THERAPEUTIC COMPOSITIONS FOR AGE-RELATED MACULAR DEGENERATION COMPRISING A COMPLEMENT FACTOR H WITH ISOLEUCINE AT RESIDUE 62 AND TYROSINE AT RESIDUE 402

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ABSTRACT

The invention relates to Factor H gene polymorphisms and haplotypes associated with an elevated or a reduced risk of AMD. The invention provides methods and reagents for diagnosis and treatment of AMD.

8 Claims, 10 Drawing Sheets
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THERAPEUTIC COMPOSITIONS FOR AGE-RELATED MACULAR DEGENERATION COMPRISING A COMPLEMENT FACTOR II WITH ISOLEUCINE AT RESIDUE 62 AND TYROSINE AT RESIDUE 402

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 11/354,559 (filed Feb. 14, 2006), which claims benefit of U.S. Provisional Application Nos. 60/660,078 (filed Feb. 14, 2005), 60/717,861 (filed Sep. 16, 2005), 60/715,503 (filed Sep. 9, 2005), and 60/735,697 (filed Nov. 9, 2005), the entire contents of which are incorporated herein by reference.

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

This invention was made with government support under NIH Eye Institute grant EY11515 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in the developed world (for reviews see Zarbin, 1998, 2004; Klein et al., 2004; Ambati et al., 2003; de Jong, 2004; van Leeuwen et al., 2003) affecting approximately 15% of individuals over the age of 60. An estimated 600 million individuals are in this age demographic. The prevalence of AMD increases with age, mild, or early forms occur in nearly 20%, and advanced forms in about 7% of the population that is 75 years and older (Klein et al., 1992; Vingerling et al., 1995a, 1995b). Clinically, AMD is characterized by a progressive loss of central vision attributable to degenerative changes that occur in the macula, a specialized region of the neural retina and underlying tissues. In the most severe, or exudative, form of the disease neovascular fronds derived from the choroidal vasculature breach Bruch’s membrane and the retinal pigment epithelium (RPE) typically leading to detachment and subsequent degeneration of the retina.

AMD, a late-onset complex disorder, appears to be caused and/or modulated by a combination of genetic and environmental factors (Seddon and Chen, 2004; Tuo et al., 2004; Klein and Francis, 2003). Familial aggregation studies have estimated the genetic component to be primarily involved in as much as 25% of the disorder (Klaver et al., 1998a). According to the prevailing hypothesis, the majority of AMD cases is not a collection of multiple single-gene disorders, but instead represents a quantitative phenotype, an expression of interaction of multiple susceptibility loci. The number of loci involved, the attributable risk conferred, and the interactions between various loci remain obscure.

Linkage and candidate gene screening analyses have provided limited insight into the genetics of AMD. Reliable association of one gene with increased risk, ABCA4 (Allikmets et al., 1997) and one gene with decreased risk, ApoE4 (Klaver et al., 1998b, Soutiel et al., 1998) for AMD have been reported. In addition, several groups have reported results of genome-wide linkage analyses (reviewed in Tuo et al., 2004; Weeks et al., 2004). Linkage of one family with AMD phenotype to a specific chromosomal region, 1q25-q31 (ARM1) has been documented (Klein et al., 1998). HEMI-CENTIN-1 has been suggested to be the causal gene (Schultz et al., 2003) although its role has not been reliably confirmed. The identification of overlapping loci on chromosome 1q in several studies (Weeks et al., 2001; Iyengar et al., 2003; Weeks et al., 2004) suggests that this locus may harbor AMD-associated gene(s).

Recent studies of drusen, the hallmark ocular lesions associated with the onset of AMD, have implicated a role for inflammation and other immune-mediated processes, in particular complement activation, in the etiology of early and late forms of AMD (Hageman et al., 1999, 2001; Mullins et al., 2000, 2001; Russell et al., 2000; Anderson et al., 2002, 2004; Johnson et al., 2000, 2001; Crabb et al., 2002; Ambati et al., 2003; Penfold et al., 2003; Espinosa-Heideman et al., 2003). These studies have revealed the terminal pathway complement components (C5, C6, C7, C8 and C9) and activation-specific complement protein fragments of the terminal pathway (C3b, iC3b, C3dg and C5b-9) as well as various complement pathway regulators and inhibitors (including Factor I, Factor I, Factor D, CD25 and CD59) within drusen, along Bruch’s membrane (an extracellular layer comprised of elastin and collagen that separates the RPE and the choroid) and within RPE cells overlying drusen (Johnson et al., 2000, 2001; Mullins et al. 2000, 2001; Crabb et al., 2002). Many of these drusen-associated molecules are circulating plasma proteins previously thought to be synthesized primarily by the liver. Interestingly, many also appear to be synthesized locally by RPE and/or choroidal cells.

Activation of the complement system plays a key role in normal host defense and in the response to injury (Kinoshita, 1991). Inappropriate activation and/or control of this system, often caused by mutations in specific complement-associated genes, can contribute to autoimmune sequelae and local tissue destruction (Holers, 2003; Liszewska and Atkinson, 1991; Morgan and Walport, 1991; Shen and Meri, 2003), as has been shown in atherosclerosis (Torzewski et al., 1997; Niculescu et al., 1999), Alzheimer’s disease (Akiyama et al., 2000) and glomerulonephritis (Schwartz, et al., 2001).

Membranoprolierative glomerulonephritis type II (MPGN II) is a rare disease that is associated with uncontrolled systemic activation of the alternative pathway of the complement cascade. The disease is characterized by the deposition of abnormal electron-dense material comprised of C3 and C3c, proteins involved in the alternative pathway of complement, within the renal glomerular basement membrane, which eventually leads to renal failure. Interestingly, many patients with MPGNII develop macular drusen, RPE detachments and choroidal neovascular membranes that are clinically and compositionally indistinguishable from those that form in AMD, although they are often detected in the second decade of life (Mullins et al., 2001; O’Brien et al., 1993; Huang et al., 2003; Colville et al., 2003; Duvall-Young et al., 1989a, 1989b; Raines et al., 1989; Leys et al., 1990; McAvoy and Silvestri, 2004; Bennett et al., 1989; Orth and Ritz, 1998; Habib et al., 1975).

In most patients with MPGNII, the inability to regulate the complement cascade is mediated by an autoantibody directed against C3bBb. Other MPGN II patients, however, harbor mutations in Factor H (Ault et al., 1997; Dragon-Durey et al., 2004) a major inhibitor of the alternative complement pathway. A point mutation in Factor H (1116R) causes MPGNII in the Yorkshire pig (Jansen et al., 1998) and Factor H deficient mice develop severe glomerulonephritis (Pickering et al., 2002). Moreover, affected individuals within some extended families with MPGNII, a related disorder, show linkage to chromosome 1q31-32 (Neary et al., 2002) a region that overlaps a locus that has been identified in genome-wide
linkage studies for AMD (see above). This particular locus contains a number of complement pathway-associated genes. One group of these genes, referred to as the regulators of complement activation (RCA) gene cluster, contains the genes that encode Factor H, five Factor H-related genes (CFHR1, CFHR2, CFHR3, CFHR4 and CFHR5), and the beta subunit of coagulation factor XIII. A second cluster of complement pathway-associated genes, including C4BPA, C4BPI, C4BPAL2, DAF (CD55) CR1, CR2, CR1L, and MCP (CD46) lies immediately adjacent to the 1q25-31 locus.

BRIEF SUMMARY OF THE INVENTION

The invention relates to polymorphisms and haplotypes in the complement Factor H gene that are associated with development of age-related macular degeneration (AMD) and membranoproliferative glomerulonephritis type 2 (MPGN II). The invention also relates to polymorphisms and haplotypes in the complement Factor H-related 5 (CFHR5) genes that are associated with development of AMD and MPGN II. The invention provides methods of diagnosing, monitoring, and treating these and other diseases.

In one aspect, the invention provides a diagnostic method for determining a subject's propensity to develop age-related macular degeneration (AMD), comprising detecting the presence or absence of a variation or variations at polymorphic sites or polymorphic sites of the Factor H gene. In one embodiment, the invention provides methods of diagnosing an increased susceptibility to developing AMD involving detecting the presence or absence of a polymorphism in the Factor H gene of an individual. The methods may include obtaining the DNA from an individual and analyzing the DNA from the individual to determine whether the DNA contains a polymorphism in the Factor H gene. Certain polymorphisms indicate the individual has an increased susceptibility to developing AMD relative to a control population. Certain polymorphisms indicate the individual has a reduced likelihood of developing AMD. Certain polymorphisms indicate the individual has neither an increased nor a reduced likelihood of developing AMD.

In one embodiment, a method of diagnosing a propensity to develop age-related macular degeneration (AMD) in a subject involves obtaining a sample of DNA from the subject and detecting in the DNA of the patient the presence or absence of a polymorphism associated with development of AMD, the presence of the polymorphism being an indication that the subject has an increased propensity to develop AMD and the absence of the polymorphism being an indication that the subject has a reduced propensity to develop AMD.

In a related aspect, the invention provides methods of diagnosing susceptibility to developing AMD involving determining an individual's Factor H haplotype. The methods include obtaining the DNA from an individual and analyzing the DNA of the individual to determine their Factor H haplotype. Certain haplotypes (risk haplotypes) indicate the individual has an increased susceptibility to develop AMD. Certain haplotypes (protective haplotypes) indicate the individual has a decreased susceptibility to develop AMD. Certain haplotypes (neutral haplotypes) indicate the individual has neither an increased nor a reduced likelihood of developing AMD.

In a related embodiment the presence or absence of a variation at a polymorphic site of the Factor H gene is determined by analysis of a gene product, such as an RNA or a Factor H protein (e.g., protein isoform) encoded by the gene. Expression of a variant protein is an indication of a variation in the Factor H gene and can indicate an increased or reduced propensity to develop AMD. Proteins can be detected using immunoassays and other methods.

In another related aspect, the invention provides methods of diagnosing susceptibility to developing AMD or other diseases by detecting a variant Factor H polypeptide in a biological sample of an individual. In one embodiment, an antibody-based assay is used to diagnose AMD or other diseases in an individual by contacting a biological sample, e.g., a serum sample, of the individual with the antibody and detecting the presence or absence of the variant Factor H polypeptide. In an embodiment, the antibody specifically interacts with an epitope specific to a variant Factor H polypeptide (i.e., not found in the wild-type Factor H polypeptide). In an embodiment, a separation-based assay (e.g., PAGE) is used to diagnose AMD or other diseases in an individual by detecting the presence or absence of the variant Factor H polypeptide in a biological sample, e.g., a serum sample, of the individual.

In one aspect, the invention provides methods of treating an individual with AMD (e.g., an individual in whom a polymorphism or haplotype indicative of elevated risk of developing symptomatic AMD is detected) or other disease involving a variant Factor H gene by modulating the type and/or amount of systemic and/or ocular levels of Factor H. The Factor H polypeptide may be a wild-type Factor H polypeptide or a variant Factor H polypeptide. The Factor H polypeptide may be a Factor H polypeptide with a sequence encoded by neutral or protective alleles rather than alleles associated with a risk haplotype. In one embodiment, the method includes administering to the individual a Factor H polypeptide in an amount effective to reduce a symptom of the disease. In one embodiment, the method includes administering to an individual a Factor H polypeptide in an amount effective to reduce the propensity to develop symptoms of the disease and delay development or progression of the disease. In one embodiment, the method includes administering blood that contains Factor H. In one embodiment, the methods include administering a nucleic acid (e.g., transgene) including a nucleotide sequence encoding a Factor H polypeptide. In one embodiment, the methods include administering cells that express a Factor H polypeptide.

In one aspect, the invention provides methods of treating an individual with AMD (e.g., an individual in whom a polymorphism or haplotype indicative of elevated risk of developing symptomatic AMD is detected) or other disease involving a variant Factor H gene. In one embodiment, the method includes administering to the patient an agent that decreases the amount of a variant Factor H or expression of a gene encoding Factor H in an amount effective to reduce a symptom of the disease in the patient. In a related embodiment a therapeutic amount of an inhibitor (e.g., inactivator) of the variant Factor H polypeptide in the individual is administered.

In one embodiment an inhibitory nucleic acid (e.g., an RNA complementary to at least a portion of the nucleotide sequence of the variant Factor H polypeptide) in the individual is administered. In one embodiment, purified antisense RNA complementary to RNA encoding a variant Factor H polypeptide is administered.

In another embodiment a therapeutic amount of an anti-CFH antibody sufficient to partially inactivate the variant Factor H polypeptide in the individual is administered.

In another embodiment, the individual is treated to remove deleterious forms of Factor H from blood (e.g., by plasmaphoresis, antibody-directed plasmaphoresis, or complexing with a Factor H binding moiety, e.g., heparin).
In one aspect, the invention provides purified DNA encoding a variant Factor H polypeptide, purified RNA encoding a variant Factor H polypeptide, purified anti-sense RNA complementary to the RNA encoding a variant Factor H polypeptide, and purified variant Factor H polypeptide. In a related aspect, the invention provides nucleic acids for expressing wild-type or variant Factor H polypeptides or biologically active fragments of Factor H.

In one aspect, the invention provides gene therapy vectors comprising nucleic acid encoding the Factor H polypeptide. The vector may include a promoter that drives expression of the Factor H gene in multiple cell types. Alternatively, the vector may include a promoter that drives expression of the Factor H gene only in specific cell types, for example, in cells of the retina or in cells of the kidney. In an aspect, pharmaceutical compositions are provided containing a gene therapy vector comprising a Factor H protein and a pharmaceutically acceptable excipient, where the composition is free of pathogens and suitable for administration to a human patient. In one embodiment the encoded Factor H polypeptide is a protective variant.

In one aspect, the invention provides a composition containing recombinant or purified Factor H polypeptide, where the polypeptide is a protective variant.

In a related aspect, the invention provides a pharmaceutical composition containing recombinant or purified Factor H polypeptide and a pharmaceutically acceptable excipient, where the composition is free of pathogens and suitable for administration to a human patient. In one embodiment the encoded Factor H polypeptide has the wild-type sequence. In one embodiment the encoded Factor H polypeptide is a protective variant.

In one aspect, the invention provides antibodies that specifically interact with a variant Factor H polypeptide but not with a wild-type Factor H polypeptide. These antibodies may be polyclonal or monoclonal and may be obtained by subtractive techniques. These antibodies may be sufficient to inactivate a variant Factor H polypeptide. In a related aspect, the invention provides pharmaceutical compositions containing an anti-Factor H antibody and a pharmaceutically acceptable excipient, where the composition is free of pathogens and suitable for administration to a human patient.

In one aspect, the invention provides methods for identifying variant Factor H proteins associated with increased or reduced risk of developing AMD. In one embodiment, the invention provides a method of identifying a protective Factor H protein by (a) identifying an individual as having a protective haplotype and (b) determining the amino acid sequences of Factor H encoded in the genomic sequence(s) of the individual to determine a protective haplotype. In one embodiment, the invention provides a method of identifying a neutral Factor H protein by (a) identifying an individual as having a neutral haplotype and (b) determining the amino acid sequence(s) of Factor H encoded in the genomic sequence(s) of the individual to determine a neutral haplotype. In a related embodiment, the invention provides a method of identifying a variant form of Factor H associated with increased or decreased risk of developing AMD comprising (a) identifying an individual as having a haplotype or haplotype associated with a decreased risk of developing AMD; (b) obtaining genomic DNA or RNA from the individual; and (c) determining the amino acid sequence(s) of Factor H encoded in the individual's genome, where a protective Factor H protein is encoded by an allele having a haplotype associated with a decreased risk of developing AMD. In an embodiment, the protective or neutral Factor H proteins do not have the amino acid sequence of the wild-type Factor H polypeptide.

In a related method, a form of Factor H associated with increased risk of developing AMD is identified by (a) identifying an individual as having a risk haplotype and (b) determining the amino acid sequence(s) of Factor H encoded in the genome of the individual, where a risk Factor H protein is encoded by an allele having a risk haplotype. In a related embodiment, the invention provides a method of identifying a variant form of Factor H associated with increased risk of developing AMD comprising (a) identifying an individual as having a haplotype or haplotype associated with an increased risk of developing AMD; (b) obtaining genomic DNA or RNA from the individual; and (c) determining the amino acid sequence(s) of Factor H encoded in the individual's genome, where a risk Factor H protein is encoded by an allele having a haplotype associated with an increased risk of developing AMD. In an embodiment, the risk Factor H proteins do not have the amino acid sequence of the wild-type Factor H polypeptide.

In one aspect, the invention provides methods of diagnosing a propensity or susceptibility to develop AMD or other diseases by detecting the ratio of full-length Factor H to truncated Factor H in a biological sample of a patient. In one embodiment, a method of diagnosing a propensity or susceptibility to develop AMD in a subject involves obtaining a sample of RNA from the subject and detecting in the RNA the ratio of expression of exon 10 (i.e., full-length Factor H) to exon 10A (i.e., truncated Factor H), the increase in ratio being an indication that the subject has an increased propensity or susceptibility to develop AMD and the decrease in ratio being an indication that the subject has a reduced propensity or susceptibility to develop AMD. In one embodiment, a method of diagnosing a propensity or susceptibility to develop AMD in a subject involves obtaining a sample of protein from the subject and detecting in the protein the ratio of expression of full-length Factor H to truncated Factor H, the increase in ratio being an indication that the subject has an increased propensity or susceptibility to develop AMD and the decrease in ratio being an indication that the subject has a reduced propensity or susceptibility to develop AMD.

In one aspect, the invention provides cells containing recombinant or purified nucleic acid encoding a Factor H protein or fragment thereof, e.g., a nucleic acid derived from the Factor H gene. The cells may be bacterial or yeast, or any other cell useful for research and drug development. Thus, the invention provides an isolated host cell or cell line expressing a recombinant variant human Factor H. In an embodiment, the variant is a risk variant and has a histidine at amino acid position 402. In an embodiment, the variant is a protective variant and has isoleucine at amino acid position 62. In an embodiment, the variant is a neutral variant. In an embodiment, the risk, protective or neutral variant Factor H proteins do not have the amino acid sequence of the wild-type Factor H polypeptide.

In one aspect, the invention provides transgenic non-human animals whose somatic and germ cells contain a transgene encoding a human variant Factor H polypeptide. Transgenic animals of the invention are used as models for AMD and for screening for agents useful in treating AMD. The animal may be a mouse, a worm, or any other animal useful for research and drug development (such as recombinant production of Factor H). In an embodiment, the Factor H is a variant human Factor H, wherein said variant has isoleucine amino acid 62 or has histidine at amino acid 402.
In one aspect, the invention provides methods of screening for polymorphic sites linked to polymorphic sites in the Factor H gene described in TABLES 1A, 1B and 1C. These methods involve identifying a polymorphic site in a gene that is linked to a polymorphic site in the Factor H gene, wherein the polymorphic form of the polymorphic site in the Factor H gene is associated with AMD, and determining haplotypes in a population of individuals to indicate whether the linked polymorphic site has a polymorphic form in equilibrium disequilibrium with the polymorphic form of the Factor H gene that associates with the AMD phenotype.

In one aspect, the invention provides diagnostic, therapeutic and screening methods for MPGNII, carried out as described above for AMD.

In one aspect, the invention provides a diagnostic method for determining a subject's propensity to develop AMD or MPGNII, comprising detecting the presence or absence of a variation or variations at polymorphic site or polymorphic sites of the CFHR5 gene. In one embodiment, the invention provides methods of diagnosing an increased susceptibility to developing AMD or MPGNII involving detecting the presence or absence of a polymorphism in the CFHR5 gene of an individual. The methods may include obtaining the DNA from an individual and analyzing the DNA from the individual to determine whether the DNA contains a polymorphism in the CFHR5 gene. Certain polymorphisms indicate the individual has an increased susceptibility to developing AMD or MPGNII. Certain polymorphisms indicate the individual has a reduced likelihood of developing AMD or MPGNII. Certain polymorphisms indicated the individual has neither an increased nor a reduced likelihood of developing AMD or MPGNII.

In one embodiment, a method of diagnosing a propensity to develop AMD or MPGNII in a subject involves obtaining a sample of DNA from the subject and detecting in the DNA of the patient the presence or absence of a polymorphism associated with development of AMD or MPGNII, the presence of the polymorphism being an indication that the subject has an increased propensity to develop AMD or MPGNII and the absence of the polymorphism being an indication that the subject has a reduced propensity to develop AMD or MPGNII.

In a related embodiment the presence or absence of a variation at a polymorphic site of the CFHR5 gene is determined by analysis of a gene product, such as an RNA or a CFHR5 protein (e.g., protein isoform) encoded by the gene. Expression of a variant protein is an indication of a variation in the CFHR5 gene and can indicate an increased or reduced propensity to develop AMD or MPGNII. Proteins can be detected using immunoassays and other methods.

In a related aspect, the invention provides methods of diagnosing susceptibility to developing AMD or MPGNII involving determining an individual's CFHR5 haplotype. The methods include obtaining the DNA from an individual and analyzing the DNA of the individual to determine their CFHR5 haplotype. Certain haplotypes (risk haplotypes) indicate the individual has an increased susceptibility to develop AMD or MPGNII relative to a control population. Certain haplotypes (protective haplotypes) indicate the individual has a decreased susceptibility to develop AMD or MPGNII. Certain haplotypes (neutral haplotypes) indicate the individual has neither an increased nor a reduced likelihood of developing AMD or MPGNII.

In another related aspect, the invention provides methods of diagnosing susceptibility to developing AMD or MPGNII or other diseases by detecting a variant CFHR5 polypeptide in a biological sample of an individual. In one embodiment, an antibody-based assay is used to diagnose AMD or MPGNII or other diseases in an individual by contacting a biological sample, e.g., a serum sample, of the individual with the antibody and detecting the presence or absence of the variant CFHR5 polypeptide. In an embodiment, the antibody specifically interacts with an epitope specific to a variant CFHR5 polypeptide (i.e., not found in the wild-type CFHR5 polypeptide). In an embodiment, a separation-based assay (e.g., PAGE) is used to diagnose MPGNII or other diseases in an individual by detecting the presence or absence of the variant CFHR5 polypeptide in a biological sample, e.g., a serum sample, of the individual. Various types of immunoassay formats can be used to assay CFH or CFHR5 polypeptide or protein in a sample. These include sandwich ELISA, radioimmunoassay, fluorimmunoassay, immunohistochemistry assay, dot-blot, dip-stick and Western Blot.

In one aspect, the invention provides methods of treating an individual with or at risk for AMD or MPGNII (e.g., an individual in whom a polymorphism or haplotype indicative of elevated risk of developing symptomatic AMD or MPGNII is detected) or other disease involving a variant CFHR5 gene by modulating the type and/or amount of systemic and/or renal levels of CFHR5. The CFHR5 polypeptide may be a CFHR5 polypeptide encoded by neutral or protective alleles rather than alleles associated with a risk haplotype. In one embodiment, the method includes administering to the individual a CFHR5 polypeptide in an amount effective to reduce a symptom of the disease. In one embodiment, the method includes administering to an individual a CFHR5 polypeptide in an amount effective to reduce the propensity to develop symptoms of the disease and delay development or progression of the disease. In one embodiment, the method includes administering blood, which contains CFHR5. In one embodiment, the methods include administering a nucleic acid (e.g., transgene) including a nucleotide sequence encoding a CFHR5 polypeptide.

In one aspect, the invention provides methods of treating an individual with AMD or MPGNII (e.g., an individual in whom a polymorphism or haplotype indicative of elevated risk of developing symptomatic AMD or MPGNII is detected) or other disease involving a variant CFHR5 gene. In one embodiment, the method includes administering to the patient an agent that decreases the amount of a variant CFHR5 or expression of a gene encoding CFHR5 in an amount effective to reduce a symptom of the disease in the patient. The CFHR5 polypeptide may be a wild-type CFHR5 polypeptide or a variant CFHR5 polypeptide.

In one embodiment an inhibitory nucleic acid (e.g., an RNA complementary to at least a portion of the nucleotide sequence of the variant CFHR5 polypeptide) in the individual is administered. In one embodiment, purified anti-sense RNA complementary to RNA encoding a variant CFHR5 polypeptide is administered.

In another embodiment a therapeutic amount of an anti-CFHR5 antibody sufficient to partially inactivate the variant CFHR5 polypeptide in the individual is administered.

In a related embodiment a therapeutic amount of an inhibitor (e.g., inactivator) of the variant CFHR5 polypeptide in the individual is administered.

In another embodiment, the individual is treated to remove deleterious forms of CFHR5 from blood (e.g., by plasmapheresis, antibody-directed plasmapheresis, or complexing with a CFHR5 binding moiety, e.g., heparin).

In one aspect, the invention provides purified DNA encoding a variant CFHR5 polypeptide, purified RNA encoding a variant CFHR5 polypeptide, purified anti-sense RNA complementary to the RNA encoding a variant CFHR5.
polypeptide, and purified variant CFHR5 polypeptide. In a related aspect, the invention provides nucleic acids for expressing wild-type or variant CFHR5 polypeptides or biologically active fragments of CFHR5.

In one aspect, the invention provides gene therapy vectors comprising nucleic acid encoding the CFHR5 polypeptide. The vector may include a promoter that drives expression of the CFHR5 gene in multiple cell types. Alternatively, the vector may include a promoter that drives expression of the CFHR5 gene in specific cell types, for example, in cells of the retina or cells of the kidney (e.g., endothelial cells, mesangial cells, podocytes). In an aspect, pharmaceutical compositions are provided containing a gene therapy vector encoding a CFHR5 protein and a pharmaceutically acceptable excipient, where the composition is free of pathogen and suitable for administration to a human patient. In one embodiment the encoded CFHR5 polypeptide is a protective variant.

In one aspect, the invention provides a composition containing recombinant or purified CFHR5 polypeptide, where the polypeptide is a protective variant.

In a related aspect, the invention provides a pharmaceutical composition containing recombinant or purified CFHR5 polypeptide and a pharmaceutically acceptable excipient, where the composition is free of pathogens and suitable for administration to a human patient. In one embodiment the encoded CFHR5 polypeptide has the wild-type sequence. In one embodiment the encoded CFHR5 polypeptide is a protective variant.

In one aspect, the invention provides antibodies that specifically interact with a variant CFHR5 polypeptide but not with a wild-type CFHR5 polypeptide. These antibodies may be polyclonal or monoclonal and may be obtained by subtractive techniques. These antibodies may be sufficient to inactivate a variant CFHR5 polypeptide. In a related aspect, the invention provides pharmaceutical compositions containing an anti-CFHR5 antibody and a pharmaceutically acceptable excipient, where the composition is free of pathogens and suitable for administration to a human patient.

In one aspect, the invention provides methods for identifying variant CFHR5 proteins associated with increased or reduced risk of developing AMD or MPGNII. In one embodiment, the invention provides a method of identifying a protective CFHR5 protein by (a) identifying an individual as having a protective haplotype and (b) determining the amino acid sequence(s) of CFHR5 encoded in the genome of the individual, where a protective CFHR5 protein is encoded by an allele having a protective haplotype. In one embodiment, the invention provides a method of identifying a neutral CFHR5 protein by (a) identifying an individual as having a neutral haplotype and (b) determining the amino acid sequence(s) of CFHR5 encoded in the genome of the individual, where a neutral CFHR5 protein is encoded by an allele having a neutral haplotype. In a related embodiment, the invention provides as method of identifying a variant CFHR5 associated with decreased risk of developing AMD or MPGNII comprising (a) identifying an individual as having a haplotype or diplostyle associated with a decreased risk of developing AMD or MPGNII; (b) obtaining genomic DNA, or RNA, from the individual; and (c) determining the amino acid sequence(s) of the CFHR5 encoded in the individual’s genome, where a protective CFHR5 protein is encoded by an allele having a haplotype associated with a decreased risk of developing AMD or MPGNII. In one embodiment, the protective or neutral CFHR5 proteins do not have the amino acid sequence of the wild-type CFHR5 polypeptide.

In a related method, a form of CFHR5 associated with increased risk of developing AMD or MPGNII is identified by (a) identifying an individual as having a risk haplotype and (b) determining the amino acid sequence(s) of CFHR5 encoded in the genome of the individual, where a risk CFHR5 protein is encoded by an allele having a risk haplotype. In a related embodiment, the invention provides a method of identifying a variant form of CFHR5 associated with increased risk of developing AMD or MPGNII comprising (a) identifying an individual as having a haplotype or diplostyle associated with an increased risk of developing AMD or MPGNII; (b) obtaining genomic DNA or RNA from the individual; and (c) determining the amino acid sequence(s) of the CFHR5 encoded in the individual’s genome, where a risk CFHR5 protein is encoded by an allele having a haplotype associated with an increased risk of developing AMD or MPGNII. In one embodiment, the risk CFHR5 proteins do not have the amino acid sequence of the wild-type CFHR5 polypeptide.

In one aspect, the invention provides cells containing recombinant or purified nucleic acid derived from the CFHR5 gene. The cells may be bacterial or yeast, or any other cell useful for research and drug development. Thus, the invention provides an isolated host cell or cell line expressing a recombinant variant human CFHR5. In one embodiment, the CFHR5 variant is a risk variant and has a serine at amino acid position 46. In one embodiment, the CFHR5 variant is a neutral variant. In one embodiment, the risk, protective or neutral variant CFHR5 proteins does not have the amino acid sequence of the wild-type CFHR5 polypeptide.

