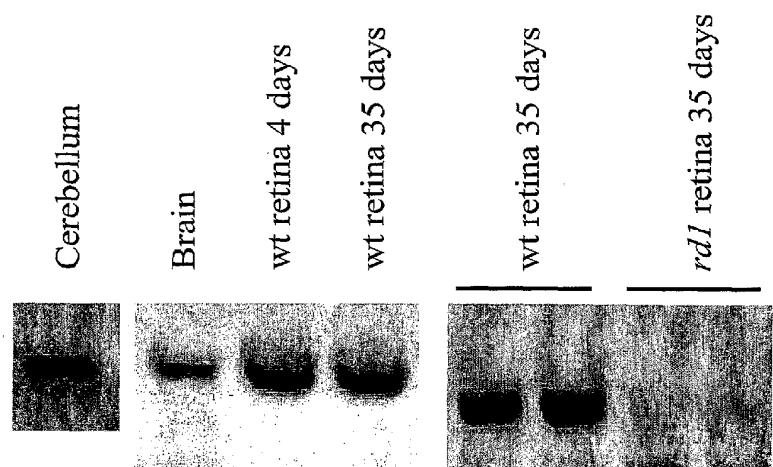
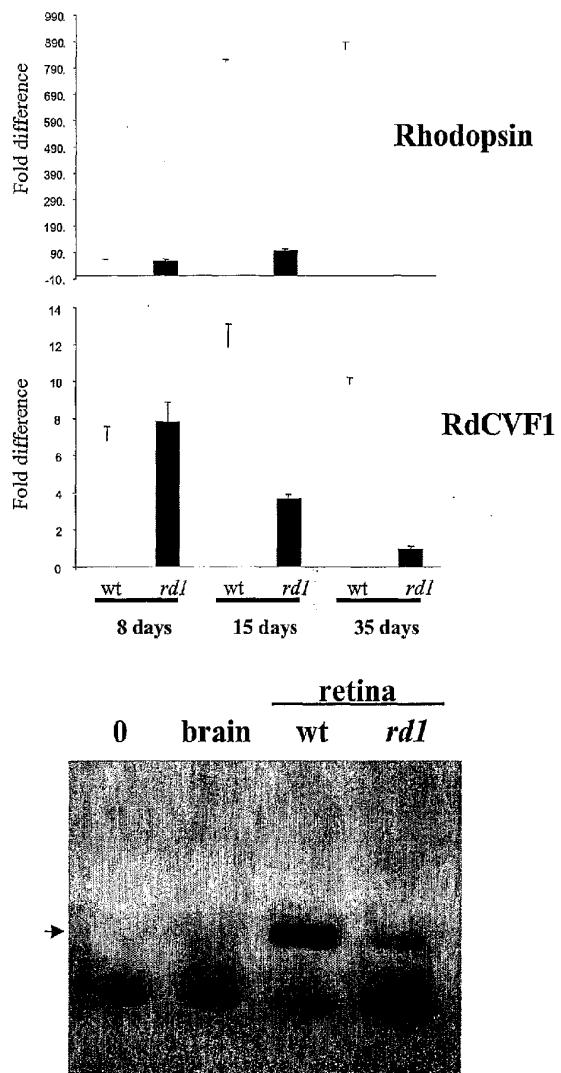


Figure 18:



EDITORIAL NOTE

APPLICATION NUMBER - 2002312794

The following Sequence Listing pages 1 to 21 are part of the description. The claims pages follow on pages 54 to 57.

