**Title:** MACULAR DEGENERATION DIAGNOSTICS

**Abstract**

Therapeutics and diagnostics based on the mapping of a gene responsible for Age related Macular Degeneration (AMD) is disclosed.
FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<table>
<thead>
<tr>
<th>Code</th>
<th>Country</th>
<th>Code</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>Armenia</td>
<td>GB</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>AT</td>
<td>Austria</td>
<td>GE</td>
<td>Georgia</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
<td>GN</td>
<td>Guinea</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GR</td>
<td>Greece</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>HU</td>
<td>Hungary</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
<td>EE</td>
<td>Ireland</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>IT</td>
<td>Italy</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>JP</td>
<td>Japan</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>KE</td>
<td>Kenya</td>
</tr>
<tr>
<td>BY</td>
<td>Belarus</td>
<td>KG</td>
<td>Kyrgyzstan</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
<td>KP</td>
<td>Democratic People’s Republic of Korea</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>KR</td>
<td>Republic of Korea</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>KZ</td>
<td>Kazakhstan</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>LI</td>
<td>Liechtenstein</td>
</tr>
<tr>
<td>CI</td>
<td>Côte d’Ivoire</td>
<td>LK</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>LR</td>
<td>Liberia</td>
</tr>
<tr>
<td>CN</td>
<td>China</td>
<td>LT</td>
<td>Lithuania</td>
</tr>
<tr>
<td>CS</td>
<td>Czechoslovakia</td>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>CZ</td>
<td>Czech Republic</td>
<td>LV</td>
<td>Latvia</td>
</tr>
<tr>
<td>DE</td>
<td>Germany</td>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>MD</td>
<td>Republic of Moldova</td>
</tr>
<tr>
<td>EE</td>
<td>Estonia</td>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>ES</td>
<td>Spain</td>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>FI</td>
<td>Finland</td>
<td>MN</td>
<td>Mongolia</td>
</tr>
<tr>
<td>FR</td>
<td>France</td>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td>GA</td>
<td>Gabon</td>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>MX</td>
<td>Mexico</td>
<td>NE</td>
<td>Niger</td>
</tr>
<tr>
<td>NL</td>
<td>Netherlands</td>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>NZ</td>
<td>New Zealand</td>
<td>PL</td>
<td>Poland</td>
</tr>
<tr>
<td>PT</td>
<td>Portugal</td>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>RU</td>
<td>Russian Federation</td>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>SE</td>
<td>Sweden</td>
<td>SG</td>
<td>Singapore</td>
</tr>
<tr>
<td>SI</td>
<td>Slovenia</td>
<td>SK</td>
<td>Slovakia</td>
</tr>
<tr>
<td>SN</td>
<td>Senegal</td>
<td>SZ</td>
<td>Swaziland</td>
</tr>
<tr>
<td>TD</td>
<td>Chad</td>
<td>TG</td>
<td>Togo</td>
</tr>
<tr>
<td>TJ</td>
<td>Tajikistan</td>
<td>TT</td>
<td>Trinidad and Tobago</td>
</tr>
<tr>
<td>UA</td>
<td>Ukraine</td>
<td>UG</td>
<td>Uganda</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
<td>UZ</td>
<td>Uzbekistan</td>
</tr>
<tr>
<td>VN</td>
<td>Viet Nam</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Macular Degeneration Diagnostics

1. Background of the Invention

Macular degeneration is a clinical term that is used to describe a variety of diseases that are all characterized by a progressive loss of central vision associated with abnormalities of Bruch’s membrane and the retinal pigment epithelium. These disorders include very common conditions that affect older patients (age related macular degeneration or AMD) as well as rarer, earlier-onset dystrophies that in some cases can be detected in the first decade of life \(^1-18\). The genes associated with some of these dystrophies have been mapped, \(^5-14\) and in three cases, blue-cone monochromasy, \(^15\) pattern dystrophy, \(^16,17\) and Sorsby fundus dystrophy, \(^18\) actually identified. However, none of the latter genes has been found to be responsible for a significant fraction of typical late-onset macular degeneration (Weber, B., 1995, personal communication; Stone and Sheffield, unpublished observations).

In developed countries, AMD is the most common cause of legal blindness in older patients. \(^19\) The hallmark of this condition is the presence of drusen, which are ophthalmoscopically visible, yellow-white hyaline excrescences of Bruch’s membrane. In some families, drusen are heritable in an autosomal dominant fashion.

