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

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C12Q 1/68 (2006.01)
G01N 33/53 (2006.01)

U.S. Cl. 435/7.92; 435/6

Field of Classification Search
None

References Cited
U.S. PATENT DOCUMENTS
5,474,796 A 12/1995 Brennan
2009/0312394 A1* 12/2009 Hughes ................. 514/44 A

FOREIGN PATENT DOCUMENTS

Other Publications

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.

30 Claims, 16 Drawing Sheets
OTHER PUBLICATIONS


* cited by examiner
FIG. 2
FIG. 3
| H Factor AA seq | 1  | MRLLAKIICLMLWAICVASDCNLELPFRRNTEILTGSWSDQTYFEGTOAIY | 50 |
| CFHR1 AA seq. | 1  | 0 |
| CFHR3 AA seq. | 1  | 0 |
| H Factor AA seq | 51 | XCRPGYRLGNVIMAVRKGDEYNVPALRKCQKRCGHPGDTFPFTTLTG | 100 | 0 |
| CFHR1 AA seq. | 1  | 0 |
| CFHR3 AA seq. | 1  | 0 |
| H Factor AA seq | 101 | CNYFEYVGKAVYCNEGQYLLGEINYRECIDGWNTDIPICEVVKCLPT | 150 |
| CFHR1 AA seq. | 1  | 0 |
| CFHR3 AA seq. | 1  | 0 |
| H Factor AA seq | 151 | APENKIVVSAEPDREYHFQAVRFVCSNGYKIEGDEEMHSDDGFWSK | 200 | MWLL 4 |
| CFHR1 AA seq. | 1  | MWLL 4 |
| CFHR3 AA seq. | 1  | MWLL 4 |
| H Factor AA seq | 201 | EKPKVREISCXSDPVINGSPISQIKYKNERFQYKNNMGYEYSEGDAV | 250 |
| CFHR1 AA seq. | 5  | VS-----------VILISRISS----------------- | 15 |
| CFHR3 AA seq. | 5  | IN-----------VILTLWVC-------------- | 15 |
| *                   | **                   | 4                  |
| H Factor AA seq | 251 | CTEGSWRPLSCHEKSCDPEIFGNYSDLKRIHKTQGDEITYQCRNGFYP | 300 |
| CFHR1 AA seq. | 16 | -VQGSAIF- | 22 |
| CFHR3 AA seq. | 16 | -ANGQVVKP- | 22 |
| H Factor AA seq | 301 | ATPNKAKCTCTGSIAPRCTLKCEYPDILKHGGHLHENRPPYPYPAVG | 350 |
| CFHR1 AA seq. | 23 | ---------------------------CFDPKINHGILYASEKYKDPSQYPT3 | 48 |
| CFHR3 AA seq. | 23 | CFDPKINHGILFHENMPYFPYPAVG | 48 |
| *                   | *                   | **                  |
| H Factor AA seq | 351 | KYSVYCFDEHFTPSGSYDHIHCTQGDWSPAVPCLRKCYFPYLENGYNQ | 400 |
| CFHR1 AA seq. | 49 | EVFYYCSEYFVPSKPFWRTITCCTEENGSPTPKLRCFLCPPEVENGHSE | 98 |
| CFHR3 AA seq. | 49 | KLYSYYCFDEHFTFSGSYYDHYHTQGDWSPAVPCLRKCYFPYLENGYNQ | 98 |
| *                   | *                   | **                  |
| H Factor AA seq | 401 | NHGRKFVPQKSIDVACHPGYALPKA-QTTTTCMENGSPTPRCIRVKTCS | 449 |
| CFHR1 AA seq. | 99 | SSGQTHLEGITVQIDIQNYRIQNN-ENNISCOVERGMSSTPF------ | 138 |
| CFHR3 AA seq. | 99 | NGYRKFDVQKSTWEVACHPGYLPVQTIVCTIENGWSTPF | 139 |
| *                   | *                   | **                  |
| H Factor AA seq | 450 | KSSIDENGIFSISESCQTVYALKKEKAKYQQCLGYVTADFTSGSIRCSDGW | 499 |
| CFHR1 AA seq. | 139 | - | 138 |
| CFHR3 AA seq. | 140 | - | 139 |
| H Factor AA seq | 500 | SAQPTCKSDFIPVFMNARTKNDFTWFKLNDLTDYECDDGYR8NTGSTG | 549 |
| CFHR1 AA seq. | 139 | - | 138 |
| CFHR3 AA seq. | 140 | - | 139 |
| H Factor AA seq | 550 | STVCQGYNWSDLDPICYERECELPKIDVHLVPDKKKDKYQKGEVJKFSCKP | 599 |
FIG. 6 (cont.)
H Factor AA seq. 1097 TEPPQCKDSTGCGPPPIDDNIDITSEFPLSVYAPASSVEYQCNLYQLEG 1146
CFHR1 AA seq. 196 TEPPQCKDSTGCGPPPPDDNNDDNTSIFPLSVYAPASSVEYQCNLYQLEG 245
CHFR3 AA seq. 199 SAQPICNSEEKCQPPIPISNQDTTSFLLKVVYPQYVEYQCSYEEQ 248