New Seq RdcVF.ST25.txt
SEQUENCE LISTING

<110> UNIVERSITE LOUIS PASTEUR/ NOVARTIS AG

<120> Disease associated protein

<130> 4-31883

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	1	
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	Phe Ser Gly Arg Ile Leu Ile Arg Asn Asn Ser Asp Gln Asp Glu Val	
	10 15 20	
25	gag aca gag gca gag ctg agc cgt agg tta gag aat cgt ctg gtg ttg	152
	Glu Thr Glu Ala Glu Leu Ser Arg Arg Leu Glu Asn Arg Leu Val Leu	
	25 30 35	
40	ctg ttc ttc ggc ggc gcc tgt ccc cag tgc cag gcc ttt gcc cca	200
	Leu Phe Phe Gly Ala Gly Ala Cys Pro Gln Cys Gln Ala Phe Ala Pro	
	40 45 50	
55	gtc ctc aaa gag ttc ttc gtg cgg ctc act gac gag ttc tac gtg ctg	248
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	55 60 65	
65	cgg gca gca cag ctg gcc ctg tat gtg tcc cag gag cct aca gag	296
	Arg Ala Ala Gln Leu Ala Leu Val Tyr Val Ser Gln Asp Pro Thr Glu	

Page 1

New Seq RdCVF.ST25.txt

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85 90 95 100		
ctg ccg ttc cat gat gaa ctg agg agg tga ggccccaggg aagaccaggg		344
Leu Pro Phe His Asp Glu Leu Arg Arg		
105		
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aagaggtatt cctc		454
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Arg Leu Val Leu Leu Phe Phe Gly Ala Gly Ala Cys Pro Gln Cys Gln		
35 40 45		
Ala Phe Ala Pro Val Leu Lys Asp Phe Phe Val Arg Leu Thr Asp Glu		
50 55 60		
Phe Tyr Val Leu Arg Ala Ala Gln Leu Ala Leu Val Tyr Val Ser Gln		
65 70 75 80		
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Lys Trp Leu Phe Leu Pro Phe His Asp Glu Leu Arg Arg		
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gcc ctg gtc tat gtt tcc cag gac cct aca gag gag caa cag gac ctc Ala Leu Val Tyr Val Ser Gln Asp Pro Thr Glu Glu Gln Gln Asp Leu 75 80 85	292
ttc ctc agg gac atg cct gaa aaa tgg ctc ttc ctg cgg ttc cat gat Phe Leu Arg Asp Met Pro Glu Lys Trp Leu Phe Leu Pro Phe His Asp 90 95 100 105	340
gaa ctg agg agg gac ctc ggg cgc cag ttc tct gtc cgt caa ctg cca Glu Leu Arg Arg Asp Leu Gly Arg Gln Phe Ser Val Arg Gln Leu Pro 110 115 120	388
gcg gtt gtt gta ctt aag cct ggt ggg gac gtt ctg aca agc gac gcc Ala Val Val Val Leu Lys Pro Gly Gly Asp Val Leu Thr Ser Asp Ala 125 130 135	436
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tac cga gta gac cgg gat gtc ggc ggg agc ggg gcg aaa cgg cgc gac Tyr Arg Val Asp Arg Asp Val Gly Gly Ser Gly Ala Lys Arg Arg Asp 190 195 200	628
tct ggt gaa ccc cag ggg gac gcg ggt aca agg gcg gag ctc tgg tga Ser Gly Glu Pro Gln Gly Asp Ala Gly Thr Arg Ala Glu Leu Trp 205 210 215	676
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 50 55 60

Phe Tyr Val Leu Arg Ala Ala Gln Leu Ala Leu Val Tyr Val Ser Gln
 65 70 75 80

Asp Pro Thr Glu Glu Gln Gln Asp Leu Phe Leu Arg Asp Met Pro Glu
 85 90 95

Lys Trp Leu Phe Leu Pro Phe His Asp Glu Leu Arg Arg Asp Leu Gly
 100 105 110

Arg Gln Phe Ser Val Arg Gln Leu Pro Ala Val Val Val Leu Lys Pro
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Gly Gly Asp Val Leu Thr Ser Asp Ala Thr Glu Glu Ile Gln Arg Leu
 130 135 140