In one aspect, the invention provides transgenic non-human animals whose somatic and germ cells contain a transgene encoding a human variant CFHR5 polypeptide. Transgenic animals of the invention are used as models for AMD or MPGNII and for screening for agents useful in treating AMD or MPGNII. The animal may be a mouse, a worm, or any other animal useful for research and drug development (such as recombinant production of CFHR5). In one embodiment, the CFHR5 is a variant human CFHR5, wherein said CFHR5 variant has serine at amino acid 46.

In one aspect, the invention provides methods of screening for polymorphic sites linked to polymorphic sites in the CFHR5 gene described in Table 14 or Table 15. These methods involve identifying a polymorphic site in a gene that is linked to a polymorphic site in the CFHR5 gene, wherein the polymorphic form of the polymorphic site in the CFHR5 gene is associated with AMD or MPGNII, and determining haplotypes in a population of individuals to indicate whether the linked polymorphic site has a polymorphic form in equilibrium disequilibrium with the polymorphic form of the CFHR5 gene that associates with the AMD or MPGNII phenotype.

In one aspect, the invention provides kits for analysis of a Factor H haplotype. The kits may be used for diagnosis of AMD in a patient. The kits may include one or more Factor H Factor H allele-specific oligonucleotides (e.g., allele-specific primers or probes), or antibodies that specifically recognize the Factor H polypeptide. The Factor H allele-specific oligonucleotides may include sequences derived from the coding (exons) or non-coding (promoter, 5' untranslated, introns or 3' untranslated) region of the Factor H gene. The Factor H-specific antibodies may recognize the normal or wild-type H polypeptide or variant Factor H polypeptides in which one or more non-synonymous single nucleotide polymorphisms (SNPs) are present in the Factor H coding region. The kits may be used to diagnose AMD, as well as other diseases associated with SNPs in the Factor H gene, such as MPGNII. The kits may include instead, or in addition, one or more
Factor H-Related 5 (CFHR5) allele-specific oligonucleotides (e.g., primers and probes), or antibodies that specifically recognize the CFHR5 polypeptide. The CFHR5 allele-specific primers and Factor H-related 5 allele-specific oligonucleotides may include sequences derived from the coding (exons) or non-coding (promoter, 5’ untranslated, introns or 3’ untranslated) region of the Factor H-related 5 gene. The Factor H-related 5-specific antibodies may recognize the normal or wild-type H polypeptide or variant Factor H-related 5 polypeptides in which one or more non-synonymous single nucleotide polymorphisms (SNPs) are present in the Factor H-related 5 coding region.

In one embodiment the kit contains probes or primers that distinguish alleles at a polymorphic site listed in TABLE 1A, TABLE 1B and/or TABLE 1C. In an embodiment the probes are primers for nucleic acid amplification of a region spanning a Factor H gene polymorphic site listed in TABLE 1A, TABLE 1B and/or TABLE 1C. In an embodiment the kit has probes or primers that distinguish alleles at more than one polymorphic site, where the polymorphic site includes: (a) rs529825; (b) rs800292; (c) rs3766404; (d) rs1061170; (e) rs1061170; (f) rs203674; (g) at least one of rs529825 and rs800292; (h) at least one of rs1061174, rs1061170 and rs203674; (i) at least one of rs529825 and rs800292; and rs3766404; and at least one of rs1061174, rs1061170 and rs203674; or (j) at least one of rs529825, rs800292, rs3766404, rs1061170 and rs203674.

In a related embodiment the kit has probes or primers that distinguish alleles at more than one polymorphic site, where the polymorphic site includes: (a) rs529825; (b) rs800292; (c) intron 2 (IVS2 or insTT) (d) rs3766404; (e) rs1061174; (f) rs1061170; (g) exon 10A; (h) rs203674; (i) rs375046; (j) rs529825 and rs800292; (k) at least two or three of rs1061174, rs1061170 and rs203674; (l) at least one of rs529825 and rs800292; and intron 2; and rs3766404; and at least one of rs1061174, rs1061170 and rs203674; and exon 10A; and, rs375046; (m) at least rs529825; rs800292; intron 2; rs3766404; rs1061170; exon 10A; rs203674; and rs375046; (n) at least two, or at least three, or at least four of rs529825, rs800292, intron 2; rs3766404, rs1061170, exon 10A, rs203674, and rs375046; (o) exon 22 (1210); or (p) exon 22 (1210) in combination with any aforementioned variation or set of variations (a-o). In an embodiment the kit has probes or primers that distinguish alleles at one or both of rs460897 and rs460184. In an embodiment the kit has probes or primers that distinguish alleles at more than one polymorphic site, where the polymorphic site are selected from: (a) rs3753394; (b) rs529825; (c) rs800292; (d) intron 2 (IVS2 or insTT); (e) rs3766404; (f) rs1061170; (g) rs1061170; (h) rs2274000; (i) rs203674; (j) rs3753396; and (k) rs1065489.

In one embodiment the kit contains probes or primers that distinguish alleles at one or both of rs460897 and rs460184. In an embodiment the kit has probes or primers that distinguish alleles at more than one polymorphic site, where the polymorphic site are selected from: (a) rs3753394; (b) rs529825; (c) rs800292; (d) intron 2 (IVS2 or insTT); (e) rs3766404; (f) rs1061170; (g) rs1061170; (h) rs2274000; (i) rs203674; (j) rs3753396; and (k) rs1065489.

In an embodiment the kit comprises probes or primers that distinguish alleles one, two or all of the following polymorphic sites: rs9427661 (–2491>C); rs9427662 (–207>T); and rs12097550 (P46S).

In one embodiment the kit contains probes or primers that distinguish alleles at a polymorphic site in the CFH gene and at a polymorphic site in the CFHR5 gene, such as CFHR5.

In one aspect, the invention provides devices for determining a subject’s haplotype. The devices are useful for, for example, the diagnosis of AMD or other diseases in a patient. In one embodiment the device contains probes or primers that distinguish alleles at a polymorphic site listed in TABLE 1A, 1B and/or 1C. In an embodiment the probes are primers for nucleic acid amplification of a region spanning a Factor H gene polymorphic site listed in TABLE 1A, 1B and/or 1C. In an embodiment the device has probes or primers that distinguish alleles at more than one polymorphic site listed in TABLE 1A, 1B and/or 1C. In an embodiment the device has probes or primers that distinguish alleles at more than one polymorphic site, where the polymorphic site includes: (a) rs529825; (b) rs800292; (c) rs3766404; (d) rs1061170; (e) rs1061170; (f) rs203674; (g) at least one of rs529825 and rs800292; (h) at least one of rs1061174, rs1061170 and rs203674; (i) at least one of rs529825 and rs800292; and rs3766404; and at least one of rs1061174, rs1061170 and rs203674; or (j) at least one of rs529825, rs800292, rs3766404, rs1061170 and rs203674.

The kits described above and their contents may also be used to identify a propensity to develop MNGPII or to determine a Factor H haplotype for any purpose.

In a related embodiment the device has probes or primers that distinguish alleles at more than one polymorphic site, where the polymorphic site includes: (a) rs529825; (b) rs800292; (c) intron 2 (IVS2 or insTT) (d) rs3766404; (e) rs1061174; (f) rs1061170; (g) exon 10A; (h) rs203674; (i) rs375046; (j) rs529825 and rs800292; (k) at least two or three of rs1061174, rs1061170 and rs203674; (l) at least one of rs529825 and rs800292; and intron 2; and rs3766404; and at least one of rs1061174, rs1061170 and rs203674; and exon 10A; and, rs375046; (m) at least rs529825; rs800292; intron 2; rs3766404; rs1061170; exon 10A; rs203674; and rs375046; (n) at least two, or at least three, or at least four of rs529825, rs800292, intron 2; rs3766404, rs1061170, exon 10A, rs203674, and rs375046; (o) exon 22 (1210); or (p) exon 22 (1210) in combination with any aforementioned variation or set of variations (a-o). In an embodiment the device has probes or primers that distinguish alleles at one or both of rs460897 and rs460184. In an embodiment the device has probes or primers that distinguish alleles at more than one polymorphic site, where the polymorphic site are selected from: (a) rs3753394; (b) rs529825; (c) rs800292; (d) intron 2 (IVS2 or insTT); (e) rs3766404; (f) rs1061170; (g) rs1061170; (h) rs2274000; (i) rs203674; (j) rs3753396; and (k) rs1065489.
In a one aspect, the invention provides devices for the diagnosis of AMD or MPGNII in a patient. In one embodiment the device contains probes or primers that distinguish alleles at a polymorphic site listed in TABLE 14 or TABLE 15. In an embodiment the probes are primers for nucleic acid amplification of a region spanning a CFHR5 gene polymorphic site listed in TABLE 14 or TABLE 15. In an embodiment the device has probes or primers that distinguish alleles at more than one polymorphic site listed in TABLE 14 or TABLE 15. Devices of the invention may contain probes or primers that distinguish between both Factor H and CFHR5 variants, including any combination of the sites described above and elsewhere in this disclosure.

The devices described above and their contents may also be used to identify a propensity to develop MPGNII or to determine a Factor H haplotype for any purpose.

In one embodiment the device contains, instead of, or in addition to, the probes or primers described above, probes, primers, antibodies and the like that distinguish polymorphic sites in the CFHR5 gene. In a one aspect, the invention provides devices for the diagnosis of AMD or MPGNII in a patient based on variation in the CFHR5 gene. The devices may include one or more CFHR5-specific probes or CFHR5 allele-specific oligonucleotides, or antibodies that specifically recognize the CFHR5 polypeptide. The CFHR5-specific primers and CFHR5 allele-specific oligonucleotides may include sequences derived from the coding (exons) or non-coding (promoter, 5’ untranscribed, introns or 3’ untranslated) region of the CFHR5 gene. The CFHR5-specific antibodies may recognize the normal or wild-type CFHR5 polypeptide or variant CFHR5 polypeptides in which one or more non-synonymous single nucleotide polymorphisms (SNPs) are present in the CFHR5 coding region. The devices may be used to diagnose AMD or MPGNII, as well as other diseases associated with SNPs in the CFHR5 gene.

In one embodiment the device contains probes or primers that distinguish alleles at a polymorphic site listed in TABLE 14 or TABLE 15. In an embodiment the device has probes or primers that distinguish alleles at more than one polymorphic site listed in TABLE 14 or TABLE 15. In an embodiment the device comprises probes or primers that distinguish alleles one, two or all of the following polymorphic sites: rs9427661 (~249T>C); rs9427662 (~20T>C); and rs12097550 (P46S).

In one embodiment the device contains probes or primers that distinguish alleles at a polymorphic site in the CHI gene and at a polymorphic site in a CFHR gene, such as CFHR5. Additional aspects of the invention will be apparent upon reading the entire disclosure.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1L show the immunolocalization of Factor H (FIGS. 1A-1H) and the terminal complement complex (C5b-9) (FIGS. 1I-1L) in the human retinal pigmented epithelium. Abbreviations: (RPE)-choroid (Chor) complex; Bruch’s membrane (BM); Retina (Ret); Drusen (Dr).

FIG. 2 shows RT-PCR analysis of Factor H gene expression (CHI and the truncated form HFL1) using RNA extracted from the human eye.

FIG. 3 is a diagram of the human Factor H gene showing the approximate locations of 12 SNPs used in the analysis, the 22 exons of the Factor H gene, the 20 short consensus repeats (SCRs), the binding sites for pathogens and other substrates, and the linkage disequilibrium (LD) blocks. The diagram, showing all 22 exons of CFH (but not introns) is not drawn to scale.

FIG. 4 is a haplotype network diagram of human Factor H gene SNPs showing the relationship between the risk (filled circles), protective (lined circles), neutral (open circles) and ancestral (indicated) haplotypes and the relative frequency of the haplotypes, as indicated by the sizes and positions of the circles.

FIG. 5 shows an association analysis of human Factor H gene haplotypes and diplotype. Eight informative SNPs were analyzed for pairwise linkage disequilibrium in AMD cases and controls. The nucleotide on the coding strand at the indicated polymorphic sites is shown, except for IVS1, where the nucleotide on the non-coding strand is shown.

FIG. 6 shows marked glomerular hypercellularity with dense intramembranous deposits that cause capillary wall thickening in a patient with MPGNII, as viewed by (A) light microscopy and (B) electron microscopy. The deposits form a segmental, discontinuous or diffuse pattern in the lamina dense of the glomerular basement membrane (GBM). By light microscopy, they are eosinophilic and retracted, stain bright with periodic acid-Schiff and are highly osmophilic, which explains their electron-dense appearance (A). Even by electron microscopy the deposits lack substructure and appear as very dark homogeneous smudges (B). The exact composition of dense deposits remains unknown (bar, 5 μm).

FIG. 7 is a diagram showing the activation and regulation of the alternative pathway of the complement cascade, which is systematically activated at a high level in patients with AMD and MPGNII. The alternative pathway of the complement cascade is systematically activated at a high level in patients with MPGN II/DDD. Normally, continuous low-level activation of C3 occurs by a process of spontaneous hydrolysis known as tick-over. C3 hydrolysis is associated with a large conformational protein change shown at the top of the diagram. The conformational change makes C3(H20) similar to C3b, a C3 cleavage product. The initial convertase, C3(H20)Bb, activates C3 to form C3b. Although C3b has a fleeting half-life, if it binds to IgG, cells or basement membranes, it is protected from immediate inactivation. (C3b)2-IgG complexes form in the fluid phase and bind properdin (P), which facilitates factor B binding and the generation of C3bBb, the convertase of the alternative pathway, shown here as a Bb(C3b)2-IgG-properdin complex. The amplification loop is depicted by the arrows. C3NeT prolongs the half-life of C3 convertase and is shown in the inset. One mechanism to degrade C3 convertase is through its interaction with complement Factor H (CFH), shown at the bottom right as H. Deficiency of and mutations in Factor H are associated with MPGN II/DDD.

FIG. 8 is a diagram showing the organization of the regulators of complement-activation (RCA) gene cluster on chromosome 1q32 and the arrangement of approximately 60-amino acid domains known as short consensus repeats (SCRs) in complement Factor H (CFH), Factor H-Like 1 (CFH1L1) 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, hepamin). Complement factor H-like I (CFH1L1) 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 CFH1L5 are similar to some of the SCRs in CFH1, as denoted by the numbers in the ovals.
For example, CFH5 has 9 SCR s, 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 CFH5 have the numbers 12-14 within the corresponding ovals because these
SCRs are similar to SCRs 12-14 of Factor H and have C3b and
heparin binding properties.

FIG. 9 shows a linkage disequilibrium plot indicating that
A507A and Y402H are in linkage disequilibrium in Factor H
and ~249T>C and ~20T>C are in linkage disequilibrium in
CFH5.

FIG. 10 shows genomic duplications in the genes for 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.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

The invention provides a collection of polymorphisms and
haplotypes comprised of multiple variations in the Factor H
gene, and in Factor H-related genes such as Factor H-Related
5 gene. These polymorphisms and haplotypes are associated with age related macular degeneration (AMD) and other
Factor H-related conditions. Certain of these polymorphisms and haplotypes result in variant Factor H polypeptides. Detection of these and other polymorphisms and sets of polymorphisms
(e.g., haplotypes) is useful in designing and performing diagnostic assays for AMD. Polymorphisms and sets of polymorphisms can be detected by analysis of nucleic acids, by analysis
of polypeptides encoded by Factor H coding sequences (including polypeptides encoded by splice variants), or by
other means known in the art. Analysis of such polymorphisms and haplotypes is also useful in designing prophylactic
and therapeutic regimes for AMD.

Factor H is a multifunctional protein that functions as a key regulator of the complement system. See Zippel, 2001, “Factor
H and disease: a complement regulator affects vital body functions” Semin Thromb Hemost. 27:191-9. The Factor H protein includes: (1) binding to C-reactive protein (CRP), (2) binding to C3b, (3) binding to heparin, (4) binding to sialic acid; (5) binding to endothelial cell surfaces, (6)
binding to cellular integrin receptors (7) binding to pathogens, including microbes (see FIG. 3), and (8) C3b co-factor
activity. The Factor H gene, known as FH1, CFH1 and FH1, is located on human chromosome 1, at position 1q32. The 1q32
particular locus contains a number of complement pathway-associated genes. One group of these genes, related to the regulators of complement activation (RCA) gene cluster, contains
the genes that encode Factor H, five Factor H-related genes (FHR-1, FHR-2, FHR-3, FHR-4 and FHR-5 or CFH/R1, CFH/R2,
CFH/R3, CFH/R4 and CFH/R5, respectively), and the gene encoding the beta subunit of coagulation
factor XII. The Factor H and Factor H related genes is composed almost entirely of short consensus repeats (SCRs).
Factor H and FH1 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 (see FIG. 14).
The order of genes, from centromere to telomere is FH/FH1, FH3, FHR-1, FHR-4, FHR-2 and FHR-5.

Factor H Gene

The reference form of human Factor H cDNA (SEQ ID NO:1) (see Ripoche et al., 1988, Biochem. J. 249:593-602) and
genomic sequences have been determined. The Factor H cDNA encodes a polypeptide 1231 amino acids in length (SEQ ID NO:2) having an apparent molecular weight of 155 kDa. There is an alternatively spliced form of Factor H is

known as FH-L (1 and also has been referred to as FH-L1 or CFHT). FH-L1 (SEQ ID NO:3) corresponds essentially to exons 1 through 9 of Factor H (see Ripoche et al., 1988, Biochem. J. 249:593-602). The FH-L1 cDNA encodes a polypeptide 449 amino acids in length (SEQ ID NO:4) having an apparent molecular weight of 45-50 kDa. The first 445 amino acids of FH1 and FH-L1 are identical, with FH-L1 having a unique C-terminal 4 amino acids (exon 10A). The alternative exon 10A is located in the intron between exon 9 and exon 10. cDNA and amino acid sequence data for human
Factor H and FH-L1 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 (GenBank accession
number Y00716 (SEQ ID NO:1)) is shown in FIG. 6, and the polypeptide sequence encoded by SEQ ID NO:1 (GenBank accession number Y00716 (SEQ ID NO:2)) is shown in FIG. 7. The 1658 base nucleotide sequence of the reference form of FH-L1, the truncated form of the human Factor H (GenBank accession number X07523 (SEQ ID NO:3)) is shown in FIG. 8, and the polypeptide sequence encoded by SEQ ID NO:3 (GenBank accession number X07523 (SEQ ID NO:4)) is shown in FIG. 9. The Factor H gene sequence (150626 bases in length) is found under 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 CHFR1, CFH-L1, CFH-L1, and FH-L1. The reference form of human FHR-1 cDNA (see Esteller et al., 1991, J. Immunol. 146:3190-3196) and genomic sequences have been determined. The FHR-1 cDNA encodes a polypeptide 330 amino acids in length having an predicted molecular weight of 39 kDa. cDNA and amino acid sequence data for human FH-L1 are found in the EMBL/GenBank Data Libraries under accession number M65292. The FHR-1 gene sequence is found under GenBank accession number AL049741.

SEQ ID NO:1 shows the 3926 base nucleotide sequence of the reference form of human Factor H cDNA (GenBank accession number Y00716). The ATG initiation codon begins at nucleotide position 74 and the TAG termination codon ends at nucleotide position 3769. SEQ ID NO:2 shows the polypeptide sequence encoded by SEQ ID NO:1 (GenBank accession number Y00716). The 1231 amino acid Factor H polypeptide includes an amino acid N-terminal signal peptide. SEQ ID NO:3 shows the 158 base nucleotide sequence of the reference form of FH-L1, the truncated form of the human Factor H (GenBank accession number X07523). The ATG initiation codon begins at nucleotide position 74 and the TGA termination codon ends at nucleotide position 402. SEQ ID NO:4 shows the polypeptide sequence of the reference form of FH-L1 (GenBank accession number X07523). The 449 amino acid FH-L1 polypeptide includes an amino acid N-terminal signal peptide. SEQ ID NO:5 shows the polypeptide sequence of an exemplary protective variant of human Factor H. This protective variant Factor H polypeptide has a isoleucine at amino acid position 62 and a tyrosine at amino acid position 402. SEQ ID NO:6 shows the polypeptide sequence of an exemplary protective variant of FH-L1, the truncated form of human Factor H. This protective variant truncated Factor H polypeptide has a isoleucine at amino acid position 62 and tyrosine at amino acid position 402. SEQ ID NO:7 shows the 2821 base nucleotide sequence of the reference form of human CFH/R5 (GenBank accession number AF295327. The ATG initiation codon begins at nucleotide position 94 and the TGA termination codon ends at nucleotide position 1803. SEQ ID NO:8 shows the
polypeptide sequence encoded by SEQ ID NO.7 (GenBank accession number AA15619). The 569 amino acid CFHR5 polypeptide includes an 18 amino acid N-terminal signal peptide.

**FHR-2 Gene**

The FHR-2 gene is also known as CHFR2, CFHL2, FHR2 and HFL3. The reference form of human FHR-2 cDNA (see Strausberg et al., Proc Natl Acad Sci USA 99:16889-16903) and genomic sequences have been determined. The FHR-2 cDNA encodes a polypeptide 270 amino acids in length having a predicted molecular weight of 31 kDa. 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 HFL4. The reference form of human FHR-3 cDNA (see Strausberg et al., Proc Natl Acad Sci USA 99:16889-16903) and genomic sequences have been determined. The FHR-3 cDNA encodes a polypeptide 330 amino acids in length having a predicted molecular weight of 38 kDa. 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 reference form of human FHR-4 cDNA (see Skerka et al., 1991, J Biol Chem 272:5627-5634) and genomic sequences have been determined. The FHR-4 cDNA encodes a polypeptide 331 amino acids in length having a predicted molecular weight of 38 kDa. cDNA and amino acid sequence data for human FHR-4 are found in the EMBL/GenBank Data Libraries under accession number X98357. 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 reference form of human CFHR5 cDNA (SEQ ID NO:7) (see McRae et al., 2001, J Biol Chem 276:6747-6754) and genomic sequences have been determined. The CFHR5 cDNA encodes a polypeptide 569 amino acids in length (SEQ ID NO:8) having an apparent molecular weight of 65 kDa. 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 (GenBank accession number AF295327 [SEQ ID NO:7] is shown in Fig. 16, and the polypeptide sequence encoded by SEQ ID NO:7 (GenBank accession number AA15619 [SEQ ID NO:8] is shown in Fig. 17. The CFHR5 gene 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.

II. 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 "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. Nucleic acids include segments of DNA, or their complements spanning or flanking any one of the polymorphic sites shown in TABLE 1A, TABLE 1B and/or TABLE 1C or otherwise known in the Factor 1 gene. The segments are usually between 5 and 100 contiguous bases, and often range from a lower limit of 5, 10, 12, 15, 20, or 25 nucleotides to an upper limit of 10, 15, 20, 25, 30, 50 or 100 nucleotides (where the upper limit is greater than the lower limit). Nucleic acids between 5-10, 5-20, 10-20, 12-30, 15-30, 10-50, 20-50 or 20-100 bases are common. The polymorphic site can occur within any position of the segment. 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 adenyne, 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 \times (number of G’s and C’s in the primer) + 2°C \times (number of A’s and T’s in the primer) for oligos <14 bases and assumes a reaction is carried out in the presence of 50 mM monovalent cations. For longer oligos, the following formula can be used: Tm = 64.9°C \times (number of G’s and C’s in the primer – 16.6)\times 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 \times 16.6°C \times \log(10[Na+4]+\log(k+0.1°C \times N)\times 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 terms “risk,” “protective,” and “neutral” are used to describe variations, SNPs, haplotypes, diplotype, and proteins in a population encoded by genes characterized by such patterns of variations. A risk haplotype is an allele form of a gene, herein Factor H or a Factor H-related gene, comprising at least one variant polymorphism, and preferably a set of variant polymorphisms, associated with increased risk for developing AMD. The term “variant” when used in reference to a Factor H or Factor H-related gene, refers to a nucleotide sequence in which the sequence differs from the sequence most prevalent in a population, herein humans of European-American descent. The variant polymorphisms can be in the coding or non-coding portions of the gene. An example of a risk Factor H haplotype is the allele of the Factor H gene encoding histidine at amino acid 402 and or cysteine at amino acid 1210. The risk haplotype can be naturally occurring or can be synthesized by recombinant techniques. A protective haplotype is an allele form of a gene, herein Factor H or a Factor H-related gene, comprising at least one variant polymorphism, and preferably a set of variant polymorphisms, associated with decreased risk of developing AMD. For example, one protective Factor H haplotype has an allele of the Factor H gene encoding isoleucine at amino acid 62. The protective haplotype can be naturally occurring or synthesized by recombinant techniques. A neutral haplotype is an allele form of a gene, herein Factor H or a Factor H-related gene, that does not contain a variant polymorphism associated in a population or ethnic group with either increased or decreased risk of developing AMD. It will be clear from the following discussion that a protein encoded in a “neutral” haplotype may be protective when administered to a patient in need of treatment or prophylaxis for AMD or other conditions. That is, both “neutral” and “protective” forms of FH or CFHR5 can provide therapeutic benefit when administered to, for example, a subject with AMD or risk for developing AMD, and thus can “protect” the subject from disease.

The term “wild-type” refers to a nucleic acid or polypeptide in which the sequence is a form prevalent in a population, herein humans of European-American descent (approximately 40% prevalence; see FIG. 8). For purposes of this disclosure, a “wild-type” Factor H protein has the sequence of SEQ ID NO:2 (FIG. 7), except that the amino acid at position 402 is tyrosine (Y; SEQ ID NO:337). For purposes of this disclosure, a Factor H gene encoding a wild-type Factor H protein has the sequence of SEQ ID NO:1 (FIG. 6), except that the codon beginning at base 1277, corresponding to the amino acid at position 402 encodes tyrosine (TAT; SEQ ID NO:336).

The term “variant” when used in reference to a Factor H or Factor H-related polypeptide, refers to a polypeptide in which the sequence differs from the normal or wild-type sequence at a position that changes the amino acid sequence of the encoded polypeptide. For example, some variations or substitutions in the nucleotide sequence of Factor H gene alter a codon so that a different amino acid is encoded (for example and not for limitation, having an alternative allele at one or more of 162V, Y402H, D936E) resulting in a variant polypeptide. Variant polypeptides can be associated with risk (e.g., having histidine at position 402), associated with protection (e.g., having isoleucine at position 62), or can be encoded by a neutral haplotype (e.g., having aspartic acid at position 936). Variant CFHR5 polypeptides can be associated with risk (e.g., having serine at position 46), associated with protection, or can be neutral.

The term “reference” when referring to a Factor H polypeptide means a polypeptide in which the amino acid sequence is identical to the sequence described by Krapoche et al., 1988, Biochem. J. 249:593-602 for full-length (FH1, SEQ ID NO:2) or truncated (FH1, SEQ ID NO:4) human Factor H. The term “reference” when referring to a CFHR5 polypeptide means a polypeptide in which the amino acid sequence is identical to the sequence described by McRae et al., 2001, J. Biol. Chem. 276:6747-6754) for full-length human CFHR5 (SEQ ID NO:8). The first identified allelic form is arbitrarily designated the reference form or allele; other allelic forms are designated as alternative or variant alleles. Wild-type and variant forms may have substantial sequence identity with the reference form (e.g., the wild-type or variant form may be identical to the reference form at least 90% of the amino acid positions of the wild-type or variant, sometimes at least 95% of the positions and sometimes at least 98% or 99% of the positions). A variant may differ from a reference form in certain regions of the protein due to a frameshift mutation or splice variation.

A single nucleotide polymorphism (SNP) occurs at a polymorphic site occupied by a single nucleotide, which is the site of variation between allelic sequences. The site is usually preceded by and followed by highly conserved sequences of the allele. A SNP usually arises due to substitution of one nucleotide for another at the polymorphic site. Replacement of one purine by another purine or one pyrimidine by another pyrimidine is called a transition. Replacement of a purine by a pyrimidine or vice versa is called a transversion. A synonymous SNP refers to a substitution of one nucleotide for another in the coding region that does not change the amino acid sequence of the encoded polypeptide. A non-synonymous SNP refers to a substitution of one nucleotide for another in the coding region that changes the amino acid sequence of the encoded polypeptide. A SNP may also arise from a deletion or an insertion of a nucleotide or nucleotides relative to a reference allele.
A “set” of polymorphisms means more than one polymorphism, e.g., at least 2, at least 3, at least 4, at least 5, at least 6, or more than 6 of the polymorphisms shown in TABLE 1A, TABLE 1B and/or TABLE 1C or otherwise known in the Factor H gene or other gene.

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 or more nucleic acid 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 “diagnose” and “diagnosis” refer to the ability to determine whether an individual has the propensity to develop disease (including with or without signs or symptoms). Diagnosis of propensity to develop disease can also be called “screening” and, as used herein, the terms diagnosis and screening are used interchangeably. It will be appreciated that having an increased or decreased propensity to develop a condition refers to the likelihood of developing the condition relative to individuals in the population without the condition.

