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Methods and reagents for treatment and diagnosis of vascular disorders and age-related macular degeneration

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(54) **Title:** METHODS AND REAGENTS FOR TREATMENT AND DIAGNOSIS OF VASCULAR DISORDERS AND AGE-RELATED MACULAR DEGENERATION
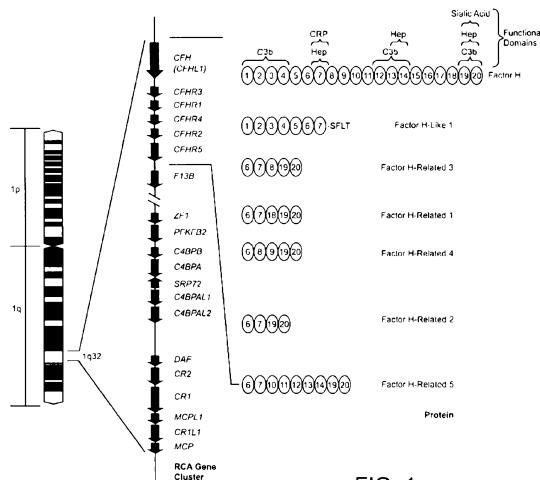
FIG. 1

(57) **Abstract:** Disclosed are screening methods for determining a human subject's propensity to develop a vascular disorder and/or age-related macular degeneration (AMD), therapeutic or prophylactic compounds for treating disease or inhibiting its development, and methods of treating patients to alleviate symptoms of the disease, prevent or delay its onset, or inhibit its progression. The inventions are based on the discovery that persons with a genome having a deletion of the CFHR-1 and/or CFHR-3 gene, which normally lie on human chromosome 1 between DNA encoding CFH and CFHR-4, are at reduced risk of developing AMD, and elevated risk of developing vascular disease such as aneurysm.

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

METHODS AND REAGENTS FOR TREATMENT AND DIAGNOSIS OF VASCULAR DISORDERS
AND AGE-RELATED MACULAR DEGENERATION

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001]   This application claims priority to U.S. Provisional Patent Application No. 60/840,073,
filed August 23, 2006. This application also claims priority to U.S. Provisional Patent Application No.
60/831,018, filed July 13, 2006. Both these applications are hereby incorporated by reference.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER
FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

FIELD OF THE INVENTION

[0003]   This invention relates to screening and therapeutic methods for complement-mediated
diseases such as age-related macular degeneration and vascular diseases. The invention finds application
in the fields of biology and medicine.

BACKGROUND OF THE INVENTION

[0004]   Complement Factor H (CFH) is a multifunctional protein that acts as a key regulator of
the complement system. See Zipfel, 2001, "Factor H and disease: a complement regulator affects vital
body functions" Semin Thromb Hemost. 27:191-9. The Factor H protein activities include: (1) binding to
C-reactive protein (CRP), (2) binding to C3b, (3) binding to heparin, (4) binding to sialic acid; (5) binding
to endothelial cell surfaces, (6) binding to cellular integrin receptors (7) binding to pathogens, including
microbes (see Figure 3 of U.S. patent publication No. 20070020647), and (8) C3b co-factor activity. The
Factor H gene, known as *HF1*, *CFH* and *HF*, is located on human chromosome 1, at position 1q32. The
1q32 locus contains a number of complement pathway-associated genes. One group of these genes,
referred to as the regulators of complement activation (RCA) gene cluster, contains the genes that encode
Factor H, five Factor H-related proteins (FHR-1, FHR-2, FHR-3, FHR-4 and FHR-5 or CFHR1, CFHR2,

CFHR3, CFHR4 and CFHR5, respectively), and the gene encoding the beta subunit of coagulation factor XIII. The Factor H and Factor H related proteins are composed almost entirely of short consensus repeats (SCRs). Factor H and FHL1 are composed of SCRs 1-20 and 1-7, respectively. FHR-1, FHR-2, FHR-3, FHR-4 and FHR-5 are composed of 5, 4, 5, 5 and 8 SCRs, respectively. The order of genes, from centromere to telomere is *FH/FHL1, FHR-3, FHR-1, FHR-4, FHR-2* and *FHR-5*.

Factor H Gene

[0005]   The Factor H cDNA encodes a polypeptide 1231 amino acids in length having an apparent molecular weight of 155 kDa (see Ripoche *et al.*, 1988, *Biochem J* 249:593-602). There is an alternatively spliced form of Factor H known as FHL-1 (and also has been referred to as HFL1 or CFHT). FHL-1 corresponds essentially to exons 1 through 9 of Factor H (see Ripoche *et al.*, 1988, *Biochem J* 249:593-602). The FHL1 cDNA encodes a polypeptide 449 amino acids in length having an apparent molecular weight of 45-50 kDa. The first 445 amino acids of FH1 and FHL1 are identical, with FHL1 having four unique C-terminal amino acids (encoded by alternative exon 10A, which is located in the intron between exon 9 and exon 10. cDNA and amino acid sequence data for human Factor H and FHL1 are found in the EMBL/GenBank Data Libraries under accession numbers Y00716 and X07523, respectively. The 3926 base nucleotide sequence of the reference form of human Factor H cDNA has GenBank accession number Y00716 and the polypeptide has GenBank accession number Y00716. The 1658 base nucleotide sequence of the reference form of HFL1, the truncated form of the human Factor H, has GenBank accession number X07523, and the polypeptide sequence has GenBank accession number X07523. The Factor H gene sequence (150626 bases in length) has GenBank accession number AL049744. The Factor H promoter is located 5' to the coding region of the Factor H gene.

FHR-1 Gene

[0006]   The FHR-1 gene is also known as *CFHR1, CFHL1, CFHL, FHR1* and *HFL1*. The FHR-1 cDNA encodes a polypeptide 330 amino acids in length having an predicted molecular weight of 39 kDa (see Estaller *et al.*, 1991, *J. Immunol.* 146:3190-3196). cDNA and amino acid sequence data for human FHR-1 are found in the EMBL/GenBank Data Libraries under accession number M65292. The FHR-1 gene sequence is found under GenBank accession number AL049741.

FHR-2 Gene

[0007]   The FHR-2 gene is also known as *CFHR2, CFHL2, FHR2* and *HFL3*. The FHR-2 cDNA encodes a polypeptide 270 amino acids in length having a predicted molecular weight of 31 kDa (see Strausberg *et al.*, *Proc. Natl. Acad. Sci USA* 99:16899-16903). cDNA and amino acid sequence data for

2

human FHR-2 are found in the EMBL/GenBank Data Libraries under accession number BC022283. The FHR-2 gene sequence is found under GenBank accession number AL139418.

FHR-3 Gene

[0008]   The FHR-3 gene is also known as *CFHR3*, *CFHL3*, *FHR3* and *HLF4*. The FHR-3 cDNA encodes a polypeptide 330 amino acids in length having a predicted molecular weight of 38 kDa (see Strausberg *et al.*, *Proc. Natl. Acad. Sci USA* 99:16899-16903). cDNA and amino acid sequence data for human FHR-3 are found in the EMBL/GenBank Data Libraries under accession number BC058009. The FHR-3 gene sequence is found under GenBank accession number AL049741.

FHR-4 Gene

[0009]   The FHR-4 gene is also known as *CFHR4*, *CFHL4* and *FHR4*. The FHR-4 cDNA encodes a polypeptide 331 amino acids in length having a predicted molecular weight of 38 kDa (see Skerka *et al.*, 1991, *J. Biol. Chem.* 272:5627-5634). cDNA and amino acid sequence data for human FHR-4 are found in the EMBL/GenBank Data Libraries under accession number X98337. The FHR-4 gene sequence is found under GenBank accession numbers AF190816 (5' end), AL139418 (3' end) and BX248415.

FHR-5 Gene

[0010]   The FHR-5 gene is also known as *CFHR5*, *CFHL5* and *FHR5*. The CFHR5 cDNA encodes a polypeptide 569 amino acids in length having an apparent molecular weight of 65 kDa (see McRae *et al.*, 2001, *J. Biol.Chem.* 276:6747-6754). cDNA and amino acid sequence data for human CFHR5 are found in the EMBL/GenBank Data Libraries under accession number AF295327. The 2821 base nucleotide sequence of the reference form of human *CFHR5* has GenBank accession number AF295327, and the polypeptide sequence has GenBank accession number AAK15619. The CFHR5 genomic sequence is found under GenBank accession numbers AL139418 (5' end) and AL353809 (3' end). The FHR-5 promoter is located 5' to the coding region of the CFHR5 gene.

BRIEF SUMMARY OF THE INVENTION

[0011]   In one aspect, the invention provides a screening method for determining a human subject's propensity to develop a vascular disorder and/or age-related macular degeneration (AMD), involving analysis of a biological sample from the subject to detect the presence or absence of a deletion in chromosome 1 between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene, wherein the presence of a deletion is evidence

that the subject is at an increased risk of developing a vascular disorder and a decreased risk of developing AMD.

[0012]   Examples of vascular disorders include aneurysms, such as abdominal aortic aneurysm (AAA) and brain intracranial aneurysm.

[0013]   In one embodiment, the method comprises detecting the presence or absence of at least a portion of the complement Factor H-related 3 (*CFHR3*) gene. In a related embodiment the entire protein coding region of the *CFHR3* gene is deleted. In a related embodiment the entire *CFHR3* gene is deleted. In a related embodiment the entire *CFHR3* gene and the region between the *CFHR3* gene and complement Factor H-related 1 (*CFHR1*) gene are deleted.

[0014]   In one embodiment, the method comprises detecting the presence or absence of at least a portion of the complement Factor H-related 1 (*CFHR1*) gene. In a related embodiment the entire protein coding region of the *CFHR1* gene is deleted. In a related embodiment the entire *CFHR1* gene is deleted. In a related embodiment the entire *CFHR1* gene and the region between the *CFHR1* gene and complement factor H-related 4 (*CFHR4*) gene are deleted. In a related embodiment the entire *CFHR1* gene and the region between the *CFHR1* gene and *CFHR3* gene are deleted.

[0015]   In one embodiment, the method comprises detecting the presence or absence of at least a portion of the *CFHR3* gene and at least a portion of the *CFHR1* gene. In a related embodiment both the entire protein coding regions of the *CFHR3* and *CFHR1* genes are deleted. In a related embodiment the entire *CFHR3* and *CFHR1* genes are deleted.

[0016]   In one embodiment, a deletion or a partial deletion of an intergenic sequence selected from: a) a sequence between the CFH gene and the CFHR3 gene; b) a sequence between the CFHR3 gene and the CFHR1 gene; c) a sequence between the CFHR1 gene and the CFHR4 gene. In yet another embodiment, at least a portion of the CFH gene is deleted (*e.g.*, at least a portion of exon 22 is deleted).

[0017]   In one embodiment, the presence or absence of the deletion is detected by assaying for a gene product encoded in chromosome 1 between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene, where the absence of the gene product, or a reduced level of expression of the gene product, indicates the presence of deletion. In another embodiment, the presence or absence of a CFHR1 gene product and/or a CFHR3 gene product is detected, where the absence of a gene product is indicative of a deletion. In one instance, the gene product is a protein. In another embodiment, detecting the presence or absence of a deletion is performed by analyzing a chromosome or nucleic acid (*e.g.*, DNA or RNA) from the subject.

[0018]   In one embodiment the presence or absence of the deletion is detected by assaying for a truncated CFHR1 or CRHR3 gene product, where detection of a truncated gene product is indicative of a

deletion. In a preferred embodiment, the CFHR1 gene is partially deleted and expresses a truncated polypeptide gene product.

[0019]   In one embodiment the subject has a genotype of T at position 1277 of the coding region of the *CFH* gene of the chromosome comprising the deletion.

[0020]   The subject may be homozygous or heterozygous for deletions. Thus, in one embodiment, deletions are present in both chromosomes 1 of the subject.

[0021]   The presence or absence of the deletion may be detected in a biological sample from a patient by, for example, analyzing a chromosome or nucleic acid (*e.g.*, DNA or RNA) sample from the subject. The presence or absence of the deletion also may be detected by, for example, determining the presence or absence of protein encoded by the (deleted) DNA in a biological sample from the subject, *e.g.*, a body fluid or tissue sample of the subject, by detecting a variant or truncated form of the CFHR1 or CFHR3 polypeptides in a body fluid or tissue sample of the subject, or by measuring the level of CFHR1 or CFHR3 polypeptides in a body fluid or tissue sample of the subject.

[0022]   The biological sample is any sample taken from a patient that is suitable for use in the invention. Examples of biological samples that include body fluids include blood, serum, urine, cerebral spinal fluid (CSF) and saliva. In one embodiment, the body fluid is blood, serum or urine. Examples of biological samples that comprise tissue samples include a skin biopsy and a cheek scraping. In one embodiment, the tissue sample is a skin biopsy.

[0023]   Proteins (amount or presence) may be detected, for example, using an immunoassay such as a sandwich immunoassay, a competitive immunoassay, a radioimmunoassay, fluorophore-labelled immunoassay, an ELISA or a Western blot. Mass spectroscopy also may be used. Variant proteins (amount or presence) may be detected, for example, using variant-specific antibodies. Truncated proteins (amount or presence) may be detected, for example, by a difference in the size of the protein by Western blot analysis or mass spectroscopy.

[0024]   In certain embodiments, the method comprises in the detecting step determining the presence of a deletion, for example, a deletion in a *CFHR1* or *CFHR3* gene, or the absence or a reduction of corresponding gene product (e.g., the amount or activity of the gene product) indicating a higher risk of the subject developing a vascular disorder.

[0025]   In other embodiments, the method comprises in the detecting step determining the absence of a deletion, for example, the presence of a *CFHR1* or *CFHR3* gene, or the presence or an increase of the corresponding gene product (e.g., the amount or activity of the gene product) indicating a lower risk of the subject developing a vascular disorder.

[0026]   In another embodiment, the method comprises in the detecting step determining the presence of a deletion, for example, a deletion in a *CFHR1* or *CFHR3* gene, or the absence or a reduction

of the corresponding gene product (e.g., the amount or activity of the gene product) indicating a lower risk of the subject developing AMD.

[0027]   In yet another embodiment, the method comprises in the detecting step determining the absence of a deletion, for example, the presence of a *CFHR1* or *CFHR3* gene, or the presence or an increase of the corresponding gene product (e.g., the amount or activity of the gene product) indicating a higher risk of the subject developing AMD. The increase in gene product, for example, can be at least 10%, at least 20%, at least 50%, or more.

[0028]   In certain embodiments, the method further comprises detecting at least one other genetic variant or biomarker indicative of AMD and/or vascular disease. Genetic variants that may be detected in the invention include genetic variants of complement factor H (*CFH*) gene, *HTRA1* gene, complement factor B (BF) gene and/or the complement component 2 (C2) gene. In an embodiment, the genetic variants include one or a plurality of polymorphic sites, such as those described herein.

[0029]   In another aspect, the invention provides a method for treating a subject having (i.e., exhibiting symptoms of), or is at risk for developing, a vascular disorder, by administering a CFHR1 polypeptide and/or a CFHR3 polypeptide to the subject. The polypeptide may be a full-length CFHR1 polypeptide or a fragment or portion thereof. The polypeptide may be a full-length CFHR3 polypeptide or a fragment or portion thereof.

[0030]   In another aspect the invention provides a pharmaceutical composition comprising a CFHR3 protein or fragment thereof and at least one pharmaceutically effective excipient. In another aspect the invention provides a pharmaceutical composition comprising a CFHR1 protein or fragment thereof and at least one pharmaceutically effective excipient.

[0031]   In another aspect the invention provides the use of a protein comprising the gene product of at least a portion of the CFHR3 and/or CFHR1 gene for the preparation of a medicament for the treatment of a vascular disorder.

[0032]   In another aspect the invention provides gene therapy vectors comprising nucleic acid encoding a CFHR3 or CFHR1 protein, or fragment thereof. The vector may include a promoter that drives expression of the *CFHR3* or *CFHR1* gene in multiple cell types. Alternatively, the vector may include a promoter that drives expression of the *CFHR3* or *CFHR1* gene only in specific cell types, for example, in cells of the retina or in cells of the kidney. In a related aspect pharmaceutical compositions are provided containing a gene therapy vector encoding a CFHR3 or CFHR1 protein or fragment thereof and a pharmaceutically acceptable excipient.

[0033]   In another aspect the invention provides a method of treating a subject having (i.e., exhibiting symptoms of), or susceptible to developing, age-related macular degeneration (AMD), by administering an agent that reduces the expression of the *CFHR1* and/or *CFHR3* genes or reduces the

activity or amount of a gene product of the *CFHR1* and/or *CFHR3* genes. Agents include antisense RNA, siRNA or ribozyme that reduces expression of the *CFHR1* and/or *CFHR3* genes. In a related aspect the level of protein is reduced, for example by using plasmaphoresis or antibody-based inhibition, for example, using an anti-CFHR1 antibody and/or an anti-CFHR3 antibody.

[0034]   In another aspect the invention provides a pharmaceutical composition comprising an anti-CFHR1 antibody and a pharmaceutically acceptable carrier. In one embodiment, an anti-CFHR1 antibody specifically binds the amino-terminus of a CFHR1 polypeptide. In another aspect the invention provides a pharmaceutical composition comprising an anti-CFHR3 antibody and a pharmaceutically acceptable carrier. In one embodiment, an anti-CFHR3 antibody specifically binds the carboxyl-terminus of a CFHR3 antibody.

[0035]   In another aspect the invention provides a diagnostic kit for diagnosing susceptibility to a vascular disorder and/or AMD in a subject, comprising nucleic acid primers or probes that detect the presence or absence of a deletion in the DNA sequence between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene on human chromosome 1.

[0036]   In another aspect the invention provides a diagnostic device comprising nucleic acid primers or probes that detect the presence or absence of a deletion in the DNA sequence between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene on human chromosome 1 immobilized on a substrate, such as a microarray.

[0037]   In another aspect the invention provides a diagnostic kit for diagnosing susceptibility to a vascular disorder and/or AMD in a subject, comprising antibodies that detect the presence or absence of the complement Factor H-related 3 (CFHR3) protein, or variant or truncated forms thereof, and/or complement Factor H related 1 (CFHR1) protein, or variant or truncated forms thereof, in a body fluid or tissue sample of the subject.

[0038]   In another aspect the invention provides a drug screening method for screening for agents for use in treating a vascular disorder. The method involves a) combining (i) a cell that expresses CFHR3 and/or CFHR1 polypeptides; and (ii) a test agent; b) measuring the level of CFHR3 and/or CFHR1 polypeptides secreted into the medium; and c) comparing the level of CFHR3 and/or CFHR1 polypeptides secreted into the medium in the presence of the test agent with a reference value, said reference value being the level of CFHR3 and/or CFHR1 polypeptides secreted into the medium in the absence of the test agent, where a higher level of CFHR3 and/or CFHR1 polypeptides secreted into the medium in the presence of the test agent indicates the test agent may be useful for treating the vascular disorder.

[0039]   In another aspect the invention provides a method for identifying a CFH protein likely to protect against AMD development, by identifying a subject with a deletion in the DNA sequence between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene on human chromosome 1; determining the sequence of the *CFH* gene encoded by the gene contained in the chromosome containing the deletion; and determining the sequence of the protein encoded by the *CFH* gene, wherein said protein is different from wild-type CFH, said protein being a CFH protein likely to protect against AMD development. The invention also provides a protective CFH protein obtained using the method.

BRIEF DESCRIPTION OF THE FIGURES

[0040]   Figure 1 is a diagram showing the organization of the regulators-of-complement-activation (RCA) gene cluster on chromosome 1q32 and the arrangement of approximately 60-amino acid domains known as short consensus repeats (SCRs) in complement Factor H (CFH), Factor H-Like 1 (CFHL1) and Factor H-Related 1, 2, 3, 4 and 5 (CFHR1, CFHR2, CFHR3, CFHR4 and CFHR5). CFH has 20 SCRs. The interacting partners with some of these SCRs has been determined and is shown on the top right (CRP, C reactive protein; Hep, heparin). Complement factor H-like 1 (CFHL1) is a splice isoform of CFH, while complement factor H-related proteins 1-5 (CFHR1-5) are each encoded by a unique gene (*CFHR1-5*). The SCRs of CFHR1-5 are similar to some of the SCRs in CFH, as denoted by the numbers in the ovals. For example, CFHR5 has 9 SCRs, with the first two being similar to SCRs 6 and 7 of Factor H and therefore having CRP and heparin binding properties. SCRs 5-7 of CFHR5 have the numbers 12-14 within the corresponding ovals because these SCRs are similar to SCRs 12-14 of Factor H and have C3b and heparin binding properties.

[0041]   Figure 2 shows regions of homology (genomic duplications) in the genes encoding CFH and the Factor H-related proteins. Exons are indicated as vertical lines. Regions labeled with the same letter (*e.g.*, A, A', and A") have substantially identical sequences.