Gly Pro Ala Cys Phe Ala Asn Trp Gln Glu Ala Ala Glu Leu Leu Asp
 145 150 155 160

Arg Ser Phe Leu Gln Pro Glu Asp Leu Asp Glu Pro Ala Arg Arg Ser
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Ile Thr Glu Pro Leu Arg Arg Lys Tyr Arg Val Asp Arg Asp Val
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gct tgt cca cag tgc cag gcc ttc gtg ccc atc ctc aag gac ttc ttc Ala Cys Pro Gln Cys Gln Ala Phe Val Pro Ile Leu Lys Asp Phe Phe 45 50 55	197
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ctg gtg tac gtg tcc cag gac tcc acg gag gag cag cag gac ctg ttc Leu Val Tyr Val Ser Gln Asp Ser Thr Glu Glu Gln Gln Asp Leu Phe 75 80 85 90	293
ctc aag gac atg cca aag aaa tgg ctt ttc ctg ccc ttt gag gat gat Leu Lys Asp Met Pro Lys Lys Trp Leu Phe Leu Pro Phe Glu Asp Asp 95 100 105	341
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 35 40 45
 Ala Phe Val Pro Ile Leu Lys Asp Phe Phe Val Arg Leu Thr Asp Glu
 50 55 60
 Phe Tyr Val Leu Arg Ala Ala Gln Leu Ala Leu Val Tyr Val Ser Gln
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 Leu Asp Thr Glu Ala Glu Val Ser Arg Arg Leu Glu Asn Arg Leu Val
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Cys Phe Ala Asn Trp Gln Glu Ala Ala Glu Val Leu Asp Arg Asn Phe	
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Cys Leu Arg Arg His Lys Tyr Arg Val Glu Lys Ala Ala Arg Gly Gly	
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Lys Trp Leu Phe Leu Pro Phe Glu Asp Asp Leu Arg Arg Asp Leu Gly
100 105 110

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130 135 140

Gly Thr Ala Cys Phe Ala Asn Trp Gln Glu Ala Ala Glu Val Leu Asp
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Arg Asn Phe Gln Leu Pro Glu Asp Leu Glu Asp Gln Glu Pro Arg Ser
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1220

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222 (365) (530)

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New Seq RdCVF.ST25.txt

New Seq RdCVF.ST25.txt

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 Phe Ala Ala Gly Arg Cys Ser Pro Ser Arg Asp Phe Thr Pro Leu Leu
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 Phe Glu Val Val Phe Val Ser Ala Asp Gly Ser Ala Glu Glu Met Leu
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New Seq RdCVF.ST25.txt

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ccc aag ctg gtg gtc atc aag cag aac gga gct gtc atc acc aac aaa	683
Pro Lys Leu Val Val Ile Lys Gln Asn Gly Ala Val Ile Thr Asn Lys	
115 120 125	
ggg cgg aag cag atc cga gag cgc ggg cta gct tgc ttt cag aac tgg	731
Gly Arg Lys Gln Ile Arg Glu Arg Gly Leu Ala Cys Phe Gln Asn Trp	
130 135 140	
gtg gaa gca gcc gat gtt ttc caa aac ttc tcg ggg tga ccagggcagt	780
Val Glu Ala Ala Asp Val Phe Gln Asn Phe Ser Gly	
145 150 155	
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ggaatgtcat tgttagtca ccatgtgtg acagagagca taaactgctc aggaaagaac	900
tacgtctgcc ccctgtgggt cctagagctc cgttgaatgt ttatttctta caccttctc	960
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<212> PRT

<213> Mus musculus

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Phe Ala Ala Gly Arg Cys Ser Pro Ser Arg Asp Phe Thr Pro Leu Leu	
35 40 45	

Cys Asp Phe Tyr Thr Glu Leu Val Ser Glu Ala Arg Arg Pro Ala Pro	
50 55 60	

Phe Glu Val Val Phe Val Ser Ala Asp Gly Ser Ala Glu Glu Met Leu	
65 70 75 80	

Asp Phe Met Arg Glu Leu His Gly Ser Trp Leu Ala Leu Pro Phe His	
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New Seq RdCVF.ST25.txt