H Factor AA seq. 1147 NKRTCRNGQWSEPCKLHPCVISREIMENYIALRWTAKQKLYSRTGES 1196
CFHR1 AA seq. 246 NKRTCRNGQWSEPCKLHPCVISREIMENYIALRWTAKKLYLRTGES 295
CHFR3 AA seq. 249 SNYVTC8NGEWSADPRCIHDLITEENMNKNKIKLANKRSDRKYAykQDT 298

H Factor AA seq. 1197 VEVVCKRGRYSRSRSHLTRTTCWDGKLEYPTCAKR 1231
CFHR1 AA seq. 296 AEFVCKRGRYSRSRSHLTRTTCWDGKLEYPTCAKR 330
CHFR3 AA seq. 299 IEFMCGLYMNANTSSILSFQAVCREGIVEYPRCE 331

FIG. 6 (cont.)
| H Factor mRNA | 1 ATGAGACITCTGAGAAATTATTTGCTATGTATTGTGGCTATTTGTGT | 50 |
| CFHR1 mRNA | 1 | 0 |
| CFHR3 mRNA | 1 | 0 |

| H Factor mRNA | 51 ACCAGAAGATTGCAATGAACTTTCCTCCAAGAAGAATAAGCGAGAAATTCG | 100 |
| CFHR1 mRNA | 1 | 0 |
| CFHR3 mRNA | 1 | 0 |

| H Factor mRNA | 101 CAGGTCTCTGATGACAAACATATCCAGAAAGGCACCAGGCTATCTAT | 150 |
| CFHR1 mRNA | 1 | 0 |
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| H Factor mRNA | 151 ATTGGCGAGCTGGATATGATCTTCTTGGAATGTAATGTGGTGGCAG | 200 |
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| H Factor mRNA | 201 GAAGGGAGATGCTGGCTCTTACTACCATTAGAAATGTCTGAGAAAAGC | 250 |
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| H Factor mRNA | 251 CCTGTGGCAGACATCCTGGGAGATGTTTACTTTTTACTTTTTACACAGGA | 300 |
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| CFHR3 mRNA | 1 | 0 |

| H Factor mRNA | 401 GCACAATGCTACTTTTACCCTTGCATGGAAGTCTGTGAAGCTTTACCAGTGACA | 450 |
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| CFHR3 mRNA | 1 | 0 |

| H Factor mRNA | 451 CGCCAGACATGAAAAATTGGTACTAGTCTCAATGGAAACCAGATCGGAG | 500 |
| CFHR1 mRNA | 1 | 0 |
| CFHR3 mRNA | 1 | 0 |

| H Factor mRNA | 501 ATACCAATTGGCAGCAAGCATCGGTGGATCTGACAGGTAGCCTACAAGA | 550 |
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**FIG. 7**
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<td>CPHR3 mRNA</td>
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</table>

**FIG. 7 (cont.)**
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FIG. 7 (cont.)
METHODS AND REAGENTS FOR TREATMENT AND DIAGNOSIS OF VASCULAR DISORDERS AND AGE-RELATED MACULAR DEGENERATION

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a Continuation of PCT/US07/73514 filed Jul. 13, 2007, which claims priority to U.S. Provisional Patent Application No. 60/840,073, filed Aug. 23, 2006, and to U.S. Provisional Patent Application No. 60/831,018, filed Jul. 13, 2006. All these applications are hereby incorporated by reference.

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

This invention was made with government support under NIH R01 EY111515 and R24 EY017404, awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

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

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 CR-2-like protein (CRP), (2) binding to C3b, (3) binding to heparin, (4) binding to sialic acid, (5) binding to endotelial cell surfaces, (6) binding to cellular integrin receptors (7) binding to pathogens, including microbes (see FIG. 3 of U.S. patent publication No. 20070020647), and (8) C3b co-factor activity. The Factor H gene, known as H1, CFH and H3, 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 R1, 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 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

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 CFHHT). 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 FHL1 and FHL1 are identical, with FHL1 having four unique C-terminal amino acids (encoded by alternative exon 10A, which is included 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 FHL1, 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

The FHR-1 gene is also known as CFHR1, CFHL1, CFHL, FHR1 and FHL1. The FHR-1 cDNA encodes a polypeptide 330 amino acids in length having an predicted molecular weight of 39 kDa (see Estall 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

The FHR-2 gene is also known as CFHR2, CFHL2, FHR2 and FHL3. 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. U.S.A. 99:16899-16903). cDNA and amino acid sequence data for 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

The FHR-3 gene is also known as CFHR3, CFHL3, FHR3 and FHL4. 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. U.S.A. 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

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

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 AF295532, and the polypeptide sequence has GenBank accession number AAIX5619. 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**

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 5' end of exon 22 of the complement factor H-related 3 (CFHR3) 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.

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

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.

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 CFHR3 gene are deleted.

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.

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).

In one embodiment, the presence or absence of the deletion is detected by assaying for a gene product encoded in chromosome 1 between the 5' 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.