In 1875, Hutchinson and Tay published a paper entitled “Symmetrical Central Choroido-Retinal Disease Occurring in Senile Persons”. \(^20\) This paper includes one of the first descriptions of the constellation of clinical findings now known as age related macular degeneration (AMD). Specifically, three of the ten patients in the report were sisters affected with whitish spots (now referred to as drusen) in the macula. In 1899, Doyne \(^21\) reported a similar disorder in which the abnormal spots were nearly confluent such that the macula had a "honeycomb" appearance. Histopathologic examination of one of Doyne’s patients \(^22\) revealed the abnormalities to be hyaline thickenings of Bruch’s membrane. In 1925, Vogt \(^23\) published the first description of the ophthalmoscopic appearance of a form of familial drusen that had been observed in
patients living in the Leventine valley in the Ticino canton of southern Switzerland. Klainguti 24 fully characterized this condition in 1932 and demonstrated its autosomal dominant inheritance. This disorder eventually became known as malattia leventinese (i.e., Leventine disease). In 1948, Waardenburg 25 stated that there was little reason to make a distinction between malattia leventinese and the condition described by Doyne. This position was strengthened when Forni and Babel 26 found that the histopathologic features of malattia leventinese were indistinguishable from those of Doyne’s honeycomb choroiditis. Piguet, Haimovici and Bird 27 recently reviewed the history of these conditions and also pointed out that the drusen in families with malattia leventinese are frequently distributed in a radical pattern (see also Figures 2 and 3). Choroidal neovascularization is uncommon in patients with radial drusen but does occur. 27 Although originally recognized in Switzerland, families affected with autosomal dominant radial drusen have been identified in Czechoslovakia, 28,29 and the United States. 30

Currently, there is no therapy that is capable of significantly slowing the degenerative progression of AMD, and treatment is limited to laser photocoagulation of the subretinal neovascular membranes that occur in 10-15% of affected patients.

2. Summary of the Invention

The instant invention features a novel age related macular degeneration causing gene which maps on the short arm of human chromosome 2. Based on this finding, in one aspect the invention features methods and kits for diagnosing a subject with macular degeneration or with a predisposition for developing macular degeneration. In a preferred embodiment, the diagnostic methods and kits utilize a set of primers for amplifying regions of the macular degeneration causing gene, and means for analyzing the macular degeneration causing gene for differences (mutations) from the normal coding sequence. In another embodiment, the diagnostic methods and kits employ antibodies to a macular degeneration causing protein (i.e. a protein encoded by the macular degeneration gene) in an immunoassay procedure to detect the presence of a macular degeneration causing protein in a subject’s bodily fluid.
The instant disclosed diagnostic methods allow an ophthalmologist to determine whether a presymptomatic individual at risk for developing macular degeneration (based on family history) will develop the disease. If the diagnosis is negative, the individual will not need to worry about the potential development of the disease over time. If the diagnosis is positive, steps may be taken to prevent or ameliorate the effects of the disease before damage, such as loss of vision, occurs.

In another aspect, the invention features macular degeneration correcting genes (i.e. "normal" genes corresponding to a mutated gene that causes macular degeneration). In one embodiment, the invention features therapies for treating or preventing macular degeneration in a subject by administering an effective amount of a macular degeneration correcting gene into the subject so that a macular degeneration correcting protein is expressed in a sufficient amount to compensate for the deficiency of functional protein that results in macular degeneration.

In a further aspect, the invention features macular degeneration correcting proteins as well as methods for producing a recombinant form of the macular degeneration correcting protein. In one embodiment, the recombinant macular degeneration correcting protein is produced in vitro in cell culture. In another embodiment, the protein is produced by a transgenic animal. In yet another embodiment, the invention features therapies for treating or preventing macular degeneration in a subject by administering to the subject an effective amount of a macular degeneration correcting protein to compensate for the inability of the subject’s macular degeneration causing genes to produce a macular degeneration correcting protein. In a further aspect, the invention features alternative therapies that address the molecular basis for macular degeneration. In yet a further aspect, the invention relates to animals expressing the macular degeneration causing gene, which may be useful, for example, as animal models for testing drugs for treating macular degeneration.

The instant disclosed protein replacement, gene and alternative therapies for treating macular degeneration correct the biochemical defect resulting in disease. Therefore the instant disclosed therapies offer a major advance over the current treatment, which involves laser photocoagulation of the subretinal neovascular membranes that only occur in 10-15% of affected patients.

Other features and advantages will be readily apparent from the
following detailed description and claims.

3. Brief Description of the Drawings

Figure 1 is a graphic representation of the family pedigrees involved in the studies described in Example 1. Individuals found to be clinically affected with radial drusen are represented by black symbols while unaffected individuals are depicted with open symbols. Individuals that are deceased are marked with a slash. All living affected patients shown were included in the linkage analysis except those marked with an asterisk. The affection status of the deceased patients and of the patients marked with an asterisk was obtained historically.

Figure 2 is a graph plotting the decline of visual acuity of macular degeneration patients with age. Each open symbol represents the visual acuity of an affected eye at one point in time. The acuity is expressed in decimal notation (20/20 = 1.0; 20/200 = 0.1). Each heavy closed symbol represents the median visual acuity for all eyes in a single decade of life. These median acuities are plotted at the centers of the relevant decades (e.g., 25 for the decade from age 20 through 29).