[0042]   Figure 3 shows a Western blot of serum proteins from seven patients using an anti-human CFH antibody. FHL-1, CFHR1 and CFHR2 indicate the positions of the truncated form of CFH, CFHR1 and CFHR2, respectively. The anti-human CFH antibody employed also cross-reacts with CFHR1 and CFHR2. No CFHR1 is detected in the serum of two patients (197-02 and 325-02) that have a homozygous deletion of the *CFHR3* and *CFHR1* genes, as determined by SSCP analysis and direct DNA sequencing.

[0043]   Figure 4 shows a SSCP analysis of the *CFH*, *CFHR3* and *CFHR1* genes. 1, 2, 3, and 4 indicate four different SSCP patterns observed using primers from exon 22 of the *CFH* gene to PCR amplify DNA. SSCP patterns 1, 2 and 3 correspond to homozygous non-deletion or heterozygous

deletion of *CFHR3* and *CFHR1*, and pattern 4 corresponds to homozygous deletion of *CFHR3* and *CFHR1*.

[0044]   Figure 5 shows a PCR analysis of the *CFH* and *CFH*-related genes 1 to 5 in leukocytes from 20 patients that are separated into four groups according to the SSCP patterns using the *CFH* exon 22 primers (patterns 1-4 are as described in Figure 4). From left to right, in each panel (gel), 5 leukocyte-derived DNA samples each from patients displaying SSCP patterns 1, 2, 3 and 4 were subjected to PCR using primers specific for *CFH*, *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4* and *CFHR5*, as indicated. When SSCP analysis and direct DNA sequencing show a homozygous deletion of the *CFHR3* and *CFHR1* genes, no PCR amplifiable *CFHR3* and *CFHR1* DNA are detected.

[0045]   Figure 6 shows an amino acid alignment of the CFH (SEQ ID NO: 2), CFHR1 (SEQ ID NO: 4), and CFHR3 (SEQ ID NO: 6) proteins.

[0046]   Figure 7 shows a nucleotide alignment of the CFH (SEQ ID NO: 1), CFHR1 (SEQ ID NO: 3), and CFHR3 genes (SEQ ID NO: 5).

DETAILED DESCRIPTION

1. Definitions

[0047]   The following definitions are provided to aid in understanding the invention. Unless otherwise defined, all terms of art, notations and other scientific or medical terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the arts of medicine and molecular biology. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not be assumed to represent a substantial difference over what is generally understood in the art.

[0048]   A "vascular disorder" is a disease or condition of the vascular system. One type of vascular disorder is an aneurysm such as abdominal aortic aneurysm or brain intracranial aneurysm. Other types of vascular disorder include hypertension, cerebral vascular accidents, trans-ischemic accidents (*e.g.*, stroke). Still other types of vascular disorders include coronary artery disease, peripheral artery disease, varicose veins, and peripheral vascular disease.

[0049]   A "nucleic acid", "polynucleotide" or "oligonucleotide" is a polymeric form of nucleotides of any length, may be DNA or RNA, and may be single- or double-stranded. Nucleic acids may include promoters or other regulatory sequences. Oligonucleotides are usually prepared by synthetic means. A reference to the sequence of one strand of a double-stranded nucleic acid defines the complementary sequence and except where otherwise clear from context, a reference to one strand of a nucleic acid also refers to its complement. For certain applications, nucleic acid (*e.g.*, RNA) molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the use

of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. Modified nucleic acids include peptide nucleic acids (PNAs) and nucleic acids with nontraditional bases such as inosine, queosine and wybutosine and acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

[0050]   "Hybridization probes" are nucleic acids capable of binding in a base-specific manner to a complementary strand of nucleic acid. Such probes include nucleic acids and peptide nucleic acids (Nielsen *et al.*, 1991). Hybridization may be performed under stringent conditions which are known in the art. For example, see, *e.g.*, Berger and Kimmel (1987) METHODS IN ENZYMOLOGY, VOL. 152: GUIDE TO MOLECULAR CLONING TECHNIQUES, San Diego: Academic Press, Inc.; Sambrook *et al.* (1989) MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Vols. 1-3, Cold Spring Harbor Laboratory; Sambook (2001) 3rd Edition; Rychlik, W. and Rhoads, R.E., 1989, *Nucl. Acids Res.* 17, 8543; Mueller, P.R. *et al.* (1993) In: CURRENT PROTOCOLS IN MOLECULAR BIOLOGY 15.5, Greene Publishing Associates, Inc. and John Wiley and Sons, New York; and Anderson and Young, QUANTITATIVE FILTER HYBRIDIZATION IN NUCLEIC ACID HYBRIDIZATION (1985)). As used herein, the term "probe" includes primers. Probes and primers are sometimes referred to as "oligonucleotides."

[0051]   The term "primer" refers to a single-stranded oligonucleotide capable of acting as a point of initiation of template-directed DNA synthesis under appropriate conditions, in an appropriate buffer and at a suitable temperature. The appropriate length of a primer depends on the intended use of the primer but typically ranges from 15 to 30 nucleotides. A primer sequence need not be exactly complementary to a template but must be sufficiently complementary to hybridize with a template. The term "primer site" refers to the area of the target DNA to which a primer hybridizes. The term "primer pair" means a set of primers including a 5' upstream primer, which hybridizes to the 5' end of the DNA sequence to be amplified and a 3' downstream primer, which hybridizes to the complement of the 3' end of the sequence to be amplified.

[0052]   Exemplary hybridization conditions for short probes and primers is about 5 to 12 degrees C below the calculated Tm. Formulas for calculating Tm are known and include: Tm = 4°C x (number of G's and C's in the primer) + 2°C x (number of A's and T's in the primer) for oligos <14 bases and assumes a reaction is carried out in the presence of 50mM monovalent cations. For longer oligos, the following formula can be used: Tm = 64.9°C + 41°C x (number of G's and C's in the primer – 16.4)/N, where N is the length of the primer. Another commonly used formula takes into account the salt concentration of the reaction (Rychlik, supra, Sambrook, supra, Mueller, supra.): Tm = 81.5°C + 16.6°C x (log10[Na+] + [K+]) + 0.41°C x (%GC) – 675/N, where N is the number of nucleotides in the oligo. The aforementioned formulae provide a starting point for certain applications; however, the

design of particular probes and primers may take into account additional or different factors. Methods for design of probes and primers for use in the methods of the invention are well known in the art.

[0053] The term "polymorphism" refers to the occurrence of two or more genetically determined alternative sequences or alleles in a population. A "polymorphic site" is the locus at which sequence divergence occurs. Polymorphic sites have at least two alleles. A diallelic polymorphism has two alleles. A triallelic polymorphism has three alleles. Diploid organisms may be homozygous or heterozygous for allelic forms. A polymorphic site may be as small as one base pair. Examples of polymorphic sites include: restriction fragment length polymorphisms (RFLPs); variable number of tandem repeats (VNTRs); hypervariable regions; minisatellites; dinucleotide repeats; trinucleotide repeats; tetranucleotide repeats; and simple sequence repeats. As used herein, reference to a "polymorphism" can encompass a set of polymorphisms (i.e., a haplotype).

[0054] A "single nucleotide polymorphism (SNP)" occurs at a polymorphic site occupied by a single nucleotide, which is the site of variation between allelic sequences. The site is usually preceded by and followed by highly conserved sequences of the allele. A SNP usually arises due to substitution of one nucleotide for another at the polymorphic site. Replacement of one purine by another purine or one pyrimidine by another pyrimidine is called a transition. Replacement of a purine by a pyrimidine or vice versa is called a transversion. A synonymous SNP refers to a substitution of one nucleotide for another in the coding region that does not change the amino acid sequence of the encoded polypeptide. A non-synonymous SNP refers to a substitution of one nucleotide for another in the coding region that changes the amino acid sequence of the encoded polypeptide. A SNP may also arise from a deletion or an insertion of a nucleotide or nucleotides relative to a reference allele.

[0055] The term "deletion," when referring to a nucleic acid sequence, has the usual meaning in genetics of an allele in which one or more bases are missing compared to a reference or wild-type sequence. Deletions may be as short as one base-pair. Deletions detected in the present invention may be longer, such as a deletion of at least 100 bp, at least 200 bp, at least 300 bp, at least 400 bp, at least 500 bp, at least 600 bp, at least 700 bp, at least 800 bp, at least 900 bp, at least 1000 bp, at least 1100 bp, at least 1200 bp, at least 1300 bp, at least 1400 bp, at least 1500 bp, at least 1600 bp, at least 1700 bp, at least 1800 bp, at least 1900 bp, at least 2000 bp, at least 2500 bp, at least 3000 bp, at least 3500 bp, at least 4000 bp, at least 4500 bp, at least 5000 bp, at least 6000 bp, at least 7000 bp, at least 8000 bp, at least 9000 bp, at least 10,000 bp, at least 15,000 bp, at least 20,000 bp, at least 30,000 bp, at least 40,000 bp, at least 50,000 bp, at least 75,000 bp, at least 100,000 bp, at least 125,000 bp, at least 150,000 bp, at least 200,000 bp or at least 250,000 bp.

[0056] The term "haplotype" refers to the designation of a set of polymorphisms or alleles of polymorphic sites within a gene of an individual. For example, a "112" Factor H haplotype refers to the

Factor H gene comprising allele 1 at each of the first two polymorphic sites and allele 2 at the third polymorphic site. A "diplotype" is a haplotype pair.

[0057]   An "isolated" nucleic acid means a nucleic acid species that is the predominant species present in a composition. Isolated means the nucleic acid is separated from at least one compound with which it is associated in nature. A purified nucleic acid comprises (on a molar basis) at least about 50, 80 or 90 percent of all macromolecular species present.

[0058]   Two amino acid sequences are considered to have "substantial identity" when they are at least about 80% identical, preferably at least about 90% identical, more preferably at least about 95%, at least about 98% identical or at least about 99% identical. Percentage sequence identity is typically calculated by determining the optimal alignment between two sequences and comparing the two sequences. Optimal alignment of sequences may be conducted by inspection, or using the local homology algorithm of Smith and Waterman, 1981, *Adv. Appl. Math.* 2: 482, using the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443, using the search for similarity method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85: 2444, by computerized implementations of these algorithms (*e.g.*, in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.) using default parameters for amino acid comparisons (*e.g.*, for gap-scoring, *etc.*). It is sometimes desirable to describe sequence identity between two sequences in reference to a particular length or region (*e.g.*, two sequences may be described as having at least 95% identity over a length of at least 500 basepairs). Usually the length will be at least about 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 amino acids, or the full length of the reference protein. Two amino acid sequences can also be considered to have substantial identity if they differ by 1, 2, or 3 residues, or by from 2-20 residues, 2-10 residues, 3-20 residues, or 3-10 residues.

[0059]   "Linkage" describes the tendency of genes, alleles, loci or genetic markers to be inherited together as a result of their location on the same chromosome. Linkage can be measured by percent recombination between the two genes, alleles, loci or genetic markers. Typically, loci occurring within a 50 centimorgan (cM) distance of each other are linked. Linked markers may occur within the same gene or gene cluster. "Linkage disequilibrium" or "allelic association" means the preferential association of a particular allele or genetic marker with a specific allele or genetic marker at a nearby chromosomal location more frequently than expected by chance for any particular allele frequency in the population. A marker in linkage disequilibrium can be particularly useful in detecting susceptibility to disease, even if the marker itself does not cause the disease.

[0060]   The terms "susceptibility," "propensity," and "risk" refer to either an increased or decreased likelihood of an individual developing a disorder (*e.g.*, a condition, illness, disorder or disease) relative to a control population. In one example, the control population may be individuals in the

population (*e.g.*, matched by age, gender, race and/or ethnicity) without the disorder, or without the genotype or phenotype assayed for. In some contexts, the terms diagnosing and screening are used interchangeably (*e.g.*, a person skilled in the art can diagnose a propensity to develop the disease).

[0061]   The term "diagnose" and "diagnosis" refer to the ability to determine or identify whether an individual has a particular disorder (e.g., a condition, illness, disorder or disease).

[0062]   The term "screen" or "screening" as used herein has a broad meaning. It includes processes intended for the diagnosis or for determining the susceptibility, propensity, risk, or risk assessment of an asymptomatic subject for developing a disorder later in life. Screening also includes the prognosis of a subject, i.e., when a subject has been diagnosed with a disorder, determinating in advance the progress of the disorder as well as the assessment of efficacy of therapy options to treat a disorder.

[0063]   The terms "portion," "fragment" and/or "truncated form" when used in reference to a Factor H-related gene product (*e.g.*, CFHR3 or CFHR1 gene product), refers to a nucleic acid or polypeptide sequence that is less than the full-length sequence (*i.e.*, a portion of the full-length gene or polypeptide). A portion or fragment or truncated form of CFHR3 or CFHR1 gene or polypeptide can be at least 25, at least 50, at least 75, at least 100, at least 150, at least 200, at least 250, or at least 300 nucleotides or amino acids in length. Typically the portion includes at least 1, often at least two, and sometimes at least 3 or 4 complete SCRs.

[0064]   As used herein, the term "gene product" means an RNA (e.g., mRNA) or protein that is encoded by the gene. A "protein coding region" is a region of DNA/RNA sequence within a gene that encodes a polypeptide or protein.

[0065]   An "assay" is a procedure wherein the presence or amount or a property of a test substance, *e.g.*, a nucleic acid or gene product, is detected or measured.

[0066]   The terms "inhibit" and "reduce" refer to any inhibition, reduction, or decrease in expression or activity including partial or complete inhibition of gene expression or gene product activity.

2. Association of Polymorphisms in the CFHR1 And CFHR3 Genes and Risk Of Developing AMD and Vascular Disorders

[0067]   A correlation between polymorphic sites and haplotypes in the *CFH* gene and the likelihood of developing AMD has been discovered. See Hageman *et al.*, 2005, *Proc. Natl. Acad. Sci. U.S.A.* 102:7227-32; Haines *et al.*, 2005, *Science* 308:419-21; Klein *et al.*, 2005, *Science* 308:385-9; Edwards *et al.*, 2005, *Science* 308:421-4 and U.S. patent publication No. 20070020647, each incorporated by reference in its entirety for all purposes. Both *CFH* risk haplotypes and *CFH* protective haplotypes are known. Polymorphisms particularly associated with increased risk include a variant allele at: rs1061170 (402H; exon 9); rs203674 (intron 10) and the polymorphism at residue 1210 (1210C; exon 22).

Polymorphisms particularly associated with decreased risk include the protective H2 haplotype, which includes a variant allele in IVS6 (intron 6, rs3766404) and the H4 haplotype, which includes a variant allele in IVS1 (intron 1, rs529825) and a variant allele (I62) (exon 2, rs800292).

[0068]   It has now been discovered that an AMD protective haplotype is genetically linked to deletions in the DNA sequence between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene on human chromosome 1 (*i.e.*, the DNA sequence encoding the CFHR1 and CFHR3 proteins). See Example 1, *infra.* The discovery that deletions at the *CFHR1* and *CFHR3* loci are associated with decreased risk of developing AMD has a number of specific applications, including screening individuals to ascertain risk of developing AMD and identification of new and optimal therapeutic approaches for individuals afflicted with, or at increased risk of developing, AMD. As discussued in Example 1, below, the deletion genotype is predominantly associated with the *CFH* H4 haplotype. See Hageman *et al.*, 2005, *Proc. Natl. Acad. Sci. U.S.A.* 102:7227-32. Thus, this deletion acts as a marker for decreased risk of conditions for which the H4 haplotype is protective.

[0069]   Moreover, it has now been discovered that deletions in the DNA sequence between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene on human chromosome 1 (*i.e.*, the DNA sequence encoding the CFHR1 and CFHR3 proteins) are associated with increased risk of developing a vascular disease such as aortic aneurysm. See Example 1, *infra.* The discovery that deletions at the *CFHR1* and *CFHR3* loci are associated with increased risk of developing a vascular disorder has a number of specific applications, including screening individuals to ascertain risk of developing a vascular disorder and identification of new and optimal therapeutic approaches for individuals afflicted with, or at increased risk of developing, vascular disorders.

3. Screening Methods

[0070]   Based on the discoveries described herein, a subject's risk for AMD or vascular disease can be assessed by determining whether or not a the subject has a deletion within the region of chromosome 1 lying between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*). The extent of the deletion may vary in different individuals or populations. For example, in one embodiment the all of most of the region between CFH exon 22 and CFHR4 exon 1 is deleted. Alternatively, a portion of the region may be deleted, such as, for example, a deletion of less than the entire region between CFH exon 22 and CFHR4 exon 1 but including the CFHR1 encoding sequence, or including the CFHR3 encoding sequence,

14

including both, or including a non-coding (e.g., intragenic) sequence. An individual may be homozygous for deletion (both chromosomes 1 have a deletion in the region) or may be heterozygous for deletion.

[0071]   For example and not limitation, the homozygous deletion of *CFHR1* and/or *CFHR3* can be detected from the absence of CFHR1 and/or CFHL3 protein in a body fluid or tissue sample (see Figure 3), by the absence of RNA encoded in the region between the 3' end of CFH exon 22 and the 5' end of CFHR4 exon 1 (e.g., absense of absence of CFHR1 and/or CFHL3 mRNAs), or by absense of genomic DNA in the region in the region between the 3' end of CFH exon 22 and the 5' end of CFHR4 exon 1. The present or absense of DNA or RNA sequences can be determined using art known methods, such as PCR. The absense of a nucleic acid sequence is deduced from the absense of an amplified PCR product in an assay of a tissue sample (see Figure 5). It will be understood that, although PCR is frequently cited herein as a method for genetic analysis, many other analytical methods are known and are suitable for detection of a deletion. For example and not limitation several are described below in the section captioned "Analysis of Nucleic Acid Samples."

[0072]   The heterozygous deletion of *CFHR1* and/or *CFHR3* can be determined, for illustration and not limitation, (1) from a reduction in the amount of protein in a body fluid or tissue sample as compared to the amount from a control having both alleles of *CFHR1* and/or *CFHR3* genes, (2) from a reduction in the amount of RNA, DNA, or amplified PCR product in a tissue sample as compared to the amount from similar sample of a homozygote without the deletion, or (3) by an assay using direct DNA sequencing, quantitative PCR or other methods known in the art. For example, the amount of a gene product may be reduced in a heterozygote by at least 10%, at least 20%, at least 30%, about 50% or or more compared to a homozygote without the deletion. Quantitative PCR and methods are available that would be able to detect a two-fold difference in mRNA or DNA in a sample.

[0073]   As noted, a deletion lies in the region between CFH exon 22 and CFHR4 exon 1 but need not span the entire region. Deletions of a portion of the CFHR1 and/or CFHR3 genes ("partial deletions") may result in truncated forms of CFHR1 and/or CFHR3 RNAs and polypeptides. Such partial deletions can be identified by a difference in size of a protein in a body fluid or tissue sample compared to the full-length protein, by detecting a size difference in the RNA, and by various methods well known in the art, including PCR amplification of DNA or RNA in a biological sample using primers selected to distinguish between a nucleic acid comprising a deletion and a nucleic acid not containg a deletion. Methods known in the art can be used to distinguish homozygotes from heterozygotes (see, e.g., Example 1).

[0074]   The selection, design and manufacture of suitable primers or probes for analysis of nucleic acid is well known in the art. A person of ordinary skill in the art can use suitable combinations of primers to detect deletions. In an embodiment, the primers or probes are designed to hybridize at any

position in the DNA sequence between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene. For instance, both primers may be located in the CHFR3 gene to detect its presence or absence. In another example. In other examples, one or more primers are located within intergenic (non-coding) sequence, *e.g.*, intergenic sequence between between *CFHR3* and *CFHR1* or between *CFHR1* and *CFHR4*.

[0075] In another embodiment, the invention includes a method of detecting a nonreciprocal transfer of genetic information, such as gene conversion. In one instance, the gene conversion results in replacement of a 3' portion of the CFH gene with a portion of the 3' CFHR1 gene, such that a chimeric protein with sequence derived from both the CFH gene and the CFHR1 gene is produced.

3.1 Analysis of Nucleic Acid Samples

[0076] Methods for detection of polymorphisms and deletions in genetic sequences are well known in the art and can be adapted for use in the present invention.