In one embodiment the presence or absence of the deletion is detected by assaying for a truncated CFHR1 or CFHR3 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.

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.

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

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.

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, cerebrospinal 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.

Proteins (amount or presence) may be detected, for example, using an immunoasay such as a sandwich immunoasay, a competitive immunoasay, a radioimmunoasay, fluorophore-labelled immunoasay, 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.

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.

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.

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.

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.

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.

In another aspect, the invention provides a method for treating a subject having (i.e., exhibiting symptoms of), or 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.

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

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 expresses 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.

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 plasmapheresis or antibody-based inhibition, for example, using an anti-CFHR1 antibody and/or an anti-CFHR3 antibody.

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.

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.

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.

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.

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.

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**

**FIG. 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-I-like 1 (CFHIL1) 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 (CFHIL1) 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 SCR's are similar to SCRs 12-14 of Factor H and have C3b and heparin binding properties.

FIG. 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.

FIG. 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 also cross-reacts with CFHR1 and CFHR2. No CFHR1 is detected in the serum of two patients (1973-02 and 325-02) that have a homozygous deletion of the CFHR3 and CFHR1 genes, as determined by SSCP analysis and direct DNA sequencing.

FIG. 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, 3 and 4 correspond to homozygous non-deletion or heterozygous deletion of CFHR3 and CFHR1, and pattern 4 corresponds to homozygous deletion of CFHR3 and CFHR1.

FIG. 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 FIG. 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.

FIG. 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.

FIG. 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

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.

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.

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.


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.

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 ×(number of G's + C's in the primer) + 2°C ×(number of A's and T's in the primer) +0°C. A reaction is carried out in the presence of 50 mM monovalent cations. For longer oligos, the following formula can be used: Tm=64.9°C +41°C ×(number of G's and C's in the primer) -16.4°C/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 +4.6°C ×log [10(natrons+)](K+)+0.41°C ×(%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.

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 homoygous 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).

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. An 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.

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.

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. 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.

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.

"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.

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).

The term "diagnosis" 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). 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, determining in advance the progress of the disorder as well as the assessment of efficacy of therapy options to treat a disorder.

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 polypep-
tide). 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.

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.

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.

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

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 (420H; exon 9); rs203674 (intron 10) and the polymorphism at residue 1210 (1210C; exon 22).

Polymorphisms particularly associated with decreased risk include the protective I2 haplotype, which includes a variant allele in IVS6 (intron 6, rs3766404) and the I4 haplotype, which includes a variant allele in IVS1 (intron 1, rs529825) and a variant allele (162) (exon 2, rs800292).

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 discussed in Example 1, below, the deletion genotype is predominantly associated with the CFH I4 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 I4 haplotype is protective.

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

Based on the discoveries described herein, a subject’s risk for AMD or vascular disease can be assessed by determining whether or not 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, 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.

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 FIG. 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., absence of absence of CFHR1 and/or CFHL3 mRNAs), or by absence 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 absence of DNA or RNA sequences can be determined using art known methods, such as PCR. The absence of a nucleic acid sequence is deduced from the absence of an amplified PCR product in an assay of a tissue sample (see FIG. 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.”

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 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.

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 containing a deletion. Methods known in the art can be used to distinguish homozygotes from heterozygotes (see, e.g., Example 1).

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

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

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. 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, Calif.). 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.

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, Kitadai 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,” Immunochemistry 1047:2095-2101) 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 deleterional determinants of α-thalassemia,” Blood 95 (1):360-362).

Polymorphisms (e.g., deletions) can also be detected using allele-specific probes; use of allele-specific primers; direct sequence analysis; denaturing gradient gel electrophoresis (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., Piatak 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. Pat. 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.

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.

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. 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.

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 allele 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.

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).

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.

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 (Truem and Noyer-Weidler, 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.

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).

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 CFIHR1-specific primer pairs are used, for instance, ("CFHL1ex6.F") [5'-AGTCGGTTTGGCAGATG-3' (SEQ ID NO: 7)] & "CFHL1ex6.R" [5'-GCACAGTTGGGATACTCC-3' (SEQ ID NO: 8)]; and/or "CFHL1ex6.F2" [5'-CATATGGTGCTTGGCAGATG-3' (SEQ ID NO: 9)] & "CFHL1ex6.R2" [5'-GCACAGTTGGGATACTCC-3' (SEQ ID NO: 10)]. In another embodiment, CFIHR3-specific primer pairs are used, for instance, ("CFHL3ex3.F") [5'-CAATTCGG-TAATGCCTCTGG-4' (SEQ ID NO: 11)]; and/or "CFHL3ex3.R" [5'-CTGGACAGACTGCTGGCC-3' (SEQ ID NO: 12)]; and/or "CFHL3ex3seq.F" [5'-TGTGAGTTATTATGCCG-3' (SEQ ID NO: 13)]; and/or "CFHL3ex3seq.R" [5'-AAAAGATCCGT-TGGC-3' (SEQ ID NO: 13)]. Absence of the correct-sized PCR product indicates the CFHL1 and/or CFHL3 gene(s) are deleted.