Figure 3 shows two point linkage data and analysis of recombinant individuals. Eighteen genetic markers from the short arm of chromosome 2 are listed on the left of the figure with the most centromeric marker at the bottom. Dots indicate markers that could be positioned on the map in an order that was greater than $10^8$ times more likely than the next most likely order. Bold type (without a dot) indicates that a marker could be ordered with greater than 1000:1 odds while plain type indicates less than 1000:1 odds for the marker order. The maximum lod score (Zmax) for all four families combined is given for each marker as well as the recombination frequency at which Zmax occurred (theta hat). Each vertical group of boxes depicts the haplotypic data from a clinically affected individual who exhibits a recombination event near the linked interval. The family designations and pedigree numbers correspond to those in Figure 1. A black box indicates that during the meiosis that gave rise to the individual, an informative recombination event occurred between the marker and the disease gene. A white box indicates that the meiosis is informative (at least with respect to the affected parent) and that no recombination occurred between the disease
gene and the marker. A gray box indicates that the meiosis is uninformative at that marker. The recombination events summarized in this figure suggest that the disease-causing mutations lie within the interval bounded by D2S1761 and D2S4444.

Figure 4 shows the results of an analysis of twenty Swiss Malattia Leventinese families, which has haplotypically narrowed the interval to approximately 1cM defined by markers D2S2352-D2S1364.

4. Detailed Description

4.1 Definitions

For convenience, the meaning of certain terms and phrases employed in the specification, examples, and appended claims are provided below.

"Cells," "host cells" or "recombinant host cells" are terms used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A "chimeric protein" or "fusion protein" is a fusion of a first amino acid sequence encoding one of the subject polypeptides with a second amino acid sequence defining a domain (e.g. polypeptide portion) foreign to and not substantially homologous with any domain of one of the polypeptides. A chimeric protein may present a foreign domain which is found (albeit in a different protein) in an organism which also expresses the first protein, or it may be an "interspecies", "intergenic", etc. fusion of protein structures expressed by different kinds of organisms.

"Complementary" sequences as used herein refer to sequences which have sufficient complementarily to be able to hybridize, forming a stable duplex.

A "delivery complex" shall mean a targeting means (e.g. a molecule that results in higher affinity binding of a gene, protein, polypeptide or peptide to a target cell surface and/or increased cellular uptake by a target cell). Examples of targeting means include: sterols (e.g. cholesterol), lipids (e.g. a cationic lipid,
virosome or liposome), viruses (e.g. adenovirus, adeno-associated virus, and retrovirus) or target cell specific binding agents (e.g. ligands recognized by target cell specific receptors). Preferred complexes are sufficiently stable in vivo to prevent significant uncoupling prior to internalization by the target cell. However, the complex is cleavable under appropriate conditions within the cell so that the gene, protein, polypeptide or peptide is released in a functional form.

As is well known, genes for a particular polypeptide may exist in single or multiple copies within the genome of an individual. Such duplicate genes may be identical or may have certain modifications, including nucleotide substitutions, additions or deletions, which all still code for polypeptides having substantially the same activity. The term "DNA sequence encoding a polypeptide" may thus refer to one or more genes within a particular individual. Moreover, certain differences in nucleotide sequences may exist between individual organisms, which are called alleles. Such allelic differences may or may not result in differences in amino acid sequence of the encoded polypeptide yet still encode a protein with the same biological activity.

As used herein, the term "gene" or "recombinant gene" refers to a nucleic acid molecule comprising an open reading frame encoding one of the polypeptides of the present invention, including both exon and (optionally) intron sequences. A "recombinant gene" refers to nucleic acid molecule encoding a polypeptide and comprising protein-encoding exon sequences, though it may optionally include intron sequences which are derived from a chromosomal gene. Exemplary recombinant genes encoding the subject polypeptides are represented in the appended Sequence Listing. The term "intron" refers to a DNA sequence present in a given gene which is not translated into protein and is generally found between exons.

"Homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the
sequences. An "unrelated" or "non-homologous" sequence shares less than 40 % identity, though preferably less than 25 % identity, with one of the sequences of the present invention.

The term "interact" as used herein is meant to include detectable interactions between molecules, such as can be detected using, for example, a yeast two hybrid assay. The term interact is also meant to include "binding" interactions between molecules. Interactions may be protein-protein or protein-nucleic acid in nature.

The term "isolated" as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively, that are present in the natural source of the macromolecule. For example, an isolated nucleic acid encoding one of the subject polypeptides preferably includes no more than 10 kilobases (kb) of nucleic acid sequence which naturally immediately flanks the gene in genomic DNA, more preferably no more than 5kb of such naturally occurring flanking sequences, and most preferably less than 1.5kb of such naturally occurring flanking sequence. The term isolated as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an "isolated nucleic acid" is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term "isolated" is also used herein to refer to polypeptides which are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides.

The "non-human animals" of the invention include mammals such as rodents, non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc. Preferred non-human animals are selected from the rodent family including rat and mouse, most preferably mouse. The term "chimeric animal" is used herein to refer to animals in which the recombinant gene is found, or in which the recombinant is expressed in some but not all cells of the animal. The term "tissue-specific chimeric animal" indicates that one of the recombinant genes is present and/or expressed or disrupted in some tissues but not others.

As used herein, the term "nucleic acid" refers to polynucleotides such
as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotides.