[0077] In one embodiment, genomic DNA is analyzed. For assay of genomic DNA, virtually any biological sample containing genomic DNA or RNA, *e.g.*, nucleated cells, is suitable. For example, genomic DNA can be obtained from peripheral blood leukocytes collected from case and control subjects (QIAamp DNA Blood Maxi kit, Qiagen, Valencia, CA). Other suitable samples include saliva, cheek scrapings, biopsies of retina, kidney, skin, or liver or other organs or tissues; amniotic fluid, cerebral spinal fluid (CSF) samples; and the like. Alternatively RNA or cDNA can be assayed. Methods for purification or partial purification of nucleic acids from patient samples for use in diagnostic or other assays are well known

[0078] Methods for detecting polymorphisms and deletions in nucleic acids include, without limitation, Southern blot analysis (see Kees *et al.*, "Homozygous Deletion of the p16/MTS1 Gene in Pediatric Acute Lymphoblastic Leukemia Is Associated With Unfavorable Clinical Outcome," *Blood* 89:4161-4166, Fizzotti *et al.*, "Detection of homozygous deletions of the cyclin-dependent kinase 4 inhibitor (p16) gene in acute lymphoblastic leukemia and association with adverse prognostic features," *Blood* 85(10):2685-2690, Kitada *et al.*, "Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism," *Nature* 392 (9):605-608); Northern Blot Analysis (see Fieschi *et al.*, "A novel form of complete IL-12/IL-23 receptor b1 deficiency with cell surface-expressed nonfunctional receptors," *Immunobiology* 104(7):2095-2101) and amplification based method such as PCR-based methods are used to detect deletions in samples. PCR primers may be designed to target DNA sequences flanking a known mutation, in which a change in PCR product size in comparison to amplification reactions using WT DNA identifies a mutant template. Primers may also be targeted to deleted sequences, wherein an absence of a PCR product identifies a mutant template (Kitada *et al.*, "Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism," *Nature* 392:605-608) including multiplex PCR (Chong *et al.*,

"Single-tube multiplex-PCR screen for common deletional determinants of α-thalassemia," *Blood* 95 (1):360-362).

[0079]   Polymorphisms (e.g., deletions) can also be detected using allele-specific probes; use of allele-specific primers; direct sequence analysis; denaturing gradient gel electropohoresis (DGGE) analysis; single-strand conformation polymorphism (SSCP) analysis; and denaturing high performance liquid chromatography (DHPLC) analysis. Other well known methods to detect polymorphisms in DNA include use of: Molecular Beacons technology (see, *e.g.*, Piatek *et al.*, 1998; *Nat. Biotechnol.* 16:359-63; Tyagi, and Kramer, 1996, *Nat. Biotechnology* 14:303-308; and Tyagi, *et al.*, 1998, *Nat. Biotechnol.* 16:49-53), Invader technology (see, *e.g.*, Neri *et al.*, 2000, *Advances in Nucleic Acid and Protein Analysis* 3826:117-125 and U.S. Patent No. 6,706,471), nucleic acid sequence based amplification (Nasba) (Compton, 1991), Scorpion technology (Thelwell *et al.*, 2000, *Nuc. Acids Res*, 28:3752-3761 and Solinas *et al.*, 2001, "Duplex Scorpion primers in SNP analysis and FRET applications" *Nuc. Acids Res*, 29:20), restriction fragment length polymorphism (RFLP) analysis, and the like.

[0080]   The design and use of allele-specific probes for analyzing polymorphisms are described by *e.g.*, Saiki *et al.*, 1986; Dattagupta, EP 235,726; and Saiki, WO 89/11548. Briefly, allele-specific probes are designed to hybridize to a segment of target DNA from one individual but not to the corresponding segment from another individual, if the two segments represent different polymorphic forms. Hybridization conditions are chosen that are sufficiently stringent so that a given probe essentially hybridizes to only one of two alleles. Typically, allele-specific probes are designed to hybridize to a segment of target DNA such that the polymorphic site aligns with a central position of the probe.

[0081]   Exemplary probes for analyzing deletions and polymorphisms are shown in Table 1 of Example 1, but many others may be designed by one of skill.

[0082]   Allele-specific probes are often used in pairs, one member of a pair designed to hybridize to the reference allele of a target sequence and the other member designed to hybridize to the variant allele. Several pairs of probes can be immobilized on the same support for simultaneous analysis of multiple polymorphisms within the same target gene sequence.

[0083]   The design and use of allele-specific primers for analyzing polymorphisms are described by, *e.g.*, WO 93/22456 and Gibbs, 1989. Briefly, allele-specific primers are designed to hybridize to a site on target DNA overlapping a polymorphism and to prime DNA amplification according to standard PCR protocols only when the primer exhibits perfect complementarity to the particular allelic form. A single-base mismatch prevents DNA amplification and no detectable PCR product is formed. The method works best when the polymorphic site is at the extreme 3'-end of the primer, because this position is most destabilizing to elongation from the primer.

17

[0084] Amplification products generated using PCR can be analyzed by the use of denaturing gradient gel electrophoresis (DGGE). Different alleles can be identified based on sequence-dependent melting properties and electrophoretic migration in solution. See Erlich, ed., PCR Technology, Principles and Applications for DNA Amplification, Chapter 7 (W.H. Freeman and Co, New York, 1992).

[0085] Alleles of target sequences can be differentiated using single-strand conformation polymorphism (SSCP) analysis. Different alleles can be identified based on sequence- and structure-dependent electrophoretic migration of single stranded PCR products (Orita *et al.*, 1989). Amplified PCR products can be generated according to standard protocols, and heated or otherwise denatured to form single stranded products, which may refold or form secondary structures that are partially dependent on base sequence.

[0086] Alleles of target sequences can be differentiated using denaturing high performance liquid chromatography (DHPLC) analysis. Different alleles can be identified based on base differences by alteration in chromatographic migration of single stranded PCR products (Frueh and Noyer-Weidner, 2003, *Clin Chem Lab Med.* 41(4):452-61). Amplified PCR products can be generated according to standard protocols, and heated or otherwise denatured to form single stranded products, which may refold or form secondary structures that are partially dependent on the base sequence.

[0087] Direct sequence analysis of polymorphisms can be accomplished using DNA sequencing procedures that are well-known in the art. See Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL (2nd Ed., CSHP, New York 1989) and Zyskind *et al.*, RECOMBINANT DNA LABORATORY MANUAL (Acad. Press, 1988).

[0088] Homozygote deletions can be identified by a variety of methods known in the art. For example, in one approach DNA samples are amplified for further analysis. In an embodiment, two CFHR1-specific primer pairs are used, for instance, ("CFHL1ex6.F" [5'- AGTCGGTTTGGACAGTG -3' (SEQ ID NO: 7)] & "CFHL1ex6R" [5'- GCACAAGTTGGATACTCC -3' (SEQ ID NO: 8)]; and/or "CHFL1ex6.F2" [5'- CATAGTCGGTTTGGACAGTG -3' (SEQ ID NO: 9)] & "CFHL1ex6.R" [5'- GCACAAGTTGGATACTCC -3' (SEQ ID NO: 8)]). In another embodiment, CFHR3-specific primer pairs are used. for instance, ("CFHL3ex3.F" [5'- TCATTGCTATGTCCTTAGG -4' (SEQ ID NO: 10)] & "CFHL3ex3.R" [5'- TCTGAGACTGTCGTCCG -3' (SEQ ID NO: 11)]; and/or "CFHL3ex3seq.F" [5'- TTTTGGATGTTTATGCG -3' (SEQ ID NO: 12)] & "CFHL3ex3seq.R" [5'- AAATAGGTCCGTTGGC -3' (SEQ ID NO: 13)]). Absence of the correct-sized PCR product indicates that the CFHL1 and/or CFHL3 gene(s) are deleted.

[0089] Similarly, heterozygote deletions can be identified by a variety of methods known in the art. For example, in one approach DNA samples are amplified for further analysis, for example with the same primers listed above, followed by direct sequencing. Heterozygotes are characterized, for instance,

by chromatograms in which one peak is approximately half the height of the second peak (in contrast to equal sized peaks) at the SNP positions (rs460897, rs16840561, rs4230, rs414628 for CFHR1; rs1061170 for CFHR3). In another embodiment, a protocol employing ParAllele genotyping data, a copy number analysis is performed, in which samples that fail to genotype key markers (MRD_3855, MRD_3856, MRD_3857, rs385390, rs389897) in the region of these two genes are identified. All samples assigned a copy number of 0 (designated CN0) allow the haplotypes that contain the deletion to be defined. Having defined a deletion haplotype, linkage disequilibrium is used to infer whether samples could not carry a deletion. Specifically, if a sample is homozygous for a different allele than one that defines the haplotype, then it does not carry a deletion.

[0090]   A wide variety of other methods are known in the art for detecting polymorphisms in a biological sample. For example and not limitation, see, *e.g.*, Ullman *et al.* "Methods for single nucleotide polymorphism detection" U.S. Pat. No. 6,632,606; Shi, 2002, "Technologies for individual genotyping: detection of genetic polymorphisms in drug targets and disease genes" *Am J Pharmacogenomics* 2:197-205; and Kwok *et al.*, 2003, "Detection of single nucleotide polymorphisms" *Curr Issues Biol.* 5:43-60).

3.2 Analysis of Protein Samples

[0091]   Methods for protein analysis that can be adapted for detection of proteins such as the CFHR1 and CFHR3 gene products and variants or fragments thereof are well known. These methods include analytical biochemical methods such as electrophoresis (including capillary electrophoresis and one- and two-dimensional electrophoresis), chromatographic methods such as high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, mass spectrometry, and various immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmnunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, Western blotting and others.

[0092]   For example, a number of well established immunological binding assay formats suitable for the practice of the invention are known (see, *e.g.*, Harlow, E.; Lane, D. ANTIBODIES: A LABORATORY MANUAL. Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory; 1988; and Ausubel *et al.*, (2004) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York NY. The assay may be, for example, competitive or non-competitive. Typically, immunological binding assays (or immunoassays) utilize a "capture agent" to specifically bind to and, often, immobilize the analyte. In one embodiment, the capture agent is a moiety that specifically binds to a variant or wild-type CFHR1 or CFHR3 polypeptide or subsequence (*e.g.*, a fragment or truncated form of CFHR1 or CFHR3). The

19

bound protein may be detected using, for example, a detectably labeled anti-CFHR1 or anti-CFHR3 antibody.

3.3 Screening Using Multiple Polymorphisms and Markers

[0093]   In diagnostic methods, analysis of *CFHR1* and/or *CFHR3* polymorphisms can be combined with analysis of polymorphisms in other genes associated with AMD or vascular disease (e.g., AAA), detection of protein markers of AMD (see, *e.g.*, Hageman *et al.*, patent publications US 20030017501; US 20020102581; WO0184149; and WO0106262; and US patent applications 11/706,154 (entitled "Protective Complement Proteins and Age-Related Macular Degeneration") and 11/706,074 (entitled "Variants in Complement Regulatory Genes Predict Age-Related Macular Degeneration"); Gorin *et al.*, US20060281120; and Hoh, WO2007/044897, each of which are incorporated herein by reference in their entirety for all purposes), assessment of other risk factors of AMD or vascular disease (such as family history).

[0094]   For example, analysis of *CFHR1* and/or *CFHR3* polymorphisms (e.g., deletions) can be combined with the analysis of polymorphisms in the Complement Factor H gene (CFH). Genetic variants of the *CFH* gene that may be detected include, but are not limited to, a genotype of a T at position 1277 of the coding region of human *CFH*, any one or more of rs529825; rs800292; rs3766404; rs1061147; rs1061170; and rs203674; any one of more of intron 2 (IVS2 or insTT); rs2274700; exon 10A; and rs375046; one or both of rs529825 and rs800292; one or more of rs1061147, rs1061170 and rs203674; at least one of rs529825 and rs800292; and rs3766404; and at least one of rs1061147, rs1061170 and rs203674; at least rs529825, rs800292, rs3766404, rs1061170, and rs203674; and/or exon 22 (R1210C). See. e.g., Hartman *et al.*, 2006, "HTRA1 promoter polymorphism in wet age-related macular degeneration" *Science* 314:989-92, incorporated herein by reference.

[0095]   In certain embodiments, the analysis of CFHR1 and/or CFHR3 polymorphisms can be combined with analysis of polymorphisms in the HTRA1 gene (also known as the PRSS11 gene), the complement factor B (BF) gene, and/or the complement component 2 (C2) gene. Genetic variants of the HTRA1 gene that may be detected include, but are not limited to, at least one of rs10490924, rs11200638, rs760336, and rs763720. Each of the single nucleotide polymorphisms (SNPs) within the HTRA1 gene are associated with increased risk of developing AMD. The genetic variants of the BF gene that may be detected include the presence of an A or G at rs641153 of the BF gene, or an R or Q at position 32 of the BF protein; and/or an A or T at rs4151667 of the BF gene, or L or H at position 9 of the BF protein. The genetic variants of the C2 protein that may be detected include a G or T at rs547154 of the C2 gene; and/or a C or G at rs9332379 of the C2 gene, or E of D at position 318 of the C2 protein. See, e.g., Gold

et al., 2006 "Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration " *Nat Genet.* 38:458-62.

[0096]   In addition, the analysis of CFHR1 and/or CFHR3 polymorphisms can be combined with an analysis of protein markers associated with AMD. The protein markers may include, but are not limited to, fibulin-3, vitronectin, β-crystallin A2, β-crystallin A3, β-crystallin A4, β-crystallin S, glucose-regulated protein 78 kD (GRP-78), calreticulin, 14-3-3 protein epsilon, serotransferrin, albumin, keratin, pyruvate carboxylase, villin 2, complement 1 q binding protein/hyaluronic acid binding protein ("complement 1 q component"), amyloid A (al amyloid A), amyloid P component, C5 and CSb-9 terminal complexes, HLA-DR, fibrinogen, Factor X, prothrombin, complements 3,5 and 9, complement reactive protein (CRP), HLA-DR, apolipoprotein A, apolipoprotein E, antichymotrypsin, p2 microglobulin, thrombospondin, elastin, collagen, ICAM-1, LFA1, LFA3, B7, IL-1, IL-6, IL-12, TNF-alpha, GM-CSF, heat shock proteins, colony stimulating factors (GM-CSF, M-CSFs), and IL-10.

4. Therapeutic Methods

[0097]   In an embodiment, the invention provides methods of treatment and/or prophylaxis of diseases associated with a deletion within a CFHR1 and/or CFHR3 gene, or with reduced amount or activity of a CFHR1 and/or CFHR3 gene product, though the administration of a CFHR1 or CFHR3 polypeptide, or at least one portion of a CFHR1 and/or a CFHR3 polypeptide, or mixtures thereof, to a subject. In one instance, the disease is vascular disease.

[0098]   In an embodiment, the invention provides methods of treatment and/or prophylaxis of diseases associated with an absence of a deletion within a CFHR1 and/or CFHR3 gene, or with unchanged or increased amount or activity of a CFHR1 and/or CFHR3 gene product, though the administration of at least one agent that reduces or inhibits CFHR1 or CFHR3 polypeptide to a subject. In one instance, the disease is AMD.

4.1 Prevention and Treatment of Vascular Disorders

[0099]   A subject identified as having an elevated likelihood of developing a vascular disorder (*e.g.*, aneurysm) can be treated by administering CFHR1 and/or CFHR3 polypeptides or biologically active fragments or variants thereof. The therapeutic polypeptide can be administered systemically (*e.g.*, by intravenous infusion) or locally (*e.g.*, directly to an organ or tissue, such as the eye or the liver). The polypeptides may have the sequence of wild-type (naturally occurring) polypeptides or may have an amino acid sequence substantially identical to the naturally occurring form.

[0100] CFHR1 and CFHR3 polypeptides or biologically active fragments or variants thereof may be isolated from blood (serum or plasma) or produced using conventional recombinant technology (see Ausubel *et al.*, 2004, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing and Wiley-Interscience, New York). Recombinant expression generally involved introducing the *CFHR1* or *CFHR3* gene into an expression vector that include a promoter to drive transcription of the DNA which is adapted for expression in prokaryotic (*e.g.*, *E. coli*) and eukaryotic (*e.g.*, yeast, insect or mammalian cells) hosts. Suitable host cells include bacteria such as *E. coli*, yeast, filamentous fungi, insect cells, and mammalian cells, which are typically immortalized, including mouse, hamster, human, and monkey cell line. Usually, the promoter is a eukaryotic promoter for expression in a mammalian cell. Usually, transcription regulatory sequences comprise a heterologous promoter and optionally an enhancer, which is recognized by the host cell. Commercially available expression vectors can be used. Expression vectors can include host-recognized replication systems, amplifiable genes, selectable markers, host sequences useful for insertion into the host genome, and the like.

[0101] In another embodiment the recombinant CFHR1 or CFHR3 is a full-length polypeptide, a variant thereof, or fragment thereof. In one embodiment the fragment is a biologically active fragment. In this context, a biologically active CFHR1 or CFHR3 polypeptide has an activity associated with wild-type CFHR1 or CFHR3. For example, in some embodiments the fragment has heparin and/or CRP and/or C3b-protein binding activity. Preferably the fragment has substantial sequence identity to at least a portion of the wild-type proteins. Biologically active fragments may comprise varying lengths of sequence substantially identical to wild-type proteins, such as, for example, at least 100, 200, 500, 700, 900 or 1100 residues. Alternatively, biologically active fragments may comprise at least one SCR substantially identical to CFHR1 or CFHR3, preferably at least 2, 3, 4, or 5 SCRs.

[0102] In specific embodiments, the biologically active fragment includes at least SCR 6-7. In another embodiment, the biologically active fragment includes at least SCR 19-20. In another embodiment, the biologically active CFH includes at least SCR1.

[0103] In certain embodiments the therapeutic Factor H polypeptides are chimeric or fusion proteins, and comprise sequence from other proteins. For example, a therapeutic Factor H polypeptide may contain portions of human CFHR1 or CFHR3 as well as portions comprised, at least in part, of SCR (or CCP) consensus domains from other proteins (e.g., CR1, MCP, DAF, C4BP, CR2, CFH) and/or artificial SCR (CCP) consensus sequences. *See* U.S. Pat. No. 5,545,619, incorporated herein by reference.

22

4.1.1 Therapeutic Compositions Containing CFHR1 or CFHR3 Polypeptides

[0104]   The invention provides therapeutic preparations of *CFHR1 or CFHR3* polypeptides,
which may be wild-type or variants (*e.g.*, neutral or protective variants), and may be full length forms,
truncated forms, or biologically active fragments, including splice variants and recombinant fusion
proteins. Therapeutic CFHR1 or CFHR3 polypeptides can be made recombinantly. Therapeutic proteins
can be recombinantly produced (*e.g.*, in cultured bacterial or eukaryotic cells) and purified using methods
well known in the art and described herein. Alternatively, CFHR1 or CFHR3 polypeptides can be
isolated from cultured RPE cells (*e.g.*, primary cultures) or other cells that express CFHR1 or CFHR3
endogenously. Recombinant or purified polypeptides subject to FDA approval must be tested for potency
and identity, be sterile, be free of extraneous material, and all ingredients in a product (*i.e.*, preservatives,
diluents, adjuvants, and the like) must meet standards of purity, quality, and not be deleterious to the
patient.

[0105]   The invention provides a composition comprising a CFHR1 polypeptide or CFHR3
polypeptide, and a pharmaceutically acceptable excipient or carrier. The term "pharmaceutically
acceptable excipient or carrier" refers to a medium that is used to prepare a desired dosage form of a
compound. A pharmaceutically acceptable excipient or carrier can include one or more solvents, diluents,
or other liquid vehicles; dispersion or suspension aids; surface active agents; isotonic agents; thickening
or emulsifying agents; preservatives; solid binders; lubricants; and the like. Remington's Pharmaceutical
Sciences, Fifteenth Edition, E.W. Martin (Mack Publishing Co., Easton, PA, 1975) and Handbook of
Pharmaceutical Excipients, Third Edition, A.H. Kibbe ed. (American Pharmaceutical Assoc. 2000),
disclose various carriers used in formulating pharmaceutical compositions and known techniques for the
preparation thereof. The pharmaceutical compositions may be formulated using slow release agents or
biodegradeable agents following techniques known in the art. In one embodiment, the pharmaceutically
acceptable excipient is not deleterious to a mammal (*e.g.*, human patient) if administered to the eye (*e.g.*,
by intraocular injection). For intraocular administration, for example and not limitation, the therapeutic
agent can be administered in a Balanced Salt Solution (BSS) or Balanced Salt Solution Plus (BSS Plus)
(Alcon Laboratories, Fort Worth, Texas, USA). In a related aspect, the invention provides a sterile
container, *e.g.* vial, containing a therapeutically acceptable CFHR1 or CFHR3 polypeptides, optionally as
a lyophilized preparation.