III. Tables

Certain tables referred to herein are provided following the Examples, infra. The following descriptions are provided to assist the reader:

TABLES 1A-1C show human Factor H gene polymorphisms and their association with age-related macular degeneration. (1A) The dbSNP no., location, sequences of the coding (top, 5’ to 3’ direction) and non-coding (bottom) strands spanning the polymorphisms, amino acid changes, allele frequencies for the control and AMD cases, and χ² and P-values for 1 SNPs in the human Factor H gene are shown. (1B) The dbSNP no., interrogated sequences, corresponding nucleotide in the chimp Factor H gene, location, amino acid changes, and sets of primers and probes for 11 SNPs in the human Factor H gene are shown. (1C) The location, sequences spanning the polymorphisms, amino acid position and amino acid change, if any, for 14 SNPs in the human Factor H gene that are not found in the dbSNP database are shown.

TABLE 2 describes a haplotype analysis of eight SNPs in the human Factor H gene in a cohort of AMD cases and controls.

TABLE 3 describes a haplotype analysis of six SNPs in the human Factor H gene and their association with AMD.

TABLE 4 shows the association of 11 human Factor H gene SNPs with age-related macular degeneration.

TABLE 5 shows the primers used for SSCP, DHPLC and DNA sequencing analysis for human Factor H.

TABLE 6 describes genotyping data of AMD patients and controls.

TABLE 7 describes the frequency of an at-risk haplotype in various ethnic groups.

TABLE 8 describes several Factor H diplotypes. Common risk and protective diplotypes are indicated.

TABLE 9 describes the sequences of primers used to amplify the Factor H coding sequence.

TABLE 10 describes the sequences of primers used to amplify the CFH5 coding sequence.

TABLE 11 describes an analysis of Factor H SNPs in 22 MPPNII patients.

TABLE 12 describes a comparison of Factor H SNP frequencies in 22 MPPNII patients and AMD-negative, ethnically matched controls.

TABLE 13 lists Factor H SNPs associated with MPPNII and their related SCR.

TABLE 14 describes an analysis of CFH5 SNPs in 22 MPPNII patients.

TABLE 15 shows a comparison of CFH5 SNP frequencies in 22 MPPNII patients and AMD-negative, ethnically matched controls.

TABLE 16 describes exemplary allele-specific probes (16A) and primers (16B) useful for detecting polymorphisms in the Factor H gene.

IV. Complement Factor H Polymorphisms

In one aspect, the invention provides new diagnostic, treatment and drug screening methods related to the discovery that polymorphic sites in the Complement Factor H(HF1) gene are associated with susceptibility to and development of AMD.

Factor H polymorphisms associated with AMD were identified as described in Example 1, by examining the coding and
adjacent intronic regions of Factor H (including exon 10A, which is transcribed for the Factor H isoform FHL1) for variants using SSCP analysis, DHEPLC analysis, and direct sequencing, according to standard protocols. Remaining polymorphisms were typed by the 5\’ nuclease (Taqman, ABI) methodology. Taqman genotyping and association analysis were performed as described (Gold et al., 2004). Primers for SSCP and DNA sequencing analyses were designed to amplify each exon and its adjacent intronic regions using MacVector software. PCR-derived amplicons were screened for sequence variation by SSCP and DHEPLC according to standard protocols. All changes detected by SSCP and DHPLC were confirmed by bidirectional sequencing according to standard protocols. Statistical analyses were performed using chi-square ($\chi^2$) and Fisher's exact tests ($p$ values).

Two independent groups of AMD cases and age-matched controls were used. All participating individuals were of European-American descent, over the age of 60 and enrolled under IRB-approved protocols following informed consent. These groups were comprised of 352 unrelated patients with clinically documented AMD (mean age 79.5±7.8 years) and 113 unrelated, control patients (mean age 78.4±7.4 years; matched by age and ethnicity) from the University of Iowa, and 550 unrelated patients with clinically documented AMD (mean age 71.3±8.9 years) and 275 unrelated, matched by age and ethnicity, controls (mean age 68.8±8.6 years) from Columbia University. Patients were examined by indirect ophthalmoscopy and slit-lamp microscopy by retina fellowship-trained ophthalmologists.

Fundus photographs were graded according to a standardized, international classification system (Bird et al., 1995). Control patients were selected and included if they did not exhibit any distinguishing signs of macular disease or have a known family history of AMD. The AMD patients were subdivided into phenotypic categories: early AMD (ARM), geographic atrophy (GA), and exudative (CNV) AMD, based upon the classification of their most severe eye at the time of their entry into the study. The University of Iowa ARM and GA cases were further subdivided into distinct phenotypes (RPE changes alone, >10 macular hard drusen, macular soft drusen, BB (circular) drusen, PED, “Cherokee” atrophy, peripheral geographic atrophy and paramacular geographic atrophy). The earliest documentable phenotype for all cases was also recorded and employed in the analyses.

As shown in Table 1A, a highly significant association of polymorphic sites in the Factor H gene with AMD was found in an examination of two independent cohorts that together included approximately 900 AMD cases and 400 matched controls. Sixteen (16) polymorphisms in the Factor H gene are listed in Tables 1A-1B. Of these twelve (12) are found in the SNP database (dbSNP) which may be found in the National Center for Biotechnology Information (NCBI). The dbSNP is a collection of SNPs in the human Factor H gene which are dispersed among the 22 coding exons of the Factor H gene and among the promoter, the 5\’ untranslated region, the introns, and the 3\’ untranslated region of the Factor H gene. Listed below are the accession numbers for 379 SNPs in the human Factor H gene that are found in the dbSNP database. These SNPs can be used in carrying out methods of the invention.

| rs17575212 | 11582839 | rs551203 | 5014735 | ns019724 | rs344797 | rs35963 |
| rs1753867 | 11580821 | rs546015 | 5014735 | rs1984994 | rs343399 | rs35544 |
| rs1684052 | 11579439 | rs546032 | 5014734 | ns239843 | ns259825 | rs85129 |
| rs16840465 | 11539862 | rs535005 | 5014730 | ns239843 | ns259825 | ns35955 |
| rs16840462 | 11398997 | rs5737976 | 5003626 | ns1887973 | ns251035 | rs85298 |
| rs16840422 | 11398998 | rs535005 | 5003625 | ns1887978 | ns251035 | rs85298 |
| rs16840419 | 11344041 | rs535563 | 5003625 | ns1831282 | ns259992 | rs35892 |
| rs16840410 | 11339120 | rs5529580 | 5002880 | ns1831282 | ns259992 | rs35892 |
| rs16840410 | 11339120 | rs5529580 | 5002880 | ns1831282 | ns259992 | rs35892 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |
| rs16840410 | 1138454 | rs5266202 | 5002876 | ns4110997 | ns551397 | rs405306 |

TABLE A
Two frequent non-synonymous variants, 162V in exons 2 and Y420H in exon 9, and a less frequent variant, R1210C in exon 22, exhibited the most significant association with AMD.

Three additional polymorphisms in TABLES 1A-1B are not found in the SNP database; a polymorphism in the promoter (promoter 1 in TABLE 1A); a polymorphism in intron 2 in which two T nucleotides are inserted; and a polymorphism in Exon 10A.

The first column in TABLE 1A lists the dbSNP number for polymorphisms in the Factor H gene. For example, rs800292 is the dbSNP designation for a polymorphism in the Factor H gene. A description of this polymorphism, as well as the other Factor H gene polymorphisms in dbSNP, are available at the NCBI database (ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp&cmd=search&term=). The second column lists the location of the polymorphism. For example, the rs800292 polymorphism is located in exon 2 of the Factor H gene. Polymorphisms not identified by a database number can be referred to by location (e.g., "intron 2"). The third column lists the nucleic acid sequence of the coding (5' to 3' direction) and non-coding (5') strands of DNA spanning the polymorphisms. For example, the rs800292 polymorphism, G or A as indicated in the brackets for the coding strand, is flanked by the 20 nucleotides shown 5' and 3' to the polymorphism. "N" in the sequence spanning the Exon 10A polymorphism indicates the insertion of a single nucleotide, either A, C, G, or T, in the variant allele. The fourth column lists the SEQ ID NO: for the sequences. The fifth column lists the amino acid change, if any, associated with the polymorphism. For example, the rs800292 polymorphism results in a change in the amino acid sequence from valine (V) to isoleucine (I) at position 62 of the Factor H polypeptide. The sixth column lists the allele frequency of the polymorphism in a control population. The numbers 1 and 2 refer to the alleles that correspond to the first and second nucleotide, respectively, at the polymorphic site in the third column. For example, for the rs800292 polymorphism, G is present in 78% and A is present in 22% of the alleles sequenced from the control population. The seventh column lists the allele frequency of the polymorphism in an AMD population. For example, for the rs800292 polymorphism, G is present in 91% and A is present in 9% of the alleles sequenced from an AMD population. The eighth column lists the chi-square and Fisher's exact tests (P values, respectively) for the comparison between the allele frequencies of the polymorphism in the control and AMD populations. For example, for the rs800292 polymorphism, the chi-square value is 16.19 and the P value is 5.74x10^-5, indicating that the G allele is associated with AMD.

The first column in TABLE 1B lists the dbSNP number for polymorphisms in the Factor H gene. For part 1, the second column lists the nucleic acid sequence spanning the polymorphisms (interrogated sequence). For the rs529825 (intron 1), rs800292 (exon 2), and rs203674 (intron 10) polymorphisms, the sequences of the non-coding strand of the human Factor H gene are shown. The third column lists the SEQ ID NOs: for the sequences. The fourth column lists the allele present in the chimp Factor H gene. The fifth column lists the location of the SNP. The sixth column lists the amino acid change, if any, associated with the polymorphism. For part 2, the second and fourth columns list the forward and reverse primers or AOD numbers for amplifying the polymorphisms. The third and fifth columns list the SEQ ID NOs: for the primers. For part 3, the second and fourth columns list probes used for detecting the polymorphisms. The third and fifth columns list the SEQ ID NOs: for the probes.

It should be understood that additional polymorphic sites in the Factor H gene, which are not listed in TABLES 1A-1B, may be associated with AMD. Exemplary polymorphic sites in the Factor H gene are listed, for example and not limitation, above. TABLE 1C lists an additional 14 polymorphic sites in the Factor H gene, which are not found in the dbSNP database, that may be associated with AMD or other diseases. The first column lists the location of the SNP. The second column lists the nucleic acid sequence spanning the polymorphisms. "notG" in the sequence spanning the Exon 5 polymorphism indicates the presence of an A, C, or T nucleotide in the variant allele. "notC" in the sequences spanning the Exon 6 polymorphisms indicates the presence of an A, G, or T nucleotide in the variant allele. "N" in the sequences spanning the Exon 21 polymorphism indicates the insertion of a single nucleotide, either A, C, G, or T, in the variant allele. The third column lists the amino acid change, if any associated with the polymorphism. The fourth column lists the SEQ ID NOs: for the sequences. These SNPs can also be used in carrying out methods of the invention. Moreover, it will be appreciated that these CFH polymorphisms are useful for linkage and association studies, genotyping clinical populations, correlation of genotype information to phenotype information, loss of heterozygosity analysis, and identification of the source of a cell sample.

TABLE 2 shows a haplotype analysis of eight SNPs in the human Factor H gene in AMD cases and controls. The at-risk haplotypes are shown in stippled boxes, with the haplotype determining SNPs (Y402H and IVS10) shown in denser stippling. The protective haplotypes are shown in diagonal-lined boxes, with the haplotype determining SNPs (IVS1, 162V and IVS6) shown indenser diagonal lines. The first column lists the allele of the polymorphism in the promoter (Prom). The second column lists the allele of the non-coding strand of the polymorphism in intron 1 (IVS1). The third column lists the
Notably, seventy percent of MPGN II (membranoproliferative glomerulonephritis type II) patients harbor this at-risk haplotype (see Table 7), indicating that propensity to develop MPGNII can be detected and treated as described herein for AMD.

Significant associations of these polymorphic sites were also found with various AMD subtypes, as disclosed in Example 1.

The non-synonymous polymorphism at amino acid position 1210 in exon 22 of the Factor H gene is strongly associated with AMD (see Table 1A). The variant allele, which encodes a cysteine instead of an arginine, is found in the heterozygous state in 5% of AMD cases, and no controls in a cohort comprised of 919 individuals ascertained at the University of Iowa. No 1210C homozygotes have been identified to date. The presence of cysteine at amino acid position 1210 of Factor H, therefore, provides a strong indication that the individual has AMD or is likely to develop AMD. Remarkably, 1210C is indicative of propensity to develop AMD or other complement mediated conditions even when detected on allele that is otherwise protective (e.g., Y402). Variation at CFH position 1210 (R1210C) is known to cause atypical hemolytic uremic syndrome (aHUS), a complement related disease with renal manifestations. By extension, other CFH variations or mutations known to cause aHUS may be associated with an increased risk for developing AMD. The most common established aHUS-causing variations include, but are not limited to, T956M, Q1076E, D1119G, W1183L, T1184R, L1189R, L1189F, S1191W, S1191L, V1197A, and R1215G (Esparza-Gordillo et al 2005; Perez-Caballerio et al 2001; Richards et al 2001; Sanchez-Corrall et al 2002); additional aHUS-causing mutations are described in Saunders (Saunders et al 2006). In one aspect of the present invention, a biological sample from a subject (e.g., protein or nucleic acid) is assayed for the presence of one or more aHUS-associated variations or mutations, the presence of which is indicative of a propensity to develop AMD.

It will be appreciated that additional polymorphic sites in the Factor H gene, which are not listed in TABLES 1A-1C, may further refine this haplotype analysis. A haplotype analysis using non-synonymous polymorphisms in the Factor H gene is useful to identify variant Factor H polypeptides. Other haplotypes associated with risk may encode a protein with the same sequence as a protein encoded by a neutral or protective haplotype, but contain an allele in a promoter or intron, for example, that changes the level or site of Factor H expression. It will also be appreciated that a polymorphism in the Factor H gene, or in a Factor H-related gene, may be linked to a variation in a neighboring gene. The variation in the neighboring gene may result in a change in expression or form of the encoded protein and have detrimental or protective effects in the carrier.

Protective Polymorphisms and Haplotypes

Unexpectedly, protective polymorphisms and haplotypes were also discovered. For example, as shown in TABLE 2 and Fig. 5, the protective H2 haplotype, including a variant allele in IVS6 (intron 6, rs3766404) occurs in 12% of controls, but only in 6% of AMD cases. The protective H4 haplotype includes the variant allele in IVS1 (intron 1, rs5928825) and the variant allele (H2) (exon 2, rs800292) and occurs in 18% of controls, but only in 12% of AMD cases. Similar data is presented in Table 3, where the haplotype 12111 occurs in 21% of controls, but only in 13% of AMD cases and the haplotype 11211 occurs in 13% of controls, but only in 6% of AMD cases. As shown in Fig. 5, homozygotes with a protective haplotype are significantly protected.
In some cases the protein encoded by a gene characterized by a protective haplotype has a sequence different from risk haplotype proteins (e.g., due to the presence of a non synonymous SNP). For example, a protective form of Factor H protein generally does not have histidine at position 402. In some embodiments a protective form has isoleucine at position 62. Additional protective forms can be identified by (1) identifying an individual or individuals with a protective haplotype and (2) determining the sequence(s) of Factor H cDNA or protein from the individuals. Other protective forms are identified as described below in Section VIII.

Neutral Polymorphisms and Haplotypes

Certain haplotypes are associated in a population with neither increased risk nor decreased risk of developing AMD and are referred to as “neutral.” Examples of neutral haplotypes identified in a Caucasian population are shown in FIG. 5 (H3 and H5). Additional or different neutral haplotypes may be identified in racially/ethnically different populations. Proteins encoded by a gene characterized by a neutral haplotype are “neutral” Factor H proteins. As explained supra, “neutral” Factor H proteins could provide therapeutic benefit when administered to patients having a risk haplotype or diagnosed with AMD. For example, exemplary proteins encoded by genes characterized by a neutral haplotype include proteins not having histidine at position 402 and/or not having isoleucine at position 62. A protein not having histidine at position 402 may have tyrosine at that position, or may have an amino acid other than histidine or tyrosine. A protein not having isoleucine at position 62 may have valine at that position, or may have an amino acid other than valine or isoleucine. A neutral form of Factor H protein generally does not have cysteine at position 1210.

V. Factor H Related 5 (CFHR5) Gene Polymorphisms

In one aspect, the invention provides new diagnostic, treatment and drug screening methods related to the discovery that polymorphic sites in the Factor H and CFHR5 genes are associated with susceptibility to and development of MPPNII.

Factor H and CFHR5 polymorphisms associated with MPPNII were identified as described in Example 2, by examining the coding and adjacent intronic regions of Factor H or CFHR5 for variants using PCR amplification, followed by agarose gel electrophoresis and bi-directional sequencing according to standard protocols to verify PCR products. Novel and reported SNPs were typed in the control population by denaturing high performance liquid chromatography (DHLPC). Primers used to amplify the Factor H and CFHR5 coding sequences are shown in TABLES 9 and 10, respectively.

The test group consisted of patients with biopsy-proven MPPNII who were ascertained in nephrology divisions and enrolled in this study under IRB-approved guidelines. The control group consisted of ethnically-matched, but not age-matched, unrelated persons in whom AMD had been excluded by ophthalmologic examination.

As shown in TABLES 11 and 12, a significant association of polymorphic sites in the Factor H gene with MPPNII was found in an examination of 22 MPPNII cases and 131 ethnically-matched controls. Eleven (11) polymorphisms in the Factor H gene are listed in TABLES 11 and 12. Of these, six (6) are found in the SNP database (dbSNP) which may be found in the National Center for Biotechnology Information (NCBI). The dbSNP is a collection of SNPs in the human genome. The SNPs in the Factor H gene are dispersed among the 22 coding exons of the Factor H gene and among the promoter, the 5' untranslated region, the introns, and the 3' untranslated region of the Factor H gene. The accession numbers for 579 SNPs in the Human Factor H gene that are found in the dbSNP database are listed above. These SNPs can be used in carrying out methods of the invention.

Five additional polymorphisms in TABLES 11 and 12 are not found in the SNP database: a polymorphism in intron 2 in which two T nucleotides are inserted (IVS2 -18InsTT); a polymorphism in intron 7 (IVS7 -53G>T); a polymorphism in intron 15 (IVS15 -30C>A); and a polymorphism in intron 18 (IVS18 -89T>C); and a polymorphism in Exon 20 (N1050Y). These polymorphisms are useful in the methods of the invention. Moreover, it will be appreciated that these CFHR5 polymorphisms are useful for linkage and association studies, genotyping clinical populations, correlation of genotype information to phenotype information, loss of heterozygosity analysis, and identification of the source of a cell sample.

The first row of TABLE 11 lists the exon or intron position of the SNP in the Factor H gene. For exon SNPs, the amino acid position and change if any, is listed. For example the exon 2 SNP is at position 62 of the Factor H polypeptide and a change from valine (V) to isoleucine (I). For intron SNPs, the nature the SNP is indicated. For example, the intron 2 SNP is an insertion of two nucleotides, T1. The second row of TABLE 11 lists dbSNP number, if any, for the polymorphism. For example, rs800292 is the dbSNP designation for a polymorphism in exon 2 in the Factor H gene. A description of this polymorphism, as well as the other Factor H (CFH) gene polymorphisms in dbSNP, is available at the NCBI database (ncbi.nlm.nih.gov/entrez/query.fcgi?db=snps&cmd=search&term=).

The third to fifth rows of TABLE 11 lists number of times a particular diplotype is present among the 22 MPPNII patients. For example, for the exon 2 SNP, GG is present in 20 patients, GA is present in 2 patient, and AA is present in no patients, with MPPNII. The sixth and seventh rows of TABLE 11 list the frequency that a particular haplotype is present among the 22 MPPNII patients. For example, for the exon 2 SNP, G is present in 95%, and A is present in 5%, of the alleles of the 22 MPPNII patients. The eighth row lists the nucleotide of the common haplotype in the Factor H gene for the 22 MPPNII patients. For example, G is the more frequent nucleotide in the exon 2 SNP, and 9 T nucleotides is more frequently observed than 11 T nucleotides in the intron 2 SNP in the Factor H gene for the 22 MPPNII patients. The remaining rows list the diplotype for the 11 SNPs in the Factor H gene for each of the 22 MPPNII patients.

It should be understood that additional polymorphic sites in the Factor H gene, which are not listed in TABLE 11 may be associated with MPPNII. Exemplary polymorphic sites in the Factor H gene are listed, for example and not limitation, above.

TABLE 12 shows a comparison of the SNP frequencies in patients with MPPNII versus AMD-negative, ethnically-matched control individuals. The first column of TABLE 12 lists the SNP in the Factor H gene. The second and third columns of TABLE 12 list the frequencies that a particular haplotype is present among the 22 MPPNII patients. The fourth and fifth columns of TABLE 12 list the frequencies that a particular haplotype is present among the 131 control individuals. The sixth column of TABLE 12 lists the P-value calculated for each data set.

As shown in TABLES 11 and 12, two frequent non-synonymous variants, 162V in exon 2 and Y420H in exon 9, a synonymous variant, A307A in exon 10, and a polymorphism in intron 2 exhibited significant association with MPPNII.
As shown in TABLES 14 and 15, a significant association of polymorphic sites in the FHR-5 gene with MPGNII was found in an examination of 22 MPGNII cases and 103 ethnically-matched controls. Five (5) polymorphisms in the CFHR5 gene are listed in TABLES 14 and 15; these are found in dbSNP in the NCBI. The SNPs in the CFHR5 gene are dispersed among the 10 coding exons of the CFHR5 gene and among the promoter, the 5' untranslated region, the introns, and the 3' untranslated region of the CFHR5 gene. Listed below are the accession numbers for 82 SNPs in the human CFHR5 gene that are found in the dbSNP database. These SNPs can be used in carrying out methods of the invention.

| Table B |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| rs18649356 | rs21106763 | rs10922131 | rs427662 | rs753593 | rs6694672 | rs1332664 |
| rs18649406 | rs21097879 | rs10801584 | rs427661 | rs7532041 | rs6692162 | rs1325926 |
| rs18649494 | rs21097550 | rs10801858 | rs427660 | rs7532068 | rs6657256 | rs170883 |
| rs1750554 | rs21097294 | rs427659 | rs7528757 | rs6571171 | rs170882 |
| rs12750576 | rs21097102 | rs10647987 | rs753407 | rs7527910 | rs779885 | rs170881 |
| rs12745733 | rs12604091 | rs10821349 | rs755391 | rs7522952 | rs748857 | rs170880 |
| rs12736097 | rs12409414 | rs10580279 | rs754575 | rs7522197 | rs1251137 | rs170879 |
| rs12736087 | rs1053271 | rs9742576 | rs755070 | rs7410675 | rs1251136 | rs170878 |
| rs12735776 | rs1183363 | rs427942 | rs750735 | rs766339 | rs185116 | rs928440 |
| rs12718488 | rs1306823 | rs427941 | rs750650 | rs768263 | rs179603 | rs928439 |
| rs12731209 | rs10922153 | rs427664 | rs754726 | rs6702340 | rs179303 | rs928439 |
| rs12439711 | rs10922152 | rs427663 | rs753788 | rs6674853 | rs1412636 |
identifying an individual or individuals with a protective haplotype and (2) determining the sequence(s) of Factor H cDNA or protein from the individuals. Some protective forms are less than full-length. Protective forms of CFH/R5 protein may be similarly identified.

Neutral Polymorphisms and Haplotypes

Certain haplotypes are associated with neither increased risk or decreased risk of developing MPPNII and are referred to as “neutral.” Proteins encoded by a gene characterized by a neutral haplotype are “neutral” Factor H or CFH5 proteins. For example, exemplary proteins encoded by genes characterized by a neutral haplotype include Factor H proteins not having histidine at position 402 or isoleucine at position 62, and CFH5 proteins not having serine at position 46.

Significance of Polymorphisms in MPPNII Patients

As shown in Example 2, it has been discovered that the same CFH5 polymorphisms associated with propensity to develop AMD are also associated with development of membranoproliferative glomerulonephritis type 2 (MPPNII). Indeed, the risk haplotypes originally found in AMD patients (Y402H and IVS10) are also found in 70% of patients tested having membranoproliferative glomerulonephritis type 2 (MPPN II), indicating that the diagnostic methods of the invention are useful to detect this condition. In addition, variations and haplotypes in the CFH5 gene were strongly associated with increased risk of having MPPNII. One conclusion that emerges from these data is that MPPNII and AMD are alternative manifestations of the same genetic lesion. Notably, patients with MPPNII develop drusen that are clinically and compositionally indistinguishable from drusen that form in AMD. The single feature that distinguishes these two fundus phenotypes is age of onset—drusen in MPPNII develop early, often in the second decade of life, while drusen in AMD develop later in life. We conclude that polymorphisms in the Factor H gene and CFH5 gene identified in either population (AMD or MPPNII) are predictive of susceptibility to both diseases. There are likely other factors that contribute to MPPNII and account for the early manifestation. Because AMD is very common and MPPNII is rare, the haplotype analysis of both CFH and CFH5 genes and other methods described herein will be useful for screening and treatment of patients with AMD, or with an increased likelihood of developing AMD.

Loss of Function

Loss of the normal or wild-type function of Factor H or CFH5 may be associated with AMD. Non-synonymous polymorphisms in the Factor H gene, such as those shown in TABLES 1A, 1B, 1C, 11, 14 and 15, showing the strongest correlation with AMD and resulting in a variant Factor H polypeptide or variant CFH5 polypeptide, are likely to have a causative role in AMD. Such a role can be confirmed by producing a transgenic non-human animal expressing human Factor H or CFH5 bearing such a non-synonymous polymorphism(s) and determining whether the animal develops AMD. Polymorphisms in Factor H or CFH5 coding regions that introduce stop codons may cause AMD by reducing or eliminating functional Factor H or CFH5 protein. Stop codons may also cause production of a truncated Factor H or CFH5 peptide with aberrant activities relative to the full-length protein. Polymorphisms in regulatory regions, such as promoters and introns, may cause AMD by decreasing Factor H or CFH5 gene expression. Polymorphisms in introns (e.g., intron 2 of CFH) may also cause AMD by altering gene splicing patterns resulting in an altered Factor H or CFH5 protein. CFH RNA or proteins can be assayed to detect changes in expression of splice variants, where said changes are indicative of a propensity to develop AMD. Alternative splice patterns have been reported for the Factor H gene itself.

The effect of polymorphisms in the Factor H gene or CFH5 gene on AMD can be determined by several means. Alternations in expression levels of a variant Factor H or CFH5 polypeptide can be determined by measuring protein levels in samples from groups of individuals having or not having AMD or various subtypes of AMD. Alternations in biological activity of variant Factor H or CFH5 polypeptides can be detected by assaying for in vitro activities of Factor H or CFH5, for example, binding to C3b or to heparin, in samples from the above groups of individuals.

VI. Polymorphisms at Sites of Genomic Duplication

As illustrated in FIG. 18, the genes for CFH and the factor H related (CFHR) 1-5 genes have regions of shared, highly conserved, sequence which are derived from genomic duplications. Certain SNPs and variations found in CFH or CFHR5, such as those described herein, are also expected in the corresponding sequences of CFHR1, CFHR2, CFHR3, and CFHR4. For example, sequences corresponding to CFH exon 22 are found in CFH, CFHR1 and CFHR2, and it is possible that polymorphisms identified in exon 22 of CFH (e.g., R1210C) are also found in CFHR1 and/or CFHR2 and these variants might be linked to propensity to development of AMD, MPPNII, and other complement related conditions. Homologous blocks of sequence flanking the polymorphic sites identified in CFH and CFHR5 can be identified by alignment of the cDNA or genomic sequences in those regions. The conserved sequences flanking the polymorphic site usually comprise at least 10 bp (on either side of the polymorphic site) and more often at least 20 bp, or at least 50 bp, or at least 100 bp with at least 95% identity at the nucleotide level, and sometimes with at least 98% identity, at least 99% identity, or even 100% identity. Identity can be determined by inspection or using well known algorithms (Smith and Waterman, 1981 or Needleman and Wunsch, 1970, both supra). The invention therefore provides methods of determining a subject's propensity to develop age-related macular degeneration (AMD) or other conditions by detecting the presence or absence of a variation at a polymorphic site of a Factor H-related gene that corresponds to a homologous polymorphic site in the CFH or CFHR5 gene.


VII. Detection and Analysis of Factor H Polymorphisms Associated with AMD

The discovery that polymorphic sites and haplotypes in the Factor H gene and CFIHR5 gene are associated with AMD (and MPGNII) 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. Without intending to be limited to a specific mechanism, polymorphisms in the Factor H gene may contribute to the phenotype of an individual in different ways. Polymorphisms that occur within the protein coding region of Factor H may contribute to phenotype by affecting the protein structure and/or function. Polymorphisms that occur in the non-coding regions of Factor H may exert phenotypic effects indirectly via their influence on replication, transcription and/or translation. Certain polymorphisms in the Factor H gene may predispose an individual to a distinct mutation that is causally related to a particular AMD phenotype. Alternatively, as noted above, a polymorphism in the CFH gene, or in a CFHR5, may be linked to a variation in a neighboring gene (including but not limited to CFHR-1, 2, 3, or 4). The variation in the neighboring gene may result in a change in expression or form of an encoded protein and have detrimental or protective effects in the carrier.