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 (MRRD_3855, MRRD_3856, MRRD_3857, rs385390, rs389897) in the region of these two genes are identified. All samples assigned a copy number of 0 (designated CNO) 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.


3.2 Analysis of Protein Samples

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, radioimmunossay (RIA), enzyme-linked immunosor- bent assays (ELISAs), immunofluorescent assays, Western blotting and others.

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. ANTIQUE: 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 N.Y. 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 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

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 U.S. patent application Ser. Nos. 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).

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 int2); 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 one of 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.

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 may be detected include, but are not limited to, at least one of rs10450924, 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 A or G 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 G or C at rs332379 of the C2 gene, or 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.

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, fibrinogen-1, 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 1q binding protein/hyaluronidase binding protein (“complement 1q component”), amyloid A (α1 amyloid A), amyloid P component, C5 and C8b-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, thrombomodulin, elastin, collagen, ICAM-1, LFA1, LFA3, β7, IL-1, IL-6, IL-12, TNF-alpha, GM-CSF, heat shock proteins, colony stimulating factors (G-CSF, M-CSF), and IL-10.

4. Therapeutic Methods

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.

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

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.

CFHR1 and CFHR3 polypeptides or biologically active fragments or variants thereof may be isolated from blood (serum or plasma) or produced using conventional recombi-
(i.e., preservatives, diluents, adjuvants, and the like) must meet standards of purity, quality, and not be deleterious to the patient.

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; ionic 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. B. 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 biodegradable 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 intracameral 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, Tex., USA). In a related aspect, the invention provides a sterile container, e.g., vial, containing a pharmaceutically acceptable CFHR1 or CFHR3 polypeptides, optionally as a lyophilized preparation.

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 500 micrograms/ml. The amount of CFHR1 or CFHR3 to be administered to an individual (e.g., 160 pound subject) can be, for example and not limited to, 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 500 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 limited to, 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.

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

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.

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, reoviruses, and papillomaviruses. Usually the viruses have been attenuated to no longer replicate (see, e.g., Kay et al. 2001, Nature Medicine 7:33-40).

The nucleic acid encoding the polypeptide is typically linked to regulatory elements, such as a promoters and 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.

Villous will be understood by those of skill in the art, gene therapy vectors contain the necessary elements for 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 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; Isumi 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).

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 oxidative stress. Hum Gene Ther. 15:960-7; and references cited therein).

Gene therapy vectors must be produced in compliance with the Good Manufacturing Practice (GMP) requirements rendering the product suitable for administration to patients.
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.

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 intravitreal 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-vecored 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 phototoxic 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-vecored gene therapy” *Novartis Found Symp.* 255:179-188, and references cited therein).

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.

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., Bejiani 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 using a vector.

4.1.3. Cell Therapy Methods

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., Okoshi 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; Serrallata 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 Gastroenterol. 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.

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 (9) therapy of hemolytic-uremic syndrome with factor H abnormality” *Pediatr Nephrol.* 18:952-5.

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 intracocular 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 intracocular implants” *Proc Natl Acad Sci USA* 103(10):3896-901). Also see et al., 2003, “Photoreceptor protection by cardiotoxin-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,* 43:4032-3298; and Hamman 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 NCT-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

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 CFHRI or/and CFHRII genes. Any method of reducing levels of CFHRI or CFHRII in the eye or systemically may be used for treatment including, for example, inhibiting transcription of a CFHRI or CFHRII gene, inhibiting translation of CFHRI or CFHRIIRNA, decreasing the amount or activity of CFHRI or CFHRII proteins (e.g., by plasmapheresis, antibody-directed plasmapheresis, or complexing with a CFHRI or CFHRII binding moiety (e.g., heparin or antibody), or by administration of inhibitory nucleic acids. In some embodiments levels of CFHRI or CFHRII 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


It is 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

In one aspect, an anti-CFHRI or anti-CFHRII binding agents (e.g., antibodies) that reduce the activity or amount of the proteins is administrated 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).

In one embodiment, an anti-CFHRI antibody specifically binds an epitope of CFHRI, in particular human CFHRI. In certain embodiments, an anti-CFHRI antibody specifically binds an epitope located within the amino-terminus of a CFHRI polypeptide. In particular, an anti-CFHRI antibody specifically binds an epitope located between amino acids 1-143 of SEQ ID NO: 4 as shown in FIG. It in other embodiments, an anti-CFHRI antibody specifically binds an epitope within the CFHRI short consensus repeats (SCRs) 6 and/or 7 as shown in FIG. 1. The amino acid sequence of CFHRI SCR6 is 35% homologous to the corresponding CFH SCR, and the amino acid sequence of CFHRI SCR7 is 45% homologous to the corresponding CFH SCR. Anti-CFHRI antibodies of the invention specifically bind CFHRI 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 CFHRI protein is within the skill of the art to determine epitopes that are most immunogenic for the generation of anti-CFHRI antibodies.