As used herein, the term "promoter" means a DNA sequence that regulates expression of a selected DNA sequence operably linked to the promoter, and which effects expression of the selected DNA sequence in cells. The term encompasses "tissue specific" promoters, i.e. promoters, which effect expression of the selected DNA sequence only in specific cells (e.g. cells of a specific tissue). The term also covers so-called "leaky" promoters, which regulate expression of a selected DNA primarily in one tissue, but cause expression in other tissues as well. The term also encompasses non-tissue specific promoters and promoters that constitutively express or that are inducible (i.e. expression levels can be controlled).

The terms "protein", "polypeptide" and "peptide" are used interchangeably herein when referring to a gene product.

The term "recombinant protein" refers to a polypeptide of the present invention which is produced by recombinant DNA techniques, wherein generally, DNA encoding a polypeptide is inserted into a suitable expression vector which is in turn used to transform a host cell to produce the heterologous protein. Moreover, the phrase "derived from", with respect to a recombinant gene, is meant to include within the meaning of "recombinant protein" those proteins having an amino acid sequence of a native protein, or an amino acid sequence similar thereto which is generated by mutations including substitutions and deletions (including truncation) of a naturally occurring form of the protein.

As used herein, the term "specifically hybridizes" or "specifically detects" refers to the ability of a nucleic acid molecule of the invention to hybridize to at least approximately 6, 12, 20, 30, 50, 100, 150, 200, 300, 350, 400 or 425 consecutive nucleotides.

"Transcriptional regulatory sequence" is a generic term used throughout the specification to refer to DNA sequences, such as initiation signals, enhancers, and promoters, which induce or control transcription of protein coding sequences with which they are operably linked. In preferred embodiments, transcription of one of the recombinant genes is under the control of a promoter sequence (or other
transcriptional regulatory sequence) which controls the expression of the recombinant gene in a cell-type in which expression is intended. It will also be understood that the recombinant gene can be under the control of transcriptional regulatory sequences which are the same or which are different from those sequences which control transcription of the naturally-occurring forms of proteins.

As used herein, the term "transfection" means the introduction of a nucleic acid, e.g., an expression vector, into a recipient cell by nucleic acid-mediated gene transfer. "Transformation", as used herein, refers to a process in which a cell's genotype is changed as a result of the cellular uptake of exogenous DNA or RNA, and, for example, the transformed cell expresses a recombinant form of a polypeptide or, in the case of anti-sense expression from the transferred gene, the expression of a naturally-occurring form of the protein is disrupted.

As used herein, the term "transgene" means a nucleic acid sequence encoding, e.g., one of the polypeptides, or an antisense transcript thereto, which is partly or entirely heterologous, i.e., foreign, to the transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, (e.g. as intron), that may be necessary for optimal expression of a selected nucleic acid.

A "transgenic animal" refers to any animal, preferably a non-human mammal, bird or an amphibian, in which one or more of the cells of the animal contain heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. In the typical transgenic animals described herein, the transgene causes cells to express a recombinant form of one of
the proteins, e.g. either agonistic or antagonistic forms. However, transgenic animals in which the recombinant gene is silent are also contemplated, as for example, the FLP or CRE recombinase dependent constructs described below. Moreover, "transgenic animal" also includes those recombinant animals in which gene disruption of one or more genes is caused by human intervention, including both recombination and antisense techniques.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of preferred vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer generally to circular double stranded DNA loops which, in their vector form are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which become known in the art subsequently hereto.

4.2 General

The instant invention is based on linkage studies that have mapped a macular degeneration causing gene to a region of human chromosome 2. As described in detail in the attached Example 1, linkage has been determined based on studies performed on eighty-six members of four families affected with radial drusen. One family was of American origin, while the other three originated in the Leventine valley of Switzerland. The pedigrees of these families are shown in Figure 1.

As reported in the following Example 1, the gene responsible for macular degeneration maps on the short arm of chromosome 2. When mutated, the gene is capable of causing the development of autosomal dominant radial drusen (mallata leventinese). All four families investigated have very similar clinical features and all four have positive lod scores (with no recombinants) with the most tightly linked markers. Thus there is no evidence of shared haplotypes between any
of the four families, suggesting that mutations that cause this disease arose more than once, or, that the families are very distantly related.

Multipoint analysis revealed a peak lod score of 12 centered on marker GATA26H10. The lod-1 confidence interval was 8 cM\(^40\). The more conservative estimate of the diseased interval (defined by observed recombinations) is 14 cM extending from marker D2S1761 (centromeric) to D2S444 telomeric.

The interval has been haplotypically narrowed by analysis of the twenty Swiss Malattia Leventinese families (Figure 5). The haplotypic interval is defined by markers D2S2352-D2S1364 (approximately 1cM). The American Malattia Leventinese families also share haplotypic identity with the Swiss families from markers CA-133 to D2S1364 < 1cM.