A. Preparation of Samples for Analysis

Polymorphisms are detected in a target nucleic acid isolated from an individual being assessed. Typically 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, in the experiments described in Example 1, genomic DNA was 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 or liver or other organs or tissues; skin biopsies; amniotic fluid or CVS samples; and the like. Alternatively DNA or cDNA can be assayed. Alternatively, as discussed below, the assay can detect variant Factor H proteins. Methods for purification or partial purification of nucleic acids or proteins from patient samples for use in diagnostic or other assays are well known.

B. Detection of Polymorphisms in Target Nucleic Acids

The identity of bases occupying the polymorphic sites in the Factor H gene and the Factor H-Related 5 gene shown in TABLES 1A, 1B, 1C, 11, 14 and 15, as well as others in the dbSNP collection that are located in or adjacent to the Factor H or CFHR5 genes (see lists above), can be determined in an individual, e.g., in a patient being analyzed, using any of several methods known in the art. Examples include: use of 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., Puitke 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. Additional methods will be apparent to the one of skill.

The design and use of allele-specific probes for analyzing polymorphisms are described by e.g., Saiki et al., 1986; Dattagupta, EP 235,726, Soiak, 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 allele-specific probes for analyzing Factor H polymorphisms are shown in TABLE 16A. Using the polymorphism dbSNP No. rs1061170 as an illustration, examples of allele-specific probes include: 5’-TTTCCCTAT-AATTTTG-3’ (SEQ ID NO:235) (variant allele probe) and 5’-TTTCTCCATATTGT-TG-3’ (SEQ ID NO:235) (variant allele probe) and 5’-TAATCGGTTTGATAATGGA-A-3’ (SEQ ID NO:232) (reference allele probe) and 5’-TAATCGGTTTGATAATGGA-A-3’ (SEQ ID NO:233) (variant allele probe). In this example, the first set of allele-specific probes hybridize to the non-coding strand of the Factor H gene spanning the exon 9 polymorphism. The second set of allele-specific probes hybridize to the coding strand of the Factor H spanning the exon 9 polymorphism. These probes are 17 bases in length. The optimum lengths of allele-specific probes can be readily determined using methods known in the art.

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 allelic form. A single-base mismatch prevents DNA amplification and no detectable PCR product is formed. The method works best when the polymorphic site is at the extreme 3′-end of the primer, because this position is most destabilizing to elongation from the primer.

Exemplary allele-specific primers for analyzing Factor H polymorphisms are shown in TABLE 16B. Using the polymorphism dbSNP No. rs1061170 as an illustration, examples
of allele-specific primers include: 5'-CAAACTTTCTTC-CAIX-3' [SEQ ID NO:294] (reference allele primer) and 5'-CAAACTTTCTTCGATG-3' [SEQ ID NO:295] (variant allele primer); and 5'-GGATATAAATCCAAAT-3' [SEQ ID NO:292] (reference allele primer) and 5'-GGATATAAAT-CAAAATCT-3' [SEQ ID NO:293] (variant allele primer). In this example, the first set of allele-specific primers hybridize to the non-coding strand of the Factor H gene directly adjacent to the polymorphism in exon 9, with the last nucleotide complementary to the reference or variant polymorphic allele as indicated. These primers are used in standard PCR protocols in conjunction with another common primer that hybridizes to the coding strand of the Factor H gene at a specified location downstream from the polymorphism. The second set of allele-specific primers hybridize to the coding strand of the Factor H gene directly adjacent to the polymorphic site in exon 9, with the last nucleotide complementary to the reference or variant polymorphic allele as indicated. These primers are used in standard PCR protocols in conjunction with another common primer that hybridizes to the non-coding strand of the Factor H gene at a specified location upstream from the polymorphism. The common primers are chosen such that the resulting PCR products can vary from about 100 to about 300 bases in length, or about 150 to about 250 bases in length, although smaller (about 50 to about 100 bases in length) or larger (about 300 to about 500 bases in length) PCR products are possible. The length of the primers can vary from about 10 to 30 bases in length, or about 15 to 25 bases in length. The sequences of the common primers can be determined by inspection of the Factor H genomic sequence, which is found under GenBank accession number AL049744.

Many of the methods for detecting polymorphisms involve amplifying DNA or RNA from target samples (e.g., amplifying the segments of the Factor H gene of an individual using Factor H-specific primers) and analyzing the amplified gene. This can be accomplished by standard polymerase chain reaction (PCR & RT-PCR) protocols or other methods known in the art. The amplifying may result in the generation of Factor H allele-specific oligonucleotides, which span the single nucleotide polymorphic sites in the Factor H gene. The Factor H-specific primer sequences and Factor H allele-specific oligonucleotides may be derived from the coding (exons) or non-coding (promoter, 5' untranslated, introns or 3' untranslated) regions of the Factor H gene.

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 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., Cold Spring Harbor Laboratory, 1989) and Zyskind et al., Recombinant DNA Laboratory Manual (Acad. Press, 1988).


It will be apparent to the skilled practitioner guided by this disclosure that various polymorphisms and haplotypes can be detected to assess the propensity of an individual to develop a Factor H related condition. The following examples and combinations, and others provided herein, are provided for illustration and not limitation. In one aspect of the invention, the allele of the patient at one of more of the following polymorphic sites in the Factor H gene is determined: rs529825; rs800029; rs3766404; rs1061147; rs1061170; and rs203674. In one embodiment the allele of the patient at rs529825 is determined. In one embodiment the allele of the patient at rs800029 is determined. In one embodiment the allele of the patient at rs3766404 is determined. In one embodiment the allele of the patient at rs1061147 is determined.

In one embodiment the allele of the patient at rs1061170 is determined. In one embodiment the allele of the patient at rs203674 is determined. In one embodiment at least one of rs529825 and rs800029 is determined. In one embodiment at least one of rs1061147, rs1061170 and rs203674 is determined. In one embodiment at least one of rs529825 and rs800029 is determined, rs3766404 is determined, and at least one of rs1061147, rs1061170 and rs203674 is determined. In one embodiment alleles at rs529825, rs800029, rs3766404, rs1061170 and rs203674 are determined. The aforementioned polymorphisms and combinations of polymorphisms are provided herein for illustration and are not intended to limit the invention in any way. That is, other polymorphisms and haplotypes useful in practicing the invention will be apparent from this disclosure.

In a related aspect of the invention, the allele of the patient at one of more of the following polymorphic sites in the Factor H gene is determined: rs529825; rs800029; intron 2 (IVS2 or insTT); rs3766404; rs1061147; rs1061170; exon 10A; rs203674; rs375046; and exon 22 (1210). In one embodiment the allele of the patient at rs529825 is determined. In one embodiment the allele of the patient at rs800029 is determined. In one embodiment the allele of the patient at rs3766404 is determined. In one embodiment the allele of the patient at rs1061147 is determined. In one embodiment the allele of the patient at rs1061170 is determined. In one embodiment the allele of the patient at exon 10A is determined.

In one embodiment the allele of the patient at rs203674 is determined. In one embodiment the allele of the patient at rs375046 is determined. In one embodiment the allele of the patient at exon 10A is determined. In one embodiment the allele of the patient at rs1210 is determined. In one embodiment the allele of the patient at exon 22 (1210) is determined. In one embodiment at least one of rs529825 and rs800029 is determined; intron 2 is determined; rs3766404 is determined; at least one of rs1061147, rs1061170 and rs203674 is determined; exon 10A is determined; rs375046 is determined; and
exon 22 (1210) is determined. In one embodiment alleles at rs298285, rs800292, intron 2; rs3766404, rs1061170, exon 10A, rs203674, rs375046, and exon 22 (1210) are determined. In one embodiment one, two, three, four, five, or more than five of the following polymorphic sites in the Factor H gene are determined: rs529825; rs800292; intron 2 (IVS2 or insTT); rs3766404; rs1061147; rs1061170; rs2274700; exon 10A; rs203674; rs375046; and exon 22 (1210). The aforementioned polymorphisms and combinations of polymorphisms are provided for illustration and are not intended to limit the invention in any way.

As discussed above, the non-synonymous polymorphism at amino acid position 1210 in exon 22 of the Factor H gene is strongly associated with AMD, and the presence of cysteine at amino acid position 1210 of Factor H, therefore, provides a strong indication that the individual has AMD or is likely to develop AMD. Remarkably, the I120C is indicative of propensity to develop AMD or other complement-mediated conditions even when detected on allele that is otherwise protective (e.g., Y402). Thus, the allele of the patient at exon 22 (1210) is highly informative with respect to risk of developing AMD or other Factor H-associated diseases.

In a related aspect of the invention, the allele of an individual at one of more of the following polymorphic sites in the CFBHR5 gene is determined: rs9427661 (–294I>C); rs9427662 (–20F>C); and rs12097550 (P468S). In one embodiment the allele of the patient at rs9427661 is determined. In one embodiment the allele of the patient at rs9427662 is determined. In one embodiment the allele of the patient at rs12097550 is determined. In one embodiment at least one of rs9427661 and rs9427662 is determined. In one embodiment at least one of rs9427661 and rs9427662 is determined, and rs12097550 is determined. In one embodiment rs9427661, rs9427662, and rs12097550 is determined. The aforementioned polymorphisms and combinations of polymorphisms are provided for illustration and are not intended to limit the invention in any way. That is, other polymorphisms and haplotypes useful in practicing the invention will be apparent from this disclosure.

C. Detection of Protein Variants

In one embodiment of the invention, a protein assay is carried out to characterize polymorphisms in a subject’s CFH or CFBHR5 genes. Methods that can be adapted for detection of variant CFH, HFL1 and CFBHR5 are well known. These methods include analytical biochemical methods such as electrophoresis (including capillary electrophoresis 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, radioimmunoassay (RIA), enzyme-linked immunosorbent 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. Antibodies: a laboratory manual. Cold Spring Harbor, N.Y.; Cold Spring Harbor Laboratory; 1988; and Ausubel et al., (2004) Current Protocols in Molecular Biology, John Wiley & Sons, New York N.Y. The assay may be, for example, competitive or non-competitive. Typically, immunological binding assays (or immunooassays) utilize a “capture agent” to specifically bind to anl, often, immobilize the analyte. In one embodiment, the capture agent is a moiety that specifically binds to a variant CFH or CFBHR5 polypeptide or subsequence. The bound protein may be detected using, for example, a detectably labeled anti-CFH/CFBHR5 antibody. In one embodiment, at least one of the antibodies is specific for the variant form (e.g., does not bind to the wild-type CFH or CFBHR5 polypeptide. In one embodiment, the variant polypeptide is detected using an immunoblot (Western blot) format.

D. Patient Screening/Diagnosis of AMD

Polymorphisms in the Factor H gene, such as those shown in TABLE 1A, TABLE 1B, TABLE 1C or identified as described herein, which correlate with AMD or with particular subtypes of AMD, are useful in diagnosing AMD or specific subtypes of AMD, or susceptibility thereto. Polymorphisms in the CFBHR5 gene, such as those shown in TABLES 14 and 15 or identified as described herein, which correlate with AMD or with particular subtypes of AMD, are useful in diagnosing AMD or specific subtypes of AMD, or susceptibility thereto. These polymorphisms are also useful for screening for MPPNII and other Factor H-associated diseases.

Individuals identified as at high risk for developing AMD can take steps to reduce risk, including frequent ophthalmological examinations and treatments described below, known in the art, or develop in the future.

As described in Example 1, an at-risk CFH haplotype in combination with a triggering event (e.g., infection) appears to be sufficient for disease manifestation. Patients identified as at-risk for AMD can receive aggressive therapy (e.g., using antibiotics, anti-inflammatory agents, treatment with protective forms of CFH/CFBHR5, or treatment with other modifiers of CHF activity) at early signs of infection.

Combined detection of several such polymorphic forms (i.e., the presence or absence of polymorphisms at specified sites), for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all of the polymorphisms in the Factor H gene listed in TABLE 1A, TABLE 1B and/or TABLE 1C, alone or in combination with additional Factor H gene polymorphisms not included in TABLES 1A-1C, may increase the probability of an accurate diagnosis. Similarly, combined detection of several polymorphic forms in the CFBHR5 gene, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all of the polymorphisms in the CFBHR5 gene listed in TABLES 14 and 15, alone or in combination with additional CFBHR5 gene polymorphisms not included in TABLES 14 and 15, may increase the probability of an accurate diagnosis. In one embodiment, screening involves determining the presence or absence of at least one polymorphism in the Factor H gene and at least one polymorphism in the CFBHR5 gene. In one embodiment, screening involves determining the presence or absence of at least 2, 3, or 4 polymorphisms in the Factor H gene in combination with and at least 2, 3, or 4 polymorphisms in the CFBHR5 gene.

The polymorphisms in the Factor H and CFBHR5 genes are useful in diagnosing AMD or specific subtypes of AMD, or susceptibility thereto, in family members of patients with AMD, as well as in the general population.

In diagnostic methods, analysis of Factor H polymorphisms and/or CFBHR5 polymorphisms can be combined with analysis of polymorphisms in other genes associated with AMD, detection of protein markers of AMD (see, e.g., Hageman et al., patent publications US20030017501; US2002012581; WO0184149; and WO0106262), assessment of other risk factors of AMD (such as family history), with ophthalmological examination, and with other assays and procedures.

E. Identification of Patients for Drug Therapy

Polymorphisms in the Factor H gene and CFBHR5 gene are also useful for identifying suitable patients for conducting clinical trials for drug candidates for AMD. Such trials are
performed on treated or control populations having similar or identical polymorphic profiles at a defined collection of polymorphic sites in the Factor H gene and/or CFHR5 gene, or having similar or identical Factor H haplotypes and/or CFHR5 haplotypes. The use of genetically matched populations eliminates or reduces variation in treatment outcome due to genetic factors, leading to a more accurate assessment of the efficacy of a potential drug.

F) Screening Donor Tissue for Transplantation

Transplantation of organs (e.g., liver) and tissues (e.g., blood, hepatocytes) is increasingly common. It is desirable, in carrying out such transplantation, to avoid introducing into the recipient a deleterious form of Factor H or a Factor H-Related Protein and thereby increasing the recipient’s risk of developing AMD. Thus, in one aspect of the invention, a donor tissue is tested to detect the presence or absence of a variation at a polymorphic site of a Factor H or CFHR5 gene to identify host tissues carrying risk haplotypes or other deleterious sequences. In addition or alternatively, organs and tissues can be tested for the expression of forms of Factor H or CFHR5 proteins, for example by using immunological assays as described herein. In one embodiment the transplanted tissue is blood or plasma (i.e., given in a blood transfusion or plasma replacement). Routine screening of donated blood to avoid administration of a protein associated with risk (e.g., the 1210C form of CFH) may avoid compromising the recipient.

G) Phenotypic Categories

Susceptibility to specific subtypes of AMD can be identified based on the association with particular haplotypes. Thus, the screening can be used to determine suitable therapies for groups of patients with different genetic subtypes of AMD.

The methods may be used for the diagnosis of AMD, which may be subdivided into phenotypic categories (for example, early AMD (ARM) geographic atrophy (GA) and exudative AMD (CNV)). The ARM and GA phenotypes may be further subdivided into distinct phenotypes (for example, RPE changes alone, >10 macular hard drusen, macular soft drusen, BB (cuticular) drusen, pigment epithelial detachment (PED), “Cherokee” atrophy, peninsular geographic atrophy and pattern geographic atrophy). For descriptions of these phenotypes see, e.g., Bird et al., 1995, Surv Ophthalmol 39, 367-74; and Klaver et al., 2001, Invest Ophthalmol Vis Sci 42, 2237-41.

H) Other Diseases

Polymorphisms in the Factor H and CFHR5 gene, such as those shown in TABLES IA, 1B, 1C, 11, 14 and 15, can also be tested for association with other diseases, (for example, Alzheimer’s disease, multiple sclerosis, lupus, and asthma) and conditions (for example, burn injuries, transplantation, and stroke) which involve dysregulation of the alternative complement pathway, that have known but hitherto unmapped genetic components. Without being limited to any particular mechanism of action, it is suggested herein that expression of variant Factor H and/or CFHR5 polypeptides is associated with dysregulation of the alternative complement pathway. The variant forms of Factor H and/or CFHR5 may have a causal effect on diseases involving a defect in the alternative complement pathway, or the presence of variant forms of Factor H and/or CFHR5 may indicate that another gene involved in the alternative complement pathway has a causal effect.

Polymorphisms in the Factor H gene may also be useful in mapping and treating diseases that map to chromosome 1q, in particular at or near 1q32 where the Factor H gene is located. This particular locus contains a number of complement path- way-associated genes. One group of these genes, referred to as the regulators of complement activation (RCA) gene cluster, contains the genes that encode Factor H, five Factor H-related genes and the beta subunit of complement factor XII. A second cluster of complement-associated genes, including C4BPA, C4BPB, C4BPA2, DAF (CD55) CR1, CR2, CR1L, and MCP (CD46) lies immediately adjacent to the 1q25-31 locus.

VIII. Prevention and Treatment of AMD

A patient with a Factor H polymorphism can be treated by administering to a patient an antagonist of the variant Factor H polypeptide and/or variant CFHR5 polypeptide. An antagonist may include a therapeutic amount of an RNA complementary to the nucleotide sequence of a variant Factor H polypeptide and/or variant CFHR5 polypeptide or an antibody that specifically interacts with and neutralizes the activity of a variant Factor H polypeptide and/or variant CFHR5 polypeptide. Alternatively, AMD associated with the Factor H polymorphism and/or CFHR5 polymorphism can be treated by administering to a patient a form of Factor H and/or CFHR5 not associated with increased risk, such as the normal or wild-type Factor H protein and/or normal or wild-type CFHR5 polypeptide. In one method of the invention, a protective variant form of Factor H and/or protective variant form of CFHR5 is administered to a patient.

Therapeutic and prophylactic approaches in subjects identified as being at high risk for AMD include, but are not limited to, (1) increasing the amount or expression of neutral or protective forms of Factor H and/or neutral or protective forms of CFHR5; (2) decreasing the amount or expression of risk-associated forms of Factor H and/or risk-associated form of CFHR5; and (3) reducing activation of the complement alternative pathway. Examples of such therapeutic and prophylactic approaches include: (1) administration of neutral or protective forms of Factor H protein or therapeutically active fragments and/or neutral or protective forms of CFHR5 or therapeutically active fragments; (2) other wise increasing expression or activity of neutral and protective forms of Factor H; (3) interfering with expression of variant Factor H and/or variant CFHR5 proteins encoded by individuals with a risk haplotype by (e.g., by administration of antisense RNA); (4) reducing the amount of activity of a detrimental variant form.

Therapeutic agents (e.g., agents that increase or decrease levels of wild-type or variant Factor H or modulate its activity and/or agents that increase or decrease levels of wild-type or variant CFHR5 or modulate its activity) can be administered systemically (e.g., by i.v. injection or infusion) or locally (e.g., to the vicinity of the ocular RPE for treatment of AMD). Methods for administration of agents to the eye are well known in the medical arts and can be used to administer AMD therapeutics described herein. Exemplary methods include intraocular injection (e.g., retrobulbar, subretinal, intravitreal and intrachoroidal), iontophoresis, eye drops, and intraocular implantation (e.g., intravitreal, sub-Tenons and sub-conjunctival). For examples, anti-VEGF antibody has been introduced into cynomolgus monkeys by intravitreal injection (see, e.g., Gaudreault et al., 2005, "Preclinical pharmacokinetics of Ranibizumab (rhuFabV2) after a single intravitreal administration" Invest Ophthalmol Vis Sci. 46:726-33), and bioactive VEGF and bFGF have been expressed in the eye via intravitreal implantation of sustained release pellets (Wong et al., 2001, "Intravitreal VEGF and bFGF produce florid retinal neovascularization and hemorrhage in the rabbit" Curr Eye Res. 22:140-7). Importantly, it has been discovered that Fac-
tor H is synthesized locally by the retinal pigment epithelium (see Example 1), indicating that local administration of agents has therapeutic benefit.

A. Administration of Therapeutic Factor H Polypeptides

Administration of neutral or protective forms of Factor H polypeptides and/or neutral or protective forms of CFHRS polypeptides to subjects at risk for developing AMD (and/or with early stage disease) can be used to ameliorate the progression of the disease.

In one approach, recombinant Factor H polypeptide is administered to the patient. In one embodiment, the recombinant Factor H is encoded by a neutral haplotype sequence, which may be full-length (CFH/H1F1), truncated (FH1L1), or alternatively spliced form, or a biologically active fragment thereof. In another embodiment the recombinant Factor H has the sequence of a protective allele, either full-length or truncated form, or a protective polyclonally active fragment thereof. Methods for production of therapeutic recombinant proteins are well known and include methods described hereinbelow. The polypeptide can be administered systemically (e.g., intravenously or by infusion) or locally (e.g., directly to an organ or tissue, such as the eye or the liver).

Some protective forms of Factor H and the CHFL1 protein are less than full-length. For example, fragments of neutral or protective forms of Factor H may be administered for treatment or prevention of AMD or MPO-NII. In particular embodiment, polypeptides encoded by CFH splice variants expressed in individuals with a protected phenotype are administered. These proteins can be identified by screening expression of CFH-related RNA in individuals homozygous for a protective or neutral haplotype.

In particular embodiments, the protective protein has a sequence corresponding to one or more exons of the CFH gene sequence. For example, the protective protein may have the sequence of full-length or truncated CFH protein, except that the amino acid residues encoded by 1, 2, 3 or more exons (which may or may not be contiguous) are deleted.

In one embodiment a protective Factor H protein of the invention has an amino acid sequence substantially identical to SEQ ID NO:2, with the proviso that the residue at position 402 is not histidine and the residue at position 1210 is not cysteine. In one embodiment the residue at position 62 is not valine. Preferably, the residue at position 62 is isoleucine. Preferably the residue at position 62 is isoleucine, the residue at position 402 is tyrosine and the residue at position 1210 is arginine. Preferably the protective Factor H protein has 95% amino acid identity to SEQ ID NO:2 or a fragment thereof; sometimes at least 95% amino acid identity, sometimes at least 98% amino acid identity, and sometimes at least 99% identity to the reference Factor H polypeptide of SEQ ID NO:2. The polypeptide sequence of an exemplary protective variant of human Factor H [SEQ ID NO:5] is shown in FIG. 10. This protective variant Factor H polypeptide has an isoleucine at amino acid position 62 and a tyrosine at amino acid position 402 (indicated in bold). The polypeptide sequence of an exemplary protective variant of HFL1, the truncated form of human Factor H (SEQ ID NO:6) is shown in FIG. 11. This protective variant truncated Factor H polypeptide has an isoleucine at amino acid position 62 and tyrosine at amino acid position 402 (indicated in bold).

In one embodiment a protective Factor H protein of the invention has an amino acid sequence substantially identical to SEQ ID NO:4 (FH1L1). In one embodiment the residue at position 62 is not valine. Preferably, the residue at position 62 is isoleucine. Preferably the protective Factor H protein has 95% amino acid identity to SEQ ID NO:4 or a fragment thereof; sometimes at least 95% amino acid identity, sometimes at least 98% amino acid identity, and sometimes at least 99% identity to the reference Factor H polypeptide of SEQ ID NO:4.

In some embodiments the protective Factor H protein has one or more activities of the reference Factor H polypeptide. In one embodiment the activity is binding to CRP. In one embodiment the activity is binding to C3b. In one embodiment the activity is binding to endothelial cell surfaces. In one embodiment the activity is C3b co-factor activity. In one embodiment, the protective Factor H protein has activity that is higher with respect to its normal function than the protein of SEQ ID NO:2. In one embodiment, the protective Factor H protein has activity with respect to its normal function that is higher than the protein of SEQ ID NO:4.

Assays for Factor H activities are well known and described in the scientific literature. For illustration and not limitation, examples of assays will be described briefly.

Binding of Protective Proteins (CFH Variants) to C3b or CRP.

Interactions between C3b and CFH proteins can be analyzed by surface resonance using a BiaCore 3000 system (Biacore AB, Uppsala, Sweden), as described previously (Manuelian et al., 2003). Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. J Clin Invest 111, 1181-90). In brief, C3b (Calbiochem, Inc), are coupled using standard amine-coupling to flow cells of a sensor chip (Carboxylated Dextran Chip CM5, Biacore AB, Uppsala, Sweden). Two cells are activated and C3b (50 μg/ml, dialyzed against 10 mM acetate buffer, pH 5.0) is injected into one flow cell until a level of coupling corresponding to 4000 resonance units is reached. Unreacted groups are inactivated using ethanolic HCl. The other cell is prepared as a reference cell by injecting the coupling buffer without C3b. Before each binding assay, flow cells will be washed thoroughly by two injections of 2 M NaCl in 10 mM acetate buffer, pH 4.6 and running buffer (PBS, pH 7.4). The Factor H protein is injected into the flow cell coupled with C3b or into the control cell at a flow rate of 5 μl/min at 25°C. Binding of Factor H to C3b is quantified by measuring resonance units over time, as described in Manuelian et al., 2003, supra.

Interactions between CRP and CHF proteins can be analyzed by surface resonance in an identical manner by substituting CRP for C3b in flow cells of a sensor chip.

Binding to Endothelial Cell Surface

Binding of CHF proteins to endothelial cell surfaces is assayed by immunofluorescence staining of HUVECs and FACS analysis. HUVEC cells are kept in serum free DMEM (DMEM/Whitacker) for 24 hrs prior to the assay. Cells are detached from the surface with DPBS/EDTA and washed twice with DPBS. 5x10^6 cells will be transferred into plastic tubes and unspecific binding sites will be blocked with 1% BSA/DPBS for 15 min prior to incubation with purified allele variants of factor H (5 μg). Controls are performed in the absence of the factor H isoform. Following binding of factor H, cells are thoroughly washed with DPBS. Polyclonal goat anti-human FH antibody is used as a primary antibody (Calbiochem) (diluted 1:100), incubating cells at 4°C for 15 minutes. Alexa-fluor 488-conjugated goat antiserum diluted 1:100 in blocking buffer is used as the secondary antibody. Cells are examined by flow cytometry (FACScalibur, Becton-Dickinson Immunocytometry, Mountain View, Calif., USA). Typically, 10,000 events are counted.

Cofactor Activity in Fluid Phase

For the fluid phase cofactor assay, C3b biotin (100 ng/reaction), Factor I (200 ng/reaction) and 100 ng of purified
factor H are used in a total volume of 30 µl. Samples taken before and after addition of Factor I are separated by SDS-PAGE under reducing conditions and analyzed by Western blotting, detecting and quantitating C3b degradation products by Streptavidin-POD-conjugation (1:10000). C3b (40 µg) (CallBiochem) is biotinylated using the Biotin Labeling Kit (Roche Diagnostics, Mannheim, Germany), according to the manufacturer’s instructions. In brief, 30 µg of C3b (CalBiochem) is labeled with D-biotinyl-episilon-aminoacrylic acid-N-hydroxysuccinimide ester for 2 hours at 25°C. Excess biotin is removed by gel filtration using a PBS equilibrated PD10 column (Amersham Biosciences). Also see Sanchez-Corrall et al., 2002, Am J Hum Genet. 71:1285-95.

Heparin Binding Assay

Binding of purified CFH proteins (CFH402Y and CFH402H) to heparin is analyzed using heparin affinity chromatography in a high-performance liquid chromatograph (HPLC) system. 10 µg of CFH protein is diluted in 350µPBS and applied to a heparin-Sepharose affinity column (HLTrap, Amersham Biosciences) at a flow rate of 0.5 ml/min. The column is extensively washed with 350µPBS, and the bound protein eluted using a linear salt gradient ranging from 75 to 500 mM NaCl, in a total volume of 10 ml at a flow rate of 0.5 ml/min. Eluted fractions are assayed by SDS-PAGE and Western blot analysis. Elution of isoforms in different fractions is indicative of specific amino acid variations in the CFH protein can modulate binding of the protein to heparin. Also see, e.g., Pangburn et al., 1991, Localization of the heparin-binding site on complement Factor H, J Biol Chem. 266:16847-53.

CFHR5 Administration

In another approach, recombinant CFHR5 polypeptide is administered to the patient. In one embodiment, the recombinant CFHR5 has a neutral-type sequence, or a biologically active fragment thereof. In another embodiment the recombinant CFHR5 has the sequence of a protective allele, or a protective biologically active fragment thereof. Methods for production of therapeutic recombinant proteins are well known and include methods described hereinbelow. The therapeutic polypeptide can be administered systemically (e.g., intravenously or by infusion) or locally (e.g., directly to an organ or tissue, such as the eye or the liver).