[0106]   The amount of CFHR1 or CFHR3 polypeptide, or biologically active fragment thereof, to
be administered to an individual can be determined. In one embodiment, exogenous CFHR1 or CFHR3
can be administered to an individual in an amount sufficient to achieve a level similar to the plasma
concentration of CFHR1 or CFHR3 in a healthy individual, *i.e.*, an amount sufficient to achieve a plasma
level of from about 50 to 600 micrograms/ml, such as from about 100 to 560 micrograms/ml. The

amount of CFHR1 or CFHR3 to be administered to an individual (e.g., a 160 pound subject) can be, for example and not for limitation, from about 10 milligrams to about 5000 milligrams per dose, from about 50 milligrams to about 2000 milligrams per dose, from about 100 milligrams to about 1500 milligrams per dose, from about 200 milligrams to about 1000 milligrams per dose, or from about 250 milligrams to about 750 milligrams per dose. The frequency with which CFHR1 or CFHR3 can be administered to an individual can be, for example and not for limitation, twice per day, once per day, twice per week, once per week, once every two weeks, once per month, once every two months, once every six months, or once per year. The amount and frequency of administration of CFHR1 or CFHR3 to an individual can be readily determined by a physician by monitoring the course of treatment.

[0107]   Alternatively, the CFHR1 or CFHR3 polypeptide, or biologically active fragment thereof, can be administered to an individual using gene therapy or cell therapy methods as described further below.

4.1.2 Gene Therapy Methods

[0108]   In another approach, CFHR1 or CFHR3 polypeptide is administered by *in vivo* expression of protein encoded by exogenous polynucleotide (*i.e.*, via gene therapy). In one example, gene therapy involves introducing into a cell a vector that expresses CFHR1 or CFHR3 polypeptides or biologically active fragments of CFHR1 or CFHR3. The cell may be an endogenous cell (*i.e.*, a cell from the patient) or engineered exogenous cell.

[0109]   Vectors can be viral or nonviral. A number of vectors derived from animal viruses are available, including those derived from adenovirus, adeno-associated virus, retroviruses, pox viruses, alpha viruses, rhadboviruses, and papillomaviruses. Usually the viruses have been attenuated to no longer replicate (see, *e.g.*, Kay *et al.* 2001, *Nature Medicine* 7:33-40).

[0110]   The nucleic acid encoding the polypeptide is typically linked to regulatory elements, such as a promoters and an enhancers, which drive transcription of the DNA in the target cells of an individual. The promoter may drive expression of the gene in all cell types. Alternatively, the promoter may drive expression of the *CFHR1* or *CFHR3* gene only in specific cell types, for example, in cells of the retina, the liver or the kidney. The regulatory elements, operably linked to the nucleic acid encoding the polypeptide, are often cloned into a vector.

[0111]   As will be understood by those of skill in the art, gene therapy vectors contain the necessary elements for the transcription and translation of the inserted coding sequence (and may include, for example, a promoter, an enhancer, other regulatory elements). Promoters can be constitutive or inducible. Promoters can be selected to target preferential gene expression in a target tissue, such as the RPE (for recent reviews see Sutanto *et al.*, 2005, "Development and evaluation of the specificity of a

24

cathepsin D proximal promoter in the eye" *Curr Eye Res.* 30:53-61; Zhang *et al.*, 2004, "Concurrent enhancement of transcriptional activity and specificity of a retinal pigment epithelial cell-preferential promoter" *Mol Vis.* 10:208-14; Esumi *et al.*, 2004, "Analysis of the VMD2 promoter and implication of E-box binding factors in its regulation" *J Biol Chem* 279:19064-73; Camacho-Hubner *et al.*, 2000, "The Fugu rubripes tyrosinase gene promoter targets transgene expression to pigment cells in the mouse" *Genesis.* 28:99-105; and references therein).

[0112]   Suitable viral vectors include DNA virus vectors (such as adenoviral vectors, adeno-associated virus vectors, lentivirus vectors, and vaccinia virus vectors), and RNA virus vectors (such as retroviral vectors). In one embodiment, an adeno-associated viral (AAV) vector is used. For recent reviews see Auricchio *et al.*, 2005, "Adeno-associated viral vectors for retinal gene transfer and treatment of retinal diseases" *Curr Gene Ther.* 5:339-48; Martin *et al.*, 2004, Gene therapy for optic nerve disease, *Eye* 18:1049-55; Ali, 2004, "Prospects for gene therapy" *Novartis Found Symp.* 255:165-72; Hennig *et al.*, 2004, "AAV-mediated intravitreal gene therapy reduces lysosomal storage in the retinal pigmented epithelium and improves retinal function in adult MPS VII mice" *Mol Ther.* 10:106-16; Smith *et al.*, 2003, "AAV-Mediated gene transfer slows photoreceptor loss in the RCS rat model of retinitis pigmentosa" *Mol Ther.* 8:188-95; Broderick *et al.*, 2005, "Local administration of an adeno-associated viral vector expressing IL-10 reduces monocyte infiltration and subsequent photoreceptor damage during experimental autoimmune uveitis" *Mol Ther.* 12:369-73; Cheng et al., 2005, "Efficient gene transfer to retinal pigment epithelium cells with long-term expression. *Retina* 25:193-201; Rex *et al.*, "Adenovirus-mediated delivery of catalase to retinal pigment epithelial cells protects neighboring photoreceptors from photo-oxidative stress. *Hum Gene Ther.* 15:960-7; and references cited therein).

[0113]   Gene therapy vectors must be produced in compliance with the Good Manufacturing Practice (GMP) requirements rendering the product suitable for administration to patients. The present invention provides gene therapy vectors suitable for administration to patients including gene therapy vectors that are produced and tested in compliance with the GMP requirements. Gene therapy vectors subject to FDA approval must be tested for potency and identity, be sterile, be free of extraneous material, and all ingredients in a product (*i.e.*, preservatives, diluents, adjuvants, and the like) must meet standards of purity, quality, and not be deleterious to the patient. For example, the nucleic acid preparation is demonstrated to be mycoplasma-free. See, e.g, Islam *et al.*, 1997, An academic centre for gene therapy research and clinical grade manufacturing capability, *Ann Med* 29, 579-583.

[0114]   Methods for administering gene therapy vectors are known. In one embodiment, CFHR1 or CFHR3 expression vectors are introduced systemically (*e.g.*, intravenously or by infusion). In one embodiment, expression vectors are introduced locally (*i.e.*, directly to a particular tissue or organ, *e.g.*, liver). In one embodiment, expression vectors are introduced directly into the eye (*e.g.*, by intraocular

injection). As will be understood by those of skill in the art, the promoter chosen for the expression vectors will be dependent upon the target cells expressing the CFHR1 or CFHR3 polypeptides. In some embodiments, a cell type-specific promoter is used and in other embodiments, a constitutive or general promoter is used. For recent reviews see, *e.g.*, Dinculescu *et al.*, 2005, "Adeno-associated virus-vectored gene therapy for retinal disease" *Hum Gene Ther.* 16:649-63; Rex *et al.*, 2004, "Adenovirus-mediated delivery of catalase to retinal pigment epithelial cells protects neighboring photoreceptors from photo-oxidative stress" *Hum Gene Ther.* 15:960-7; Bennett, 2004, "Gene therapy for Leber congenital amaurosis" *Novartis Found Symp.* 255:195-202; Hauswirth *et al.*, "Range of retinal diseases potentially treatable by AAV-vectored gene therapy" *Novartis Found Symp.* 255:179-188, and references cited therein).

[0115]   Thus in one aspect, the invention provides a preparation comprising a gene therapy vector encoding a CFHR1 or CFHR3 polypeptide, optionally a viral vector, where the gene therapy vector is suitable for administration to a human subject and in an excipient suitable for administration to a human subject (*e.g.*, produced using GLP techniques). Optionally the gene therapy vector comprises a promoter that is expressed preferentially or specifically in retinal pigmented epithelium cells.

[0116]   Nonviral methods for introduction of *CFHR1* or *CFHR3* gene sequences, such as encapsulation in biodegradable polymers (*e.g.*, polylactic acid (PLA); polyglycolic acid (PGA); and co-polymers (PLGA) can also be used (for recent reviews see, *e.g.*, Bejjani *et al.*, 2005, "Nanoparticles for gene delivery to retinal pigment epithelial cells" *Mol Vis.* 11:124-32; Mannermaa *et al.*, 2005, "Long-lasting secretion of transgene product from differentiated and filter-grown retinal pigment epithelial cells after nonviral gene transfer" *Curr Eye Res.* 2005 30:345-53; and references cited therein). Alternatively, the nucleic acid encoding a CFHR1 or CFHR3 polypeptide may be packaged into liposomes, or the nucleic acid can be delivered to an individual without packaging without using a vector.

4.1.3. Cell Therapy Methods

[0117]   In another approach, CFHR1 or CFHR3 polypeptide is administered by *in vivo* expression of protein encoded by endogenous or exogenous CFHR1 or CFHR3 polynucleotide (*i.e.*, via cell therapy). For example, hepatocyte transplantation has been used as an alternative to whole-organ transplantation to support many forms of hepatic insufficiency (see, *e.g.*, Ohashi *et al.*, Hepatocyte transplantation: clinical and experimental application, *J Mol Med.* 2001 79:617-30). According to this method, hepatocytes or other CFHR1- or CFHR3-expressing cells are administered (*e.g.*, infused) to a patient in need of treatment. These cells migrate to the liver or other organ, and produce the therapeutic protein. Also see, *e.g.*, Alexandrova *et al.*, 2005, "Large-scale isolation of human hepatocytes for therapeutic application" *Cell Transplant.* 14(10):845-53; Cheong *et al.*, 2004,"Attempted treatment of

factor H deficiency by liver transplantation" *Pediatr Nephrol.* 19:454-8; Ohashi *et al.*, 2001, "Hepatocyte transplantation: clinical and experimental application" *J Mol Med.* 79:617-30; Serralta *et al.*, 2005, "Influence of preservation solution on the isolation and culture of human hepatocytes from liver grafts" *Cell Transplant.* 14(10):837-43; Yokoyama *et al.*, 2006, "In vivo engineering of metabolically active hepatic tissues in a neovascularized subcutaneous cavity" *Am. J. Transplant.* 6(1):50-9; Dhawan *et al.*, 2005, "Hepatocyte transplantation for metabolic disorders, experience at King's College hospital and review of literature." *Acta Grastroenterol. Belg.* 68(4):457-60; Bruns *et al.*, 2005, "Injectable liver: a novel approach using fibrin gel as a matrix for culture and intrahepatic transplantation of hepatocytes" *Tissue Eng.* 11(11-12):1718-26. Other cell types that may be used include, for illustration and not limitation, kidney and pancreatic cells. In one embodiment, the administered cells are engineered to express a recombinant form of the CFHR1 or CFHR3 protein.

[0118]   In another, related approach, therapeutic organ transplantation is used. Most of the body's systemic CFHR1 and CFHR3 is produced by the liver, making transplantation of liver tissue the preferred method. See, Gerber *et al.*, 2003, "Successful (?) therapy of hemolytic-uremic syndrome with factor H abnormality" *Pediatr Nephrol.* 18:952-5.

[0119]   In another approach, a CFHR1 or CFHR3 protein is delivered to the back of the eye by injection into the eye (*e.g.*, intravitreal) or via encapsulated cells. Neurotech's Encapsulated Cell Technology (ECT), as an example, is a unique technology that allows for the sustained, longterm delivery of therapeutic factors to the back of the eye. See (http://www.neurotech.fr). ECT implants consist of cells that have been genetically modified to produce a specific therapeutic protein that are encapsulated in a semi-permeable hollow fiber membrane. The cells continuously produce the therapeutic protein that diffuses out of the implant and into the eye (Bush *et al.*, 2004, "Encapsulated cell-based intraocular delivery of ciliary neurotrophic factor in normal rabbit: dose-dependent effects on ERG and retinal histology" *Invest Ophthalmol Vis Sci.* 45:2420-30.). CNTF delivered to the human eye by ECT devices was recently shown to be completely successful and associated with minimal complications in 10 patients enrolled in a Phase I clinical trial (Sieving *et al.*, 2006, "Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants" *Proc Natl Acad Sci USA* 103(10):3896-901). Also see Song *et al.*, 2003, "Photoreceptor protection by cardiotrophin-1 in transgenic rats with the rhodopsin mutation s334ter" *IOVS*, 44(9):4069-75; Tao *et al.*, 2002, "Encapsulated Cell-Based Delivery of CNTF Reduces Photoreceptor Degeneration in Animal Models of Retinitis Pigmentosa" *IOVS*, 43 10:3292-3298; and Hammang *et al.*, U.S. Pat. No. 6,649,184. In one embodiment of the present invention, a form of CFHR1 or CFHR3 is expressed in cells and administered in an encapsulated form. In one embodiment, the cells used are the NTC-201 human RPE

line (ATCC # CRL-2302) available from the American Type Culture Collection P.O. Box 1549, Manassas, VA 20108.

4.2 Prevention and Treatment of AMD

[0120]   A subject identified as having an elevated likelihood of developing AMD, exhibiting symptoms of AMD, or susceptible to AMD, can be treated by reducing the expression, activity or amount of a gene product of the *CFHR1* and/or *CFHR3* genes. Any method of reducing levels of CFHR1 or CFHR3 in the eye or systemically may be used for treatment including, for example, inhibiting transcription of a *CFHR1* or *CFHR3* gene, inhibiting translation of CFHR1 or CFHR3 RNA, decreasing the amount or activity of CFHR1 or CFHR3 proteins (*e.g.*, by plasmaphoresis, antibody-directed plasmaphoresis, or complexing with a CFHR1 or CFHR3 binding moiety (*e.g.*, heparin or antibody), or by administration of inhibitory nucleic acids. In some embodiments levels of CFHR1 or CFHR3 are preferentially reduced in the eye (*e.g.*, RPE) relative to other tissues. For illustration and not limitation, several methods are briefly described below.

4.2.1 Inhibitory Nucleic Acids

[0121]   Inhibitory nucleic acids are known and include antisense nucleic acids, interfering RNAs, ribozymes and others (see, *e.g.*, Gomes *et al.*, 2005, "Intraocular delivery of oligonucleotides" *Curr Pharm Biotechnol.* 6:7-15; and Henry *et al.*, 2004, "Setting sights on the treatment of ocular angiogenesis using antisense oligonucleotides" Trends Pharmacol Sci 25:523-7; PCT Publications WO 98/53083; WO 99/32619; WO 99/53050; WO 00/44914; WO 01/36646; WO 01/75164; WO 02/44321; and U.S. Patent No. 6,107,094; Sui *et al.*, 2002, "A DNA vector-based RNAi technology to suppress gene expression in mammalian cells" *Proc Natl Acad Sci USA* 99:5515-20; and Kasahara and Aoki, 2005, "Gene silencing using adenoviral RNA vector in vascular smooth muscle cells and cardiomyocytes" *Methods Mol Med.*112:155-72; US Patent Nos. US6180399; US5869254; US6025167; US5854038; US5591610; US5667969; US5354855;US5093246; US5180818; US5116742; US5037746; and US4987071; Dawson *et al.*, 2000, "Hammerhead ribozymes selectively suppress mutant type I collagen mRNA in osteogenesis imperfecta fibroblasts" *Nucleic Acids Res.* 28:4013-20; Blalock et al., 2004 "Hammerhead ribozyme targeting connective tissue growth factor mRNA blocks transforming growth factor-beta mediated cell proliferation" *Exp Eye Res.* 78:1127-36; Kuan *et al.*, 2004, "Targeted gene modification using triplex-forming oligonucleotides" *Methods Mol Biol.* 262:173-94.

[0122]   It will be understood that inhibitory nucleic acids can be administered as a pharmaceutical composition or using gene therapy or cell therapy methods.

4.2.2 Antibodies and Antibody Therapy

[0123]   In one aspect, an anti-CFHR1 or anti-CFHR3 binding agents (e.g., antibodies) that reduce the activity or amount of the proteins is administered to an individual with or at risk for AMD. The antibody can be administered systemically or locally (see, e.g., Gaudreault et al., 2005, "Preclinical pharmacokinetics of Ranibizumab (rhuFabV2) after a single intravitreal administration" Invest Ophthalmol Vis Sci. 46:726-33).

[0124]   In one embodiment, an anti-CFHR1 antibody specifically binds an epitope of CFHR1, in particular human CFHR1. In certain embodiments, an anti-CFHR1 antibody specifically binds an epitope located within the amino-terminus of a CFHR1 polypeptide. In particular, an anti-CFHR1 antibody specifically binds an epitope located between amino acids 1-143 of SEQ ID NO: 4 as shown in Figure 6. In other embodiments, an anti-CFHR1 antibody specifically binds an epitope within the CFHR1 short consensus repeats (SCRs) 6 and/or 7 as shown in Figure 1. The amino acid sequence of CFHR1 SCR6 is 35% homologous to the corresponding CFH SCR, and the amino acid sequence of CFHR SCR7 is 45% homologous to the corresponding CFH SCR. Anti-CFHR1 antibodies of the invention specifically bind CFHR1 and do not cross-react with CFH or other factor H related proteins including CFHT, CFHR2, CFHR3, CFHR4, or CFHR5. A variety of immunoassay formats may be used to select antibodies that are specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with an antigen. See Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York. Epitope mapping of the CFHR1 protein is within the skill of the art to determine epitopes that are most immunogenic for the generation of anti-CFHR1 antibodies.

[0125]   In another embodiment, an anti-CFHR3 antibody specifically binds an epitope of CFHR3, in particular human CFHR3. In certain embodiments, an anti-CFHR3 antibody specifically binds an epitope located within the carboxyl-terminus of a CFHR3 polypeptide. For example, an anti-CFHR3 antibody may specifically bind to an epitope between amino acids 144-330 of SEQ ID NO: 6 as shown in Figure 6. In other embodiments, an anti-CFHR3 antibody specifically binds an epitope within the CHFR3 SCRs 8, 19 and/or 20 as shown in Figure 1. The amino acid sequence of CFHR3 SCR8 is 63% homologous to the corresponding CRH SCR, the amino acid sequence of CFHR3 SCR19 is 62% homologous to the corresponding CFH SCR, and the amino acid sequence of CFHR3 SCR20 is 36% homologous to the corresponding CFH SCR. Anti-CFHR3 antibodies of the invention specifically bind CFHR3 and do not cross-react with CFH or other factor H related proteins including CFHT, CFHR1, CFHR2, CFHR4, or CFHR5. Epitope mapping of the CFHR3 protein is within the skill of the art to determine epitopes that may be immunogenic for the generation of anti-CFHR3 antibodies.

[0126] It is understood that each of the antibodies discussed above can be an intact antibody, for example, a monoclonal antibody. Alternatively, the binding protein can be an antigen binding fragment of an antibody, or can be a biosynthetic antibody binding site. Antibody fragments include Fab, Fab', (Fab')$_2$ or Fv fragments. Techniques for making such antibody fragments are known to those skilled in the art. A number of biosynthetic antibody binding sites are known in the art and include, for example, single Fv or sFv molecules, described, for example, in U.S. Patent Nos. 5,476,786. Other biosynthetic antibody binding sites include bispecific or bifunctional binding proteins, for example, bispecific or bifunctional antibodies, which are antibodies or antibody fragments that bind at least two different antigens. For example, bispecific binding proteins can bind CFHR1, CFHR3, and/or another antigen. Methods for making bispecific antibodies are known in art and, include, for example, by fusing hybridomas or by linking Fab' fragments. See, e.g., Songsivilai *et al.* (1990) CLIN. EXP. IMMUNOL. 79: 315-325; Kostelny *et al.* (1992) J. IMMUNOL. 148: 1547-1553.

[0127] Anti-CFHR1 and anti-CFHR3 antibodies can be produced using techniques well known in the art. Monoclonal antibodies can be produced using standard fusion techniques for forming hybridoma cells. See G. Kohler, et al., *Nature*, 256:456 (1975). Alternatively, monoclonal antibodies can be produced from cells by the method of Huse, et al., Science, 256:1275 (1989).

[0128] It is understood that the CDRs of the antibodies described herein confer the binding specificity to CFHR1 or CFHR3. The antibodies described herein can be used as diagnostic and/or therapeutic agents. It is understood that the antibodies of the invention can be modified to optimize performance depending upon the intended use of the antibodies. For example, when the antibody is being used as a therapeutic agent, the antibody can be modified to reduce its immunogenicity in the intended recipient. Alternatively or in addition, the antibody can be fused or coupled to another protein or peptide, for example, a growth factor, cytokine, or cytotoxin. Such modifications can be achieved by using routine gene manipulation techniques known in the art.

[0129] Various techniques for reducing the antigenicity of antibodies and antibody fragments are known in the art. These techniques can be used to reduce or eliminate the antigenicity of the antibodies of the invention. For example, when the antibodies are to be administered to a human, the antibodies preferably are engineered to reduce their antigenicity in humans. This process often is referred to as humanization. Preferably, the humanized binding proteins have the same or substantially the same affinity for the antigen as the original non-humanized binding protein it was derived from.