Genes that fall in the haplotypically identical Swiss interval are set forth in the following Table 1:

<table>
<thead>
<tr>
<th>Marker</th>
<th>Gen Bank Accession Number</th>
<th>Expression (from Unigene)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGC35022</td>
<td>H72600</td>
<td>fetal liver/spleen</td>
<td>dual specificity protein kinase</td>
</tr>
<tr>
<td>WI-11560</td>
<td>R08151</td>
<td>fetal liver/spleen</td>
<td></td>
</tr>
<tr>
<td>SGC32447</td>
<td>RO9316</td>
<td>brain</td>
<td></td>
</tr>
<tr>
<td>SGC34889</td>
<td>M66771</td>
<td>fetal liver/spleen</td>
<td></td>
</tr>
<tr>
<td>WI-6704</td>
<td>Z38691</td>
<td>infant brain, fetal heart</td>
<td></td>
</tr>
<tr>
<td>WI6613</td>
<td></td>
<td>embryo retina, infant brain, fetal liver/spleen</td>
<td></td>
</tr>
<tr>
<td>WI12526</td>
<td></td>
<td>similar to spectrin B-g chain</td>
<td></td>
</tr>
<tr>
<td>B-fodrin</td>
<td></td>
<td>brain</td>
<td></td>
</tr>
<tr>
<td>WI-11791</td>
<td>R28010</td>
<td>placenta, fetal liver/spleen, melanocytes</td>
<td></td>
</tr>
<tr>
<td>WI-11399</td>
<td>T87762</td>
<td>fetal liver/spleen</td>
<td></td>
</tr>
</tbody>
</table>
(Physical and genetic mapping for each marker information can be obtained from the Whitehead Institute for Genome Research (http://www-genome.wi.mit.edu).)

70% of the β fodrin gene has been screened and found to contain no mutations. In addition, the 3' UTR of WI-6613 has been screened. In WI-6613, a polymorphism has been identified, which segregates in all affecteds in the Swiss families, possibly implicating it as the disease-causing gene.

Of the genes known to map in or near the linked interval, beta-fodrin is the most promising candidate for involvement in dominant drusen.\textsuperscript{44,45} Also known as nonerythrocytic spectrin, this protein is known to be expressed in the brain and is a membrane associated protein thought to be important in the maintenance of cell shape. This is the same function proposed for two other proteins involved in retinal degeneration: peripherin (RDS)\textsuperscript{46} and ROM-1.\textsuperscript{47}

Isolation of a macular degeneration causing gene makes macular degeneration testing a reality. Diagnostic testing can now be performed on presymptomatic individuals, who are at risk of developing macular degeneration based on family history. In addition, tests can be performed on postsymptomatic individuals diagnosed with macular degeneration based on an ophthalmologic examination. Further a diagnostic test be performed on DNA obtained from a fetus in utero, although macular degeneration would not appear to be a sufficiently life threatening or disabling disease to warrant prenatal identification.

Macular degeneration testing can be performed on a nucleic acid sample obtained from a subject by standard techniques. For example a patient’s bodily fluid (e.g. blood) can be obtained by known techniques (e.g. venipuncture). Alternatively, nucleic acid tests have been performed on dry samples (e.g. hair or skin). Fetal nucleic acid samples can be obtained from maternal blood as described in International Patent Application No. WO91/07660 to Bianchi; alternatively amniocytes or chorionic villi may be obtained for performing prenatal testing.

It may be useful or essential to first amplify the complement of nucleic acid present in a sample prior to analysis using one of many possible means. For example, the nucleic acid may be amplified using a procedure such as the polymerase chain reaction (PCR) (Saiki R. et al., (1988) Science 239:487-49; Sheffield, V.C. et al., (1989) Proc. Natl. Acad. Sci. U.S.A. 86: 232-236); or Q-beta replicase.

In addition to being used diagnostically, isolation of a macular
degeneration gene allows production of transgenic animals expressing a macular degeneration gene. These animals can be used in developing drugs for treating macular degeneration.

In addition, isolation of a macular degeneration gene allows identification of a macular degeneration correcting gene (i.e. a "normal" gene corresponding to the mutated gene that expresses a macular degeneration causing gene product which results in macular degeneration). Identification of a macular degeneration correcting gene and protein (e.g. glycosylated or unglycosylated protein, polypeptide or protein) makes protein replacement and gene therapy treatments for macular degeneration possible.

A macular degeneration correcting protein can be made by introducing (preferably in a suitable expression cassette, containing an appropriate promoter and optional enhancer sequence) into cells in culture using standard techniques (e.g., via calcium phosphate or calcium chloride co-precipitation, DEAE dextran mediated transfection, lipofection, or electroporation). Recombinant cells encoding the macular degeneration correcting gene can then be cultured in vitro in a manner that allows expression and preferably also secretion, and the recombinant factor can be purified using well known techniques. Either prokaryotic or eukaryotic cells may be useful "host cells" for producing recombinant macular degeneration correcting protein in vitro. Preferred host cells are mammalian cells (e.g., COS, Baby hamster kidney (BHK) and C127 cells), yeast cells and insect cells.