Asp Pro Tyr Arg His Glu Leu Lys Lys Arg Tyr Glu Ile Thr Ala Ile
 100 105 110

Pro Lys Leu Val Val Ile Lys Gln Asn Gly Ala Val Ile Thr Asn Lys
 115 120 125

Gly Arg Lys Gln Ile Arg Glu Arg Gly Leu Ala Cys Phe Gln Asn Trp
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Val Glu Ala Ala Asp Val Phe Gln Asn Phe Ser Gly
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<211> 1472

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<222> (331)..(738)

<223>

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cggggcaccgc cggcgctcgc cgcgcctcc cgcgggtga tcatccctc gcagggttcc	300					
tcgggtctca ggtggctgcg tgcgcggc atg gtt gac att ctg ggc gag cgg	354					
Met Val Asp Ile Leu Gly Glu Arg						
1 5						
cac ctg gtg acc tgg aag ggc gcg acg gtg gag gcc gag gcg gcg ctg	402					
His Leu Val Thr Cys Lys Gly Ala Thr Val Glu Ala Glu Ala Ala Leu						
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Gln Asn Lys Val Val Ala Leu Tyr Phe Ala Ala Arg Cys Ala Pro						
25 30 35 40						
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Ser Arg Asp Phe Thr Pro Leu Leu Cys Asp Phe Tyr Thr Ala Leu Val						
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gcc gag gcg ccg cgg ccc gcg ccc ttc gaa gtg gtc ttc gtg tca gcc	546					
Ala Glu Ala Arg Arg Pro Ala Pro Phe Glu Val Val Phe Val Ser Ala						
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New Seq RdCVF.ST25.txt

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Ala Trp Leu Ala Leu Pro Phe His Asp Pro Tyr Arg Gln Arg Ser Leu	
90 95 100	
gct ctg ttg ccc agg ctg gag tgc agt ggc gtg atc tta gct cac tgc	690
Ala Leu Leu Pro Arg Leu Glu Cys Ser Gly Val Ile Leu Ala His Cys	
105 110 115 120	
aac ctt tgc ctc ctg ggt tca agt gat tct cta gcc tta gcc tcc tga	738
Asn Leu Cys Leu Leu Gly Ser Ser Asp Ser Leu Ala Leu Ala Ser	
125 130 135	
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Phe Ala Ala Ala Arg Cys Ala Pro Ser Arg Asp Phe Thr Pro Leu Leu	
35 40 45	

New Seq RdCVF.ST25.txt

Cys Asp Phe Tyr Thr Ala Leu Val Ala Glu Ala Arg Arg Pro Ala Pro
 50 55 60

Phe Glu Val Val Phe Val Ser Ala Asp Gly Ser Cys Gln Glu Met Leu
 65 70 75 80

Asp Phe Met Arg Glu Leu His Gly Ala Trp Leu Ala Leu Pro Phe His
 85 90 95

Asp Pro Tyr Arg Gln Arg Ser Leu Ala Leu Leu Pro Arg Leu Glu Cys
 100 105 110

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Asp Ser Leu Ala Leu Ala Ser
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<211> 702

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<211> 387

<212> DNA

<213> *Rattus norvegicus* New Seq RdCVF.ST25.txt

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<210> 17

<211> 759

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<213> *Mus musculus*

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<211> 443

<212> DNA

<213> *Rattus norvegicus*

New Seq RdcVF.ST25.txt

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 gtctatgtt cccaggaccc tacagaggag caacaggacc ttgcctccg ggacatgcct 360
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 <211> 889
 <212> DNA
 <213> Homo sapiens

<400> 19
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New Seq RdCVF.ST25.txt

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cagccgacgg	cagctccag	gagatgttgg	acttcatgcg	cgagctgtatg	gcccggctt	300
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New Seq RdCVF.ST25.txt

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New Seq RdCVF.ST25.txt

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<210> 29
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Page 19

New Seq RdCVF.ST25.txt

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<213> Artificial Sequence New Seq RdCVF.ST25.txt

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22