[0130] In one well known humanization approach, chimeric proteins are created in which immunoglobulin constant regions of antibodies from one species, e.g., mouse, are replaced with immunoglobulin constant regions from a second, different species, e.g., a human. In this example, the

resulting antibody is a mouse-human chimera, where the human constant region sequences, in principle, are less immunogenic than the counterpart murine sequences. This type of antibody engineering is described, for example, Morrison, *et al.* (1984) *Proc. Nat. Acad. Sci.* 81: 6851-6855, Neuberger *et al.*, 1984, *Nature* 312: 604-608; U.S. Patent Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and 4,816,567 (Cabilly).

[0131]   In another approach, known as CDR grafting, the CDRs of the light and heavy chain variable regions of an antibody of interest are grafted into frameworks (FRs) from another species. For example, murine CDRs can be grafted into human FR sequences. In some embodiments, the CDRs of the light and heavy chain variable regions of an anti-CFHR1 antibody or an anti-CFHR3 antibody are grafted into human FRs or consensus human FRs. In order to create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. CDR grafting is described, for example, in U.S. Patent Nos. 7,022,500 (Queen); 6,982,321 (Winter); 6,180,370 (Queen); 6,054,297 (Carter); 5,693,762 (Queen); 5,859,205 (Adair); 5,693,761 (Queen); 5,565,332 (Hoogenboom); 5,585,089 (Queen); 5,530,101 (Queen); Jones *et al.* (1986) NATURE 321: 522-525; Riechmann *et al.* (1988) NATURE 332: 323-327; Verhoeyen *et al.* (1988) SCIENCE 239: 1534-1536; and Winter (1998) FEBS LETT 430: 92-94.

[0132]   In addition, it is possible to create fully human antibodies in mice. In this approach, human antibodies are prepared using a transgenic mouse in which the mouse's antibody-producing genes have been replaced by a substantial portion of the human antibody producing genes. Such mice produce human immunoglobulin instead of murine immunoglobulin molecules. See, e.g., WO 98/24893 (Jacobovitz et al.) and Mendez *et al.*, 1997, *Nature Genetics* 15: 146-156. Fully human anti-CFHR1 and/or anti-CFHR3 monoclonal antibodies can be produced using the following approach. Transgenic mice containing human immunoglobulin genes are immunized with the antigen of interest, e.g., CFHR1 or CFHR3. Lymphatic cells from the mice then are obtained from the mice, which are then fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. The hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to CFHR1 or CFHR3.

5. Drug Screening/Antagonists of Risk Variant Factor H or Variant CFHR5

[0133]   The invention provides a drug screening method for screening for agents for use in treating vascular disorders. The method involves combining (i) a cell that expresses CFHR3 and/or CFHR1 polypeptides; and (ii) a test agent; b) measuring the level of CFHR3 and/or CFHR1 gene expression in the cell and c) comparing the level of CFHR3 and/or CFHR1 gene expression in the cell with a reference value, where the reference value is the level of CFHR3 and/or CFHR1 gene expression in

the absence of the test agent, where a higher level of CFHR3 and/or CFHR1 gene expression in the presence of the test agent indicates the test agent may be useful for treating the vascular disorders. Compounds from natural product libraries or synthetic combinatorial libraries may be screened. The level of CFHR3 and/or CFHR1 gene expression using a variety of approaches including measuring protein levels, measuring mRNA levels or other methods.

[0134] In one embodiment the method involves combining (i) a cell that expresses CFHR3 and/or CFHR1 polypeptides; and (ii) a test agent; b) measuring the level of CFHR3 and/or CFHR1 polypeptides produced by the cell (e.g., secreted into the medium); and c) comparing the level of CFHR3 and/or CFHR1 polypeptides secreted into the medium in the presence of the test agent with a reference value, said reference value being the level of CFHR3 and/or CFHR1 polypeptides produced (or secreted into the medium) in the absence of the test agent, where a higher level of CFHR3 and/or CFHR1 polypeptides secreted into the medium in the presence of the test agent indicates the test agent may be useful for treating the vascular disorders. Compounds from natural product libraries or synthetic combinatorial libraries may be screened.

6. Identifying Protective Forms of Complement Factor H Proteins

[0135] As described above, deletions at the *CFHR1* and *CFHR3* loci are linked to the presence of a protective haplotype. Protective haplotypes and protective forms of CFH proteins are described in Hageman *et al.*, 2005, *Proc. Natl. Acad. Sci. U.S.A.* 102:7227-32 and U.S. patent publication No. 20070020647. In one aspect, the invention provides a method for identifying a CFH protein likely to protect against development of AMD when administered to a subject having, or at risk of developing, AMD. The method involves identifying a subject with a deletion in the DNA sequence between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene on human chromosome 1; determining the sequence of the CFH gene encoded by the gene contained in the chromosome containing the deletion; and determining the sequence of the protein encoded by the *CFH* gene, wherein said protein is different from wild-type CFH, said protein being a CFH protein likely to protect against AMD development. The invention also provides a protective CFH protein obtained using the method. U.S. patent publication No. 20070020647 discloses the use of protective forms of CFH protein to protect against AMD development and to treat AMD.

7. Kits and Diagnostic Devices

[0136] The invention provides reagents, devices and kits for detecting *CFHR1* or *CFHR3* deletions. A number of assay systems are known in the art, and it is within the skill of the art to arrive at means to determine the presence of variations associated with vascular disorders or AMD. The kit

reagents, such as multiple primers, multiple probes, combinations of primers, or combinations of probes, may be contained in separate containers prior to their use for diagnosis or screening. In an embodiment, the kit contains a first container containing a probe, primer, or primer pair for a first *CFHR1* or *CFHR3* allele described herein, and a second container containing a probe, primer, or primer pair for a second *CFHR1* or *CFHR3* allele described herein.

[0137]   The kits may contain one or more pairs of *CFHR1* and/or *CFHR3* allele-specific oligonucleotides hybridizing to different forms of a polymorphism. The allele-specific oligonucleotides may be provided immobilized on a substrate.

[0138]   The invention also provides devices and reagents useful for diagnostic, prognostic, drug screening, and other methods are provided. In one aspect, a device comprising immobilized primer(s) or probe(s) specific for detecting deletions in the *CFHR1* and/or *CFHR3* genes and optionally also including immobilized primer(s) or probe(s) specific for detecting polymorphic sites in *CFH* that are associated with AMD. Exemplary probes and polymorphic sites are described in U.S. patent publication No. 20070020647.

[0139]   In one aspect, a device comprising immobilized primer(s) or probe(s) specific for one or more Factor H and/or CFHR5 and/or CFHR1 and/or CFHR3 gene products (polynucleotides or proteins) is provided. The primers or probes can bind polynucleotides (*e.g.*, based on hybridization to specific polymorphic sites) or polypeptides (*e.g.*, based on specific binding to a variant polypeptide).

[0140]   In one embodiment, an array format is used in which a plurality (at least 2, usually at least 3 or more) of different primers or probes are immobilized. The term "array" is used in its usual sense and means that each of a plurality of primers or probes, usually immobilized on a substrate, has a defined location (address) *e.g.*, on the substrate. The number of primers or probes on the array can vary depending on the nature and use of the device. For example, a dipstick format array can have as few as 2 distinct primers or probes, although usually more than 2 primers or probes, and often many more, will be present. One method for attaching the nucleic acids to a surface is by making high-density oligonucleotide arrays (see, Fodor *et al.*, 1991, *Science* 251:767-73; Lockhart *et al.*, 1996, *Nature Biotech* 14:1675; and U.S. Pat. Nos. 5,578,832; 5,556,752; and 5,510,270). It is also contemplated that, in some embodiments, a device comprising a single immobilized probe can be used.

[0141]   In one embodiment, an array format is used in which a plurality (at least 2, usually at least 3 or more) of different primers or probes are immobilized. The term "array" is used in its usual sense and means that each of a plurality of primers or probes, usually immobilized on a substrate, has a defined location (address) *e.g.*, on the substrate. The number of primers or probes on the array can vary depending on the nature and use of the device.

[0142]   In one embodiment, the immobilized probe is an antibody or other CFHR1 or CFHR3 binding moiety.

[0143]   It will be apparent to the skilled practitioner guided by this disclosure than various polymorphisms and haplotypes can be detected, and used in combination with a deletion in the DNA sequence between the 3' end of exon 22 of the complement factor H (*CFH*) gene and the 5' end of exon 1 of complement Factor H-related 4 (*CFHR4*) gene on human chromosome 1, to assess the propensity of an individual to develop a Factor H related condition. Examples of *CFH* polymorphisms that may be assayed for include the following SNPs and combinations of SNPs: rs529825; rs800292; rs3766404; rs1061147; rs1061170; rs203674; and optionally including exon 22 (R1210C). In one embodiment the array includes primers or probes to determine the allele at at least one of the following polymorphic sites: rs529825; rs800292; intron 2 (IVS2 or insTT); rs3766404; rs1061147; rs1061170; exon 10A; rs203674; rs375046; and optionally including exon 22 (R1210C). In an embodiment the array includes primers or probes to determine the allele at at least one of the following polymorphic sites: (a) rs3753394; (b) rs529825; (c) rs800292; (d) intron 2 (IVS2 or insTT); (e) rs3766404; (f) rs1061147; (g) rs1061170; (h) rs2274700; (i) rs203674; (j) rs3753396; (j) rs1065489; and optionally including exon 22 (R1210C). In one embodiment, the array includes primers or probes to determine the allele at at least one of the following polymorphic sites: rs800292 (I62V); IVS 2 (-18insTT); rs1061170 (Y402H); and rs2274700 (A473A). In one embodiment, the array includes primers or probes to determine the allele at at least one of the following polymorphic sites: rs9427661 (-249T>C); rs9427662 (-20T>C); and rs12097550 (P46S).

[0144]   The array can include primers or probes to determine the allele at two of the above sites, at least three, at least four, at least five or at least six. In one embodiment the primers or probes distinguish alleles at rs529825. In one embodiment the primers or probes distinguish alleles at rs800292. In one embodiment the primers or probes distinguish alleles at rs3766404. In one embodiment the primers or probes distinguish alleles at rs1061147. In one embodiment the primers or probes distinguish alleles at rs1061170. In one embodiment the primers or probes distinguish alleles at rs203674. In one embodiment the primers or probes distinguish alleles at exon 22 (R1210C). In one embodiment the primers or probes distinguish alleles at rs529825 and rs800292. In one embodiment the primers or probes distinguish alleles at two or three of rs1061147, rs1061170 and rs203674. In one embodiment the primers or probes distinguish alleles at rs529825 and rs800292, at rs3766404, two or three of rs1061147, rs1061170 and rs203674. In one embodiment the primers or probes distinguish alleles at rs529825, rs800292, rs3766404, rs1061170 and rs203674. In one embodiment, the primers or probes distinguish alleles at exon 22 (R1210C) and at rs529825; at rs800292; at rs3766404; at rs1061147; at rs1061170; at rs203674; at rs529825 and rs800292; at two or three of rs1061147, rs1061170 and rs203674; at rs529825 and rs800292, rs3766404, and two or three of rs1061147, rs1061170 and rs203674; or at rs529825,

rs800292, rs3766404, rs1061170 and rs203674. In one embodiment, the primers or probes distinguish alleles at (a) any one or more of rs529825; rs800292; rs3766404; rs1061147; rs1061170; and rs203674; (b)any one of more of intron 2 (IVS2 or insTT); rs2274700; exon 10A; and rs375046; (c) one or both of rs529825 and rs800292; (d) one or more of rs1061147, rs1061170 and rs203674; (e) at least one of rs529825 and rs800292; and rs3766404; and at least one of rs1061147, rs1061170 and rs203674; (f) at least rs529825, rs800292, rs3766404, rs1061170, and rs203674; (g) exon 22 (R1210C); (h) exon 22 (R1210C) and any of (a)-(g); or (i) any one or more of rs529825; rs800292; rs3766404; rs1061147; rs1061170; rs203674; intron 2 (IVS2 or insTT); rs2274700; exon 10A; rs375046; and exon 22 (R1210C) and any one or more of rs9427661, rs9427662 and rs12097550.

[0145]   The array can include primers or probes to determine the allele at two of the above sites, at least three, at least four, at least five or at least six. In one embodiment the primers or probes distinguish alleles at rs529825. In one embodiment the primers or probes distinguish alleles at rs800292. In one embodiment the primers or probes distinguish alleles at intron 2 (IVS2 or insTT). In one embodiment the primers or probes distinguish alleles at rs3766404. In one embodiment the primers or probes distinguish alleles at rs1061147. In one embodiment the primers or probes distinguish alleles at rs1061170. In one embodiment the primers or probes distinguish alleles at exon 10A. In one embodiment the primers or probes distinguish alleles at rs2274700. In one embodiment the primers or probes distinguish alleles at rs203674. In one embodiment the primers or probes distinguish alleles at rs375046. In one embodiment the primers or probes distinguish alleles at exon 22 (R1210C). In one embodiment the primers or probes distinguish alleles at rs529825 and rs800292. In one embodiment the primers or probes distinguish alleles at two or three of rs1061147, rs1061170 and rs203674. In one embodiment the primers or probes distinguish alleles at of rs529825 and rs800292, at intron 2, at rs3766404, at two or three of rs1061147, rs1061170 and rs203674, at exon 10A, at rs2274700, and at rs375046. In one embodiment the primers or probes distinguish alleles at rs529825, rs800292, intron 2 (IVS2 or insTT), rs3766404, rs1061170, exon 10A, rs2274700, rs203674, and rs375046. In one embodiment, the primers or probes distinguish alleles at exon 22 (R1210C) and at either at rs529825; at rs800292; at intron 2 (IVS2 or insTT); at rs3766404; at rs1061147; at rs1061170; at rs2274700, at exon 10A; at rs203674; at rs375046; at rs529825 and rs800292; at two or three of rs1061147, rs1061170 and rs203674; at rs529825 and rs800292, intron 2 (IVS2 or insTT), rs3766404, two or three of rs1061147, rs1061170 and rs203674, rs2274700, exon 10A, and rs375046; or at rs529825, rs800292, intron 2 (IVS2 or insTT), rs3766404, rs1061170, rs2274700, exon 10A, rs203674, and rs375046. In one embodiment, the device distinguishes any combination of allelles at the sites listed above in the context of kits.

[0146]   In one embodiment, the substrate comprises fewer than about 1000 distinct primers or probes, often fewer than about 100 distinct primers or probes, fewer than about 50 distinct primers or

probes, or fewer than about 10 distinct primers or probes. As used in this context, a primer is "distinct" from a second primer if the two primers do not specifically bind the same polynucleotide (*i.e.*, such as cDNA primers for different genes). As used in this context, a probe is "distinct" from a second probe if the two probes do not specifically bind the same polypeptide or polynucleotide (*i.e.*, such as cDNA probes for different genes). Primers or probes may also be described as distinct if they recognize different alleles of the same gene (*i.e.*, *CFH* or *CFHR5*). Thus, in one embodiment diagnostic devices of the invention detect alleles of *CFH* only, *CFHR5* only, *CFH* and *CFHR5* only, or *CFH*, *CFHR5* and up to 20, preferably up to 10, or preferably up to 5 genes other than *CFH* and/or *CFHR5*. That is, the device is particularly suited to screening for AMD and related complement-associated diseases. In one embodiment, the device comprises primers or probes that recognize *CFH* and/or one or more of *CFHR1-5* only. In a related embodiment, the device contains primers and probes for up to 20, preferably up to 10, or preferably up to 5 other genes than *CFH* or *CFHR1-5*.

[0147]   In one embodiment, the immobilized primer(s) is/are an allele-specific primer(s) that can distinguish between alleles at a polymorphic site in the Factor H or *CHRF5* gene. Exemplary allele-specific primers to identify alleles at polymorphic sites in the Factor H gene are shown in TABLE 16A of U.S. patent publication No. 20070020647, incorporated by reference in its entirety for all purposes. The immobilized allele-specific primers hybridize preferentially to nucleic acids, either RNA or DNA, that have sequences complementary to the primers. The hybridization may be detected by various methods, including single-base extension with fluorescence detection, the oligonucleotide ligation assay, and the like (see Shi, M.M., 2001, Enabling large-scale pharmacogenetic studies by high-throughput mutation detection and genotyping technologies" *Clin. Chem.* 47(2):164-172). Microarray-based devices to detect polymorphic sites are commercially available, including Affymetrix (Santa Clara, CA), Protogene (Menlo Park, CA), Genometrix (The Woodland, TX), Motorola BioChip Systems (Northbrook, IL), and Perlegen Sciences (Mountain View, CA).

[0148]   The invention provides reagents and kits for detecting CFHR1 and/or CFHR3 proteins. A number of assay systems are known in the art, and it is within the skill of the art to arrive at means to determine the presence or absence of CFHR1 and/or CFHR3, or variant or truncated forms thereof, associated with vascular disorders or AMD. The kit reagents, such as anti-CFHR3 or CFHR1 antibodies or other CFHR3 or CFHR1 binding moieties, may be contained in separate containers prior to their use for diagnosis or screening. In an embodiment, the kit contains a first container containing an antibody or binding moiety that specifically binds to CFHR1 protein, or a variant or truncated form thereof, and a second container containing an antibody or binding moiety that specifically binds to CFHR3 protein, or a variant or truncated form thereof. In some embodiments the binding moieties is an aptamer, such as a nucleic acid aptamer. Aptamers are RNA or DNA molecules selected *in vitro* from vast populations of

random sequence that recognize specific ligands by forming binding pockets. Aptamers are nucleic acids that are capable of three dimensional recognition that bind specific proteins or other molecules. See, e.g., US20050176940 "Aptamers and Antiaptamers".

[0149]   Thus, the invention provides reagents for conducting the screening methods of the invention, comprising a binding moiety capable of specifically binding CFHR1 and/or CFHR3 protein or a portion thereof (e.g., a labeled binder that reacts preferentially with CFHR1 and/or CFHR3 protein or a portion thereof or a labeled binder that reacts preferentially with CFHR1 mRNA and/or CFHR3 mRNA or a portion thereof, or a labeled binder that reacts preferentially with CFHR1 DNA and/or CFHR3 DNA). The binding moiety may comprise, for example, a member of a ligand-receptor pair, i.e., a pair of molecules capable of having a specific binding interaction (such as antibody-antigen, protein-protein, nucleic acid-nucleic acid, protein-nucleic acid, or other specific binding pair known in the art). Optionally the binding moiety is labled (e.g., directly labeled) or is accompanied by a labeled molecule that reacts with the binding moiety (indirectly labeled). Detectable labels can be directly attached to or incorporated into the detection reagent by chemical or recombinant methods. Examples of detectable labels include, but are not limited to, radioisotopes, fluorophores, chromophores (e.g., colored particles), mass labels, electron dense particles, magnetic particles, spin labels, and molecules that emit chemiluminescence. Methods for labeling are well known in the art.

[0150]   The kits may contain an instruction manual with instructions how to use the anti-CFHR3 or CFHR1 antibodies or other CFHR3 or CFHR1 binding moieties to detect CFHR3 or CFHR1 proteins in body fluids or in tissue samples.

[0151]   The kits may contain a control antibody or binding moiety. An example of a control antibody or binding moiety is an antibody that specifically binds to CFH protein.

[0152]   The kits may contain one or more pairs of antibodies or binding moieties that specifically bind to different (i.e., not wild-type or full-length) forms (e.g., variant or truncated) of CFHR1 or CFHR3 proteins.

[0153]   In one embodiment, the antibodies or binding moieties are immobilized to a solid support such as an ordered array.

[0154]   In one embodiment, the antibodies or binding moieties are used in Western blots.

EXAMPLES

Example 1:

[0155]   Polymerase chain reaction (PCR) amplification, single-strand conformation polymorphism (SSCP) analysis and direct DNA sequencing were used to characterize a deletion in the *CFHR3* and *CFHR1* genes located between the *CFH* and *CFHR4* genes on chromosome 1. Examples of primers that can be used for PCR amplification of the *CFH* gene and *CFH*-related genes 1 to 5 are shown in Table 1A. Examples of primers that can be used for SSCP analysis of the *CFH* and *CFHR3* genes are shown in Table 1B. Examples of primers that can be used for direct DNA sequencing of the *CFH*, *CFHR1* and *CFHR3* genes are shown in Table 1C and 1D.