As an alternative to production by in vitro culture, recombinant macular degeneration correcting protein can also be produced in vivo, for example in a transgenic animal. Preferably in vivo production is carried out in a manner that is well tolerated by the animal host. Transgenic methods for producing recombinant proteins are well known in the art and include for example using gene constructs, which include a milk protein specific promoter for production and secretion in mammalian milk (see e.g., U.S. Patent No. 4,873,316 entitled "Isolation of Exogenous Recombinant Proteins From The Milk of Transgenic Mammals" to Meade et al.).

It may be advantageous to use a functional fragment or derivative of the macular degeneration correcting protein, for example in developing an appropriate pharmaceutical composition or for generating antibodies. Various fragments and
derivatives can be tested for biological activity (i.e., ability to prevent macular degeneration symptoms, such as production of drusen) using an appropriate activity assay. In addition, variously glycosylated forms of a macular degeneration correcting protein can be tested for example for increased circulatory life using an appropriate activity assay.

A subject (e.g. a human or animal) can be treated for macular degeneration by administration of an "effective amount" of a macular degeneration correcting protein alone or in conjunction with a pharmaceutically acceptable carrier or diluent according to any method that allows access into a subject's blood stream and enables contact with the subject's eye tissue. An effective amount is an amount sufficient to reduce or eliminate the symptoms associated with macular degeneration. The effective amount can be determined by one of skill in the art using no more than routine experimentation and may take into account such factors as the type and severity of symptoms being treated, the weight and/or age of the subject, the previous medical history of the subject, and the selected route for administration. Exemplary modes of administration include topically, intraocularly, subcutaneously, intravenously, intraperitoneally, intramuscularly, parenterally, submucosally, orally, transdermally or other appropriate manner. If necessitated by a particular mode of administration, a macular degeneration correcting protein can be encapsulated within a material that protects it from enzymatic degradation.

Alternatively, a preparation of a macular degeneration correcting gene can be incorporated into a suitable vector for delivering the gene to appropriate cells in a subject suspected or known to have macular degeneration. Since macular degeneration is an optical disorder, gene therapy vectors preferably are capable of delivering a macular degeneration correcting gene to the subject's eye. For use in clinical treatment, appropriate vectors must also be appropriately maintained in host cells and be safe. Preferred vectors for performing gene therapy include retrovirus, adenovirus, adeno-associated virus and lipid based vectors.

A subject can be treated by administration of an "effective amount" of a macular degeneration correcting gene alone or in conjunction with a pharmaceutically acceptable carrier or diluent according to any method that allows access to a subject's eye tissue. An "effective amount" of a macular degeneration correcting gene is an amount sufficient to result in manufacture of sufficient macular
degeneration correcting protein to reduce or eliminate the symptoms associated with macular degeneration. The effective amount can be determined by one of skill in the art using no more than routine experimentation and may take into account such factors as the type and severity of symptoms being treated, the weight and/or age of the subject, the previous medical history of the subject, and the selected route for administration. Exemplary modes of administration of a macular degeneration correcting gene therapy vector include topically, intraocularly, subcutaneously, intravenously, intraperitoneally, intramuscularly, parenterally, submucosally, orally, transdermally or other appropriate manner. If necessitated by a particular mode of administration, a macular degeneration correcting gene can be encapsulated within a material that protects it from enzymatic degradation.

In addition to the gene and protein replacement therapies described above, alternative therapies can be developed that prevent or compensate for a step along the biochemical that results in macular degeneration. The identification of a single gene known to be responsible for AMD can also improve understanding of the types and classes of genes that can cause related disorders.

In addition, the identification of one gene product causing a disorder can make it possible to identify other genes which can cause a similar phenotype. For example, the identification of the dystrophin gene has led to the isolation of dystrophin related glycoproteins, at least one of which plays a role in other forms of muscular dystrophy. Also, a gene capable of causing a Mendelian disorder, may contribute to the inheritance of a multifactorial form of the disorder. A striking example of this has been the identification of genes involved in various forms of cancer (e.g. colon cancer) by studying familial forms of cancer (e.g. hereditary nonpolyposis colon cancer and familial adenomatous polyposis). Groden, J.A. et al.,(1991) Cell 66:589-600 ; Aaltonen, L.A. (1993) Science 260:812-816). It is possible that AMD is allelic to some other macular degeneration (e.g. Doyne's macular dystrophy) and/or that alleles of the AMD contribute to the portion of macular degeneration that has a multifactorial etiology.

The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are
hereby expressly incorporated by reference.

Example 1 Genetic Linkage of a Macular Degeneration Causing Gene to The Short Arm of Human Chromosome 2.

A total of 86 family members at 50% risk for autosomal dominant radial drusen were studied (Figure 1). These individuals belonged to four families that were known to be related. Genealogical investigation revealed that family A has lived in the United States since at least the late 1700's. The two branches of this family (A1 and A2) were found to be connected by a sibship that lived in West Virginia in the 1790's. Families B-D all originated from the Leventine valley of southern Switzerland. Members of family B have been the subjects of previous reports by Vogt, Klainguti, Forni and Babel, and Scarpatetti, Forni, and Niemeyer.