Therapeutic Compositions Containing CFH or CFHR5 Polypeptides

The invention provides therapeutic preparations of Factor H polypeptides, which may be wild-type or variants (e.g., neutral or protective variants), and may be full length forms, truncated forms, or biologically active fragments of the variant Factor H polypeptides. As described herein, protective Factor H proteins (and genes encoding them) can be identified by identifying an individual as having a protective haplotype and determining the amino acid sequence(s) of Factor H encoded in the genome of the individual, where a protective Factor H protein is encoded by an allele having a protective haplotype. Biologically active fragments may include any portion of the full-length Factor H polypeptide which confers a biological function on the variant protein. In some cases, a protective haplotype will be associated with expression of a less-than full length form of Factor H (i.e., in addition to FH1-1) due, for example, to the presence of a premature stop codon in the gene.

Therapeutically active fragments can also be identified by testing the effect of the protein on expression of AMD biomarkers. Exemplary AMD biomarkers include complement pathway components (for example, Factor I, Factor H, C3, C3a), C reactive protein, haptoglobin, apolipoprotein E, immunoglobulin heavy or light chain(s), alpha 1 antitrypsin, alpha 2 macroglobulin, transthreutin, creatinine, and others described incopending provisional application No. 60/715, 503 entitled “Biomarkers Associated With Age-Related Macular Degeneration.”

The invention provides therapeutic preparations of CFHR5 polypeptides, which may be wild-type or variants (e.g., neutral or protective variants), and may be full length forms or biologically active fragments of the variant CFHR5 polypeptides. As described herein, protective CFHR5 proteins (and genes encoding them) can be identified by identifying an individual as having a protective haplotype and determining the amino acid sequence(s) of CFHR5 encoded in the genome of the individual, where a protective CFHR5 protein is encoded by an allele having a protective haplotype. Biologically active fragments may include any portion of the full-length CFHR5 polypeptide which confers a biological function on the variant protein. Therapeutically active fragments can also be identified by testing the effect of the protein on expression of AMD biomarkers as described above for Factor H.

Some forms of Factor H and CFHR5 can be isolated from the blood of genotyped donors, from cultured or transformed RPE cells derived from genotyped ocular donors, or from cell lines (e.g., glial or hepatic) that express endogenous Factor H. Alternatively, therapeutic proteins can be recombinantly produced (e.g., in cultured bacterial or eukaryotic cells) and purified using methods well known in the art and described herein. As noted above, some forms of Factor H and CFHR5 have been recombinantly expressed for research purposes. However, such research preparations are not suitable for therapeutic use. The present invention provides recombinant polypeptides suitable for administration to patients including polypeptides that are produced and tested in compliance with the Good Manufacturing Practice (GMP) requirements. For example, recombinant polypeptides subject to FDA approval must be tested for potency and identity, be sterile, be free of extraneous material, and all ingredients in a product (i.e., preservatives, diluents, adjuvants, and the like) must meet standards of purity, quality, and not be deleterious to the patient.

The invention provides a composition comprising a Factor H polypeptide or CFHR5 polypeptide, and a pharmaceutically acceptable excipient or carrier. The term “pharmaceutically acceptable excipient or carrier” refers to a medium that is used to prepare a desired dosage form of a compound. A pharmaceutically acceptable excipient or carrier can include one or more solvents, diluents, or other liquid vehicles; dispersion or suspension aids; surface active agents; isotonic agents; thickening or emulsifying agents; preservatives; solid binders; lubricants; and the like. Remington’s Pharmaceutical Sciences, Fifteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1975) and Handbook of Pharmaceutical Excipients, Third Edition, A. H. Kibbe ed. (American Pharmaceutical Assoc. 2000), disclose various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. 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 intracocular injection). For intracocular 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 therapeutically acceptable Factor H protein, optionally a lyophilized preparation. Therapeutic Factor H proteins or CFHR5 polypeptides can be made recombinantly, as described above.
tively, Factor H protein or CFHR5 polypeptide can be isolated from cultured RPE cells (e.g., primary cultures) or other cells that express Factor H or CFHR5 endogenously.

The amount of neutral or protective forms of Factor H or truncated Factor H, or biologically active fragments thereof, or neutral or protective forms of CFHR5, or biologically active fragments thereof, to be administered to an individual can be determined. The normal plasma concentration of Factor H varies between 116 and 562 micrograms/mL and the half-life of Factor H in plasma is about 6½ days (for a recent review, see Esparza-Gordillo et al., 2004 “Genetic and environmental factors influencing the human factor H plasma levels” Immunogenetics 56:77-82). In one embodiment, exogenous Factor H can be administered to an individual in an amount sufficient to achieve a level similar to the plasma concentration of Factor H in a healthy individual, i.e., an amount sufficient to achieve a plasma level of from 50 to 600 mg/mL, such as from 100 to 560 mg/mL. The amount of Factor H to be administered to an individual (e.g., a 160 pound subject) can be, for example and not for limitation, from 10 milligrams to 5000 milligrams per dose, from 50 milligrams to 2000 milligrams per dose, from 100 milligrams to 1500 milligrams per dose, from 200 milligrams to 1000 milligrams per dose, or from 250 milligrams to 750 milligrams per dose. The frequency with which Factor H can be administered to an individual can be, for example and not for limitation, once per day, twice per day, once per week, twice 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 Factor H to an individual can be readily determined by a physician by monitoring the course of treatment.

B) Gene Therapy Methods

In another approach, Factor H protein or CFHR5 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 Factor H polypeptide or biologically active fragment or CFHR5 polypeptide or biologically active fragment.

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, rhadoviruses, and papovaviruses. 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 Factor H polypeptide or CFHR5 polypeptide is typically linked to regulatory elements, such as a promoters and an enhancers, which drive transcription of the DNA in the target cells of an individual. The promoter may drive expression of the Factor H gene or CFHR5 gene in all cell types. Alternatively, the promoter may drive expression of the Factor H gene or CFHR5 gene only in specific cell types, for example, in cells of the retina or the kidney. The regulatory elements, operably linked to the nucleic acid encoding the Factor H polypeptide or CFHR5 polypeptide, are often cloned into a vector.

As will be understood by those of skill in the art, gene therapy vectors contain the necessary elements for the transcription and translation of the inserted coding sequence (and may include, for example, a promoter, an enhancer, other regulatory elements). Promoters can be constitutive or inducible. Promoters can be selected to target preferential gene expression in a target tissue, such as the RPE (for recent reviews see Sutanto et al., 2005 “Development and evaluation of the specificity of a cathepsin D proximal promoter in the eye” Curr Eye Res. 30:53-61; Zhang et al., 2004, “Concurrent enhancement of transcriptional activity and specificity of a retinal pigment epithelial cell-preferential promoter” Mol Vis. 10:208-14; Esumi et al., 2004, “Analysis of the VMD2 promoter and implication of E-box binding factors in its regulation” J Biol Chem 279:19064-73; Camacho-Hubner et al., 2000, “The Fugu rubripes tyrosinase gene promoter targets transegenic expression to pigment cells in the mouse” Genesis. 28:99-105; and references therein).


Gene therapy vectors must be produced in compliance with the Good Manufacturing Practice (GMP) requirements rendering the product suitable for administration to patients. The present invention provides gene therapy vectors suitable for administration to patients including gene therapy vectors that are produced and tested in compliance with the GMP requirements. Gene therapy vectors subject to FDA approval must be tested for potency and identity, be sterile, be free of extraneous material, and all ingredients in a product (i.e., preservatives, diluents, adjuvants, and the like) must meet standards of purity, quality, and not be deleterious to the patient. For example, the nucleic acid preparation is demonstrated to be mycoplasma-free. See, e.g., Islam et al., 1997, An academic centre for gene therapy research and clinical grade manufacturing capability. Ann Med 29, 579-595. Methods for administering gene therapy vectors are known. In one embodiment, Factor H or CFHR5 expression vectors are introduced systemically (e.g., intravenously or by infusion). In one embodiment, Factor H or CFHR5 expression vectors are introduced locally (i.e., directey to a particular tissue or organ, e.g., liver). In one preferred embodiment, Factor H or CFHR5 expression vectors are introduced directly into the eye (e.g., by ocular injection). For recent reviews see, e.g., Dinculescu et al., 2005, “Adeno-associated virus-vectorated gene therapy for retinal disease” Hum Gene Ther 16:649-63; Rex et al., 2004, “Adenovirus-mediated delivery of catalase to retinal pigment epithelial cells protects neighboring photoreceptors from photo-oxidative stress” Hum Gene Ther. 15:960-7; Bennett, 2004, “Gene therapy for Leber congenital amaurosis” Novartis Found Symp. 255:195-202; Hauswirth et al., “Range of retinal diseases potentially

Thus in one aspect, the invention provides a preparation comprising a gene therapy vector encoding a Factor H protein or CFHR5 polypeptide, optionally a viral vector, where the gene therapy vector is suitable for administration to a human subject and in an ex vivo format for administration to a human subject (e.g., produced using GLP techniques). Optionally the gene therapy vector comprising a promoter that is expressed preferentially or specifically in retinal pigmented epithelial cells.

Nonviral methods for introduction of Factor H or CFHR5 sequences, such as encapsulation in biodegradable polymers (e.g., polyactic acid (PLA); polyglycolic acid (PGA); and co-polymers (PLGA) can also be used (for recent reviews see, e.g., Beijani et al., 2005, “Nanoparticles for gene delivery to retinal pigment epithelial cells” Mol Vis. 11: 124-32; Mannergaa 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 Factor H polypeptide or CFHR5 polypeptide may be packaged into liposomes, or the nucleic acid can be delivered to an individual without packaging without using a vector.

C) DNA Repair

In another approach, subjects at risk for developing AMD (and/or with early stage disease) can have a risk form of Factor H or CFHR5 replaced by a neutral or protective form of Factor H or CFHR5 by DNA repair. In one embodiment, triplex forming oligonucleotides designed to specifically bind to polymorphic sites in the Factor H or CFHR5 gene associated with a risk haplotype can be administered to an individual by viral or nonviral methods. Triplex-forming oligonucleotides bind to the major groove of duplex DNA in a sequence-specific manner and provoke DNA repair, resulting in the targeted modification of the genome (for a recent review see Kuan et al., 2004, “Targeted gene modification using triplex-forming oligonucleotides” Methods Mol Biol. 262:173-94). A triplex-forming oligonucleotide that binds to a sequence spanning a polymorphism associated with a risk haplotype provokes DNA repair, resulting in the modification of the sequence from a risk allele to a neutral or protective allele and can ameliorate the development or progression of disease.

D) Introduction of Cells, Tissues, or Organs Expressing a Neutral or Protective Form of Factor H Protein or CFHR5 Polypeptide

In another approach, cells expressing neutral or protective forms of Factor H or Factor H-Related proteins (e.g., CFHR5) are administered to a patient. In an embodiment, the recipient is heterozygous or, more often, homozygous for a risk haplotype. For example, hepatocyte transplantation has been used as an alternative to whole-organ transplantation to support many forms of hepatic insufficiency (see, e.g., Ohashi et al., Hepatocyte transplantation: clinical and experimental application, J Mol Med. 2001 79:617-30). According to this method, hepatocytes or other CFH or CFHR5-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; Serralet 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 protein.

In another, related approach, therapeutic organ transplantation is used. Most of the body’s systemic Factor H is produced by the liver, making transplantation of liver tissue the preferred method. See, Gerber et al., 2003, “Successful (?) therapy of hemolytic-uremic syndrome with factor H abnormality?” Pediatr Nephrol. 18:1952-5.

In another approach, a protective form of CFH 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). CN1T 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 2005). Also see Song et al., 2003; Tao 2002, and Hammang et al., U.S. Pat. No. 6,649,184. In one embodiment of the present invention, a protective form of Factor H (including the so-called neutral form) is expressed in cells and administered in an encapsulated form. In one embodiment, the cells used are the NTC-201 human RPE line (ATCC # CRL-2302) available from the American Type Culture Collection P.O. Box 1549, Manassas, Va. 20108.

E) Therapy to Decrease Levels of Risk Variant of Factor H or CFHR5

Loss of the normal or protective function of Factor H or CFHR5 may be associated with AMD. Non-synonymous polymorphisms in the Factor H and CFHR5 genes, such as those shown in TABLES 1A, 1B, 1C, 11, 14 and 15, showing the strongest correlation with AMD and resulting in a variant Factor H polypeptide or CFHR5 polypeptide, are likely to have a causative role in AMD. For example, the variant Factor H or CFHR5 may act as a so-called “dominant-negative” mutant interfering with normal Factor H or CFHR5 function. Any method of reducing levels of the risk forms of Factor H or CFHR5 in the eye or systemically may be used for treatment including, for example, inhibiting transcription of a Factor H or CFHR5 gene, inhibiting translation of Factor H or CFHR5 RNA, increasing the amount or activity of a neutral or protective form of Factor H or truncated Factor H, or biologically active fragment thereof, increasing the amount or activity of a neutral or protective form of CFHR5 polypeptide, or biologically active fragment thereof, or decreasing the amount or activity of Factor H protein or CFHR5 polypeptides (e.g., by phosphomorphosis, antibody-directed phosphomorphosis, or complexing with a Factor H or CFHR5 binding moiety, e.g., heparin or variant specific antibody). In some
embodiments levels of Factor H or CFHR5 are preferentially reduced in the eye (e.g., RPE) relative to other tissues. For illustration and not limitation, several methods are briefly described below.

In one approach, a subject identified as being at risk for AMD is treated by administration of heparin. Heparin and heparin derivatives (including heparinoids) may have promising therapeutic properties for the treatment of various complement-associated diseases, including MPPGNII (Floeg et al., 1993; Girardi, 2005; Diamond and Karmovsky, 1986; Striker, 1999; Rops et al., 2004). In view of the association between AMD and MPPGNII disclosed herein, heparin and heparin derivatives (including heparinoids) may be efficacious for the treatment of AMD. In a clinical trial of patients with chronic proliferative glomerulonephritis receiving daily subcutaneous injections of heparin for over one year, Cade and colleagues reported improved creatinine clearance and a regression of glomerular hypercellularity (Cade et al., 1971). Both heparin and low molecular weight heparin (Enoxaparin) have been shown to prevent the progression of antiphospholipid antibody syndrome in mice by blocking the alternative and classical pathways of the complement cascade (Girardi et al., 2004). The anti-complement activity of heparin includes blockade of the formation of C3bBb, the amplification conversion by the alternative pathway; fluid phase heparin prevents the generation of C3bBb by inhibiting the interaction of C3b with factor B and factor D (Weiler et al., 1976).

F) Administration of Inhibitory Nucleic Acids

Antisense Nucleic Acids

Antisense nucleic acids, such as purified anti-sense RNA complementary to the RNA encoding a variant Factor H polypeptide can be used to inhibit expression of a Factor H gene associated with a risk haplotype. For recent reviews see, e.g., Giannes et al., 2005, “Intracellular delivery of oligonucleotides” Curr Pharm Biotechnol. 6:7-15; and Henry et al., 2004, “Setting sights on the treatment of ocular angiogenesis using antisense oligonucleotides” Trends Pharmacol Sci 25:523-7; and references cited therein.

RNA Interference

Double stranded RNA (dsRNA) inhibition methods can also be used to inhibit expression of HF1. The RNA used in such methods is designed such that at least a region of the dsRNA is substantially identical to a region of the HF1 gene; in some instances, the region is 100% identical to the HF1 gene. For use in mammals, the dsRNA is typically about 19-30 nucleotides in length (i.e., short interfering RNAs are used (siRNA or RNAi), and most often about 21 nucleotides in length. Methods and compositions useful for performing dsRNAi and siRNA are discussed, for example, in PCT Publications WO 98/53083; WO 99/32649; WO 99/53050; WO 00/44914; WO 01/36646; WO 01/75164; WO 02/44321; and U.S. Pat. No. 6,107,094. siRNA can be synthesized in vitro and administered to a patient. Alternatively, RNAi strategies can be successfully combined with vector-based approaches to achieve synthesis in transfected cells of small RNAs from a DNA template (see, e.g., Sui et al., 2002, “A DNA vector-based RNAi technology to suppress gene expression in mammalian cells” Proc Natl Acad Sci USA 99:5515-20; and Kasa- hara and Aoki, 2005, “Gene silencing using adenoviral RNAi vector in vascular smooth muscle cells and cardiomyocytes” Methods Mol Med. 112:155-72; and references cited therein).

Ribozymes

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of the sequence encoding human Factor H. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the sequences such as, GU, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Properties of ribozymes are well known in the art; for a general description see patents by Ceech (U.S. Pat. No. 6,180,399; U.S. Pat. No. 5,869,254; U.S. Pat. No. 6,025,167; U.S. Pat. No. 5,854,034; U.S. Pat. No. 5,591,610; U.S. Pat. No. 5,667,069; U.S. Pat. No. 5,354,855; U.S. Pat. No. 5,093,246; U.S. Pat. No. 5,180,818; U.S. Pat. No. 5,116,742; U.S. Pat. No. 5,037,746; and U.S. Pat. No. 4,987,071). Ribozymes and other inhibitory nucleic acids can be designed to preferably inhibit expression of a gene having a sequence associated with a risk haplotype. Thus, a ribozyme that recognizes the sequence spanning the polymorphism and cleaving adjacent to GUA recognizes the risk form but not the neutral or protective form, allowing selective cleavage (Dawson et al., 2000, “Hammerhead ribozymes selectively suppress mutant type I collagen mRNA in osteogenesis imperfecta fibroblasts” Nucleic Acids Res. 28:4013-20; Blalock et al., 2004 “Hammerhead ribozyme targeting connective tissue growth factor mRNA blocks transforming growth factor-beta mediated cell proliferation” Exp Eye Res. 78:1127-36).

Triplex-Forming Oligonucleotides

Triplex-forming oligonucleotides bind to the major groove of duplex DNA in a sequence-specific manner and provoke DNA repair, resulting in the targeted modification of the genome (for a recent review see Kuan et al., 2004, “Targeted gene modification using triplex-forming oligonucleotides” Methods Mol Biol. 262:173-94). Oligonucleotides can be designed to specifically bind to polymorphic sites in the Factor H gene associated with a risk haplotype. A triplex-forming oligonucleotide that binds to a sequence spanning a polymorphism associated with a risk haplotype provokes DNA repair, resulting in the modification of the sequence from a risk allele to a neutral or protective allele. Similar antisense nucleic acid, RNA interference, ribozyme and triplex-forming pliconucleotide methodologies as described above may be used to reduce levels of risk forms of CFHR5 in the eye or systemically for treatment of AMD.

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

G) Antibody Therapy

In one aspect, an anti-HF1 antibody that specifically interacts with and neutralizes the activity of a variant Factor H polypeptide is administered to an individual with or at risk for AMD. In one embodiment, the antibody recognizes both wild-type and variant Factor H protein. In one embodiment, the antibody recognizes the variant but not the wild-type Factor H protein. In another aspect, an anti-CFHR5 antibody that specifically interacts with and neutralizes the activity of a variant CFHR5 polypeptide is administered to an individual with or at risk for AMD. In one embodiment, the antibody recognizes both wild-type and variant CFHR5 protein. In one embodiment, the antibody recognizes the variant but not the
wild-type CFHR5 protein. The antibody can be administered systemically or locally (see, e.g., Guadarrama et al., 2005, "Preclinical pharmacokinetics of Rambizumab (rhuFabV2) after a single intravitreal administration" Invest Ophthalmol Vis Sci. 46:726-33). Methods for making anti-HF1 and anti-CFHR5 antibodies are known in the art, and include methods described below. In a related aspect, an agent that preferentially interacts with and reduces the activity of a variant Factor H polypeptide and/or CFHR5 polypeptide is administered to an individual with or at risk for AMD.

II) Modulators of the Alternative Pathway

In one aspect the invention provides methods for treating AMD by administering an agent (e.g., native protein, recombinant protein, antibody, or small molecule) directed at modulating the alternative pathway (AP) of the complement cascade, either locally in the eye or at the systemic level. In one embodiment, the treatment comprises administering an agent that modulates the AP directly. In one embodiment, the treatment comprises administering an agent that modulates the triggering of the AP (e.g., microvesicles). In one embodiment, the treatment comprises administering an agent that modulates pathways downstream from the AP. Exemplary agents that modulate the AP are known in the art and include, but are not limited to, DFP, PR226, BCX-1470, FUT-175, sMCP, PS-oligo, Comstatin, Fucan, and GCRI (see, e.g., Makrides, 1998, "Therapeutic inhibition of the complement system" Pharmacol Rev. 50:59-87; Holland et al., 2004, "Synthetic small molecule complement inhibitors" Curr Opin Investig Drugs 5:1163-73; Holers et al., 2004, "The alternative pathway of complement in disease: opportunities for therapeutic targeting" Mol Immunol. 41:147-52). AP modulators can be administered systemically or by intravitreal injection or other methods known for delivery of compounds to the eye.

J) Drug Screening/Antagonists of Risk Variant Factor H or Variant CFHR5

The invention provides a method of screening for an agent effective for treatment of AMD by contacting a variant protein, host cell or transgenic animal expressing a Factor H or CFHR5 variant, and monitoring binding, expression, processing or activity of the variant. In an embodiment, the Factor H variant has valine at amino acid 62 and/or has histidine at amino acid 402 and/or has cysteine at amino acid 1210. In an embodiment, the CFHR5 variant has a serine at amino acid 46.

Antagonists of variant Factor H polypeptides (e.g., variants associated with risk haplotypes) can be used to treat AMD. Antagonists may suppress expression of variant Factor H, suppress activity, or reduce RNA or protein stability. Antagonists can be obtained by producing and screening large combinatorial libraries, which can be produced for many types of compounds in a step-wise and high throughput fashion. Such compounds include peptides, poly-peptides, beta-turn mimetics, polysaccharides, phospholipids, hormones, prosta-lindins, steroids, aromatic compounds, heterocyclic compounds, benzodiazepines, oligomeric N-substituted glycines and oligocarbonates, and the like. Large combinatorial libraries of the compounds can be constructed by methods known in the art. See, e.g., WO 95/12608; WO 93/06121; WO 94/08051; WO 95/35503; WO 95/30642 and WO 91/18980.

Libraries of compounds are initially screened for specific binding to the variant Factor H polypeptide. Compounds with in vitro binding activity can also be assayed for their ability to interfere with a biological activity of the variant Factor H polypeptide, for example, binding to C3b or to heparin. Antagonist activity can be assayed in either a cell-based system or in a transgenic animal model in which exogenous variant Factor H polypeptide is expressed.

Antagonists of variant CFHR5 polypeptides (e.g., variants associated with risk haplotypes) can be used to treat AMD and can be obtained as described above for variant Factor H antagonists.

J) Patient Specific Therapy

Customized therapies can be devised for groups of patients with different genetic subtypes of AMD, based upon the presence of certain polymorphisms in the Factor H gene or CFHR5 gene having causative roles in AMD and having elucidated the effect of these polymorphisms on the expression level and/or biological activity of variant Factor H polypeptides or CFHR5 polypeptides. For example, if a polymorphism in Factor H or CFHR5 causes AMD in an animal model by increasing the expression level and/or biological activity of a variant Factor H polypeptide or CFHR5 polypeptide, AMD associated with the Factor H or CFHR5 polymorphism can be treated by administering to a patient an antagonist of the variant Factor H polypeptide or variant CFHR5 polypeptide.

K) Assessing Therapeutic Efficacy Using AMD Biomarkers

As noted above, therapeutic efficacy of particular fragments of CFH or CFHR5 proteins can also be determined by testing the effect of the protein on expression of AMD biomarkers. Exemplary AMD biomarkers include those described hereinabove. These AMD-associated proteins (biomarkers) are present in individuals with AMD at different (elevated or reduced) levels compared to healthy individuals. The invention provides methods of assessing the efficacy of treatment of AMD and monitoring the progression of AMD by determining a level of a biomarker(s) in an individual with AMD being treated for the disease and comparing the level of the biomarker(s) to an earlier determined level or a reference level of the biomarker. As described in copending provisional application No. 60/715,503 the level of the biomarker(s) can be determined by any suitable method, such as conventional techniques known in the art, including, for example and not for limitation, separation-based methods (e.g., gel electrophoresis), immunoassay methods (e.g., antibody-based detection) and function-based methods (e.g., enzymatic or binding activity). In one embodiment, a method of assessing the efficacy of treatment of AMD in a individual involves obtaining a sample from the individual and determining the level of the biomarker(s) by separating proteins by 2-dimensional difference gel electrophoresis (DIGE).

VIII. Factor H and CFHR5 Nucleic Acids

A) Primers and Probes

The invention provides nucleic acids adjacent to or spanning the polymorphic sites. The nucleic acids can be used as probes or primers (including Invader, Molecular Beacon and other fluorescence resonance energy transfer (FRET) type probes) for detecting Factor H polymorphisms. In one embodiment, the probes or primers recognize the insertion in intron 2 but do not recognize the wild-type sequence. Exemplary nucleic acids comprise sequences that span at least one of the polymorphisms listed in TABLES 1A, 1B, 1C, 11, 14 and 15 in which the polymorphic position is occupied by an alternative base for that position. The base for that position, which is found more frequently in the control population, is denoted the normal or wild-type sequence, whereas the alternative base for that position, which is found less frequently in the control population, is denoted the variant sequence. The nucleic acids also comprise sequences that span other polymorphisms known in the Factor H and CFHR5 genes, such as polymorphisms identified in Tables A and B above.
B) Expression Vectors and Recombinant Production of Factor H and CFHR5 Polypeptides

The invention provides vectors comprising nucleic acid encoding the Factor H polypeptide. The Factor H polypeptide may be wild-type or a variant (e.g., a protective variant) and may be a full-length form (e.g., HF1) or a truncated form. The nucleic acid may be DNA or RNA and may be single-stranded or double-stranded.

Some nucleic acids encode full-length, variant forms of Factor H polypeptides. The variant Factor H polypeptide may differ from normal or wild-type Factor H at an amino acid encoded by a codon including one of any non-synonymous polymorphic position known in the Factor H gene. In one embodiment, the variant Factor H polypeptides differ from normal or wild-type Factor H polypeptides at an amino acid encoded by a codon including one of the non-synonymous polymorphic positions shown in TABLE 1A, TABLE 1B and/or TABLE 1C, that position being occupied by the amino acid shown in TABLE 1A, TABLE 1B and/or TABLE 1C. It is understood that variant Factor H genes may be generated that encode variant Factor H polypeptides that have alternate amino acids at multiple polymorphic sites in the Factor H gene.

The invention provides vectors comprising nucleic acid encoding the CFHR5 polypeptide. The CFHR5 polypeptide may be wild-type or a variant (e.g., a protective variant). The nucleic acid may be DNA or RNA and may be single-stranded or double-stranded.

Some nucleic acids encode full-length, variant forms of CFHR5 polypeptides. The variant CFHR5 polypeptide may differ from normal or wild-type CFHR5 at an amino acid encoded by a codon including one of any non-synonymous polymorphic position known in the CFHR5 gene. In one embodiment, the variant CFHR5 polypeptides differ from normal or wild-type CFHR5 polypeptides at an amino acid encoded by a codon including one of the non-synonymous polymorphic positions shown in TABLES 14 and 15, that position being occupied by the amino acid shown in TABLES 14 and 15. It is understood that variant CFHR5 genes may be generated that encode variant CFHR5 polypeptides that have alternate amino acids at multiple polymorphic sites in the CFHR5 gene.

Expression vectors for production of recombinant proteins and peptides are well known (see Ausubel et al., 2004, Current Protocols In Molecular Biology, Greene Publishing and Wiley-Interscience, New York). Such expression vectors include the nucleic acid sequence encoding the Factor H polypeptide linked to regulatory elements, such as a promoter, which drive transcription of the DNA and are adapted for expression in prokaryotic (e.g., E. coli) and eukaryotic (e.g., yeast, insect or mammalian cells) hosts. A variant Factor H or CFHR5 polypeptide can be expressed in an expression vector in which a variant Factor H or CFHR5 gene is operably linked to a promoter. Usually, the promoter is a eukaryotic promoter for expression in a mammalian cell. Usually, transcription regulatory sequences comprise a heterologous promoter and optionally an enhancer, which is recognized by the host cell.Commercially available expression vectors can be used. Expression vectors can include host-recognized replication systems, amplifiable genes, selectable markers, host sequences useful for insertion into the host genome, and the like.

Suitable host cells include bacteria such as E. coli, yeast, filamentous fungi, insect cells, and mammalian cells, which are typically immortalized, including mouse, hamster, human, and monkey cell lines, and derivatives thereof. Host cells may be able to process the variant Factor H or CFHR5 gene product to produce an appropriately processed, mature polypeptide. Such processing may include glycosylation, ubiquitination, disulfide bond formation, and the like.