Table 1. Primers Used for Detecting the *CFH* and *CFHR1-5* Genes

A. PCR Primers

	Forward 5'-3'	Reverse 5'-3'	Product Size (bp)
CFH ex22	GGTTTGGATAGTGTGTTTTGAG (SEQ ID NO:14)	ACCGTTAGTTTTCCAGG (SEQ ID NO:15)	521
CFHR1 ex6	AGTCGGTTTGGACAGTG (SEQ ID NO:7)	GCACAAGTTGGATACTCC (SEQ ID NO:8)	321
CFHR2 ex4	TGTGTTCATTCAGTGAG (SEQ ID NO:16)	ATAGACATTTGGTAGGC (SEQ ID NO:17)	510
CFHR3 ex3	TCATTGCTATGTCCTTAGG (SEQ ID NO:10)	TCTGAGACTGTCGTCCG (SEQ ID NO:11)	263
CFHR4 ex3	CTACAATGGGACTTTCTTAG (SEQ ID NO:18)	TTCACACTCATAGGAGGAC (SEQ ID NO:19)	378
CFHR5 ex2	AACCCTTTTCCCAAG (SEQ ID NO:20)	CACATCCTTCTCTATTCAC (SEQ ID NO:21)	193

B. SSCP Primers

	Forward 5'-3'	Reverse 5'-3'	Product Size (bp)
CFH ex22	GGTTTGGATAGTGTGTTTTGAG (SEQ ID NO:14)	ATGTTGTTCGCAATGTG (SEQ ID NO:22)	283
CFHR3 ex3	TCATTGCTATGTCCTTAGG (SEQ ID NO:10)	TCTGAGACTGTCGTCCG (SEQ ID NO:11)	263

C. Sequencing Primers

	Forward 5'-3'	Reverse 5'-3'	Product Size (bp)
CFH ex22	GGTTTGGATAGTGTGTTTTGAG (SEQ ID NO:14)	ACCGTTAGTTTTCCAGG (SEQ ID NO:15)	521
CFHR3 ex3 seq	TTTTGGATGTTTATGCG (SEQ ID NO:12)	AAATAGGTCCGTTGGC (SEQ ID NO:13)	420
CFHR1 ex6	AGTCGGTTTGGACAGTG (SEQ ID NO:7)	GCACAAGTTGGATACTCC (SEQ ID NO:8)	321

D. Primers used for detecting the *CFH* and *CFHR1-5* genes and results

	Forward 5'- 3'	Reverse 5'- 3'	Product
CFH (ex22)	GGTTTGGATAGTGTGTTTTGAG (SEQ ID NO:14)	ATGTTGTTCGCAATGTG (SEQ ID NO:22)	Yes
CFH (ex22)	GGTTTGGATAGTGTGTTTTGAG (SEQ ID NO:14)	ACCGTTAGTTTTCCAGG (SEQ ID NO:15)	Yes
IVS 5' to CFHR3	CACGCTATTTGAAAGACAAACTT (SEQ ID NO:23)	AAGCAACCCTGCTCTACAATGT (SEQ ID NO:24)	Yes
IVS 5' to CFHR3	GGAACCACATGGGTCAAATG (SEQ ID NO:25)	GCACAACAAATAAAAACTAGCAAATCAT (SEQ ID NO:26)	Yes
IVS 5' to CFHR3	ATTGCTGCAATCTCAGAAGAAAA (SEQ ID NO:27)	TCAAAACGAACAAACAAACAAACAGG (SEQ ID NO:28)	No
CFHR3 (ex2)	TGCGTAGACCATACTTTCCAG (SEQ ID NO:29)	CTCTCTTTAATCTTTTAAAGTTTTATACACATGTG (SEQ ID NO:30)	No
CFHR3 (ex3)	TTTTGGATGTTTATGCG (SEQ ID NO:12)	AAATAGGTCCGTTGGC (SEQ ID NO:13)	No
CFHR3 (ex3)	TCATTGCTATGTCCTTAGG (SEQ ID NO:10)	TCTGAGACTGTCGTCCG (SEQ ID NO:11)	No

39

CFHR1 (ex2)	TAAAGTGCTGTGTTTGTATTTGC (SEQ ID NO:31)	GTGATTATTTGTTACCAACAGC (SEQ ID NO:32)	No
CFHR1 (ex6)	AGTCGGTTTGGACAGTG (SEQ ID NO:7)	GCACAAGTTGGATACTCC (SEQ ID NO:8)	No
CFHR1 (ex6)	CATAGTCGGTTTGGACAGTG (SEQ ID NO:9)	GCACAAGTTGGATACTCC (SEQ ID NO:8)	No
CFHR2	TCCTTTTCTAGTTCATTAACATA (SEQ ID NO:33)	AGTGATATGACACATGCTGAC (SEQ ID NO:34)	Yes
CFHR2	CTACAGACTAACTTTCAATAATTT (SEQ ID NO:35)	GATACTTTTACATTTCTTATGAT (SEQ ID NO:36)	Yes
CFHR2	ACATAGTTATATGATCGTTTTGAGT (SEQ ID NO:37)	ACAGAGAAAGAACTTACTAATTG (SEQ ID NO:38)	Yes
CFHR2	TGTGTTCATTCAGTGAG (SEQ ID NO:16)	ATAGACATTTGGTAGGC (SEQ ID NO:17)	Yes
CFHR4	AGTATTAAATTGTTCAGTCCAG (SEQ ID NO:39)	AAACTAGTGTAAGAATGTATGAT (SEQ ID NO:40)	Yes
CFHR4	TAAGTTGAAAGAGATCTAAACAC (SEQ ID NO:41)	ACTGTATGTAAGATTATGAAAGTAT (SEQ ID NO:42)	Yes
CFHR4	CTACAATGGGACTTTCTTAG (SEQ ID NO:18)	TTCACACTCATAGGAGGAC (SEQ ID NO:19)	Yes
CFHR5	AACCCTTTTTCCCAAG (SEQ ID NO:20)	CACATCCTTCTCTATTCAC (SEQ ID NO:21)	Yes

[0156]   In a study directed toward further characterization of *CFH* and its associated haplotypes on chromosome 1q, a complete deletion of the entire *CFHL1* and *CFHL3* genes was identified. In examining SSCP gels generated using *CFH* exon 22 primers (Table 1), several additional patterns of variation were observed due to the amplification of *CFHR1* in addition to *CFH*. By designing another set of CFH-specific primers, it was determined that there were no variations in exon 22 of *CFH*. *CFHR1*-specific primers were generated and used to identify a deletion of *CFHR1*. Further analysis of the *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4* and *CFHR5* genes and intervening sequence 5' to CFHR3 (Table 1D) using specific primers revealed a deletion that extends across the entire length of the *CFHR1* and *CFHR3* genes. The precise boundaries of the complete deletion have not be determined, but the mapping of the boundaries is within the skill of the art.

[0157]   SSCP analysis and direct DNA sequencing was used to determine the frequency of the homozygous deletion of the *CFHR3* and *CFHR1* genes in a set of 1074 patients with and without a clinical history of AMD. The cohort included patients who had other systemic diseases, including vascular diseases, irrespective of their AMD status. As shown in Table 2, homozygous deletion of the *CFHR1* and *CFHR3* genes was found in ~2.7% of the persons tested.

Table 2. Frequency of homozygous deletion of *CFHR1* and *CFHR3* genes

Genotype*	Count	Percent
+/+, +/Δ	1046	97.3%
Δ/Δ	28	2.7%
+/+, +/Δ, Δ/Δ	1074	100%

*Genotype refers to the deletion (Δ) or non-deletion (+) of the *CFHR1* and *CFHR3* genes by SSCP analysis and direct sequencing.

[0158]   Initial analysis suggested that the deletion homozygotes were more common in control individuals than in AMD cases. To determine whether there was an association of the homozygous deletion of the *CFHR3* and *CFHR1* genes with AMD, a subset of the above patient population was analyzed by SSCP analysis and direct DNA sequencing. As shown in Table 3, in a study of 576 AMD patients and 352 age-matched non-AMD control patients, deletion homozygotes make up 5.1% of controls and 1.2% of cases. The homozygous deletion of *CFHR1* and *CFHR3* is strongly associated with controls, with $\chi2 = 10.2$ and P value = 0.0014, demonstrating a highly significant protective effect of the homozygous *CFHR1/CFHR3* deletion for AMD.

Table 3. Association of homozygous deletion of *CFHR1* and *CFHR3* genes with non-AMD

	Genotype	Non-AMD patients	AMD patients
Count	+/+, +/Δ	352	576
Count	Δ/Δ	18	7
Frequency	+/+, +/Δ	0.951	0.988
Frequency	Δ/Δ	0.049	0.012

*Genotype refers to the deletion (Δ) or non-deletion (+) of the *CFHR1* and *CFHR3* genes by SSCP analysis and direct sequencing.

[0159]   To determine whether there was an association of the homozygous deletion of the *CFHR3* and *CFHR1* genes with vascular disorders, two subsets of the above patient population were analyzed by SSCP analysis and direct DNA sequencing. As shown in Table 4A, a study of 26 patients with abdominal aortic aneurysm (AAA) and 133 non-AAA patients revealed that the homozygous deletion of *CFHR1* and *CFHR3* was strongly associated with AAA, with $\chi2$ = 6.982329 and P = 0.0082. As shown in Table 4B, a second study of 86 patients with abdominal aortic aneurysm (AAA) and 221 non-AAA patients revealed that the homozygous deletion of *CFHR1* and *CFHR3* was associated with AAA, with $\chi2$ = 4.05 and P = 0.0442.

Table 4. Association of homozygous deletion of *CFHR1* and *CFHR3* genes with AAA

A. Study 1

	Genotype	Controls	AAA
Count	+/+, +/Δ	126	19
Count	Δ/Δ	7	7
Total	+/+, +/Δ, Δ/Δ	133	26

*Genotype refers to the deletion (Δ) or non-deletion (+) of the *CFHR1* and *CFHR3* genes by SSCP analysis and direct sequencing.

B. Study 2

	Genotype	Controls	AAA
Count	+/+, +/Δ	221	86
Count	Δ/Δ	12	11
Total	+/+, +/Δ, Δ/Δ	233	97

*Genotype refers to the deletion (Δ) or non-deletion (+) of the *CFHR1* and *CFHR3* genes by SSCP analysis and direct sequencing.

[0160]   To determine whether previously identified protective haplotypes in the *CFH* gene were associated with the del (Δ) *CFHR1* allele, haplotype analysis was performed. As shown in Tables 5A-5E, the relationship between the del (Δ) *CFHR1* allele and SNPs in the *CFH* gene revealed strong linkage

disequilibrium. The SNPs used in this haplotype analysis are described in U.S. patent publication No. 20070020647. In the table, letters refer to genotypes and numbers refer to SSCP shift patterns.

Table 5. *CFH* gene haplotype analysis in subjects with the del/del (Δ/Δ) *CFHR1* allele

A. Promoter 1 to Exon 3

	Promoter 1	Promoter 4	Exon 2	Exon 3a	Exon 3
		rs3753394	rs800292		same SNP as 3a
			I62V		
		C-257T	G184A	IVS2-18insTT	
1	AA	TT	GG	SS	SS
2	AA	CC	GG	SS	SS
3	AA	CT	GG	SS	SS
4	AA	CC	GG	SS + G100R het	SS + G100R het
5	AA	CT	GG	SS	SS
6	AA	CT	GG	SS	SS
7	AA	CC	GG	SS	SS
8	AA	TT	GG	SS	SS
9	AA	CT	GG	SS	SS
10	AA	CC	GG	SS	SS
11	AA	CC	GG	SS	SS
12	AA	CC	GG	SS	SS
13	AA	CT	GG	SS	SS
14			GG		SS
15			GG		SS
16			GG		SS
17			GG		SS
18			GG		SS
19			GG		SS
20			GG		SS
21			GA		SS
22			GG		SS
23					SS
24					SS
25					SS
26					SS

B. IVS 6 to Exon 7b

	IVS 6	IVS 6	IVS6	IVS6	Exon 7b
	shift	N or Del	rs16840419	rs3766404	rs1061147
					A307A
					A921C
1	3	NN	GA	CT	CC
2	5	NDel	5 (GG)	5 (CC?)	CC
3	2	NN	GG	CC	CC

	IVS 6	IVS 6	IVS6	IVS6	Exon 7b
	shift	N or Del	rs16840419	rs3766404	rs1061147
4	2	NN	GG	CC	CC
5	3	NN	GA	CT	CC
6	1	NN	AA	TT	AC
7	5	NDel	5 (GG)	5 (CC?)	CC
8	1	NN	AA	TT	CC
9	3	NN	GA	CT	CC
10	2	NN	GG	CC	CC
11	No DNA (3)	NN	No DNA (GA)	No DNA (CT)	AC
12	2	NN	GG	CC	CC
13	1	NN	AA	TT	CC
14					
15					
16					
17					
18					AA
19					
20					
21					
22					
23					
24					
25					
26					

C. Exon 9 to Exon 16b

	Exon 9	Exon 10A	Exon 10a	Exon 13b	Exon 16b
	rs1061170		rs2274700	rs3753396	rs375046
	Y402H		A473A	Q672Q	IVS15
	C1204T	CFHtrunc	G2016A	A2089G	
1	TT	1	AA	AA	
2	TT			AA	AA
3	TT	1	AA	AA	AA
4	TT	1	AA	AA	AA
5	TT	1	AA	AA	4
6	CT	1	GA	AA	
7	TT				AA
8	TT	1	AA	AA	AA
9	TT	1	AA	AA	AA
10	TT	1	AA	AA	AC?
11	CT	1	GA	AA	
12	TT			AA	
13	TT				
14	TT	1	AA		
15	TT	1	AA		
16	CT	1	GA		

44

	Exon 9	Exon 10A	Exon 10a	Exon 13b	Exon 16b
	rs1061170		rs2274700	rs3753396	rs375046
	Y402H		A473A	Q672Q	IVS15
	C1204T	CFHtrunc	G2016A	A2089G	
17	TT	1	AA	AA	CC
18	CC	1	GG		
19	TT	1	AA		
20	TT	1	AA		
21	TT	1	AA		
22	TT	1	AA		
23	TT				
24	TT				
25	TT				
26	TT				

D. Exon 17a to Exon 19a

	Exon 17a	Exon 17b	Exon 18a	Exon 18b	Exon 19a
			rs1065489	rs1065489	rs534399
		A892V	E936D	E936D	V1007L
		C2748T	G2881T	G2881T	G3092T
1	1	CC	GG	GG	GG
2	1	CC			
3	1	CC	GG	GG	GG
4	1	CC	GG	GG	GG
5	1	CC	GG	GG	GG
6	3	CC	GG	GG	TT
7	1				
8	1	CC	GG	GG	GG
9	1	CC	GG	GG	GG
10	1	CC	GG	GG	GG
11	1	CC	GG	GG	GG
12	1	CC			
13	1	CC			
14				GG	
15				GG	
16				GG	
17				GG	
18	1	CC	GG	GG	GG
19				GG	
20				GG	
21				GG	
22				GG	
23				GG	
24				GG	
25				GG	

	Exon 17a	Exon 17b	Exon 18a	Exon 18b	Exon 19a
			rs1065489	rs1065489	rs534399
		A892V	E936D	E936D	V1007L
		C2748T	G2881T	G2881T	G3092T
26				GG	

E. Exon 20b to Exon22 split (detects both *CFH* and *CFHR1*)

	Exon 20b	Exon 22b	Exon 22split
		1191/1197/1210	1197
1	4	4	4
2			4
3	4	4	4
4	2	4	4
5	4	4	4
6	4	4	4
7			4
8	4	4	4
9	4	4	4
10	6	4	4
11	4	4	4
12			4
13			4
14		4	
15	4	4	
16	4	4	
17		4	
18	4	4	
19	4	4	
20	6	4	
21	4	4	
22	4	4	
23	4	4	
24	4	4	
25	4	4	
26	4	4	

[0161]   As shown in Table 6, in two studies it was found that the deletion of the *CFHR1* and *CFHR3* genes was associated with 402T-containing haplotypes. This deletion is almost never found on the same 402C-containing haplotype as the major *CFH* risk allele, Y402H. The del (Δ) *CFHR1* mutation is predominantly associated with the *CFH* H4 haplotype, a haplotype with T at position 1277 of the coding region of CFH (codon 402) shown previously shown to be protective for AMD. However, not every del (Δ) *CFHR1* chromosome is on H4, and the protection of del/del (Δ/Δ) *CFHR1* homozygotes for

46

AMD is even stronger than H4 homozygotes. Heterozygous deletion of the *CFHR3* and *CFHR1* genes was detected by direct DNA sequencing of the *CFH*, *CFHR1* and *CFHR3* genes using a *CFH* exon 22 primer.

Table 6. Association of homozygous deletion of *CFHR1* and *CFHR3* genes with the TT genotype at position 1277 of the coding region of CFH (codon 402)

A. Study 1

			CFH402 Genotype	
	Genotype	TT	TC	CC
Count	+/+, +/Δ	102	209	150
Count	Δ/Δ	11	2	0
Count	+/+, +/Δ, Δ/Δ	113	211	150

*Genotype refers to the deletion (Δ) or non-deletion (+) of the *CFHR1* and *CFHR3* genes by SSCP analysis and direct sequencing.
**CFH402 Genotype refers to the nucleotide on both alleles at position 1277 of the coding region of human CFH. A T results in a tyrosine at codon 402, whereas a C results in a histidine at codon 402.
***Of the 474 patients, approximately 22+/-4% are heterozygous (+/Δ) for the deletion of the *CFHR1* and *CFHR3* genes, as determined by direct DNA sequencing.

B. Study 2

			CFH402 Genotype	
	Genotype	TT	TC	CC
Count	+/+, +/Δ	192	393	283
Count	Δ/Δ	23	3	0
Count	+/+, +/Δ, Δ/Δ	215	396	283

*Genotype refers to the deletion (Δ) or non-deletion (+) of the CFHR1 and CFHR3 genes by SSCP analysis and direct sequencing.
**CFH402 Genotype refers to the nucleotide on both alleles at position 1277 of the coding region of human CFH. A T results in a tyrosine at codon 402, whereas a C results in a histidine at codon 402.

[0162] By Western blotting, it was determined that CFHR1 protein, normally an abundant serum protein, is absent in sera derived from individuals homozygous for the *CFHR1/CFHR3* deletion. Figure 3 shows a representative Western blot of serum proteins from seven (out of a sample set of 52) patients using an anti-human CFH antibody. Serum proteins were separated by one-dimensional SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. After transfer, the membrane was blocked with 5% non-fat dry milk, washed, and then incubated with a goat anti-human CFH (Calbiochem, 1:1000 dilution). After incubation, the membrane was washed, and then incubated

with horse radish peroxidase-conjugated rabbit anti-goat Ig antibody (Abcam, 1:4000 dilution). After incubation, the membrane was washed, and then incubated with extravidin (1:1500 dilution). Samples 197-02 and 325-02 were from patients with a TT 402 genotype (protective CFH H4 haplotype) and have homozygous deletion of *CFHR1* and *CFHR3* genes, as determined by SSCP analysis and direct sequencing. Figure 3 shows that no CFHR1 is detected in the serum from patients having a homozygous deletion of the *CFHR1* and *CFHR3* genes.

[0163]   Western blotting using the same anti-human CFH antibody was used to detect CFH and CFHR1 in serum from an additional 40 patients, separated according to SSCP patterns using the *CFH* exon 22 primers. Patterns 1-3 correspond to homozygous, or heterozygous for, non-deletion of *CFHR1* and *CFHR3* (+/+, +/Δ), and pattern 4 corresponds to homozygous deletion of *CFHR1* and *CFHR3* (Δ/Δ) (see Figure 4). All 10 of the serum samples from patients displaying SSCP pattern 4 show no CFHR1, whereas all 30 of the serum samples from patients displaying SSCP patterns 1-3 show at least some CFHR1 (data not shown). Thus, analysis of serum from individuals with a *CFHR1* del/del (Δ/Δ) genotype shows that they lack any detectable CFHR1 protein. This protein analysis confirms that these individuals lack both the *CFHR1* gene and encoded protein. Individuals who are heterozygous for deletion of *CFHR1* and *CFHR3* can be recognized by protein analysis of serum samples by virtue of the intensity of the band corresponding to CFHR1 being roughly half the intensity in heterozygous (+/Δ) patients as compared to homozygous non-deletion (+/+) patients.

[0164]   PCR experiments using leukocyte-derived DNA were performed to confirm that patients having a homozygous deletion of *CFHR1* and *CFHR3* do not have *CFHR1* and *CFHR3* DNA. Figure 5 shows a PCR analysis of *CFH* and *CFHR1-5* from DNA samples from 20 patients, separated into four groups according to SSCP patterns using the *CFH* exon 22 primers mentioned above. Patterns 1-3 correspond to homozygous non-deletion or heterozygous deletion of *CFHR1* and *CFHR3* (+/+, +/Δ), and pattern 4 corresponds to homozygous deletion of *CFHR1* and *CFHR3* (Δ/Δ). From left to right, 5 samples each from patients displaying SSCP patterns 1, 2, 3 and 4 were subjected to PCR using primers specific for *CFH, CFHR1, CFHR2, CFHR3, CFHR4* and *CFHR5*, as indicated. This figure shows that *CFH, CFHR4* and *CFHR5* DNA are amplified in all of the samples, whereas *CFHR1* and *CFHR3* DNA are amplified in samples from patients displaying SSCP patterns 1-3, but not from patients displaying SSCP pattern 4. The *CFHR2* DNA was amplified in some, but not all, of the samples. Thus, when SSCP and direct sequencing show a homozygous deletion of the *CFHR1* and *CFHR3* genes, no PCR amplifiable *CFHR1* and *CFHR3* DNA are detected in samples.