Informed consent was obtained from study participants. Seventy-one patients had complete eye examinations (visual acuity, slit lamp examination, indirect ophthalmoscopy and retinal biomicroscopy). The medical records and fundus photographs of 15 additional patients were reviewed. Throughout the study, the clinicians remained masked to the evolving genotypic data. Patients were judged to be affected if: 1) they were found to have unmistakable evidence of radial drusen on clinical examination; or, 2) if they were found to have large disciform scars but had children affected with radial drusen. Blood samples were obtained from all of the affected family members as well as 19 spouses of affected patients with children. Seven to ten milliliters of blood were obtained from each patient in EDTA-containing glass tubes. DNA was prepared from the blood using a non-organic method. Oligonucleotide primers complementary to sequences flanking 380 short tandem repeat polymorphisms (STRPs) distributed across the entire autosomal genome were obtained from Research Genetics (Marker Sets 6 and 6A). The majority of these STRPs were tetranucleotide repeat polymorphisms developed by the Cooperative Human Linkage Center (CHLC). Fifty nanograms of each patient’s DNA were used as template in a 8.35 μl polymerase chain reaction (PCR) containing 1.25 μl 10X buffer (100mM Tris-HCL pH 8.8, 500mM KC1, 15mM MgCl2, 0.01% w/v gelatin, 200μm of each dCTP, dATP, dGTP and dTTP, 1 pmole of each primer and 0.25U Taq polymerase (Perkin-Elmer Cetus). Samples were incubated in a DNA
thermocycler (Omnigene) for 35 cycles under the following conditions: 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec. After amplification, 5µl of stop solution (95% formamide, 10mM NaOH, 0.05% Bromophenol Blue, 0.05% Xylene Cyanol) were added to each sample. Amplification products were then denatured and electrophoresed on 6% polyacrylamide gels at 60W for approximately 3 hours. Following electrophoresis, gels were silver stained as previously described.34,35 Permanent records were created by placing a sheet of Silver Sequence film (Promega) against the dried gel and placing it on a light box for 5-9 sec. The film was then processed in Dektol developer.

Because of the variable expressivity of the disease, only affected patients and informative spouses were included in the linkage analysis. Pairwise linkage analysis was performed with the MLINK and LODSCORE programs as implemented in the FASTLINK (v2.3) version36,37 of the LINKAGE program package.38 Multipoint linkage of three markers (D2S337, D2S378 and D2S119) and the disease locus was performed with the fast version of LINKMAP from FASTLINK (v2.3). The distances between the three markers were held fixed while the disease locus was moved through the map. The genetic maps used for the multipoint analysis and analysis of recombinants were constructed in the following manner. Genotypic data from the CEPH reference panel were obtained electronically from two primary sources: CEPH (ftp.cephp.fr) and CHLC (ftp.chlc.org). The potential informativeness of each marker in the CEPH reference panel was estimated by conducting pairwise linkage analysis of each marker against itself using the CLODSCORE module of the LINKAGE (v5.1) package. The two most informative markers were used as the ordered loci for a CRI-MAP BUILD run (CRI-MAP v2.2).39 Markers were then incorporated into the map in decreasing order of informativeness. The map building process continued until no further markers could be placed into the map while retaining a predetermined level of significance over the next most likely order. To obtain an order with a high level of confidence for the multipoint analysis of the disease locus, odds of at least $10^8$ to 1 were required. The map used to place the recombination events within the families was constructed with odds of $10^3$ to 1. Markers that could not be incorporated into the map with at least 10 to 1 odds were placed into the map in a location most consistent with the observed recombinants. For the data given in Figure 3, the allele frequencies were assumed
to be equal for each marker. The true population allele frequencies for each marker could not be reliably estimated from the small number of spouses in the families. In order to show that the assumption of the equal allele frequencies would not significantly affect the linkage results, the lod scores were calculated using allele frequencies for the "affected" allele of two of the most tightly linked markers (GATA26H01 and D2S378) ranging from 0.01 to 0.5. In family A alone, the Zmax remained greater than 4 for each of these markers for all allele frequencies in this range. In the six spouses of family A that were studied, the frequency of the "affected" allele of GATA26H01 was 16% and for D2S378 was 0%.

Results

Fifty-six patients were found to have fundus abnormalities consistent with the diagnosis of autosomal dominant radial drusen. The affected patients ranged in age from 15 to 85 years. Most were asymptomatic until the fourth or fifth decade, at which point they began experiencing a variety of symptoms including decreased visual acuity (especially after moving from a brightly lit room to a dim one), paracentral scotomas, photophobia and metamorphopsia. The visual acuity of most patients declined steadily between the ages of 50 and 80 such that the median visual acuity in affected patients between 70 and 79 years of age was less than 20/200 (Figure 2).

Linkage of radial drusen of 2p was first suspected when all 29 affected individuals from family A were found to share a common allele of marker D2S1352. Genotyping of all 4 families with 18 different STRPs that map to 2p revealed significant linkage (lod > 3.0) in family A with 14 different markers and in family B with two different markers (D2S391 and GATA26H10). The maximum lod score observed in a single family was 7.0 (theta=0) and was obtained with marker D2S378 in family A. The maximum lod score obtained by combining all 4 families was 10.5 (theta=0) and was obtained with marker D2S378. The lod scores obtained with 18 different chromosome 2 markers are given in Figure 3. The analysis of patients who exhibited recombination events near the linked interval is also shown in Figure 3. These recombination events show the disease-causing gene to lie within the 14cM interval between markers D2S1761 (telomeric) and D2S444 (centromeric).