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

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 ScFv molecules, described, for example, in U.S. Pat. No. 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 CFHRI, CFHRII, and/or another antigen. Methods for making bispecific antibodies are known in the art and include, for example, by fusing hybridomas or by linking Fab' fragments. See, e.g., Sengsivilai et al. (1990) Clin Exp Immunol. 79: 315-325; Kostely et al. (1992) J. Immunol. 148: 1547-1553.


It is understood that the CDRs of the antibodies described herein confer the binding specificity to CFHRI or CFHRII.
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.

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.

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. Pat. Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and 4,816,567 (Cabilly).

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-CFHR3 antibody or an anti-CFHR1 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. Pat. Nos. 7,022,590 (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; Verhooyen et al. (1988) Science 239:1534-1536; and Winter (1998) FEBS LETT 430:92-94.

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. Lympthocytes from the mice are then 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

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.

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 in 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 in 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

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.
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 least one of the following polymorphic sites: rs529825; rs800292; intron 2 (IVS2 or ins1T); rs3766404; rs1061147; rs1061170; exon 10A; rs203674; rs375046; and optionally including exon 22 (R1210C). In one embodiment the array includes primers or probes to determine the allele at least one of the following polymorphic sites: rs529825; rs800292; (d) rs800292; (g) intron 2 (IVS2 or ins1T); (e) rs3766404; (f) rs1061147; (g) rs3753396; (h) rs2274700; (i) rs3766404; (j) rs3753396; (k) rs1065489; and optionally including exon 22 (R1210C). In one embodiment, the array includes primers or probes to determine the allele at least one of the following polymorphic sites: rs800292 (162V); IVS2 (-1ins1T); rs1061170 (Y402H); and rs2274700 (A473A). In one embodiment, the array includes primers or probes to determine the allele at least one of the following polymorphic sites: rs4927661 (−249T>C); rs9427662 (−201T>C); and rs12097550 (P46S).

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 allele at rs203674. In one embodiment the primers or probes distinguish alleles at exon 22 (R210C). 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 and rs800292, at rs3766404, 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 (a) one or more of rs529825; rs800292; rs3766404; rs1061147; rs1061170; and rs203674; (b) any one of more of intron 2 (IVS2 or ins1T); 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 one of rs529825, rs800292, rs3766404, rs1061170, and rs203674; (g) exon 22 (R1210C); (h) exon 22 (R1210C) and any of (a)-(g); or (i) any one or...
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 CHF5 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. 20070020640, 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, Calif.), Proteogene (Menlo Park, Calif.), Genometrix (The Woodland, Tex.), Motorola BioChip Systems (Northbrook, Ill.), and Perlegen Sciences (Mountain View, Calif.).

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 Anti-aptamers”.

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 labeled (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.
The kits may contain an instruction manual with instructions on 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.

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.

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.