Example 2: Production of Anti-CFHR1 and Anti-CFHR3 Monoclonal Antibodies

[0165]   Mice will be immunized with recombinant human CFHR1 or CFHR3. Two mice with sera displaying the highest anti-CFHR1 and anti-CFHR3 activity by Enzyme Linked Immunosorbent Assay (ELISA) will be chosen for subsequent fusion and spleens and lymph nodes from the appropriate mice will be harvested. B-cells will be harvested and fused with an myeloma line. Fusion products will be serially diluted on one or more plates to near clonality. Supernatants from the resulting fusions will be screened for their binding to hCFHR1 or hCFHR3 by ELISA. Supernatants identified as containing antibodies to CFHR1 or CFHR3 will be further characterized by *in vitro* functional testing as discussed below. A panel of hybridomas will be selected and the hybridomas will be subcloned and expanded. The monoclonal antibodies will then be purified by affinity chromatography on Protein A/G resin under standard conditions.

[0166]   Anti-CFHR1 and anti-CFHR3 antibodies may be further characterized by *in vitro* functional testing using complement activation assays well known in the art. For example, complement activation assays may be conducted in solution (e.g., fluid phase in blood) or on immobilized surfaces. Exemplary assays may measure the ability of the anti-CFHR1 and/or anti-CFHR3 antibodies to block or reduce CFH, C3b, heparin and/or C-reactive protein (CRP) binding to a substrate.

***

[0167]   Although the present invention has been described in detail with reference to specific embodiments, those of skill in the art will recognize that modifications and improvements are within the scope and spirit of the invention, as set forth in the claims which follow. All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents (patents, published patent applications, and unpublished patent applications) is not intended as an admission that any such document is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description is for purposes of illustration and not limitation of the following claims.

WHAT IS CLAIMED IS:

1.   A screening method for determining a human subject's propensity to develop a vascular disorder and/or age-related macular degeneration (AMD) comprising:

detecting the presence or absence in chromosome 1 of the subject of a deletion that encompasses at least a portion of the complement Factor H-related 3 (CFHR3) gene and at least a portion of the Factor H-related 1 (CFHR1) gene

wherein the presence of the deletion indicates the subject is at increased risk of developing a vascular disorder and is at decreased risk of developing AMD.

2.   The method of claim 1 wherein entire protein coding region of the CFHR3 gene is deleted and wherein entire protein coding region of the CFHR1 gene is deleted.

3.   The method of claim 1 or claim 2 wherein the method comprises assaying for a CFHR1 gene product, where the absence of a gene product, or a reduced level of expression of the gene product, is indicative of the deletion.

4.   The method of any preceding claim wherein the method comprises assaying for a CFHR3 gene product, where the absence of a gene product, or a reduced level of expression of the gene product, is indicative of the deletion.

5.   The method of claim 3 or claim 4 wherein the gene product is a protein.

6.   The method of claim 5 wherein the protein is detected using an immunoassay or mass spectroscopy.

7.   The method of claim 1 wherein the method comprises assaying for a truncated CFHR1 gene product, where detection of a truncated gene product is indicative of the deletion.

8.   The method of claim 1 or claim 7 wherein the method comprises assaying for a truncated CFHR3 gene product, where detection of a truncated gene product is indicative of the deletion.

9.   The method of claim 1 or claim 2 wherein the presence or absence of the deletion is detected by analyzing a chromosome or nucleic acid from the subject.

10.   The method of claim 9 wherein the nucleic acid is DNA or RNA.

11.   The method of claim 1, further comprising detecting a deletion of an intragenic sequence selected from a sequence between the CFHR3 gene and the CFHR1 gene and a sequence between the CFHR1 gene and the CFHR4 gene.

12.   The method of claim 1 wherein the subject is homozygous for the deletion.

13.   The method of claim 1 wherein the subject has been diagnosed with AMD.

14.   The method of claim 1 wherein the method comprises analyzing a blood, serum, urine or tissue sample obtained from the patient.

15.   The method of claim 1 wherein the subject has a genotype of T at position 1277 of the coding region of the CFH gene of the chromosome comprising the deletion.

16.   The method of claim 1 further comprising detecting genetic variants of complement factor H (CFH) gene comprising detecting one or a plurality of polymorphic sites selected from the group consisting of:

a)   any one or more of rs529825; rs800292; rs3766404; rs1061147; rs1061170; and rs203674;

b)   any one or more of intron 2 (IVS2 or insTT); rs2274700; exon 10A; and rs375046;

c)   one or both of rs529825 and rs800292;

d)   one or more of rs1061147, rs1061170 and rs203674;

e)   at least one of rs529825 and rs800292; and rs3766404; and at least one of rs1061147; rs1061170; and rs203674;

f)   at least rs529825, rs800292, rs3766404, rs1061170 and rs203674;

g)   exon 22 (R1210C); and

h)   exon 22 (R1210C) and any of (a)-(g).

17.   The method of claim 1 further comprising detecting a macular degeneration-associated molecule selected from the group consisting of fibulin-3, vitronectin, β-crystallin A2, β-crystallin A3, β-crystallin A4, β-crystallin S, glucose-regulated protein 78kD (GRP-78), calreticulin, 14-3-3 protein epsilon, serotransferrin, albumin, keratin, pyruvate carboxylase, villin 2, complement 1 q binding protein/hyaluronic acid binding protein ("complement 1 q complement"), amyloid A (al amyloid A), amyloid P component, C5 and C5b-9 terminal complexes, HLA-DR, fibrinogen, Factor X, prothrombin, complements 3, 5 and 9, complement reactive protein (CRP), HLA-DR, apolipoprotein A, apolipoprotein E, antichymotrypsin, p2 microgobulin, thrombospondin, elastin, collagen, ICAM-1, LFA1, LFA3, B7, IL-1, IL-6, IL-12, TNF-alpha, GM-CSF, heat shock proteins, colony stimulating factors (GM-CSF, M-CSFs), and IL-10.

18.   The method of claim 1 further comprising detecting genetic variants of the HTRA1 gene comprising detecting a polymorphic site selected from the group consisting of: at least one of rs10490924; rs11200638, rs760336, and rs763720.

19.   The method of claim 1 further comprising detecting genetic variants of the complement factor B (BF) gene and/or the complement component 2 (C2) gene comprising detecting a polymorphic site selected from the group consisting of:

a)   A or G at rs641153 of the BF gene, or R or Q at position 32 of the BF protein;

b)   A or T at rs4151667 of the BF gene, or L or H at position 9 of the BF protein;

c)   G or T at rs547154 of the C2 gene; and

52

d)   C or G at rs9332379 of the C2 gene, or E or D at position 318 of the C2 protein.

20.   A kit or device when used for assessing a human subject's propensity to develop a vascular disorder and/or age-related macular degeneration (AMD), said kit or device comprising:

(i)   a primer pair; or

(ii)   a nucleic acid probe; or

(iii)   an antibody

capable of detecting the presence or absence in chromosome 1 of the subject of a deletion of a region between the 3' end of exon 22 of the complement factor H (CFH) gene and the 5' end of exon 1 of the complement Factor H-related 4 (CFHR4) gene in chromosome 1, wherein the deletion encompasses at least a portion of the complement Factor H-related 3 (CFHR3) gene and at least a portion of the Factor H-related 1 (CFHR1) gene, wherein the presence of the deletion indicates the subject is at increased risk of developing a vascular disorder and is at decreased risk of developing AMD.

21.   The kit or device of claim 20 comprising a primer pair or a nucleic acid probe capable of detecting a deletion in the CFHR1 gene, the CFHR3 gene, or an intergenic region.

22.   The kit or device of claim 21 wherein:

20

(a)   the primer pair amplifies a sequence in the region between the 3' end of exon 22 of the CFH gene and the 5' end of exon 1 of the CFHR4 gene and the absence of, or a reduced level of, an amplification product is indicative of a deletion; or

(b)   the probe hybridizes to a sequence in the region between the 3' end of exon 22 of the CFH gene and the 5' end of exon 1 of the CFHR4 gene and the absence of, or a reduced level of, hybridization is indicative of a deletion.

25

53

23.   The kit or device of claim 20 comprising;

(i)   two primer pairs; or

(ii)   two nucleic acid probes; or

(iii)   two antibodies

capable of detecting the presence or absence in chromosome 1 of the subject of a deletion of a region between the 3' end of exon 22 of the complement factor H (CFH) gene and the 5' end of exon 1 of complement Factor H-related 4 (CFHR4) gene.

24.   The kit or device of claim 23 comprising:

(a)   two primer pairs wherein the first primer pair amplifies a region of the CFHR1 gene and the second primer pair amplifies a region of the CFHR3 gene; or

(b)   two probes wherein the first probe hybridizes to a sequence in the CFHR1 gene and the second probe hybridizes to a sequence in the CFHR3 gene; or

(c)   a first antibody and a second antibody, wherein the first antibody is specific for the protein product of the CFHR1 gene and the second antibody is specific for the protein product of the CFHR3 gene.

25.   The kit or device of claim 23 or claim 24, wherein each of said two primer pairs, each of said two nucleic acid probes, or each of said two antibodies are contained in separate containers.

26.   The kit or device of any one of claims 20-25, further comprising primers or probes capable of detecting the presence or absence of a polymorphic sequence in the complement factor H (CFH) gene, the HTRA1 gene, the complement factor B (BF) gene and/or the complement component 2 (C2) gene.

27.   The kit or device of claim 26 wherein the polymorphic sequence is selected from the group consisting of: rs529825; rs800292; rs3766404; rs1061147; rs1061170;

54

rs203674; rs2274700; rs375046; rs10490924; rs11200638; rs760336; rs763720; rs4151667; rs547154; and rs9332379.

28.   The kit or device of any one of claims 20-27, wherein:

(a)   the primer is 15 to 30 nucleotides in length; and/or

(b)   the primer, probe or antibody is immobilized on a substrate.

29.   The kit or device of any one of claims 20-28:

(a)   wherein the primer, probe or antibody is detectably labeled; or

(b)   further comprising a labeled molecule that reacts with the primer, probe or antibody.

30.   One of the following reagents:

(i)   a primer pair; or

(ii)   a nucleic acid probe; or

(iii)   an antibody

when used for assessing a human subject's propensity to develop a vascular disorder and/or age-related macular degeneration (AMD), by detecting the presence or absence in chromosome 1 of the subject of a deletion of a region between the 3' end of exon 22 of the complement factor H (CFH) gene and the 5' end of exon 1 of the complement Factor H-related 4 (CFHR4) gene in chromosome 1, wherein the deletion encompasses at least a portion of the complement Factor H-related 3 (CFHR3) gene and at least a portion of the Factor H-related 1 (CFHR1) gene, wherein the presence of the deletion indicates the subject is at increased risk of developing a vascular disorder and is at decreased risk of developing AMD.

31.   The reagent of claim 30, comprising a primer pair or a nucleic acid probe capable of detecting a deletion in the CFHR1 gene, the CFHR3 gene, or an intergenic region.

32.   The reagent of claim 30 or 31, comprising:

(i)   two primer pairs; or

(ii)   two nucleic acid probes; or

(iii)   two antibodies

capable of detecting the presence or absence in chromosome 1 of the subject of a deletion of a region between the 3' end of exon 22 of the complement factor H (CFH) gene and the 5' end of exon 1 of complement Factor H-related 4 (CFHR4) gene.

33.   The reagent of claim 32 wherein:

(a)   said two primer pairs comprise a first primer pair that amplifies a region of the CFHR1 gene and a second primer pair that amplifies a region of the CFHR3 gene; or

(b)   said two probes comprise a first probe that hybridizes to a sequence in the CFHR1 gene and a second probe that hybridizes to a sequence in the CFHR3 gene; or

(c)   said two antibodies comprises a first antibody specific for the protein product of the CFHR1 gene, and a second antibody specific for the protein product of the CFHR3 gene; or

(d)   said two primer pairs each amplifies a region of the CFHR1 gene or each amplifies a region in the CFHR3 gene.

34.   The reagent of any one of claims 30 to 33, wherein:

(a)   the primer is 15 to 30 nucleotides in length; and/or

20

(b)   the primer, probe or antibody is immobilized on a substrate.

35.   The reagent of any one of claims 30 to 34:

(a)   wherein the primer, probe or antibody is detectably labeled; or

56

(b)   further comprising a labeled molecule that reacts with the primer, probe or antibody.

36.   The reagent of any one of claims 30 to 35 further comprising primers, antibodies or probes capable of detecting the presence or absence of a polymorphic sequence in the complement factor H (CFH) gene, the HTRA1 gene, the complement factor B (BF) gene and/or the complement component 2 (C2) gene.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

5 / 15

FIG. 5

6 / 15

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H Factor AA seq     1 MRLLAKIICLMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIY    50
CFHR1 AA seq.       1                                                          0
CHFR3 AA seq.       1                                                          0


H Factor AA seq    51 KCRPGYRSLGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDTPFGTFTLTG   100
CFHR1 AA seq.       1                                                          0
CHFR3 AA seq.       1                                                          0


H Factor AA seq   101 GNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVT   150
CFHR1 AA seq.       1                                                          0
CHFR3 AA seq.       1                                                          0


H Factor AA seq   151 APENGKIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSK   200
CFHR1 AA seq.       1                                                 MWLL      4
CHFR3 AA seq.       1                                                 MLLL      4


H Factor AA seq   201 EKPKCVEISCKSPDVINGSPISQKIIYKENERFQYKCNMGYEYSERGDAV   250
CFHR1 AA seq.       5 VS-----------VILISRISS--------------------------    15
CHFR3 AA seq.       5 IN-----------VILTLWVSC--------------------------    15
                                      **   .*


H Factor AA seq   251 CTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKHRTGDEITYQCRNGFYP   300
CFHR1 AA seq.      16 ---------------------------------VGGEATF--------    22
CHFR3 AA seq.      16 ---------------------------------ANGQVKP--------    22
                                                              .  .


H Factor AA seq   301 ATRGNTAKCTSTGWIPAPRCTLKPCDYPDIKHGGLYHENMRRPYFPVAVG   350
CFHR1 AA seq.      23 -----------------------CDFPKINHGILYDEEKYKPFSQVPTG    48
CHFR3 AA seq.      23 -----------------------CDFPDIKHGGLFHENMRRPYFPVAVG    48
                                             **.* * ** *. *  .*. * *


H Factor AA seq   351 KYYSYYCDEHFETPSGSYWDHIHCTQDGWSPAVPCLRKCYFPYLENGYNQ   400
CFHR1 AA seq.      49 EVFYYSCEYNFVSPSKSFWTRITCTEEGWSPTPKCLRLCFFPFVENGHSE    98
CHFR3 AA seq.      49 KYYSYYCDEHFETPSGSYWDYIHCTQNGWSPAVPCLRKCYFPYLENGYNQ    98
                      . * *. .* .** *.* * ** . ****.  *** *.**..***  .


H Factor AA seq   401 NHGRKFVQGKSIDVACHPGYALPKA-QTTVTCMENGWSPTPRCIRVKTCS   449
CFHR1 AA seq.      99 SSGQTHLEGDTVQIICNTGYRLQNN-ENNISCVERGWSTPP---------   138
CHFR3 AA seq.      99 NYGRKFVQGNSTEVACHPGYGLPKVRQTTVTCTENGWSPTP---------   139
                      *.  ..*  .   . *. ** *     .....* * *** *


H Factor AA seq   450 KSSIDIENGFISESQYTYALKEKAKYQCKLGYVTADGETSGSIRCGKDGW   499
CFHR1 AA seq.     139 -------------------------------------------------   138
CHFR3 AA seq.     140 ------------------------------------------------   139


H Factor AA seq   500 SAQPTCIKSCDIPVFMNARTKNDFTWFKLNDTLDYECHDGYESNTGSTTG   549
CFHR1 AA seq.     139 -------------------------------------------------   138
CHFR3 AA seq.     140 ------------------------------------------------   139


H Factor AA seq   550 SIVCGYNGWSDLPICYERECELPKIDVHLVPDRKKDQYKVGEVLKFSCKP   599
CFHR1 AA seq.     139 -------------------------------------------------   138
CHFR3 AA seq.     140 ------------------------------------------------   139
```

FIG. 6

```
H Factor AA seq    600  GFTIVGPNSVQCYHFGLSPDLPICKEQVQSCGPPPELLNGNVKEKTKEEY  649
CFHR1 AA seq.      139  -------------------------------------------------  138
CHFR3 AA seq.      140  -------------------------------------------------  139


H Factor AA seq    650  GHSEVVEYYCNPRFLMKGPNKIQCVDGEWTTLPVCIVEESTCGDIPELEH  699
CFHR1 AA seq.      139  -------------------------------------------------  138
CHFR3 AA seq.      140  -------------------------------------------------  139


H Factor AA seq    700  GWAQLSSPPYYYGDSVEFNCSESFTMIGHRSITCIHGVWTQLPQCVAIDK  749
CFHR1 AA seq.      139  -------------------------------------------------  138
CHFR3 AA seq.      140  -------------------------------------------------  139


H Factor AA seq    750  LKKCKSSNLIILEEHLKNKKEFDHNSNIRYRCRGKEGWIHTVCINGRWDP  799
CFHR1 AA seq.      139  --KCRST------------------------------------------  143
CHFR3 AA seq.      140  --RCIR-------------------------------------------  143
                          .*


H Factor AA seq    800  EVNCSMAQIQLCPPPPQIPNSHNMTTTLNYRDGEKVSVLCQENYLIQEGE  849
CFHR1 AA seq.      144  -------------------------------------------------  143
CHFR3 AA seq.      144  -------------------------------------------------  143


H Factor AA seq    850  EITCKDGRWQSIPLCVEKIPCSQPPQIEHGTINSSRSSQESYAHGTKLSY  899
CFHR1 AA seq.      144  -------------------------------------------------  143
CHFR3 AA seq.      144  -------------------------------------------------  143


H Factor AA seq    900  TCEGGFRISEENETTCYMGKWSSPPQCEGLPCKSPPEISHGVVAHMSDSY  949
CFHR1 AA seq.      144  -------------------------------------------------  143
CHFR3 AA seq.      144  -------------------------------------------------  143


H Factor AA seq    950  QYGEEVTYKCFEGFGIDGPAIAKCLGEKWSHPPSCIKTDCLSLPSFENAI  999
CFHR1 AA seq.      144  -------------------------------------------------  143
CHFR3 AA seq.      144  -------------------------------------------------  143


H Factor AA seq   1000  PMGEKKDVYKAGEQVTYTCATYYKMDGASNVTCINSRWTGRPTCRDTSCV 1049
CFHR1 AA seq.      144  ----------------------------------------DTSCV  148
CHFR3 AA seq.      144  ----------------------------------------DRTCS  148
                                                                         *  .*


H Factor AA seq   1050  NPPTVQNAYIVSRQMSKYPSGERVRYQCRSPYEMFGDEE---VMCLNGNW 1096
CFHR1 AA seq.      149  NPPTVQNAYIVSRQMSKYPSGERVRYQCRSPYEMFGDEE---VMCLNGNW  195
CHFR3 AA seq.      149  KSDIEIENGFISESSSIYILNKEIQYKCKPGYATADGNSSGSITCLRNGW  198
                         .*     * *   .   ..*.*.   *         .  ** ..*


H Factor AA seq   1097  TEPPQCKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVEYQCQNLYQLEG 1146
CFHR1 AA seq.      196  TEPPQCKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVEYQCQNLYQLEG  245
CHFR3 AA seq.      199  SAQPICINSSEKCGPPPPISNGDTTSFLLKVYVPQSRVEYQCQSYYELQG  248
                         .  * *   *. *******  *** *** * ** * * ****** *.*.*


H Factor AA seq   1147  NKRITCRNGQWSEPPKCLHPCVISREIMENYNIALRWTAKQKLYSRTGES 1196
CFHR1 AA seq.      246  NKRITCRNGQWSEPPKCLHPCVISREIMENYNIALRWTAKQKLYLRTGES  295
CHFR3 AA seq.      249  SNYVTCSNGEWSAPPRCIHPCIITEENMNKNNIKLKGRSDRKYYAKTGDT  298
                         .** **.** **.*.***.*. *   **  *.  . .* *  .**..