Expression constructs containing a variant Factor H or CFHR5 gene are introduced into a host cell, depending upon the particular construction and the target host. Appropriate methods and host cells, both prokaryotic and eukaryotic, are well-known in the art. Recombinant full-length human Factor H has been expressed for research purposes in Sf9 insect cells (see Sharma and Pangburn, 1994, Biologically active recombinant human complement factor H: synthesis and secretion by the baculovirus system, Gene 143:301-2). Recombinant fragments of human Factor H have been expressed for research purposes in a variety of cell types (see, e.g., Cheng et al., 2005, “Complement factor H as a marker for detection of bladder cancer” Clin Chem. 5:856-63; Vaziri-Sani et al., 2005, “Factor H binds to washed human platelets” J Thromb Haemost. 3:154-62; Gordon et al., 1995, “Identification of complement regulatory domains in human factor H” J Immunol. 155:348-56). Recombinant full-length human CFHR5 has been expressed for research purposes in Sf9 insect cells (see McRae et al., 2001, Human Factor H-related Protein 5 (CFHR75). J Biol. Chem. 276:6747-6754).

A variant Factor H or CFHR5 polypeptide may be isolated by conventional means of protein biochemistry and purification to obtain a substantially pure product. For general methods see Jacoby, Methods in Enzymology Volume 104. Academic Press, New York (1984); Scopes, Protein Purification, Principles and Practice, 2nd Edition, Springer-Verlag, New York (1987); and Deutscher (ed) Guide to Protein Purification, Methods in Enzymology, Vol. 182 (1990). Secreted proteins, like Factor H or CFHR5, can be isolated from the medium in which the host cell is cultured. If the variant Factor H or CFHR5 polypeptide is not secreted, it can be isolated from a cell lysate.

In one embodiment the vector is an expression vector for production of a variant Factor H protein having a sequence having non-wildtype sequence at one or more of the polymorphic sites shown in TABLES 1A, 1B and/or 1C.

In one embodiment the vector is an expression vector for production of a variant Factor H protein having a sequence of a protective variant of Factor H.

In one embodiment the vector is an expression vector for production of a variant CFHR5 protein having a sequence having non-wildtype sequence at one or more of the polymorphic sites shown in TABLES 14 and 15.

In one embodiment the vector is an expression vector for production of a variant CFHR5 protein having a sequence of a protective variant of Factor H.

C) Gene Therapy Vectors

Methods for expression of Factor H polypeptides or CFHR5 polypeptides for gene therapy are known and are described in Section IV(A) above.

XI. Antibodies

The invention provides Factor H-specific antibodies that may recognize the normal or wild-type Factor H polypeptide or a variant Factor H polypeptide in which one or more non-synonymous single nucleotide polymorphisms (SNPs) are present in the Factor H coding region. In one embodiment, the invention provides antibodies that specifically recognize variant Factor H polypeptides or fragments thereof, but not Factor H polypeptides not having a variation at the polymorphic site.

The invention also provides CFHR5-specific antibodies that may recognize the normal or wild-type CFHR5 polypep-
tide or a variant CFHR5 polypeptide in which one or more non-synonymous single nucleotide polymorphisms (SNPs) are present in the CFHR5 coding region. In one embodiment, the invention provides antibodies that specifically recognize variant CFHR5 polypeptides or fragments thereof, but not CFHR5 polypeptides not having a variation at the polymorphic site.

The antibodies can be polyclonal or monoclonal, and are made according to standard protocols. Antibodies can be made by injecting a suitable animal with a variant Factor H or variant CFHR5 polypeptide, or fragment thereof, or synthetic peptide fragments thereof. Monoclonal antibodies are screened according to standard protocols (Kociker and Milstein 1975, Nature 256:495; Dower et al.,WO 91/17271 and McCafferty et al., WO 92/01047; and Vaughan et al., 1996, Nature Biotechnology, 14: 309; and references provided below). In one embodiment, monoclonal antibodies are assayed for specific immunoactivity with the variant Factor H or CFHR5 polypeptide, but not the corresponding wild-type Factor H or CFHR5 polypeptide, respectively. Methods to identify antibodies that specifically bind to a variant polypeptide, but not to the corresponding wild-type polypeptide, are well-known in the art. For methods, including antibody screening and subtraction methods; see Harlow & Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Press, New York (1988); Current Protocols in Immunology (J. E. Coligan et al., eds., 1999, including supplements through 2005); and Goding, Monoclonal Antibodies, Principles and Practice (2d ed.) Academic Press, New York (1986); Burioni et al., 1998, “A new subtraction technique for molecular cloning of rare antiviral antibody specificities from phage display libraries” Res Virol. 149(5):327-30; Ames et al., 1994, Isolation of neutralizing anti-C5a monoclonal antibodies from a filamentous phage monovalent Fab display library. J Immunol. 152 (9):4572-81; Shinohara et al., 2002, Isolation of monoclonal antibodies recognizing rare and dominant epitopes in plant vascular cell walls by phage display subtraction. J Immunol Methods 264(1-2):187-94. Immunization or screening can be directed against a full-length variant protein or, alternatively (and often more conveniently), against a peptide or polypeptide fragment comprising an epitope known to differ between the variant and wild-type forms. Particular variants include the Y402H or 162V variants of CFH and HFL1, the R1210C variant of CFH, the P46S variant of CFHR5, and truncated forms of CFH. In one embodiment the HFL1 is measured. As discussed above, in one embodiment the ratio of HFL1 and CHF is measured. Monoclonal antibodies specific for variant Factor H or CFHR5 polypeptides (i.e., which do not bind wild-type proteins, or bind at a lower affinity) are useful in diagnostic assays for detection of the variant forms of Factor H or CFHR5, or as an active ingredient in a pharmaceutical composition.

The present invention provides recombinant polypeptides suitable for administration to patients including antibodies that are produced and tested in compliance with the Good Manufacturing Practice (GMP) requirements. For example, recombinant antibodies 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.

The invention provides a composition comprising an antibody that specifically recognizes a Factor H or CFHR5 polypeptide (e.g., a normal or wild-type Factor H polypeptide or a variant Factor H polypeptide, a normal or wild-type CFHR5 polypeptide or a variant CFHR5 polypeptide) and a pharmaceutically acceptable excipient or carrier.

In a related aspect, the invention provides a sterile container, e.g., vial, containing a therapeutically acceptable Factor H-specific or CFHR5-specific antibody. In one embodiment it is a lyophilized preparation.

In a related aspect, the invention provides pharmaceutical preparations of human or humanized anti-Factor H or anti-CFHR5 antibodies for administration to patients. Humanized antibodies have variable region framework residues substantially from a human antibody (termed an acceptor antibody) and complementarity determining regions substantially from a mouse antibody, (referred to as the donor immunoglobulin). See, Peterson, 2005, Advances in monoclonal antibody technology: genetic engineering of mice, cells, and immunoglobulins, ILAR J. 46:314-9, Kashmiri et al., 2005, SDR grafting—a new approach to antibody humanization, Methods 356:25-34, Queen et al., Proc Natl Acad Sci USA 86:10029-10033 (1989), WO 90/07861, U.S. Pat. No. 5,693,762, U.S. Pat. No. 5,693,761, U.S. Pat. No. 5,855,089, U.S. Pat. No. 5,530,101, and Winter, U.S. Pat. No. 5,225,539. The constant region(s), if present, are also substantially or entirely from a human immunoglobulin. The human variable domains are usually chosen from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable region domains from which the CDRs were derived. The heavy and light chain variable region framework residues can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies or can be consensus sequences of several human antibodies. See Carter et al., WO 92/22653. Certain amino acids from the human variable region framework residues are selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is by modeling, examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutation of particular amino acids.

For example, when an amino acid differs between a murine variable region framework residue and a selected human variable region framework residue, the human framework amino acid should usually be substituted by the equivalent framework amino acid from the mouse antibody when it is reasonably expected that the amino acid: (1) noncovalently binds antigen directly, (2) is adjacent to a CDR region, (3) otherwise interacts with a CDR region (e.g. is within about 6 Å of a CDR region), or (4) participates in the VL-VH interface.

Other candidates for substitution are acceptor human framework amino acids that are unusual for a human immunoglobulin at that position. These amino acids can be substituted with amino acids from the equivalent position of the mouse donor antibody or from the equivalent positions of more typical human immunoglobulins. Other candidates for substitution are acceptor human framework amino acids that are unusual for a human immunoglobulin at that position. The variable region frameworks of humanized immunoglobulins usually show at least 85% sequence identity to a human variable region framework sequence or consensus of such sequences.

IX. Identification of Risk, Protective, and Neutral Variations and Haplotypes

The invention provides methods of screening for polymorphic sites linked to polymorphic sites in the Factor H gene and/or CFHR5 gene described in Tables 1A, 1B, 1C, 11, 14 and 15. These methods involve identifying a polymorphic site in a gene that is linked to a polymorphic site in the Factor H
gene or CFHR5 gene, wherein the polymorphic form of the polymorphic site in the Factor H gene or CFHR5 gene is associated with AMD (e.g., increased or decreased risk), and determining haplotypes in a population of individuals to indicate whether the linked polymorphic site has a polymorphic form in equilibrium or disequilibrium with the polymorphic form of the Factor H gene or CFHR5 gene that correlates with the AMD phenotype.

Polymorphisms in the Factor H gene or CFHR5 gene, such as those shown in TABLES 1A, 1B, 1C, 11, 14 and 15, can be used to establish physical linkage between a genetic locus associated with a trait of interest and polymorphic markers that are not associated with the trait, but are in physical proximity with the genetic locus responsible for the trait and co-segregate with it. Mapping a genetic locus associated with a trait of interest facilitates cloning the gene(s) responsible for the trait following procedures that are well-known in the art.

Polymorphisms in the Factor H gene or CFHR5 gene, such as those shown in TABLES 1A, 1B, 1C, 11, 14 and 15, can be used in familial linkage studies to determine which polymorphisms co-segregate with a phenotypic trait, to determine individuals who require therapy, and to determine the effects of therapy.

Linkage is analyzed by calculation of a LOD (log of the odds) score, which is the log₁₀ of the ratio of the likelihood of obtaining observed segregation data for a marker and a genetic locus when the two are located at a recombination fraction theta, versus the situation in which the two are not linked (segregating independently). See Thompson & Thompson, Genetics in Medicine (5th ed., W.B. Saunders Company, Philadelphia, 1991) and Strachan, “Mapping the human genome” in The Human Genome (BOS Scientific Publishers Ltd., Oxford) Chapter 4). A LOD score of 3 indicates a 1000 to 1 odds against an apparent observed linkage being a coincidence. A LOD score of +3 or greater is considered definitive evidence that two loci are linked, whereas LOD score of -2 or less is considered definitive evidence against linkage.

X. Transgenic Non-Human Animals

The invention provides transgenic non-human animals capable of expressing human variant Factor H or CFHR5 polypeptides. Transgenic non-human animals may have one or both of alleles of the endogenous Factor H or CFHR5 gene inactivated. Expression of an exogenous variant Factor H or CFHR5 gene is usually achieved by operably linking the gene to a promoter and optionally an enhancer, and then microinjecting the construct into a zygote following standard protocols. See Hogan et al., “Manipulating the Mouse Embryo, A Laboratory Manual,” Cold Spring Harbor Laboratory. The endogenous Factor H or CFHR5 genes can be inactivated by methods known in the art (Cancevic, 1989). Factor H deficient mice are available for the introduction of exogenous human variant Factor H genes. Transgenic animals expressing human or non-human variant Factor H or CFHR5 polypeptides provide useful drug screening systems and as models of AMD and other complement related diseases. Transgenic animals may also be used for production of recombinant CFH and CFHR5 proteins of the invention (see, e.g., U.S. Pat. Nos. 6,066,725; 6,013,857; 5,994,616; and 5,959,171; Lillico et al., 2005; Houdébine, 2000).

XI. Kits

The invention provides reagents, devices and kits detecting Factor H or CFHR5 polymorphisms and haplotypes. Although particularly suited for screening for risk of developing AMD and/or for identifying appropriate therapy for preventing or ameliorating AMD in a subject, it will be understood that in certain embodiments these reagents, devices and kits can be used for analysis of Factor H and CFHR5 polymorphisms and haplotypes for any purpose, including but not limited to determining risk of developing MPO-NII or any other complement associated condition.

A number of assay systems are known in the art, and it is within the skill of the art to arrive at means to determine the presence of variations associated with AMD. The kit reagents, such as multiple primers, multiple probes, combinations of primers, or combinations of probes, may be contained in separate containers prior to their use for diagnosis or screening. In an embodiment, the kit contains a first container containing a probe, primer, or primer pair for a first CFH or CFHR5 allele described herein, and a second container containing a probe, primer, or primer pair for a second CFH or CFHR5 allele described herein.

In one embodiment, the invention provides kits comprising at least one Factor H or CFHR5 allele-specific oligonucleotide that hybridizes to a specific polymorphism in the Factor H or CFHR5 gene. The kits may contain one or more pairs of Factor H or CFHR5 allele-specific oligonucleotides hybridizing to different forms of a polymorphism. The Factor H or CFHR5 allele-specific oligonucleotides may include sequences derived from the coding (exons) or non-coding (promoter, 5' untranslated, introns or 3' untranslated) region of the Factor H or CFHR5 gene. The Factor H or CFHR5 allele-specific oligonucleotides may be provided immobilized on a substrate. The substrate may comprise either Factor H or CFHR5 allele-specific oligonucleotide probes for detecting at least 2, 3, 4, 5, more than 5, (e.g., at least 6, 7, or 8) or all of the polymorphisms shown in TABLES 1A, 1B, 1C, 11, 14 and 15 and/or other polymorphisms in the Factor H or CFHR5 gene (e.g., including polymorphisms listed above that are found in the SNP database). In one embodiment the kit is used to diagnose AMD. In a related embodiment, the kit is used to screen for another disease associated with variation in the Factor H or CFHR5 gene.

The kit may include at least one Factor H- or CFHR5-specific primer that hybridizes spanning or adjacent to a specific polymorphism in the Factor H or CFHR5 gene. The Factor H- or CFHR5-specific primers may include sequences derived from the coding (exons) or non-coding (promoter, 5' untranslated, introns or 3' untranslated) region of the Factor H or CFHR5 gene. Often, the kits contain one or more pairs of Factor H- or CFHR5-specific primers that hybridize to opposite strands of nucleic acid adjacent to a specific polymorphism in the Factor H or CFHR5 gene. In the presence of appropriate buffers and enzymes, the Factor H- or CFHR5-specific primer pairs are useful in amplifying specific polymorphisms in the Factor H or CFHR5 gene.

It will be apparent to the skilled practitioner guided by this disclosure that various polymorphisms and haplotypes can be detected to assess the propensity of an individual to develop a Factor H related condition. The following examples and combinations are provided for illustration and not limitation. In some cases, the assay identifies the allele at least one, at least two, at least three, at least four, at least five or at least six polymorphic sites in the Factor H or CFHR5 gene. In some cases, the assay identifies the allele at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all of the polymorphisms in the Factor H or CFHR5 gene listed in TABLES 1A, 1B, 1C, 11, 14 and 15. In one embodiment, the sites are selected from: rs5298225; rs800292; rs7705440; rs1061147; rs1061170; rs203674; and optionally including exons 22 (R120C). In one embodiment, the sites are
selected from rs529825; rs800292; intron 2 (IVS2 or ins/TT); rs3766404; rs1061147; rs1061170; exon 10A; rs203674; rs375046; and optionally including exon 22 (R1210C). In one embodiment, the sites are selected from: rs3753394; rs529825; rs800292; intron 2 (IVS2 or ins/TT); rs3766404; rs1061147; rs1061170; rs2274700; rs203674; rs3753396; rs1065489; and optionally including exon 22 (R1210C). In one embodiment, the sites are selected from: rs800292 (162V); IVS 2 (-18insTT); rs1061170 (Y402H); and rs2274700 (A473A). In one embodiment, the sites are selected from: rs9427661 (-249T>C); rs9427662 (-207C>T); and rs12097550 (P465S). In a preferred embodiment, a diagnostic/screening assay of the invention identifies the allele at least two polymorphic sites in the Factor H or CFHR5 gene. In a preferred embodiment, a diagnostic/screening assay of the invention identifies the allele at least three polymorphic sites in the Factor H or CFHR5 gene. In a preferred embodiment, a diagnostic/screening assay of the invention identifies the allele at least four polymorphic sites in the Factor H or CFHR5 gene.

In some cases, the kit includes primers or probes ("oligonucleotides") to identify the allele at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all of the polymorphisms in the Factor H or CFHR5 gene listed in TABLES 1A, 1B, 1C, 11, 14 and 15. In one embodiment the kit includes primers or probes to determine the allele at least one of the following polymorphic sites: rs529825; rs800292; rs3766404; rs1061147; rs1061170; and rs203674; and optionally including exon 22 (R1210C). In one embodiment the kit includes primers or probes to determine the allele at least one of the following polymorphic sites: rs529825; rs800292; intron 2 (IVS2 or ins/TT); rs3766404; rs1061147; and rs203674; and optionally including exon 22 (R1210C). In one embodiment the kit includes primers or probes to determine the allele at least one of the following polymorphic sites: rs529825; rs800292; intron 2 (IVS2 or ins/TT); rs3766404; rs1061147; rs1061170; rs2274700; rs203674; rs3753396; rs1065489; and optionally including exon 22 (R1210C). In one embodiment, the sites are selected from: rs9427661 (-249T>C); rs9427662 (-207C>T); and rs12097550 (P465S).

The kit 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 kit includes primers or probes distinguish alleles at rs529825 and rs800292. In one embodiment the kit includes primers or probes distinguish alleles at rs3766404. In one embodiment the kit includes primers or probes distinguish alleles at rs1061147. In one embodiment the kit includes primers or probes distinguish alleles at rs1061170. In one embodiment the kit includes primers or probes distinguish alleles at rs203674. In one embodiment the kit contains probes or primers for detecting at least one variation in the Factor H gene as well as probes or primers for detecting at least one variation in the CHFR-5 gene. In this embodiment, the kit optionally contains probes or primers for detecting more than one variation in either or both of the Factor H and CHFR-5 genes, such as two, three or more than three variations.
63

64


In one embodiment, the primers or probes distinguish alleles at (a) any one of more of rs529825; rs800292; rs3766404; rs1061147; rs1061170; and rs203674; (b) any one of more of intron 2 (IVS2 or insTT); rs2274700; exon 10A; and rs375046; (c) one or both of rs529825 and rs800292; (d) one or more of rs1061147, rs1061170 and rs203674; (e) at least one of rs529825 and rs800292; and rs3766404; and at least one of rs1061147, rs1061170 and rs203674; (f) at least 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 of more of rs529825; rs800292; rs3766404; rs1061147; rs1061170; rs203674; intron 2 (IVS2 or insTT); rs2274700; exon 10A; rs375046; and exon 22 (R1210C) and any one of more of rs9427661, rs9427662 and rs12097550. In one embodiment, the kits include oligonucleotides sufficient to distinguish any combination of alleles at the sites listed below in the context of devices.

The kits may include antibodies that specifically recognize the Factor H or CFHRS5 polypeptide. The Factor H- or CFHRS5-specific antibodies may recognize the normal, functional Factor H or CFHRS5 polypeptide or variant Factor H or CFHRS5 polypeptides in which one or more non-synonymous single nucleotide polymorphisms (SNPs) are present in the Factor H or CFHRS5 coding region.

XII. Diagnostic Devices

Devices and reagents useful for diagnostic, prognostic, drug screening, and other methods are provided. In one aspect, a device comprising immobilized primer(s) or probe(s) specific for one or more Factor H and/or CFHRS5 polymorphic sites is provided. In an embodiment the Factor H and/or CFHRS5 polymorphic sites are those described herein associated with AMD.

In one aspect, a device comprising immobilized primer(s) or probe(s) specific for one or more Factor H and/or CFHRS5 gene products (polynucleotides or proteins) is provided. The primers or probes can bind polynucleotides (e.g., based on hybridization to specific polymorphic sites) or polypeptides (e.g., based on specific binding to a variant polypeptide).

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

In one embodiment, the immobilized probe is an antibody or other Factor H or CFHRS5 binding moiety.

It will be apparent to the skilled practitioner guided by this disclosure that various polymorphisms and haplotypes can be detected to assess the propensity of an individual to develop a Factor H related condition. The following examples and combinations are provided for illustration and not limitation. In some cases, the array includes primers or probes to identify the allele at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all of the polymorphisms in the Factor H or CFHRS5 gene listed in TABLES 1A, 1B, 1C, 11, 14 and 15. In one embodiment the array includes primers or probes to determine the allele at least one of the following polymorphic sites: 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 insTT); rs3766404; rs1061147; rs1061170; exon 10A; rs203674; and 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: (a) rs3753394; (b) rs529825; (c) rs800292; (d) intron 2 (IVS2 or insTT); (e) rs3766404; (f) rs1061147; (g) rs1061170; (h) rs2274700; (i) rs203674; (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 (1624); IVS 2 (18insTT); 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: rs9427661 (~249T-C); rs9427662 (~201T-C); and rs12097550 (P468S).

The array can include primers or probes to determine the allele at two of the above sites, at least three, at least four, at least five or at least six. In one embodiment the primers or probes distinguish alleles at rs529825. In one embodiment the primers or probes distinguish alleles at rs800292. In one embodiment the primers or probes distinguish alleles at rs3766404. In one embodiment the primers or probes distinguish alleles at rs1061147. In one embodiment the primers or probes distinguish alleles at rs1061170.

In one embodiment the primers or probes distinguish alleles at rs203674. In one embodiment the primers or probes distinguish alleles at rs529825 and rs800292. In one embodiment the primers or probes distinguish alleles at rs203674. In one embodiment the primers or probes distinguish alleles at rs1061170.

In one embodiment, the primers or probes distinguish alleles at exon 22 (R1210C) and at rs529825; at rs800292; at rs3766404; at rs1061147; at rs1061170; at rs203674; at rs529825 and rs800292; at two or three of rs1061147, rs1061170 and rs203674; at rs529825 and rs800292, rs3766404, and two or three of rs1061147, rs1061170 and rs203674; or at rs529825, rs800292, rs3766404, rs1061147 and rs1061170; and
rs203674; (b) any one of more of intron 2 (IVS2 or insTT); rs2274700; exon 10A; and rs375046; (c) one or both of rs529625 and rs800029; (d) one or more of rs1061147, rs1061170 and rs203674; (e) at least one of rs529825 and rs800029; and rs3766404; and at least one of rs1061147, rs1061170 and rs203674; (f) at least one of rs529825, rs800029, rs3766404, rs1061170, and rs203674; (g) exon 22 (R1210C); (h) exon 22 (R1210C) and any of (a)-(g); or (i) any one or more of rs529825; rs800029; rs3766404; rs1061147; rs1061170; rs203674; intron 2 (IVS2 or insTT); rs2274700; exon 10A; rs375046; and exon 22 (R1210C) and any one or more of rs9427661, rs9427662 and rs12097550.

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 rs800029. In one embodiment the primers or probes distinguish alleles at intron 2 (IVS2 or insTT). In one embodiment the primers or probes distinguish alleles at rs3766404. In one embodiment the primers or probes distinguish alleles at rs1061147. In one embodiment the primers or probes distinguish alleles at rs1061170. In one embodiment the primers or probes distinguish alleles at rs203674. In one embodiment the primers or probes distinguish alleles at rs375046. In one embodiment the primers or probes distinguish alleles at exon 22 (R1210C). In one embodiment the primers or probes distinguish alleles at rs529825 and rs800029. In one embodiment the primers or probes distinguish alleles at two or three of rs1061147, rs1061170 and rs203674. In one embodiment the primers or probes distinguish alleles at of rs529825 and rs800029, at intron 2, at rs3766404, at two or three of rs1061147, rs1061170 and rs203674, at exon 10A, at rs2274700, and at rs375046. In one embodiment the primers or probes distinguish alleles at rs529825, rs800029, intron 2 (IVS2 or insTT), rs3766404, rs1061170, exon 10A, rs2274700, rs203674, and rs375046. In one embodiment, the primers or probes distinguish alleles at exon 22 (R1210C) and at either rs529825; at rs800029; at intron 2 (IVS2 or insTT); at rs3766404; at rs1061147; at rs1061170; at rs2274700, at exon 10A; at rs203674; at rs375046; at rs529825 and rs800029; or at two or three of rs1061147, rs1061170 and rs203674; at rs529825 and rs800029, intron 2 (IVS2 or insTT), rs3766404, two or three of rs1061147, rs1061170 and rs203674; intron 2 (IVS2 or insTT), rs3766404, rs1061170, rs2274700, exon 10A, and rs375046; or at rs529825, rs800029, intron 2 (IVS2 or insTT), rs3766404, two or three of rs1061147, rs1061170 and rs203674; intron 2 (IVS2 or insTT), rs3766404, rs1061170, rs2274700, exon 10A, and rs375046. In one embodiment, the device distinguishes any combination of alleles at the sites listed above in the context of kits.

In one embodiment, the substrate comprises fewer than about 1000 distinct primers or probes, often fewer than about 100 distinct primers or probes, fewer than about 50 distinct primers or probes, or fewer than about 10 distinct primers or probes. As used in this context, a primer is “distinct” from a second primer if the two primers do not specifically bind the same polynucleotide (i.e., such as cDNA primers for different genes). As used in this context, a probe is “distinct” from a second probe if the two probes do not specifically bind the same polypeptide or polynucleotide (i.e., such as cDNA probes for different genes). Primers or probes may also be described as distinct if they recognize different alleles of the same gene (i.e., CFH or CFH5). Thus, in one embodiment diagnostic devices of the invention detect alleles of CFH1 only, CFH5 only, CFH1 and CFH5 only, or CFH, CFH5 and up to 20, preferably up to 10, or preferably up to 5 genes other than CFH and/or CFH5. That is, the device is particularly suited to screening for AMD and related complement-associated diseases. In one embodiment, the device comprises primers or probes that recognize CFH and/or one or more of CFH1-5 only. In a related embodiment, the device contains primers and probes for up to 20, preferably up to 10, or preferably up to 5 other genes than CFH or CFH1-5.

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 CFHR5 gene. Exemplary allele-specific primers to identify alleles at polymorphic sites in the Factor H gene are shown in TABLE 16A. 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 Catar, Calif.), Protogene (Menlo Park, Calif.), Genomatrix (The Woodland, Tex.), Motorola BioChip Systems (Northbrook, Ill.), and Perlegen Sciences (Mountain View, Calif.).

In one embodiment, the immobilized probe(s) is/are an allele-specific probe(s) that can distinguish between alleles at a polymorphic site in the Factor H or CFH5 gene. Exemplary allele specific probes to identify alleles at polymorphic sites in the Factor H gene are shown in TABLE 16B. The immobilized allele-specific probes hybridize preferentially to nucleic acids, either RNA or DNA, that have sequences complementary to the probes. The hybridization may be detected by various methods, including fluorescence of hybridized nucleic acids (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 Catar, Calif.), Protogene (Menlo Park, Calif.), Genomatrix (The Woodland, Tex.), Motorola BioChip Systems (Northbrook, Ill.), and Perlegen Sciences (Mountain View, Calif.).

In certain embodiments probes or primers specific for particular SNPs and variations are excluded from a kit or a device of the invention. For example, in some embodiments, a kit or device does not include one or more of the following SNPs can be excluded: (i) rs529825; (ii) rs900292; (iii) intron 2 (IVS2 or insTT); (iv) rs3766404; (v) rs1061147; (vi) rs1061170; (vii) rs2274700; (viii) exon 10A; (ix) rs306374; (x) rs375046; (xi) rs3755396; (xii) rs1065489; or (xiii) exon 22 (R1210C).

XIII. Examples

Example 1

Common Haplotype in the Factor H Gene
(HF1/CFH) Predisposes Individuals to Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is the most frequent cause of irreversible blindness in the elderly in developed countries, affecting more than 50 million individuals worldwide. Our previous studies implicated activation of the alternative complement pathway in the formation of ocular
drusen, the hallmark lesion of AMD. We have also shown that macular drusen in AMD patients are indistinguishable from those that form at an early age in individuals with membra-noproliferative glomerulonephritis type 2 (MPGNII), a disease characterized by uncontrolled activation of the alternative pathway of the complement cascade. Here we show that Factor H protein (FH1), the major inhibitor of the alternative complement pathway, accumulates within drusen, and is synthesized locally by the retinal pigment epithelium. Previous linkage analyses identified chromosome 1q25-32, which harbors the Factor H gene (FH1/CFH), as a major AMD susceptibility locus. We analyzed FH1 for genetic variation in two independent cohorts comprised of approximately 900 AMD cases and 400 matched controls. We find a highly significant association of 8 common FH1 SNPs with AMD in these cohorts; two common missense variants exhibit highly significant associations (I62V, \( \chi^2 = 360.1, p = 3.2 \times 10^{-11} \) and Y402H, \( \chi^2 = 5.4, p = 1.6 \times 10^{-10} \)). Haplotype analysis suggests that multiple FH1 variants confer either an elevated or a reduced risk of AMD. One common at-risk haplotype is present at a frequency of 49% in AMD cases and 26% in controls (OR=2.67, 95% CI [1.80-2.85]). Homozygotes for this haplotype account for 22.1% of cases and 5.1% of controls (OR=5.26, 95% CI [2.84-9.76]). Several protective haplotypes are also identified (OR=0.44-0.55). Further strengthening these data is the finding of the risk haplotype in 70% of MPGNII patients. We propose that genetically pre-determined variation in regulators of the complement system, when combined with triggering events such as infection, underlie a major proportion of AMD in the human population.

Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in the developed world (Klein et al., 2004; van Leeuwen et al., 2003), affecting 15% of individuals over the age of 60 or an estimated 600 million individuals. AMD is characterized by a progressive loss of central vision attributable to degenerative and neovascular changes which occur at the interface between the neural retina and the underlying choroid. At this location lie the photoreceptors, the adjacent retinal pigmented epithelium (RPE), a basement membrane complex known as Bruch’s membrane BM), and a network of choroidal capillaries.

The prevailing view is that AMD is a complex disorder attributable to the interaction of multiple genetic and environmental risk factors (Klein et al., 2003; Tuo et al., 2004). Familial aggregation studies indicate that a genetic component can be identified in up to 25% of the cases (Klaver et al., 1998). As such, AMD appears to be a product of the interaction between multiple susceptibility loci rather than a collection of single-gene disorders. The number of loci involved, the attributable risk conferred, and the interactions between various loci remain obscure.

Linkage analyses and candidate gene screening have provided limited insight into the genetics of AMD. Reliable associations of one gene with increased risk, ABCA4 (Allikmets et al., 1997; Allikmets, 2000), and one gene with decreased risk, ApoE4 (Klaver et al., 1998; Souied et al., 1998), have been reported. Several groups have reported results from genome-wide linkage analyses (Tuo et al., 2004; Weeks et al., 2001). To date, linkage of one AMD phenotype (ARM1; MIM 603075) to a specific chromosomal region, 1q25-q31, has been documented (Klein et al., 1998); HEMICENTIN-1, also known as FBIB6, has been tentatively identified as the causal gene (Schultz et al., 2003), although it does not account for a significant disease load (Abeasis et al., 2004; Hayashi et al., 2004). The identification of overlapping loci on chromosome 1q by several groups (Weeks et al., 2001; Lyngar et al., 2003) indicates that this locus is likely to harbor a major AMD-associated gene.

In AMD, as well as many other diseases such as Alzheimer’s disease (Akiyama et al., 2000), atherosclerosis (Torzewski et al., 1997), and glomerulonephritis (Schwartz et al., 2000), characteristic lesions and deposits contribute to the pathogenesis and progression of the disease. Although the molecular pathogenesis of these diseases may be diverse, the deposits contain many shared molecular constituents that are attributable, in part, to local inflammation and activation of the complement cascade, a key element of host defense in the innate immune system. Drusen are the hallmark deposits associated with early AMD, and recent studies have implicated local inflammation and activation of the complement cascade in their formation as well (Hageman et al., 1999; Espinou-Heidmann et al., 2003). Drusen contain a variety of complement activators, inhibitors, activation-specific complement fragments, and terminal pathway components including the membrane attack complex (MAC), the lytic complex formed as a consequence of complement activation. The MAC is potentially lethal to host cells and tissues as well as foreign pathogens.

Many individuals with membranoproliferative glomerulonephritis type II (MPGNII), a rare kidney disease characterized by uncontrolled systemic activation of the alternative activation pathway of complement, also develop ocular drusen in the macula that are indistinguishable in composition and appearance from those in AMD (Mullins et al., 2001; O’Brien et al., 1993; MacAvoy et al., 2004). Furthermore, one patient diagnosed with MPGNII harbors a mutation in FH1 (FH1), a major inhibitor of the alternative pathway of complement activation (Zipfel, personal communication). Additionally, individuals in a few extended families with MPGNII, a related disorder, show linkage to a region of chromosome mapped in 1q31-32 (Neary et al., 2002) that overlaps the locus identified in genome-wide linkage studies for AMD. Collectively, these findings provided the impetus for examining whether FH1 is involved in the development of AMD and MPGNII.

In this investigation, we determined the frequencies of FH1 sequence variants in AMD and MPGNII patients and matched controls, and analyzed their association with disease phenotype. We also examined FH1 transcription and the distribution of FH1 protein in the macular RPE-choroid complex from normal and AMD donors.

Methods

Patients, Phenotyping and DNA

Two independent groups of AMD cases and age-matched controls were used for this study. All participating individuals were of European-American descent, over the age of 60, and enrolled under IRB approved protocols following informed consent. These groups were comprised of 404 unrelated patients with clinically documented AMD (mean age 79.5±7.8) and 131 unrelated, control individuals (mean age 78.4±7.4; matched by age and ethnicity) from the University of Iowa, and 550 unrelated patients with clinically documented AMD (mean age 71.32±8.9 years), and 275 unrelated, matched by age and ethnicity, controls (mean age 68.84±8.6 years) from the Columbia University. Patients were examined by indirect ophthalmoscopy and slit-lamp microscopy by retina fellowship-trained ophthalmologists.

Dr. Caroline Klaver, and later individuals trained by Dr. Klaver, graded fundus photographs at both institutions according to a standardized, international classification system (Bird et al., 1995). Control patients were selected and included if they did not exhibit any distinguishing signs of macular disease or have a known family history of AMD.
AMD patients were subdivided into phenotypic categories—early AMD (eAMD), geographic atrophy (GA) and exudative (CNV) AMD—based on the classification of their most severe eye at the time of their entry into the study. The University of Iowa eAMD and GA cases were further subdivided into distinct phenotypes (RPE changes alone, >10 macular hard drusen, macular soft drusen, BB (cuticular) drusen, PED, “Cherokee” atrophy, peninsular geographic atrophy and pattern geographic atrophy). The earliest documented phenotype for all cases was also recorded and employed in the analyses.

Genomic DNA was generated from peripheral blood leukocytes collected from case and control subjects using QIAamp DNA Blood Maxi kits (Qiagen, Valencia, Calif.).

Rapanui
Following an informed consent process approved by the Unidad de Bioetica, Ministerio de Salud (Santiago, Chile), 447 (66% female; 34% male) Easter Island inhabitants were provided a complete eye examination that included a dilated funduscopic examination. Medical, family and ophthalmic histories were taken and records and assistance from local physicians and community leaders were used to classify the ethnicity of the subjects. 49% of those patients examined were pure Rapanui, 9% were admixed (mixture of Rapanui and European, Chilean, Mapuchi and/or recent Polynesian), and 42% were continental (largely Chilean European). Peripheral venous blood and sera were collected from 201 of the older individuals; 114 of these individuals were pure Rapanui (108 were >50 years old; 89 were >60 years old). DNA from 60 of the pure Rapanui inhabitants and 13 of the Chilean residents over the age of 65 was used in this study.

Human Donor Eyes
Human donor eyes were obtained from the Iowa Lions Eye Bank (Iowa City, Iowa), the Oregon Lions Eye Bank (Portland, Oreg.) and the Central Florida Lions Eye and Tissue Bank (Tampa, Fla.) within five hours of death. Gross pathologic features of these eyes, as well as fundus photographs and angiograms, when available, were read and classified by retinal specialists. Fundi were graded according to a modified version of the International AMD grading system (Bird et al., 1995) by at least two individuals.

Total RNA was prepared from retina, RPE/choroid, and RPE cells derived from eyes using an RNeasy Mini Kit (Qiagen, Valencia, Calif.). Genomic DNA was sheared using a QiaShredder (Qiagen, Valencia, Calif.) and residual genomic DNA digested with DNase (Promega). RNA integrity was assessed using an Agilent BioAnalyzer.

DNA derived from 38 unrelated donors with clinically documented AMD (mean age 81.5±8.6) and 19 unrelated, control donors (mean age 80.5±8.8; matched by age and ethnicity) were employed for SSCP analyses and to assess potential genotype-phenotype correlations.

Immunohistochemistry

Wedges of posterior poles, including the ora serrata and macula were fixed and processed as described previously (Hageman et al., 1999). Some posterior poles were embedded directly in OCT without prior fixation. Tissues were sectioned to a thickness of 6-8 µm on a cryostat and immunolabeling was performed as described previously (Hageman et al., 1999). Adjacent sections were incubated with secondary antibody alone, to serve as controls. Some immunolabeled specimens were prepared and viewed by confocal laser scanning microscopy, as described previously (Anderson et al., 2002).

Polymerase Chain Reaction (PCR)
First strand cDNA was synthesized from total RNA using Superscript reverse transcriptase (Gibco BRL) and random hexamers. PCR reactions were carried out using the following primer sets: FHI ( exon 8 to exon 10) 5'-GAA-CATTTTGAGACTCGGT-3' [SEQ ID NO:324] and 5'-CACATCCATCATTCCACC-3' [SEQ ID NO:325]; HFL1 (exon 9 to exon 10) 5'-TCTTGGTACGCTTCTTC-3' [SEQ ID NO:326] and 5'-ACATCCATCATTCCACC-3' [SEQ ID NO:327]; HFL3 (exon 8 to exon 10) 5'-TCGCCAGGAATTTGACTG-3' [SEQ ID NO:327] and 5'-AGTCAC- CATACGAGGACCC-3' [SEQ ID NO:328]; HFL1 (exon 9 to exon 10) 5'-GGCTACGCTTCTTCCAAGAG-3' [SEQ ID NO:329] and 5'-AGTCACATGACGAGGACCC-3' [SEQ ID NO:330]. PCR primers (IDT, Coralville, Iowa) were designed using MacVector software (San Diego, Calif.). Reaction parameters were one cycle at 94°C for 3 minutes, 40 cycles at 94°C for 45 sec, 51.4°C (FHI) / 55°C (HFL1) for 1 min, 72°C for 1 min, and one cycle at 72°C for 3 min. The PCR products were run on 2% agarose gels and recorded using a Gel Doc 2000™ Documentation System accompanied by Quantity One® software (Bio-Rad, Hercules, Calif.).

Microarray Analyses:

DNA microarray analyses were performed using total RNA extracted from native human RPE or the RPE-Choroid complex (RNasey minikit, Qiagen, Valencia, Calif.) collected within <5 hours of death. Three different platforms were used: an 18,380 non-redundant DNA microarray (Incyte Pharmaceuticals; St. Louis, Mo.); the Affymetrix gene chip system; and a Whole Human Genome or Human 1A V2 oligo-microarray (Agilent Inc., Palo Alto, Calif.). The individual protocols followed each of the manufacturer’s instructions. For the Incyte analyses, cDNA derived from 6 mm punches of macular and mid-peripheral regions was labeled with 33-P in a random-primer reaction, purified and hybridized to the Nylon-based arrays containing 18,380 non-redundant cDNAs. The membranes were phosphoimaged, signals were normalized and data were analyzed using the Genome Discovery Software package. For the Affymetrix analyses, RPE and RPE/choroid (from 6-8 mm macular and peripheral punches) cDNA was hybridized directly to Affymetrix GeneChips (HG-U133A) using standardized protocols. These procedures were conducted in the University of Iowa DNA core facility, which is equipped with a fluids station and a Gene-Array scanner. The Agilent data was obtained from punches of the macula and mid-periphery. CY3 and CY5 labeled amplified cDNA derived from macular and peripheral RPE/choroid was generated using an Agilent Low Input RNA Amplification Kit using the macular and peripheral RNA from the same donor. The Agilent array data were collected from 3 normal young donors, 3 AMD donors, and 3 age-matched non-AMD controls using a VersArray Scanner; data were quantified using the VersArray Analyzer Software (Bio-Rad). The median net intensity of each spot was calculated using global background subtraction and the data was normalized using the local regression method.

Mutation Screening and Analysis

Coding and adjacent intronic regions of HFI1 (including exon 10A that is transcribed to generate the truncated FHL1 isoform) were examined for variants using single-strand conformation polymorphism (SSCP) analyses, denaturing high performance liquid chromatography (DHPLC) and direct sequencing. The remaining SNPs were typed by the 5′ nuclease (Taqman, ABI) methodology. Taqman genotyping and association analyses were performed as described (Gold et al., 2004). Primers for SSCP, DHPLC and DNA sequencing analyses (TABLE 5) were designed to amplify each exon and its adjacent intronic regions using MacVector software (San Diego, Calif.). PCR-derived amplicons were screened for sequence variation by SSCP and DHPLC, as described previously (Allikmets et al., 1997; Hiyashi et al., 2004). All
changes detected by SSCP and DHPLC were confirmed by
directional sequencing according to standard protocols.
Statistical analyses were performed using chi-square and
Fisher's exact tests.

Results

Factor H at the RPE-Choroid Interface

The distribution of Factor H protein in the RPE/choroid
complex from the macula and extramacula was assessed in
eyes obtained from six donors with a history of early AMD
and three donors of similar age without AMD or drusen
(FigS. 1A-1L). In donors with AMD, intense HF1 immu-
noreactivity (IR) is present in drusen, beneath the RPE (i.e.
the sub-RPE space), and around the choroidal capillaries
(FigS. 1A-1D, 1E, 1G). Absence of the primary anti-
body, labeling in the RPE/choroid is absent (Fig. 1F). All
of the Factor H antibodies labeled drusen to some degree, in
a homogenous fashion (FigS. 1C and 1E). One antibody also
labeled substructural elements within drusen (FigS. 1A and
1B). Such structures are also labeled using antibodies to
the activated complement component C3b/iC3b that binds HF1
(Anderson et al., 2004; Johnson et al., 2001). Factor H immu-
noreactivity is more robust in donors with AMD compared to
age-matched controls; it is also more pronounced in the
maculas of AMD donors than in the periphery (FigS. 1G and
1H). The anti-HF1 pattern in the macula (Fig. 1G) is highly
similar to the anti-C5b-9 pattern (FigS. 1I and 1K); in both
cases, labeling typically includes the choroidal capillaries.
Extramacular locations show much less anti-C5b-9 immu-
noreactivity (Fig. 1J). Little or no C5b-9 immunoreactivity in
the RPE-choroid is observed in donors under the age of 50
and without AMD (Fig. 1L).

Description of Fig. 1

(A-B) High magnification confocal immunofluorescence images from an 84 year old male donor diagnosed with atro-
phic AMD. Anti-HF1 (Advanced Research Technologies)
labeled of substructural elements (white arrows) in drusen
and the sub-RPE space is imaged on the Cy2/fluorescein
channel. The sub-RPE space is the extracellular compartment
between the basal RPE surface and the inner collagenous
layer of Bruch’s membrane. Such elements also display
immunoreactivity (IR) using monoclonal antibodies directed
against C3 fragments (iC3b, C3d, C3dg) that bind covalently
to complement activating surfaces (Johnson et al., 2001;
2003). The intense anti-Factor H labeling in the lumens of
choroidal capillaries (asterisks) from this donor most likely
reflects the high circulating levels of HF1 in the bloodstream.

Autofluorescent lipofuscin granules in the RPE cytoplasm
are labeled on the Cy3/Texas Red channel. Magnification bars.
A) 5 μm; B) 3 μm.

(C-D) Immunofluorescence localization of HF1 in drusen and
the sub-RPE space in an 83 year old male with
AMD using a different HF1 polyvalent antibody (Quidel)
(Cy2/fluorescein channel; green). C) In this donor eye, the
drusen (Dr) labeling pattern is homogeneous. D) Low magni-
figure of the RPE-choroid. Anti-HF1 IR is present
throughout the choroid and in the sub-RPE space (arrows),
the anatamical compartment where drusen and other deposits
associated with aging and AMD form. Lipofuscin autofluo-
rescence (Cy3 channel; red). Magnification bars. C) 10 μm;
D) 20 μm.

(E-F) Immunohistochemical localization of HF1 in drusen.
E) Anti-HF1 monoclonal antibody (Quidel) labeling, sig-
nified by the purple alkaline phosphatase reaction product, is
apparent in drusen, along Bruch’s membrane, and on the
choroidal capillary walls (arrows). F) Control section from
the same eye. In the absence of the primary antibody, no
labeling is present. Brown pigmentation in the RPE cyto-
plasm and choroid is melanin. Magnification bars=10 μm.

(G-H) Immunolocalization of HF1 in the macula. G) Extensive labeling is present along BM, the choroidal capi-
llary walls, and the intercapillary pillars (arrows) in a donor
with AMD. H) Control section from the macula of a donor
without AMD; much less labeling is apparent in same struc-
tures. Magnification bars=20 μm.

(I-J) Immunohistochemical localization of the comple-
ment membrane attack complex (C5b-9) in the RPE-choroid
underlying the macula (Fig. 1I) and extramacula (Fig. 1J)
from the same AMD donor eye. In the macula, intense anti-
C5b-9 labeling is associated with drusen, Bruch’s membrane,
and the choroidal capillary endothelium. Anti-C5b-9 labeling
outside the macula is restricted to a narrow zone in the vic-
inity of Bruch’s membrane. Brown pigment in the RPE cyto-
plasm and choroid represents melanin pigmentation. Magni-
fication bars=20 μm.

(K-L) Immunohistochemical location of C5b-9 in the
macula from a donor with AMD (Fig. 1K) and from a second
donor without AMD (Fig. 1L). Brown pigmentation in the
RPE cytoplasm and choroid represents melanin.

Labeled C5b-9 is associated primarily with the choroidal
kapillary walls (black arrows) and the intercapillary pillars
white arrows). Labeling is much more intense in the AMD
eye. Note the strong similarity to the anti-HF1 labeling pat-
tern in the macula from the same donor, as shown in Figure G.
Magnification bars. K=15 μm; L=20 μm.

The Retinal Pigment Epithelium is a Local Source of Factor
H

Expression of HF1 and FHL1 in the RPE, RPE/choroid and
retina was assessed by RT-PCR and DNA microarray analy-
sis. Appropriately sized PCR products for both gene products
are present in freshly isolated RPE and the RPE/choroid
complex, but not normal retina, in human eyes derived from
donors with and without AMD (Fig. 2). Primers were chosen
within exons 8, 9, 10A (the exon employed to generate the
truncated isoform FHL1) and 10 of the HF1 coding sequence.

The PCR reactions were performed with cDNA prepared from
RNA extracted from human neurosensory retina (lanes 2),
RPE and choroid (lanes 3), and freshly isolated RPE cells
(lanes 4) derived from a donor with a clinically documented
history of AMD. Genomic DNA was employed as a template
for amplification (lanes 5); no template was added to the
mixtures depicted in lanes 6. Lanes 1 contains the 100 bp
ladder. Amplifiers spanning from exon 8 to exon 10 (left
panel), and from exon 9 to exon 10 (right panel), of HF1 and
from exon 8 to exon 10A (left panel), and exon 8 to exon 10A
(right panel), of FHL1 were of the expected sizes (376, 210,
424 and 248 bp, respecively). Transcripts for FHRs 1-5 are
not detected in RPE or RPE/choroid, but FHRs 1-4 are
detected in neural retina by RT-PCR (data not shown).

Gene expression array data derived from three platforms
confirm that HF1 and FHL1 transcripts, but few if any of the
HF1-related protein transcripts (FHR1 being the possible
exception), are expressed locally by RPE and choroid cells.
Data derived from Incyte arrays probed with RPE/choroid
cDNA derived from nine donors with AMD and three age-
matched controls show elevated levels that average 2-3 times
that of HF1 mRNA in the donors with AMD. There is also a
trend toward slightly higher levels in the macula regions as
compared to the extramacular regions, although the differ-
ence is not statistically significant. The data generated from
the examination of isolated RPE and adjacent RPE/choroid
preparations from two donors with AMD and two age-
matched control donors using Affymetrix arrays confirm the
presence of HF1 transcripts in these tissues and shows that a
significant proportion of the HFI message is present within
the RPE layer (data not shown). Variants in HFI are
Associated with AMD and MGPNI

To test whether allele variants of HFI gene are associated
with AMD, all 22 coding exons and 50-100 bp flanking
intron sequences were screened, in a cohort of 404 AMD
patients and 131 matched controls at the University of
Wisconsin. A total of 26 sequence variants are detected; 17
SNPs in the coding region (cSNPs), including 5 synonymous
and 12 non-synonymous substitutions, and 9 intronic SNPs
(some of the variants shown in Fig. 3). Fig. 3 shows the
approximate locations of 11 SNPs used in the analysis, the 20 short
consensus repeats (SCRs), and the linkage disequilibrium (LD)
blocks, and the approximate binding sites for pathogens and
other substrates are depicted below the diagram based on
previously published data (Zipfel et al., 2002; Rodriguez de
Cordoba et al., 2004). cSNPs included previously described
common non-synonymous variants, such as 162V in exon 2,
Y402H in exon 9, and D936E in exon 18 (Fig. 3). An example of
a common intronic SNP with a potentially functional effect is
the IVS2–1insT variant. Five rare (<0.5%) variants are
also detected (data not shown) in both AMD patients and
controls, excluding the possibility of the disease phenotype
being caused by rare HFI alleles (i.e., disease-causing
mutations). Detailed genotyping data were obtained for 6 SNPs in
some or all of the 404 patients and 131 controls (TABLES 4
and 6A-6C) and association analyses were performed using a
case-control study design. Highly significant associations are
found with several individual variants, including 162V
(\(150\), p=1.1\times10^{-4}) and Y402H (\(149.4\), p=2.1\times10^{-5}).

The strongest association with AMD in this cohort is
observed with a synonymous A473A variant in exon 10,
resulting in an odds ratio (OR) of 3.42 (95% confidence
interval (CI) [2.7-5.15]).

These results were confirmed in an independent cohort of
AMD patients (n=550) and matched controls (n=275)
obtained at Columbia University, New York (TABLE 4). The
same two non-synonymous SNPs are also highly associated
with AMD in this second cohort (162V: \(1.5\), p=3.2\times10^{-7}
and Y402H: \(1.5\), p=1.6\times10^{-11}). In addition, several other
intronic SNPs were selected based on frequency and the
availability of commercial assays (for a total of 11 SNPs). The
strongest association in this cohort is observed with SNP
rs203674 in intron 10 (\(1.6\), p=4.29\times10^{-11}). This variant
shows an OR of 2.44 with AMD (95% CI=1.97-3.03). Although
the OR is modest, the variant is very common; 30.5% of the
cases are homozygous for allele B, but only
12.9% of the controls. The Q672Q and D936E alleles in exon
13 and 18 show no statistically significant association, sug-

The association is highly significant when the entire AMD
case and control cohorts are compared (TABLE 4). When
the major sub-phenotypes of AMD, such as the early AMD
eAMD, characterized by macular drusen and/or pigmen-
tary abnormalities), CNV (neovascular membranes and/or dis-
ciform scars), and GA (geographic atrophy) are analyzed sepa-

Factor H Polymorphisms and AMD

The data presented here links a major proportion of AMD
cases in two independent cohorts to specific polymorphisms
in the complement regulatory gene, Factor H (HFI/CFH)
(Zipfel, 2001; Rodriguez de Cordoba et al., 2004). Haplo-
type analysis shows the most frequent at-risk haplotype to be
present in almost half of individuals with AMD, compared to
approximately 25% of controls. The frequencies and extent of
SNP associations are very similar in the two cohorts and,
genotyped SNPs show highly significant association with
AMD in each. The associations are especially prominent in
cases of early AMD or choroidal neovascularization, less so
for geographic atrophy. The magnitude of the observed asso-

Linkage disequilibrium (LD) analysis showed extensive
LD across the entire HFI gene (TABLE 2 and Fig. 3). Three
SNPs in the exon 2-3 region are in virtually complete LD as
are the A307A and Y402H variants in exons 7 and 9, and the
Q672Q and D936E variants in exon 13 and 18 (TABLE 6 and
Fig. 5). Haplotype estimation in cases and controls identified
the most frequent at-risk haplotype in 49% of cases versus
only in 26% of controls (OR=2.95 95% CI [2.29-3.74]).

Homozygotes for this haplotype are present in 22.1% of the
Columbia cases and 5.1% of the controls (OR=5.26, 95% CI
[2.84-9.76]). Two common protective haplotypes are found in
30% of controls and 18% of cases (OR=0.476 95% CI [0.349-
0.650] and OR=0.472, 95% CI [0.320-0.698]). These haplo-
types differ only in the exon 2-3 locus SNPs and the intron 10
SNP. As shown in Fig. 4 and TABLE 2, these protective
haplotypes are closely related to each other and are both at
least five steps away from the risk haplotype. Interestingly,
the 3 SNPs (promoter -257C>G, A473A, and D936E) pre-
viously shown to be associated with HUS are all on one
relatively common haplotype (12%) that is neutral for AMD
risk (see the discussion below). For each SNP we identified
the base present in the consensus chimpanzee genome.
The haplotype generated represents the likely ancestral human
haplotype and is closely related to the protective haplotypes
(data not shown).

SNPs IVS2–1insTT and Y402H were also genotyped in
20 unrelated MGPNI patients, 52 Rapanui natives and small
cohorts of Hispanic Americans, African Americans and Euro-
American (TABLE 7). The frequency of the at-risk haplotype
was estimated in samples from different populations
from genotypes of the Y402H variant and/or the IVS10
locus. These include Rapanui natives over the age of 65
(AMD is extremely rare, and most likely absent, in this Easter
Island population), controls (~65 years of age) from Colum-
bia University, Hispanics general population, controls (~65
years of age) from the University of Iowa, African Americans
general population, AMD cases from Columbia University,
European Americans general population, AMD cases from the
University of Iowa and individuals with MGPNNI.
N-number of individuals. In the MGPNNI cohort, the fre-

Discussion
cation of specific HFI haplotypes with AMD is striking when compared to those genetic abnormalities previously linked to AMD.

Further support for the conclusion that specific HFI haplotypes confer increased risk for an AMD disease phenotype was obtained by genotyping of SNPs IVS2 –18insTT and Y402H in 20 unrelated patients with MPGNII, a renal disease associated with HFI mutations in which patients develop early onset macular drusen, and in 52 Rapanui natives, a race with a remarkably low incidence of AMD, if any. Approximately 70% of MPGN II patients and 19% of Rapanui were found to harbor the HFI at-risk haplotype in this study. Analysis of larger sample sets will be required to confirm these results, but the data do suggest that the HFI association with AMD may not be restricted to European-derived populations. Protective haplotypes we also identified, further implicating HFI function in the pathogenetic mechanisms underlying AMD.

Functional Implications of Factor H Polymorphisms

Factor H deficiencies in humans are associated with MPGN II and atypical hemolytic uremic syndrome (aHUS) (Zipfel et al., 2001). HFI deficiency arises from mutations leading to protein truncations or amino acid substitutions that result in protein retention in the endoplasmic reticulum. Reduced levels of plasma HFI ensue, leading to uncontrolled activation of the alternative complement pathway with concomitant consumption of C3 and other complement components. The HFI mutations that lead to aHUS, in contrast, are typically missense mutations that limit the complement-inhibitory functions of FF1 at the cell surface. Recent studies have revealed an association between three common SNPs and aHUS in individuals both with and without FF1 mutations (Caprioli et al., 2003). Furthermore, insults such as infection have been documented to trigger the manifestation of aHUS.

Most of the genotyped SNPs of HFI are located within important functional domains of the encoded protein (FIG. 3), which consists of 20 short consensus repeats (SCR) of 60 amino acids. The SCR repeats bind sites (SCR1-4, SCR12-14 and SCR19-20), heparin, sialic acid (SCR7, SCR13 and SCR19-20) and C-reactive protein (CRP) (SCR7). Therefore, SNPs located within the functional domains, although common, presumably affect protein function through variability in expression levels, binding efficiency, and other molecular properties. For example, the exon 2 162V variant is located in SCR2, which is included in the first C3b binding site, and the exon 9 Y402H variant is within SCR7 domain, which binds both heparin and CRP. Intronic SNPs, such as the IVS2 –18insTT variant, can affect splicing. For example, the analysis of the effect of the TT insertion (on the https://splice.cshl.edu/server) suggested a creation of a new cryptic splice acceptor 6 bp upstream of the natural acceptor site (data not shown). It is also possible that some of the studied SNPs affect the expression of HFI isoforms. For example, I62V is present in a predicted exon splice enhancer (Wang et al., 2004) (data not shown).

The functional consequences of common SNPs may be modest, since they are involved in late-onset phenotypes and not subjected to (rigorous) evolutionary constraint. HFI variant with more substantial effects, i.e., disease-causing mutations, are implicated in early-onset, severe (recessive) diseases, such as HFI deficiency and aHUS (Zipfel et al., 2002; Rodríguez de Córdoba et al., 2004; Caprioli et al., 2003; Zipfel, 2001). Of interest is the fact that true disease-causing mutations have been identified in only about 25% to 35% of HUS patients after complete screening of the HFI gene (Caprioli et al., 2003). At the same time, a disease-associated haplotype defined by variants -257C>T (promoter), A473A (exon 13) and D936E (exon 18) has been identified in HUS patients, predominantly in those persons with no disease-causing mutations (Caprioli et al., 2003). Moreover, the same study identified several families where affected probands had inherited a mutated allele from one parent and a susceptibility allele, from another. By contrast, healthy siblings of affected probands, carriers of the disease-causing mutation, had inherited a protective allele. In affected individuals from these families a disease-triggering effect, specified as bacterial or viral infection in >60% of cases, was identified in >80% of all cases and in >90% of cases with no apparent disease-associated mutation (Caprioli et al., 2003).