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

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

### EXAMPLES

Example 1

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>
<thead>
<tr>
<th>Primers Used for Detecting the CFH and CFHR1-5 Genes</th>
<th>Forward 5'-3'</th>
<th>Reverse 5'-3'</th>
<th>Product Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. PCR Primers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFH ex22</td>
<td>GCTTTGGATAGTGTCACCOTAGTTTTCG</td>
<td>TTTUAG</td>
<td>AGG</td>
</tr>
<tr>
<td>CFHR1 ex6</td>
<td>AGTCGTTTGGACACGCACAGTTTGGATA</td>
<td>GTG</td>
<td>CTCC</td>
</tr>
<tr>
<td>CFHR2 ex4</td>
<td>TGTGTTTCATTGACTATAG</td>
<td>GAG</td>
<td>GSC</td>
</tr>
<tr>
<td>CFHR3 ex3</td>
<td>TCATTGCTATAGTCCCTCTGAGACTCTGCT</td>
<td>TTAGG</td>
<td>CGG</td>
</tr>
<tr>
<td>CFHR4 ex3</td>
<td>CTACAATGCACTCGGCAAGCCATTGGCTGAG</td>
<td>TTTAGG</td>
<td>AGGAC</td>
</tr>
<tr>
<td>CFHR5 ex2</td>
<td>AACCCCTTTTTTCCACTACCTCCTCTTAAG</td>
<td>TTACCA</td>
<td>(SEQ ID NO: 20)</td>
</tr>
<tr>
<td><strong>B. SSCP Primers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFH ex22</td>
<td>GCTTTGGATAGTGTCACCOTAGTTTTCG</td>
<td>TTTUAG</td>
<td>GTG</td>
</tr>
<tr>
<td>CFHR3 ex3</td>
<td>TCATTGCTATAGTCCCTCTGAGACTCTGCT</td>
<td>TTAGG</td>
<td>CGG</td>
</tr>
<tr>
<td><strong>C. Sequencing Primers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFH ex22</td>
<td>GCTTTGGATAGTGTCACCOTAGTTTTCG</td>
<td>TTTUAG</td>
<td>AGG</td>
</tr>
<tr>
<td>CFHR3 ex3 seq</td>
<td>TTTGAGATGTTTTATTAATAGCTCC</td>
<td>GCG</td>
<td>GC</td>
</tr>
<tr>
<td>CFHR1 ex6</td>
<td>AGTCGTTTGGACACGCACAGTTTGGATA</td>
<td>GTG</td>
<td>CTCC</td>
</tr>
<tr>
<td>Forward 5'−3'</td>
<td>Reverse 5'−3'</td>
<td>Product</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td><strong>CFH (ex22)</strong></td>
<td>GGTGTTGAATAGTG TTTGOG</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>CFH (ex22)</strong></td>
<td>GGTGTTGAATAGTG ACCUTGATTTCC</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>IVS 5' to CFHR3</strong></td>
<td>CAGCCTATGTGAAA AAGGAAATCCGCTC</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>IVD 5' to CFHR3</strong></td>
<td>GGGGCCACCATGCTG GCACACACACAAAGTAA</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>IVD 5' to CFHR3</strong></td>
<td>ATTCTGCGAATCTC TCAAACGACAAAGAG</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR3 (ex2)</strong></td>
<td>TGCTGATACCATAC ATTTTTATATTT</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR3 (ex3)</strong></td>
<td>TTGGATATGTTAT AAATGATCCTG</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR3 (ex3)</strong></td>
<td>TCATTGCACTTC TCTGAGACTGCTGT</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR3 (ex3)</strong></td>
<td>TAAAGCTGCTTTTT GTGATTATTATTT</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR1 (ex2)</strong></td>
<td>AGCGCGTTGGTACA GCACCAGTGTGCTA</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR1 (ex6)</strong></td>
<td>CATATGCGGTTGG GCACAGTTGCTA</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR2</strong></td>
<td>TCTTTTCTATTC AGTTATATGACACCA</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR2</strong></td>
<td>CTACCACTAACCT TCLATTTTACATT</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR2</strong></td>
<td>ACATGTTTATGG ACCAGGAGGACCT</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR2</strong></td>
<td>TGGCTGACTGCTG GTAGCAGTCTG</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR4</strong></td>
<td>GCTTATGGAATATTG AACACGTCGAAGA</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR4</strong></td>
<td>TAAATGTTGAAAGA ACTTATGATATGAC</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>CFHR4</strong></td>
<td>CTACAATGGGACCTT TCCACACTTATGG</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>
In a study directed toward further characterization of CFH and its associated haplotypes on chromosome 1q, a complete deletion of the entire CFHIL1 and CFHIL3 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 CFHRI. 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 been determined, but the mapping of the boundaries is within the skill of the art.

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>
<thead>
<tr>
<th>Genotype*</th>
<th>Count</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>++/+Δ, +/+Δ</td>
<td>1046</td>
<td>97.3%</td>
</tr>
<tr>
<td>Δ/Δ</td>
<td>28</td>
<td>2.7%</td>
</tr>
<tr>
<td>++/+, Δ/Δ</td>
<td>1074</td>
<td>100%</td>
</tr>
</tbody>
</table>

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

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 χ2=10.2 and P=0.0014, demonstrating a highly significant protective effect of the homozygous CFHR1/CFHR3 deletion for AMD.

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 application No. 20070206747. In the table, letters refer to genotypes and numbers refer to SSCP shift patterns.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>AAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>++/+Δ, +/+Δ</td>
<td>126</td>
<td>19</td>
</tr>
<tr>
<td>ΔΔ</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Total ++/+Δ, ΔΔ</td>
<td>133</td>
<td>26</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Count</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>++/+Δ, +/+Δ</td>
<td>221</td>
<td>86</td>
</tr>
<tr>
<td>ΔΔ</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Total ++/+Δ, ΔΔ</td>
<td>233</td>
<td>97</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Non-AMD patients</th>
<th>AMD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>++/+Δ, +/+Δ</td>
<td>352</td>
<td>576</td>
</tr>
<tr>
<td>ΔΔ</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Frequency ++/+Δ, +/+Δ</td>
<td>0.951</td>
<td>0.988</td>
</tr>
<tr>
<td>Frequency ΔΔ</td>
<td>0.049</td>
<td>0.012</td>
</tr>
</tbody>
</table>

*Genotype refers to the deletion (Δ) or non-deletion (+) of the CFHR1 and CFHR3 genes by SSCP analysis and direct sequencing.
### TABLE 5-continued

**CFH gene haplotype analysis in subjects with the del/del (ΔΔ) CFHR1 allele**

<table>
<thead>
<tr>
<th></th>
<th>CFHR1</th>
<th>11</th>
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<th>CC</th>
<th>GG</th>
<th>SS</th>
<th>SS</th>
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<td>AA</td>
<td>CC</td>
<td>GG</td>
<td>SS</td>
<td>SS</td>
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<td></td>
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<tr>
<td>13</td>
<td>AA</td>
<td>CT</td>
<td>GG</td>
<td>SS</td>
<td>SS</td>
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</tr>
<tr>
<td>14</td>
<td>TT</td>
<td>GC</td>
<td>SS</td>
<td>SS</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>15</td>
<td>GG</td>
<td>SS</td>
<td>SS</td>
<td></td>
<td></td>
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<td>16</td>
<td>GG</td>
<td>SS</td>
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</tr>
<tr>
<td>17</td>
<td>GG</td>
<td>SS</td>
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<td></td>
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<tr>
<td>22</td>
<td>SS</td>
<td></td>
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**E. Exon 20b to Exon 22b split (detects both CFH and CFHR1)**