Multipoint analysis was performed with the genotypic data from three markers D2S337, D2S378 and D2S119) pooled from all four families. This analysis
revealed a peak lod score of 12, centered on marker D2S378. The lod-1 confidence interval was 8cM. Table 2 shows the set of marker alleles (haplotype) linked to the disease phenotype in each of the four families. For all nine markers in the linked interval, the two main branches of family A were found to have the same allele linked to the disease phenotype. In contrast, none of the three Swiss families (B-D) showed this degree of haplotypic similarity with each other, or with family A.

**Table 2 Alleles** linked to disease phenotype

<table>
<thead>
<tr>
<th>Families</th>
<th>A1</th>
<th>A2</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 D2S391 (5)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>D2S123 (6)</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>D2S1352 (4)</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>GATA26H10 (10)</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>15 D2S378 (7)</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>D2S1364 (5)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>D2S357 (7)</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>D2S370 (6)</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

* For each marker, the allele with the greatest number of repeats is designated as allele 1.
** The number in parentheses is the total number of different alleles observed in the four families in this study.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

References


22) Collins T. A pathological report upon a case of Doyne's choroiditis ("honeycomb" or "family choroiditis"). *Ophthalmoscope* (1913); Vol. 11: pp. 537-538.


30) Gass JDM. Diseases causing choroidal exudative and hemorrhagric localized


Claims

1. A macular degeneration causing gene substantially corresponding to a region of the short arm of human chromosome 2 obtained from a subject affected with radial drusen, said region being bordered by marker D2S2352 and D2S1364.

2. A nucleic acid fragment antisense to the gene of Claim 1.

3. A probe comprising the antisense nucleic acid fragment of claim 2 and a label.

4. A method for diagnosing a subject with macular degeneration or with having a predisposition to macular degeneration comprising the steps of:
   a) obtaining a nucleic acid containing sample from the subject;
   b) amplifying the nucleic acid with appropriate primers;
   c) analyzing the amplification products to determine the presence of a macular degeneration causing gene.

5. A kit for diagnosing macular degeneration comprising:
   a) primers;
   b) reagents for performing amplification; and
   c) reagents for analyzing the amplified nucleic acid.

6. A macular degeneration causing protein encoded by the gene of claim 1.

7. An antibody to the macular degeneration causing protein of claim 6.

8. An antibody of claim 7, which is a monoclonal antibody.

9. A method for diagnosing a subject for macular degeneration or a predisposition to macular degeneration comprising performing an immunoassay on a sample obtained from the subject using the antibody of claim 7, wherein detection of the presence of bound antibody indicates that the subject has macular degeneration or a predisposition to macular degeneration.
10. A kit for diagnosing macular degeneration comprising:
   a) an antibody of claim 7; and
   b) reagents for performing an immunoassay.

11. An animal model for macular degeneration comprising a transgenic animal
   encoding the gene of claim 1.

12. A macular degeneration correcting gene substantially corresponding to a
    region of chromosome 2 obtained from a human not having macular degeneration,
    said region being bordered by marker D2S2352 and D2S1364.

13. A macular degeneration correcting protein encoded by the gene of claim 12.

14. A method for obtaining the protein of claim 13 comprising the steps of:
    a) transfeciting a host cell with the gene of claim 12;
    b) culturing the host cell in a manner that allows expression and
       secretion of the protein into a culture media; and
    c) isolating the protein from the culture media.

15. A method for obtaining the protein of claim 13, comprising the steps of:
    a) developing a transgenic animal comprised of cells encoding the gene
       of claim 12;
    b) maintaining the animal in a manner that allows expression of the
       gene and secretion of the encoded protein into a body fluid; and
    c) isolating the protein from the body fluid.

16. A pharmaceutical composition useful in treating macular degeneration
    comprising an effective amount of a macular degeneration correcting protein and a
    therapeutically acceptable carrier.

17. A method for treating or preventing macular degeneration in a subject
    comprising administering an effective amount of a pharmaceutical composition of
    claim 16 to the subject.
18. A composition comprising the macular degeneration correcting gene of claim 12 and an expression vector.

19. A pharmaceutical composition useful in treating macular degeneration comprising an effective amount of the composition of claim 18 and a therapeutically acceptable carrier.

20. A method for treating or preventing macular degeneration in a subject comprising administering an effective amount of a pharmaceutical composition of Claim 19 to the subject.

21. A pharmaceutical composition useful in treating macular degeneration comprising an effective amount of the nucleic acid fragment of claim 12, an expression vector and a therapeutically acceptable carrier.

22. A method for treating or preventing macular degeneration in a subject comprising administering an effective amount of a pharmaceutical composition of claim 21 to the subject.