H Factor AA seq   1197  VEFVCKRGYRLSSRSHTLRTTCWDGKLEYPTCAKR 1231
CFHR1 AA seq.      296  AEFVCKRGYRLSSRSHTLRTTCWDGKLEYPTCAKR  330
CHFR3 AA seq.      299  IEFMCKLGYNANTSILSFQAVCREGIVEYPRCE    331
                         **.** **   .   ... * .* .*** *
```

```
H Factor mRNA      1 ATGAGACTTCTAGCAAAGATTATTTGCCTTATGTTATGGGCTATTTGTGT   50
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA     51 AGCAGAAGATTGCAATGAACTTCCTCCAAGAAGAAATACAGAAATTCTGA  100
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA    101 CAGGTTCCTGGTCTGACCAAACATATCCAGAAGGCACCCAGGCTATCTAT  150
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA    151 AAATGCCGCCCTGGATATAGATCTCTTGGAAATGTAATAATGGTATGCAG  200
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA    201 GAAGGGAGAATGGGTTGCTCTTAATCCATTAAGGAAATGTCAGAAAAGGC  250
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA    251 CCTGTGGACATCCTGGAGATACTCCTTTTGGTACTTTTACCCTTACAGGA  300
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA    301 GGAAATGTGTTTGAATATGGTGTAAAAGCTGTGTATACATGTAATGAGGG  350
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA    351 GTATCAATTGCTAGGTGAGATTAATTACCGTGAATGTGACACAGATGGAT  400
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA    401 GGACCAATGATATTCCTATATGTGAAGTTGTGAAGTGTTTACCAGTGACA  450
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA    451 GCACCAGAGAATGGAAAAATTGTCAGTAGTGCAATGGAACCAGATCGGGA  500
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0


H Factor mRNA    501 ATACCATTTTGGACAAGCAGTACGGTTTGTATGTAACTCAGGCTACAAGA  550
CFHR1 mRNA    .    1                                                       0
CFHR3 mRNA         1                                                       0
```

FIG. 7

```
H Factor mRNA     551 TTGAAGGAGATGAAGAAATGCATTGTTCAGACGATGGTTTTTGGAGTAAA  600
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA     601 GAGAAACCAAAGTGTGTGGAAATTTCATGCAAATCCCCAGATGTTATAAA  650
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA     651 TGGATCTCCTATATCTCAGAAGATTATTTATAAGGAGAATGAACGATTTC  700
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA     701 AATATAAATGTAACATGGGTTATGAATACAGTGAAAGAGGAGATGCTGTA  750
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA     751 TGCACTGAATCTGGATGGCGTCCGTTGCCTTCATGTGAAGAAAAATCATG  800
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA     801 TGATAATCCTTATATTCCAAATGGTGACTACTCACCTTTAAGGATTAAAC  850
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA     851 ACAGAACTGGAGATGAAATCACGTACCAGTGTAGAAATGGTTTTTATCCT  900
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA     901 GCAACCCGGGGAAATACAGCCAAATGCACAAGTACTGGCTGGATACCTGC  950
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA     951 TCCGAGATGTACCTTGAAACCTTGTGATTATCCAGACATTAAACATGGAG 1000
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA    1001 GTCTATATCATGAGAATATGCGTAGACCATACTTTCCAGTAGCTGTAGGA 1050
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0


H Factor mRNA    1051 AAATATTACTCCTATTACTGTGATGAACATTTTGAGACTCCGTCAGGAAG 1100
CFHR1 mRNA      .   1                                                      0
CFHR3 mRNA          1                                                      0
```

```
H Factor mRNA   1101 TTACTGGGATCACATTCATTGCACACAAGATGGATGGTCGCCAGCAGTAC 1150
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1151 CATGCCTCAGAAAATGTTATTTTCCTTATTTGGAAAATGGATATAATCAA 1200
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1201 AATCATGGAAGAAAGTTTGTACAGGGTAAATCTATAGACGTTGCCTGCCA 1250
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1251 TCCTGGCTACGCTCTTCCAAAAGCGCAGACCACAGTTACATGTATGGAGA 1300
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1301 ATGGCTGGTCTCCTACTCCCAGATGCATCCGTGTCAAAACATGTTCCAAA 1350
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1351 TCAAGTATAGATATTGAGAATGGGTTTATTTCTGAATCTCAGTATACATA 1400
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1401 TGCCTTAAAAGAAAAAGCGAAATATCAATGCAAACTAGGATATGTAACAG 1450
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1451 CAGATGGTGAAACATCAGGATCAATTAGATGTGGGAAAGATGGATGGTCA 1500
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1501 GCTCAACCCACGTGCATTAAATCTTGTGATATCCCAGTATTTATGAATGC 1550
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1551 CAGAACTAAAAATGACTTCACATGGTTTAAGCTGAATGACACATTGGACT 1600
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0


H Factor mRNA   1601 ATGAATGCCATGATGGTTATGAAAGCAATACTGGAAGCACCACTGGTTCC 1650
CFHR1 mRNA       .   1                                                   0
CFHR3 mRNA           1                                                   0
```

```
H Factor mRNA    1651 ATAGTGTGTGGTTACAATGGTTGGTCTGATTTACCCATATGTTATGAAAG 1700
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    1701 AGAATGCGAACTTCCTAAAATAGATGTACACTTAGTTCCTGATCGCAAGA 1750
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    1751 AAGACCAGTATAAAGTTGGAGAGGTGTTGAAATTCTCCTGCAAACCAGGA 1800
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    1801 TTTACAATAGTTGGACCTAATTCCGTTCAGTGCTACCACTTTGGATTGTC 1850
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    1851 TCCTGACCTCCCAATATGTAAAGAGCAAGTACAATCATGTGGTCCACCTC 1900
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    1901 CTGAACTCCTCAATGGGAATGTTAAGGAAAAAACGAAAGAAGAATATGGA 1950
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    1951 CACAGTGAAGTGGTGGAATATTATTGCAATCCTAGATTTCTAATGAAGGG 2000
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    2001 ACCTAATAAAATTCAATGTGTTGATGGAGAGTGGACAACTTTACCAGTGT 2050
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    2051 GTATTGTGGAGGAGAGTACCTGTGGAGATATACCTGAACTTGAACATGGC 2100
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    2101 TGGGCCCAGCTTTCTTCCCCTCCTTATTACTATGGAGATTCAGTGGAATT 2150
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0


H Factor mRNA    2151 CAATTGCTCAGAATCATTTACAATGATTGGACACAGATCAATTACGTGTA 2200
CFHR1 mRNA     .    1                                                 0
CFHR3 mRNA          1                                                 0
```

```
H Factor mRNA   2201 TTCATGGAGTATGGACCCAACTTCCCCAGTGTGTGGCAATAGATAAACTT 2250
CFHR1 mRNA    .    1                                                   0
CFHR3 mRNA         1                                                   0


H Factor mRNA   2251 AAGAAGTGCAAATCATCAAATTTAATTATACTTGAGGAACATTTAAAAAA 2300
CFHR1 mRNA    .    1                                                   0
CFHR3 mRNA         1                                                   0


H Factor mRNA   2301 CAAGAAGGAATTCGATCATAATTCTAACATAAGGTACAGATGTAGAGGAA 2350
CFHR1 mRNA    .    1                                                   0
CFHR3 mRNA         1                                                   0


H Factor mRNA   2351 AAGAAGGATGGATACACACAGTCTGCATAAATGGAAGATGGGATCCAGAA 2400
CFHR1 mRNA    .    1                                                   0
CFHR3 mRNA         1                                                   0


H Factor mRNA   2401 GTGAACTGCTCAATGGCACAAATACAATTATGCCCACCTCCACCTCAGAT 2450
CFHR1 mRNA    .    1                                                   0
CFHR3 mRNA         1                                                   0


H Factor mRNA   2451 TCCCAATTCTCACAATATGACAACCACACTGAATTATCGGGATGGAGAAA 2500
CFHR1 mRNA    .    1                                                   0
CFHR3 mRNA         1                                                   0


H Factor mRNA   2501 AAGTATCTGTTCTTTGCCAAGAAAATTATCTAATTCAGGAAGGAGAAGAA 2550
CFHR1 mRNA    .    1                                                   0
CFHR3 mRNA         1                                                   0


H Factor mRNA   2551 ATTACATGCAAAGATGGAAGATGGCAGTCAATACCACTCTGTGTTGAAAA 2600
CFHR1 mRNA    .    1                                                   0
CFHR3 mRNA         1                                                   0


H Factor mRNA   2601 AATTCCATGTTCACAACCACCTCAGATAGAACACGGAACCATTAATTCAT 2650
CFHR1 mRNA    .    1                                                   0
CFHR3 mRNA         1                                                   0


H Factor mRNA   2651 CCAGGTCTTCACAAGAAAGTTATGCACATGGGACTAAATTGAGTTATACT 2700
CFHR1 mRNA    .    1              ATGTGGCTCCTGGTCAGTGTAATTCTAAT   29
CFHR3 mRNA         1              ATGTTGTTACTAATCAATGTCATTCTGAC   29
                                 ***     *     * * *     ** *


H Factor mRNA   2701 TGTGAGGGTGGTTTCAGGATATCTGAAGAAAATGAAACAACATGCTACAT 2750
CFHR1 mRNA    .   30 CTCACGGATATCCTCTG-----TTGGGGGAGAAGCAACATTTTGTGATTT   74
CFHR3 mRNA        30 CTTGTGGGTTTCCTGTG-----CTAATGGACAAGTGAAACCTTGTGATTT   74
                     ** *     *   *        *   * * * *  * *    ** *  *
```

```
H Factor mRNA   2751 GGGAAAATGGAGTTCTCCACCTCAGTGTGAAGGCCTTCCTTGTAAATCTC 2800
CFHR1 mRNA      .  75 TCCAAAAATAAACCATGGAATTCTATATGATGAAGAAAAATATAAGCCAT 124
CFHR3 mRNA         75 TCCAGACATTAAACATGGAGGTCTATTTCATGAGAATATGCGTAGACCAT 124
                        *  *      *      *    *  **  * * * *       **   *

H Factor mRNA   2801 CACCTGAGATTTCTCATGGTGTTGTAGCTCACATGTCAGACAGTTATCAG 2850
CFHR1 mRNA      . 125 TTTCCCAGGTTCCTACAGGGGAAGTTTTCTA------------TTACTCC 162
CFHR3 mRNA        125 ACTTTCCAGTAGCTGTAGGAAAATATTACTC------------CTATTAC 162
                        *  **  **                              **

H Factor mRNA   2851 TATGGAGAAGAAGTTACGTACAAATGTTTTGAAGGTTTTGGAATTGATGG 2900
CFHR1 mRNA      . 163 TGTGAATATAATTTTGTGT----CTCCTTCAAAATCATT-------TTGG 201
CFHR3 mRNA        163 TGTGATGAACATTTTGAGA----CTCCGTCAGGAAGTTA-------CTGG 201
                        * **   *   *  **  *       *   *        *     ***

H Factor mRNA   2901 GCCTGCAATTGCAAAATGCTTAGGAGAAAAATGGTCTCACCCTCCATCAT 2950
CFHR1 mRNA      . 202 ACTCGCA----TAACATGCACAGAAGAAGGATGGTCACCAACACCAAAGT 247
CFHR3 mRNA        202 GATTACA----TTCATTGCACACAAAATGGGTGGTCACCAGCAGTACCAT 247
                        **       ***  *  *  *    *****  *    *     *   *

H Factor mRNA   2951 GCATAAAAACAGATTGTCTCAGTTTACCTAGCTTTGAAAATGCCATACCC 3000
CFHR1 mRNA      . 248 GTCT-----CAGACTGTGTTTCTTTCCTTTTGTGGAAAATGGTCATTCTG 292
CFHR3 mRNA        248 GTCT-----CAGAAAATGTTATTTTCCTTATTTGGAAAATGGATATAATC 292
                        *  *     ****   *  *  *** *  *    *   *** *  **

H Factor mRNA   3001 ATGGGAGAGAAGAAGGATGTGTATAAGGCGGG--TGAGCAAGTGACTTAC 3048
CFHR1 mRNA      . 293 AATCTTCAGGACAAACACATCTGGAAGGTGATACTGTGCAAATTATTTGC 342
CFHR3 mRNA        293 AAAATTATGGAAGAAAGTTTGTACAGGGTAACTCTACAGAAGTTGCCTGC 342
                        *     *  *  *      *  *  * **      *    ** *     * *

H Factor mRNA   3049 A-CTTGTGCAACATATTACAAAATGGATGGAGCCAGTAATGTAACATGCA 3097
CFHR1 mRNA      . 343 AACACAGGATACAGACTTCAAAACAATGAGAAC----AACATTTCATGTG 388
CFHR3 mRNA        343 CATCCTGGCTACGGTCTTCCAAAAGTCCGTCAG-ACCACAGTTACATGTA 391
                        *  **    *  * ***      *    *        *    *  ****

H Factor mRNA   3098 TTAATAGCAGATGGACAGGAAGGCCAACATGCAGA------GACACCTCC 3141
CFHR1 mRNA      . 389 TAGAACGGGGCTGGTCCACCCCTCCCAAATGCAGGTCCACTGACACTTCC 438
CFHR3 mRNA        392 CGGAGAATGGCTGGTCTCCTACTCCCAGATGCATCC---GAGACAGAACA 438
                        *      *  ***  *       ** * *****      ****   *

H Factor mRNA   3142 TGTGTGAATCCGCCCACAGTACAAAATGCTTATATAGTGTCGAGACAGAT 3191
CFHR1 mRNA      . 439 TGTGTGAATCCGCCCACAGTACAAAATGCTTATATAGTGTCGAGACAGAT 488
CFHR3 mRNA        439 TGCTCAAAATCAGATATAGAAATTGAAATGGATTCATTTCTGAATCTTC 488
                        **    **  *    * **  *    *    *    *  * **    *

H Factor mRNA   3192 GAGTAAATATCCATCTGGTGAGAGAGTACGTTATCAATGTAGGAGCCCTT 3241
CFHR1 mRNA      . 489 GAGTAAATATCCATCTGGTGAGAGAGTACGTTATCAATGTAGGAGCCCTT 538
CFHR3 mRNA        489 CTCTATTTATATTTTAAATAAAGAAATACAATATAAATGTAAACCAGGAT 538
                        **  ***    *    * *   * *** *** ****** *         *

H Factor mRNA   3242 ATGAAATGTT---TGGGGAT------GAAGAAGTGATGTGTTTAAATGGA 3282
CFHR1 mRNA      . 539 ATGAAATGTT---TGGGGAT------GAAGAAGTGATGTGTTTAAATGGA 579
CFHR3 mRNA        539 ATGCAACAGCAGATGGAATTCTTCAGGATCAATTACATGTTTGCGAAAT 588
                        *** **      ***  **       * *   *  *   *****
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```
H Factor mRNA    3283 AACTGGACGGAACCACCTCAATGCAAAGATTCTACAGGAAAATGTGGGCC 3332
CFHR1 mRNA      . 580 AACTGGACGGAACCACCTCAATGCAAAGATTCTACGGGAAAATGTGGGCC  629
CFHR3 mRNA        589 GGATGGTCAGCACAACCAATTTGCATTAATTCTTCAGAAAAGTGTGGACC  638
                      *** * * ** ***    ****   ***** * * *** ***** **


H Factor mRNA    3333 CCCTCCACCTATTGACAATGGGGACATTACTTCATTCCCGTTGTCAGTAT 3382
CFHR1 mRNA      . 630 CCCTCCACCTATTGACAATGGGGACATTACTTCATTCCCGTTGTCAGTAT  679
CFHR3 mRNA        639 TCCTCCACCTATTAGCAATGGTGATACCACCTCCTTTCTACTAAAAGTGT  688
                      *********** ****** ** *  ** ** ** *    *   *** *


H Factor mRNA    3383 ATGCTCCAGCTTCATCAGTTGAGTACCAATGCCAGAACTTGTATCAACTT 3432
CFHR1 mRNA      . 680 ATGCTCCAGCTTCATCAGTTGAGTACCAATGCCAGAACTTGTATCAACTT  729
CFHR3 mRNA        689 ATGTGCCACAGTCAAGAGTCGAGTACCAATGCCAGTCCTACTATGAACTT  738
                      *** ***    ***   *** ** ************** ** *** *****


H Factor mRNA    3433 GAGGGTAACAAGCGAATAACATGTAGAAATGGACAATGGTCAGAACCACC 3482
CFHR1 mRNA      . 730 GAGGGTAACAAGCGAATAACATGTAGAAATGGACAATGGTCAGAACCACC  779
CFHR3 mRNA        739 CAGGGTTCTAATTATGTAACATGTAGTAATGGAGAGTGGTCGGCACCACC  788
                      *****   **   ********** ****** * ***** * ******


H Factor mRNA    3483 AAAATGCTTACATCCGTGTGTAATATCCCGAGAAATTATGGAAAATTATA 3532
CFHR1 mRNA      . 780 AAAATGCTTACATCCGTGTGTAATATCCCGAGAAATTATGGAAAATTATA  829
CFHR3 mRNA        789 TAGATGCATACATCCATGTATAATAACTGAAGAAAACATGAATAAAAATA  838
                      * **** ******* *** ***** *    *****   *** * **   ***


H Factor mRNA    3533 ACATAGCATTAAGGTGGACAGCCAAACAGAAGCTTTATTCGAGAACAGGT 3582
CFHR1 mRNA      . 830 ACATAGCATTAAGGTGGACAGCCAAACAGAAGCTTTATTTGAGAACAGGT  879
CFHR3 mRNA        839 ACATAAAGTTAAAAGGAAGAAGTGACAGAAAATATTATGCAAAAACAGGG  888
                      *****   ****   * * *    *    **   ****    * ******


H Factor mRNA    3583 GAATCAGTTGAATTTGTGTGTAAACGGGGATATCGTCTTTCATCACGTTC 3632
CFHR1 mRNA      . 880 GAATCAGCTGAATTTGTGTGTAAACGGGGATATCGTCTTTCATCACGTTC  929
CFHR3 mRNA        889 GATACCATTGAATTTATGTGTAAATTGGGATATAATGCAAATACATCAAT  938
                      ** *   ******* ******** *******  *       **


H Factor mRNA    3633 TCACACATTGCGAACAACATGTTGGGATGGGAAACTGGAGTATCCAACTT 3682
CFHR1 mRNA      . 930 TCACACATTGCGAACAACATGTTGGGATGGGAAACTGGAGTATCCAACTT  979
CFHR3 mRNA        939 TCTATCATTTCAAGCAGTGTGTCGGGAAGGGATAGTGGAATACCCCAGAT  988
                      **   **** * * **   *** **** **** * **** ** ** *  *


H Factor mRNA    3683 GTGCAAAAAGATAGAATCAAT-CATAAAGTGCACACCTTTATTCAGAACT 3731
CFHR1 mRNA      . 980 GTGCAAAAAGATAGAATCAAT-CATAAAATGCACACCTTTATTCAGAACT 1028
CFHR3 mRNA        989 GCG-AATAAGGCAGCATTGTTACCCTAAATGTATGTCCAACTTCCACTTT 1037
                      * * ** *** ** **   * *   ** ** *   *    ***     *


H Factor mRNA    3732 TTAGTATTAAATCAGTTCTCAATTTCATTTTTTATGTATTGTTTTACTCC 3781
CFHR1 mRNA      . 1029 TTAGTATTAAATCAGTTCTTAATTTAATTTTTAA-GTATTGTTTTACTCC 1077
CFHR3 mRNA        1038 TCCACTTCTCACTCTTATGGTCTCAAAGCTTGCAAAGATAGCTTCTGATA 1087
                       *     *    *     *     *   *  ** *    ** *  **


H Factor mRNA    3782 TTTTTATTCATACGTAAAATTTTGGATTAATTTGTGAAAATGTAATTATA 3831
CFHR1 mRNA      . 1078 TTTTTATTCATACGTAAAATTTTGGATTAATTTGTGAAAATGTAATTATA 1127
CFHR3 mRNA        1088 TTGTTGTA-ATTTCTACTTTATTTCAAAGAAAATTAATATAATAGTTTCA 1136
                       ** **  * **   ** **   * **  *      * * *   ** ** *


H Factor mRNA    3832 AGCTGAGACCGGTGGCTCTCTT                             3853
CFHR1 mRNA      . 1128 AGCTGAGACCGGTGGCTCTCTTCTTAAAAGCACCATATTAAAACTTGGAA 1177
```

```
CFHR3 mRNA        1137 ATTTGCAAC---TTAATATATTCTCAAAAATATATTAAAACAAACTAAAT 1183
                       *  **  **    *   * * **

H Factor mRNA     3854                                                 3853
CFHR1 mRNA      . 1178 AACTGGAAAACT                                   1189
CFHR3 mRNA        1184 TATTGCTTATGCTTGTACTAAAATAATAAAAACTACTCTTATAAAAAAAA 1233


H Factor mRNA     3854              3853
CFHL1 mRNA      . 1190              1189
CFHL3 mRNA        1234 AAAAAAAAAAAAA 1246
```