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As shown in Table 6, in two studies it was found that the deletion of the CFHRI 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) CFHRI 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 to be protective for AMD. However, not every del (Δ) CFHRI chromosome is on H4, and the protection of del/del (Δ/Δ) CFHRI homozgyotes for AMD is even stronger than H4 homozygotes. Heterozygous deletion of the CFHRI and CFHR3 genes was detected by direct DNA sequencing of the CFH, CFHR1 and CFHR3 genes using a CFH exon 22 primer.

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

**CFHRI420 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.

***CFHRI and CFHR3 patients, approximately 22 +/- 4% are heterozygous (Δ/Δ) for the deletion of the CFHRI and CFHR3 genes, as determined by direct DNA sequencing.

By Western blotting, it was determined that CFHRI protein, normally an abundant serum protein, is absent in sera derived from individuals homozygous for the CFHRI/CFHRI deletion. FIG. 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 (Cubiochem, 1:1000 dilution). After incubation, the membrane was washed, and then incubated with horse radish peroxidase-conjugated rabbit anti-goat IgG 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 CFHRI and CFHR3 genes, as determined by SSCP analysis and direct sequencing. FIG. 3 shows that no CFHRI is detected in the serum from patients having a homozygous deletion of the CFHRI and CFHR3 genes.

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

PCR experiments using leukocyte-derived DNA were performed to confirm that patients having a homozygous deletion of CFHRI and CFHR3 do not have CFHR1 and CFHR3 DNA. FIG. 5 shows a PCR analysis of CFHRI and CFHR3-5 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 CFHRI and CFHR3 (+/+, +/Δ), and pattern 4 corresponds to homozygous deletion of CFHRI 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, CFHRI, CFHR1, CFHR2, CFHR3, CFHR4, and CFHR5, as indicated. This figure shows that CFHRI, 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 CFHR2DNA was amplified in some, but not all, of the samples. Thus, when SSCP and direct sequencing show a homozygous deletion of the CFHRI and CFHR3 genes, no PCR amplifiable CFHRI and CFHR3 DNA are detected in samples.

Example 2

Production of Anti-CFHRI and Anti-CFHR3 Monoclonal Antibodies

Mice will be immunized with recombinant human CFHRI or CFHR3. Two mice with sera displaying the highest anti-CFHRI and anti-CFHR3 activity by Enzyme Linked Immunoabsorbent 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 hCFHRI or hCFHR3 by ELISA. Supernatants identified as containing antibodies to CFHRI 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.

Anti-CFHRI 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-CFHRI and/or anti-CFHR3 antibodies to block or reduce CFH, C3b, heparin and/or C-reactive protein (CRP) binding to a substrate.

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.
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Val Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys 180 185 190
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Ser Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gin Lys 210 215 220
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Arg Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile 260 265 270
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Glu Ile Thr Tyr Gin Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly 290 295 300
Asn Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys 305 310 315 320
Thr Leu Lys Pro Cys Asp Tyr Pro Asp Ile Lys His Gly Gly Leu Tyr 325 330 335
His Glu Asn Met Arg Asp Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr 340 345 350
Tyr Ser Tyr Tyr Cys Asp Gin Ser Glu Thr Pro Ser Gin Ser Tyr Ser 355 360 365
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Leu Tyr Asp Gln Glu Gln Tyr Gln Pro Phe Ser Gin Val Pro Thr Gly
35 40 45
Glu Val Phe Tyr Tyr Ser Cys Glu Tyr Asn Phe Val Ser Pro Ser Lys
50 55 60
Ser Phe Trp Thr Arg Ile Thr Cys Thr Gln Glu Gly Trp Ser Pro Thr
65 70 75 80
Pro Lys Cys Gln Arg Leu Gln Phe Phe Pro Phe Gin Glu Gin Gly His
85 90 95
Ser Gln Ser Glu Ser Glu Gin Thr His Leu Gln Gly Asp Thr Val Gin Ile
100 105 110
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Leu Phe His Glu Asn Met Arg Arg Pro Tyr Phe Pro Val Ala Val Gly
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Lys Tyr Tyr Ser Tyr Tyr Cys Asp Glu His Phe Glu Thr Pro Ser Gly
50  55  60

Ser Tyr Trp Asp Tyr Ile His Cys Thr Glu Asn Gly Trp Ser Pro Ala
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Val Pro Cys Leu Arg Lys Cys Tyr Phe Pro Tyr Leu Arg Asp Gly Tyr
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Ala Cys His Pro Gly Tyr Gly Leu Pro Lys Val Arg Glu Thr Thr Val
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Thr Cys Thr Glu Asn Gly Trp Ser Pro Thr Pro Arg Cys Ile Arg Asp
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Arg Thr Cys Ser Lys Ser Asp Ile Glu Asp Gly Phe Ile Ser
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Glu Ser Ser Ser Ile Tyr Leu Asn Lys Glu Ile Glu Tyr Lys Cys
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Lys Pro Gly Tyr Ala Thr Ala Asp Gly Asn Ser Gly Ser Ile Thr
180 185 190

Cys Leu Arg Asn Gly Trp Ser Ala Glu Pro Ile Cys Ile Asn Ser Ser
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Glu Lys Cys Gly Pro Pro Pro Pro Ile Ser Asn Gly Asp Thr Thr Ser
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Phe Leu Leu Lys Val Tyr Val Pro Glu Ser Arg Val Glu Tyr Glu Cys
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Glu Ser Tyr Tyr Glu Leu Glu Gly Ser Asn Tyr Val Thr Cys Ser Asn
245 250 255

Gly Glu Trp Ser Ala Pro Arg Cys Ile His Pro Cys Ile Ile Thr
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<223> OTHER INFORMATION: Synthetic PCR primer CFHR2 reverse

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What is claimed is:

1. A screening method for determining a human subject’s propensity to develop an abdominal aortic aneurysm and/or age-related macular degeneration (AMD) comprising:
   analyzing a biological sample from the subject to detect the presence or absence of a deletion of at least 1000 bp in the region of chromosome 1 between the 3' end of exon 22 of the complement factor H (CFH-1) gene and the 5' end of exon 1 of complement Factor H-related 4 (CFHR4) gene
   wherein the presence of a deletion indicates the subject is at increased risk of developing an abdominal aortic aneurysm and is at decreased risk of developing AMD.

2. The method of claim 1 wherein 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.

3. The method of claim 2 wherein the presence or absence of a complement Factor H-related 1 (CFHR1) gene product and/or a complement Factor H-related 3 (CFHR3) gene product is detected, where the absence of a gene product is indicative of a deletion.

4. The method of claim 2 wherein the gene product is a protein.

5. The method of claim 3 wherein entire protein coding region of the CFHR3 gene is deleted.

6. The method of claim 3 wherein entire protein coding region of the CFHR1 gene is deleted.

7. The method of claim 1 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.

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

9. The method of claim 1 wherein the biological sample is blood, serum, urine or a tissue sample.

10. The method of claim 4 wherein the detection step comprises detecting a gene product using an immunoassay or mass spectroscopy.

11. The method of claim 1 wherein the presence or absence of the deletion is detected by assaying for a truncated CFHR1 or CFHR3 gene product, where detection of a truncated gene product is indicative of a deletion.

12. The method of claim 1 wherein the step comprising detecting the presence or absence of a deletion is performed by analyzing a chromosome or nucleic acid from the subject.

13. The method of claim 12 wherein the nucleic acid is DNA or RNA.

14. 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.

15. The method of claim 1 further comprising detecting genetic variants of complement factor H (CFH) gene com
prising 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).

16. The method of claim 1 further comprising detecting in a sample from the subject 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 78 kDa (GRP-78), calreticulin, 14-3-3 protein epsilon, seryltransferrrin, albumin, keratin, pyruvate carboxylase, villin 2, complement 1 q binding protein/hyaluronic acid binding protein ("complement 1 q component"), amyloid A (a1 amyloid A), amyloid P component, C5 and C9 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.

17. The method of claim 1 further comprising detecting in a sample from the subject genetic variants of the HTRAP gene comprising detecting a polymorphic site selected from the group consisting of at least one of rs10490924, rs11200638, rs760336, and rs763720.

18. The method of claim 1 further comprising detecting in a sample from the subject 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
d) C or G at rs9332379 of the C2 gene, or E or D at position 318 of the C2 protein.

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

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

21. The method of claim 1 wherein the subject has a deletion of at least 10,000 bp in the genomic sequence encoding CFHR1 and/or CFHR3.

22. The method of claim 21 wherein the entire genomic sequence encoding CFHR3 is deleted.

23. The method of claim 21 wherein the entire genomic sequence encoding CFHR1 is deleted.

24. The method of claim 21 wherein at least a portion of the genomic sequence encoding CFHR1 and at least a portion of the genomic sequence encoding CFHR3 is deleted.

25. A screening method for determining a human subject's propensity to develop age-related macular degeneration (AMD) and/or abdominal aortic aneurysm (AAA) comprising analyzing the subject's genome to detect the presence or absence of a deletion of at least 10,000 bp in the genomic sequence encoding CFHR1 and/or CFHR3, wherein the presence of said deletion indicates the subject is at increased risk of developing an abdominal aortic aneurysm and is at decreased risk of developing AMD.

26. The method of claim 25 wherein the entire genomic sequence encoding CFHR3 is deleted.

27. The method of claim 25 wherein the entire genomic sequence encoding CFHR1 is deleted.

28. The method of claim 25 wherein at least a portion of the genomic sequence encoding CFHR1 and at least a portion of the genomic sequence encoding CFHR3 is deleted.

29. The method of claim 26 wherein the subject is homozygous for said deletion.

30. The method of claim 4 wherein the protein is CFHR1.