Together, these data strongly suggest that an at-risk HFI haplotype in combination with an infectious triggering event is sufficient for disease manifestation. Interestingly, the at-risk HFI haplotype in HUS (mainly C-terminal) does not overlap with that in AMD and/or MPGNII (TABLE 2), suggesting different triggers in HUS as opposed to MPGNII and AMD. Observation of early-onset drusen in MPGNII and those of the same composition in AMD, but not in HUS, support a different etiology for these diseases.

Disease-causing mutations in HFI are rare in MPGN II and have not been reported in AMD, nor did we find them after extensive screening in this study. However, we observed the same at-risk haplotype at a frequency of 70% of patients with MPGN II and approximately 80% in patients with AMD. These data are consistent with an at-risk HFI haplotype that, if triggered by an infectious agent, substantially increase one’s susceptibility to disease. The combined effect of these factors determines the severity the resulting phenotype, ranging from AMD to MPGNII.

Evolutionary analysis of the HFI haplotype indicates that the risk haplotype has evolved significantly from the ancestral haplotype found in the chimpanzee. FIG. 4 depicts a haplotype network diagram of HFI SNPs which shows the relationship between the haplotypes and the size of the circles is proportional to the frequency of the haplotype. The large filled-in circle represents the major risk haplotype, the vertically-lined circles are the two significant protective haplotypes, and the large open circle is the haplotype that contains the three SNPs associated with atypical hemolytic uremic syndrome (HUS), which is neutral for AMD risk. The putative ancestral haplotype is also indicated. It is possible that different forms of the HFI gene emerged in response to pathogens that activate the alternative complement pathway. Weakly acting HFI haplotypes could provide reduced complement inhibition and stronger protection against bacterial infection. However, such weak alleles could have the consequence of predisposing individuals to the consequences of complement system dysfunction. It is interesting that the AMD risk haplotype is principally different in the 5’ end of the gene that produces both the full length HFI and the FHII protein. In contrast the HUS mutations cluster in the 3’ portion of the gene that is found only in HFI. Therefore, it will be important to determine the role of these two forms of the protein in disease.

Biological Model of Factor H Dysfunction in AMD

One of the primary functions of the complement system is to provide defense against such infectious agents. It mediates the opsonization and lysis of microorganisms, removal of foreign particles, recruitment of inflammatory cells, regulation of antibody production, and elimination of immune complexes (Morgan et al., 1991; Kinoshita, 1991). Activation of the system triggers a sequential, amplifying, proteolytic cascade that gives rise to modifications of activating surfaces, to the release of soluble anaphylatoxins that stimulate inflam-
matory cells, and ultimately, to formation of the membrane attack complex (MAC), a macromolecular complex that promotes cell lysis through the formation of transmembrane pores. Uncontrolled activation of complement can lead to bystander damage to host cells and tissues. As a result, HF1, as well as other circulating and membrane-associated proteins, have evolved to modulate the system (Morgan, 1999).

A spectrum of complement components has been identified either within drusen (and/or the RPE cells that flank or overlie them), along Bruch's membrane, and/or on the choroidal endothelial cell membrane (Hageman et al., 2001; Mullins et al., 2000; Mullins et al., 2001; Anderson et al., 2002; Johnston et al., 2001; Crabb et al., 2002; Johnson et al., 2002; Mullins et al., 1997). These include terminal pathway complement components, activation-specific fragments of the terminal pathway, as well as various complement modulators. There is evidence that cell-mediated events may also contribute to this process (Penfold et al., 2001; Seddon et al., 2004; Miller et al., 2004).

We now show that HF1 is also a constituent of drusen in human donors with a prior history of AMD. Secondly, we show that HF1 co-localizes with alterations in genes that contain substructural elements within drusen, further implicating these structures as candidate complement activators (Anderson et al., 2004; Johnson et al., 2002). We also demonstrate that HF1 and the MAC, as shown by C5b-9 immunoreactivity, co-distribute at the RPE-choroid interface and that these deposits are more robust in eyes from donors with prior histories of AMD. Finally, HF1 and C5b-9 immunoreactivities are more intense in the macula compared to more peripheral locations from the same eye. All of these findings are consistent with the fact that AMD pathology is manifested primarily in the macula and with the conclusion that complement activation at the level of Bruch's membrane is a key element in the process of drusen formation as well as a contributing factor in the pathogenesis of AMD (Hageman et al., 2001; Anderson et al., 2002).

The distribution of HF1 at the RPE-choroid interface is strikingly similar to that of C5b-9, implying that significant amounts of the MAC are generated and deposited at the RPE-choroid interface. This suggests that the protein associated with the at-risk HF1 haplotype(s) may undergo a reduction in its normal ability to attenuate complement activation. Thus, the HF1 variants associated with AMD may put RPE and choroidal cells at sustained risk for alternative pathway-mediated complement activation, drusen formation and the disruption of Bruch's membrane integrity that is associated with late-stage AMD. Since Bruch's membrane is significantly thinner in the macula than elsewhere (Chong et al., 2005), it may be more susceptible to subsequent neovascular invasion. Because Bruch's membrane is significantly thinner in the macula than in the periphery, it may be more likely to become degraded to an extent that it is susceptible to neovascular invasion.

In summary, the results of this investigation provide strong evidence that common haplotypes in HF1 predispose individuals to AMD. We propose that alterations in genes that regulate the alternative pathway of the complement cascade, in combination with events that activate the system, underlie a major proportion of AMD in the human population.

Example 2

Variations in the Complement Regulatory Genes Factor II (CFH) and Factor H Related 5 (CFHR5) are Associated with Membranoproliferative Glomerulonephritis Type II (Dense Deposit Disease)

Introduction

Membranoproliferative glomerulonephritides are diseases of diverse and often obscure etiology that account for 4% and 7% of primary renal causes of nephrotic syndrome in children and adults, respectively (Orth et al., 1998). Based on renal immunopathology and ultrastructural studies, three subtypes are recognized. Membranoproliferative glomerulonephritis (MPGN) types I and III are variants of immune complex-mediated disease; MPGNII, in contrast, has no known association with immune complexes (Appel et al., 2005).

MPGNII accounts for less than 20% of cases of MPGN in children and only a fractional percentage of cases in adults (Orth et al., 1998; Habib et al., 1975; Habib et al., 1987). Both sexes are affected equally, with the diagnosis usually made in children between the ages of 5-15 years who present with non-specific findings like hematuria, proteinuria, acute nephritic syndrome or nephrotic syndrome (Appel et al., 2005). More than 80% of patients with MPGNII are also positive for serum C3 nephritic factor (C3NeF), an autoantibody directed against C3bBb, the convertase of the alternative pathway of the complement cascade (Schwertz et al., 2001). C3NeF is found in up to one-half of persons with MPGN types I and III and also in healthy individuals, making the electron microscopic demonstration of dense deposits in the glomerular basement membrane (GBM) necessary for a definitive diagnosis of MPGN II (Appel et al., 2005). This morphological hallmark is so characteristic of MPGN II that the disease is more accurately referred to as Dense Deposit Disease (MPGNII/DDD) (FIG. 12).

Spontaneous remissions of MPGNII/DDD are uncommon (Habib et al., 1975; Habib et al., 1987; Cameron et al., 1983; Barbiano di Belgiojoso et al., 1977). The more common outcome is chronic deterioration of renal function leading to end-stage renal disease (ESRD) in about half of patients within 10 years of diagnosis (Barbiano di Belgiojoso et al., 1977; Swainson et al., 1983). In some patients, rapid fluctuations in proteinuria occur with episodes of acute renal deterioration in the absence of obvious triggering events; in other patients, the disease remains stable for years despite persistent proteinuria.

C3NeF persists throughout the disease course in more than 50% of patients with MPGNII/DDD (Schwertz et al., 2001). Its presence is typically associated with evidence of complement activation, such as a reduction in CH50, decrease in C3, increase in C3dg/C3d and persistently high levels of activation of the alternative pathway of the complement cascade. C3, the most abundant complement protein in serum (~1.2 mg/ml), normally undergoes low levels of continuous auto-activation by hydrolysis of its thioester in a process known as tick-over. C3 hydrolysis induces a large conformational protein change, making C3(H2O) similar to C3b, a cleavage product of C3. C3(H2O) associates with factor B to form C3(H2O) Bb, which cleaves C3 to C3b in an amplification loop that consumes C3 and produces C3bBb (~ FIG. 13).

In MPGNII/DDD, C3NeF binds to C3bBb (or to the assembled convertase) to prolong the half-life of this enzyme, resulting in persistent C3 consumption that overwhelms the normal regulatory mechanisms to control levels of C3bBb and complement activation (Appel et al., 2005). Normal control involves at least seven proteins, four of which are present in serum (complement Factor H (CFH), complement factor H-like protein 1 (CFHL1), complement factor l (CFI) and C4 binding protein (C4BP)) and three of which are cell membrane-associated (membrane co-factor protein (MCP, CD46), decay accelerating factor (DAF, CD55) and complement receptor 1 (CR1, CD35)) (Appel et al., 2005; Meri et al., 1994; Pascual et al., 1994).
Of particular relevance to MPGNII/DDD is Factor H, one of 7 proteins in the Factor H family. In pigs and mice, its deficiency is associated with the development of renal disease that is similar at the light and electron microscopic level to MPGNII/DDD, and in humans its deficiency as well as mutations in the Factor H gene have been reported in patients with MPGNII/DDD (Mert et al., 1994; Dringen-Durey et al., 2004; Zipfel et al., 2005) (FIG. 14). The other 6 members of the Factor H family include FH1L, which is a splice isoform of Factor H, and five CFHR-related proteins encoded by distinct genes (CFHR1-5). There is little known about the latter five proteins, although they do show varying degrees of structural similarity to Factor H (Appel et al., 2005). Most interesting in this group with respect to MPGNII/DDD is CFHR5, because it shows the highest similarity to Factor H and has been demonstrated in renal biopsies of patients with other types of glomerulonephritis (Appel et al., 2005; Murphy et al., 2002). In vitro studies have also shown that V is present on surfaces exposed to complement attack, suggesting a possible role in the complement cascade (Murphy et al., 2002).

A possible relationship between Factor H/CFHR5 and MPGNII/DDD is further strengthened by the observation that patients with MPGNII/DDD develop an ocular phenotype called drusen. Drusen result from the deposition of abnormal extracellular deposits in the retina within the ocular Bruch’s membrane beneath the retinal pigment epithelium. The drusen of MPGNII/DDD are clinically and compositionally indistinguishable from drusen that form in age-related macular degeneration (AMD) (Mullins et al., 2001; Anderson et al., 2002), which is the most common form of visual impairment in the elderly (Klein et al., 2004; van Leeuwen et al., 2003). The single feature that distinguishes these two types of drusen is age of onset—drusen in MPGNII/DDD develop early, often in the second decade of life, while drusen in AMD are found in the elderly.

Four recent studies have implicated specific allele variants of Factor with AMD, suggesting that subtle differences in Factor H-mediated regulation of the alternative pathway of complement may play a role in a substantial proportion of AMD cases (Hageman et al., 2005; Edwards et al., 2005; Haines et al., 2005; Klein et al., 2005). One of these studies also showed that MPGNII/DDD and AMD patients segregate several of the same Factor H risk alleles (Hageman et al., 2005). In this study, we sought to refine the association of allele variations of Factor H and CFHR5 with MPGNII/DDD.

Materials and Methods

Patients and Controls.

Patients with biopsy-proven MPGNII/DDD and 131 persons without AMD participated in this study. Mean age of the control group was 78.4 years, reflecting our ascertainment criterion to exclude AMD.

Factor H, CFHR5 and MPGNII/DDD.

Allele frequencies of four of seven Factor H SNPs genotyped in the MPGNII/DDD patient group and the control population showed a significant association with the MPGNII/DDD disease phenotype at p<0.05. These SNPs included exon 2 J62V, IVS 2-18insTT, exon 9 Y402H and exon 10 A473A. Allele frequencies for exon 7 A307A, exon 13 Q672Q and exon 18 D936E were not significantly different between groups (TABLES 11 to 13).

Five CFHR5 SNPs were genotyped in the MPGNII/DDD patient group and control population, including one non-synonymous SNP (exon 2 P465S), two promoter SNPs (~249T>C, ~20T>C) and two intronic SNPs (IVS1+75T>A, IVS2+58C>T). Allele frequencies of these three SNPs~exon 2 P465S, ~249T>C and ~20T>C—were significantly different between groups at p<0.05 (TABLES 14 and 15).

Haploblocks.

Haplotypes that showed A307A and Y402H are in linkage disequilibrium in Factor H while ~249T>C and ~20T>C are in linkage disequilibrium in CFHR5 (FIG. 15).

Discussion.

The alternative pathway of complement represents an elegant system to protect humans from pathogens. Its central component, C3, circulates in plasma and is distributed throughout body fluids (Walport: 2001). Its activation creates a toxic local environment that damages foreign surfaces and results in the elimination of microbes. To prevent unrestricted complement activation, host cells and tissue surfaces down-regulate the amplification loop using a combination of surface-attached and membrane-bound regulators of complement. Some host cells express a single membrane-bound regulator of complement in high copy number, while other cells express several membrane-bound regulators and also attach soluble fluid-phase regulators. A few tissues lack membrane-bound regulators and depend exclusively on the attachment of soluble regulators (Appel et al., 2005).
In the kidney, endothelial and mesangial cells express two membrane-bound regulators of complement, MCP and DAF (van den Dobbelsteen et al., 1994; Timmerman et al., 1996). Podocytes express four: MCP, DAF, CR1 and CD59. Both mesangial cells and podocytes also secrete the soluble regulator, Factor H, which is up-regulated in membranous nephropathy in response to complement activation and inflammation (Angaku et al., 1998; Bao et al., 2002). Factor H acts in an autocrine fashion by binding directly to the secreting mesangial cells and podocytes.

The GBM, in contrast, is unique. It lacks endogenous membrane-bound regulators to protect it from complement-mediated injury, however it is highly negatively charged surface binds and absorbs Factor H (Zipfel et al., 2005). The dependency of the GBM on Factor H for local complement control is consistent with the finding of pathologic mutations in Factor H in a few patients with MPGNII/IDD (Ault et al., 1997; DeJong-Durey et al., 2004).

Our data identifying several allele variants of Factor H and CFIH5 associated with MPGNII/IDD is consistent with the hypothesis that complement control plays a role in the pathogenesis of this disease. A comparison of our data with reported AMD data adds additional support, as the allele frequency for each of the identified at-risk SNP variants we observed in Factor H is higher in the MPGNII/IDD patient cohort than in the AMD patient cohort, and strong evidence implicates Factor H in AMD (Hageman et al., 2005; Edwards et al., 2005; Haines et al., 2005; Klein et al., 2005). Although it is not known whether the amino acid changes in exons 2 and 9 of Factor H impact function, these changes are found in domains that interact with C3b and heparin, and differences in C3b/C3d and heparin binding have been demonstrated with several amino acid changes in Factor H that are associated with another renal disease, atypical hemolytic uremic syndrome (Manuelian et al., 2005) (TABLES 12 and 13).

With the exception of Factor H, the function of other members of the Factor H-related family is largely unknown and their expression patterns have not been explored, however studies of CFH5 have shown that it has properties similar to Factor H, including heparin, CRP and C3b binding (Murphy et al., 2002) (FIG. 14). This similarity suggests that like Factor H, CFH5 plays a role in MPGNII/IDD. Consistent with this is our finding of CFH5 expression in renal biopsies from two patients with MPGNII/IDD (data not shown).

Our genotyping data show that some allele variants of CFH5 preferentially associate with the MPGNII/IDD disease phenotype. Included are two SNPs in the promoter region of CFH5 which could affect transcription, one by removing a binding site for C/EBPbeta and the other by adding a GATA-1 binding site. The other significant association change a proline to serine in exon 2. Since exons 1 and 2 of CFH5 encode a domain homologous to short consensus repeat 6 (SCR6) of Factor H, which is integral to heparin and CRP binding, this change could affect complement activation and control.

Example 3

Production of Proteic Form of Factor H Protein

An exemplary protective form of human complement factor H (CFH) was prepared based on haplotype H2 (FIG. 5). Briefly, RNA was isolated from ocular tissues (RPE/choroid complexes) of four donors. The RNA was amplified by reverse transcription-polymerase chain reaction using the following primers:

5'-GAGAATTGGAAGAACTTTCTCCAGG-3' [SEQ ID NO: 331]
5'-AGATCTGAAATAGGAGTGC-3' [SEQ ID NO: 332]

RT-PCR reactions were performed using Superscript III One-Step High Fidelity with Platinum Taq, as described by the manufacturer (Invitrogen, Carlsbad, Calif.). The appropriate sized products (3,769 bp) were excised from agarose gels and isolated using spin columns.

The PCR products were cloned using the TOPO TA cloning system, as recommended by the manufacturer (Invitrogen) in the vector pCR2.1-TOPO. The complete genetic sequences of the clones derived from each of the four patients were determined by direct sequencing. The DNA derived from one patient (patient #498-01) had the fewest number of nucleotide polymorphisms relative to that of an exemplary protective reference sequence (H2), although this DNA encoded the risk sequence at amino acid position 402 (histidine) and encoded valine at amino acid position 62. To prepare a gene encoding a protective form of CFH we changed the bases encoding amino acid 62 such that it coded for isoleucine and those at position 402 such that they encoded a tyrosine, using the QuickChange Mutagenesis system (Stratagene, La Jolla, Calif.), resulting in SEQ ID NO:335. The amino acid at position 1210 of this protein is arginine. The oligonucleotides employed were as follows (plus the appropriate antisense version):

5'-GATAGATAAAATTATGGAAGAAGTTGCTGAC-3' [SEQ ID NO: 333]
5'-ATGATAAATGGAATATGGAAGAAGTTGCTGAC-3' [SEQ ID NO: 334]

The fidelity of the introduced mutations were confirmed by direct sequencing of the entire gene. The resulting protective gene was cloned into the eukaryotic expression vector pcDNA3.1 (Invitrogen) under control of the cytomegalovirus promoter. This expression vector was transfected into the human lung carcinoma cell line A549 (ATCC, Manassas, Va.) using the Exgen 500 transfection reagent (Fermentas, Hanover, Md.). Subsequent to transfection, cells were grown in serum-free media (Hybridoma-STM, Invitrogen).

Supernatants were collected 48 hours after transfection and subjected to Western blot analyses. The presence of the appropriate-sized product (approximately 150 kDa) was confirmed using monoclonal and polyclonal antibodies directed against human CFH (Quidel, San Diego, Calif.).
### TABLE 1A

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<th>Sequence Spanning Polymorphism</th>
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<th>AlleleFreq.</th>
<th>AlleleFreq.</th>
<th>ChI2 &amp; P Value</th>
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(*) Shows the non-coding strand of the genomic sequence.

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### TABLE 1C

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### TABLE 2

Haplotype Analysis of H1 SNPs in AMD Cases and Controls

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The frequency of allele 1 and allele 2 from each SNP was compared between cases and controls and the Yates Chi squared (X2) and P values were calculated along with the Odds Ratio (OR) and 95% confidence interval (95% CI). The actual counts of each genotype are given in Tables 6A-6C.

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| Count Allele 2 | 174 | 333 |

| Yates $\chi^2$/P value | 49.4 | 2.08746E-12 |
| OR/95% CI              | 2.82 | 2.11-3.78   |

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| Count allele 1 | 349 | 593 |
| Count allele 2 | 175 | 591 |

| Yates $\chi^2$/P value | 59.6 | 1.16235E-14 |
| OR/95% CI              | 2.34 | 1.89-2.91   |

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| Count Allele 1 | 74  | 355 |
| Count Allele 2 | 62  | 87  |

| Yates $\chi^2$/P value | 35.14 | 3.06833E-09 |
| OR/95% C              | 3.42  | 2.27-5.15   |

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| Count allele 1 | 285 | 771 |
| Count allele 2 | 243 | 313 |

| Yates $\chi^2$/P value | 45.4 | 1.60634E-11 |
| OR/95% CI              | 2.10 | 1.09-2.61   |

|        |       |   |        | 2.44 | 1.97-3.03 |
### TABLE 6-continued

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### TABLE 7

**Frequency of the At-risk Allele in Various Ethnic Groups with or without AMD**

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<th>Risk Haplotype</th>
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<th>Columbia Controls</th>
<th>Hispanic</th>
<th>Iowa Controls</th>
<th>African American Controls</th>
<th>African American Cases</th>
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<th>European American</th>
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The frequency of the at-risk haplotype was estimated in samples from different populations from genotypes of the Y480H variant and/or the IVS1030locus. These include Rapamani natives over the age of 65 (AMD is extremely rare, and most likely absent, in this Easter Island population), controls (<65 years of age) from Columbia University, Hispanics general population, controls (>65 years of age) from the University of Iowa, African Americans general population, AMD cases from Columbia University, European Americans general population, AMD cases from the University of Iowa and individuals with MPGNI.

N = number of individuals.

### TABLE 8

**Factor H Diplotypes**

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**Factor H Diplotypes**

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**CFH SNPs in 22 Patients Segregating with MPGNII**

(Angle Frequencies (F1 and F2) and Number of Patients by Genotype are Shown)

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### TABLE 12

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<th>p-value</th>
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<td>2 (A)</td>
<td>194 (A)</td>
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### TABLE 12-continued

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<th>p-value</th>
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### TABLE 13

**Coding SNPs Associated with MPGNII and the Related Short Consensus Repeat (SCR) of Factor H**

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<th>SCR Function of SCR</th>
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<td>Exon 2</td>
<td>1 Interaction with C3b</td>
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<td>Exon 9</td>
<td>2 Heparin binding</td>
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<td>A473A</td>
<td>8 Interaction with C reactive protein</td>
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### TABLE 14

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### TABLE 14-continued

CFHRS SNP in 22 Patients Segregating with MPGNII
(Allele Frequencies (f1 and f2) and Number of Patients by Genotype are Shown)

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### TABLE 16A

Probes

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<th>SEQ ID</th>
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**TABLE 16B**

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TABLE 16B-continued

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XV. References

Full citations are provided below for references cited in this text. The author and date are provided above:


113


Straub et al. “Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences.” *Proc Natl Acad Sci USA* 2002; 99(26):16899-16903.


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 the admission that any such document is pertinent prior art, nor does it constitute an 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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Glu Gly Phe Gly Ile Asp Gly Pro Ala Ile Ala Lys Cys Leu Gly Glu
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Gln Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe
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180 185 190
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<td>GLU GLU TYR GLY HIS ASP GLU VAL GLU TYR ASP CYS ASP PRO ASP</td>
<td>225 230 235 240</td>
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<td>PHE ILE ILE ASP GLY PRO LYS ILE GLN CYs VAL ASP GLY GLU TRP</td>
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<td>325 330 335</td>
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<td>340 345 350</td>
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Met Thr Thr Thr Val Asn Tyr Glu Asp Gly Glu Lys Val Ala Val Leu
405 410 415

Cys Lys Glu Asn Tyr Leu Leu Pro Glu Ala Lys Glu Ile Val Cys Lys
420 425 430

Asp Gly Arg Trp Glu Ser Leu Pro Arg Cys Val Glu Ser Thr Ala Tyr
435 440 445

Cys Gly Pro Pro Pro Ser Ile Asn Gly Asp Thr Thr Ser Phe Pro
450 455 460

Leu Ser Val Tyr Pro Pro Gly Ser Val Thr Val Tyr Arg Cys Gln Ser
465 470 475 480

Phe Tyr Lys Leu Gln Gly Ser Val Thr Val Thr Cys Arg Asn Lys Gln
485 490 495

Trp Ser Glu Pro Pro Arg Cys Leu Asp Pro Cys Val Val Ser Glu Glu
500 505 510

Asn Met Asn Lys Asn Asn Ile Gln Leu Lys Trp Arg Asn Asp Gly Lys
515 520 525

Leu Tyr Ala Lys Thr Gly Asp Ala Val Glu Phe Gln Cys Lys Phe Pro
530 535 540

His Lys Ala Met Ile Ser Ser Pro Pro Phe Arg Ala Ile Cys Gln Glu
545 550 555 560

Gly Lys Phe Glu Tyr Pro Ile Cys Glu
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Factor H gene polymorphism
<400> SEQUENCE: 9

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<210> SEQ ID NO 10
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism
<400> SEQUENCE: 10

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<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism
<400> SEQUENCE: 11

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<210> SEQ ID NO 12
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism
<400> SEQUENCE: 12

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42

aaaggaatacc atttaggact yatttagaaggt tagtgtaaacc a
41

caacccggg gaatacacagmaatgacaaaggtactggtctg a
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aaatggata taatcaaaat yatggaagaaagttggtaaca g
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tatgccttaa aagaaaaagc raaatatcaatgcacactag g
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<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism

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<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism

<400> SEQUENCE: 26
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<210> SEQ ID NO: 27
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (19)...(19)
<223> OTHER INFORMATION: Residues 18-19 may be optionally absent

<400> SEQUENCE: 27
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<210> SEQ ID NO: 28
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism

<400> SEQUENCE: 28
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<210> SEQ ID NO: 29
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism

<400> SEQUENCE: 29
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<210> SEQ ID NO: 30
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism

<400> SEQUENCE: 30
ggataatac aaatytatg aagagaaa g 31

<210> SEQ ID NO: 31
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism

<400> SEQUENCE: 31
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<210> SEQ ID NO 32
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism

<400> SEQUENCE: 32
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<210> SEQ ID NO 33
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism

<400> SEQUENCE: 33
accaataaaattctcagattttggtttgag g

<210> SEQ ID NO 34
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Factor H gene polymorphism

<400> SEQUENCE: 34
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<210> SEQ ID NO 35
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 35
acctgctcctcctctctc ac

<210> SEQ ID NO 36
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 36
cctctttttgc tgtgagacctc t

<210> SEQ ID NO 37
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 37
tgasaacgtagattgctgaaatgag t

<210> SEQ ID NO 38
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 38

caggtatcca gccagtacct gt 22

<210> SEQ ID NO 39
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 39
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<210> SEQ ID NO 40
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 40
ggcaggccac gcttactagat ttacc 25

<210> SEQ ID NO 41
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 41
tcaccatctg ctggtacata tctagt 27

<210> SEQ ID NO 42
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 42
tgggattttt tctgaatctc agtatacata tgc 33

<210> SEQ ID NO 43
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 43
aatcagcga aatgc 16

<210> SEQ ID NO 44
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 44
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<210> SEQ ID NO 45
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 45
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<210> SEQ ID NO 46
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 46
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<210> SEQ ID NO 47
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 47
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<210> SEQ ID NO 48
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 48
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<210> SEQ ID NO 49
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 49
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<210> SEQ ID NO 50
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 50
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<210> SEQ ID NO 51
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<221> NAME/KEY: misc_feature
<222> LOCATION: (21)...(21)
<223> OTHER INFORMATION: Residue 21 is G or H
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<210> SEQ ID NO 52
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)...(21)
<223> OTHER INFORMATION: Residue is C or D

<400> SEQUENCE: 52
tatctataa ggaagtaagaa ngatttcaat ataatgtaa c

<210> SEQ ID NO 53
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)...(21)
<223> OTHER INFORMATION: Residue is C or D

<400> SEQUENCE: 53
cactgaaact ggtggtgctt ggtgtgcttc atgtgaag

<210> SEQ ID NO 54
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)...(21)
<223> OTHER INFORMATION: Residue 21 is optionally absent

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<210> SEQ ID NO 55
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 55
acatatttc gcacctccac atcagattcc caatctccac a

<210> SEQ ID NO 56
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 56
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<210> SEQ ID NO 57
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 57

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<210> SEQ ID NO 58
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 58
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<210> SEQ ID NO 59
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 59
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<210> SEQ ID NO 60
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 60
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<210> SEQ ID NO 61
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 61
aacaactctcg tgtgatcag cccacagtc aaat 35

<210> SEQ ID NO 62
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<222> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 62
ctttgtatca ctggagggta nancaagcga atacatgta gasa 44

<210> SEQ ID NO 63
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polymorphism

<400> SEQUENCE: 63

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<210> SEQ ID NO 64
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 64

gcaaagtttt tgtataggc

<210> SEQ ID NO 65
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 65

aaccttcact tcgtgtaaac

<210> SEQ ID NO 66
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 66

ttagatagc ctgtgactg

<210> SEQ ID NO 67
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 67

tcaggtcataa ttgctac

<210> SEQ ID NO 68
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 68

actgttccu ccactc

<210> SEQ ID NO 69
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 69

ctcttttctc tatgactac
<210> SEQ ID NO 70
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 70
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<210> SEQ ID NO 71
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 71
acacctttcc tctgtgaagg

<210> SEQ ID NO 72
<211> LENGTH: 17
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 72
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<210> SEQ ID NO 73
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 73
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<210> SEQ ID NO 74
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 74
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<210> SEQ ID NO 75
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 75
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<210> SEQ ID NO 76
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 76
atacgagtaatgagc

<210> SEQ ID NO 77
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 77
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<210> SEQ ID NO 78
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 78
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<210> SEQ ID NO 79
<211> LENGTH: 18
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<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 79
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<210> SEQ ID NO 80
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 80
gtcctggctacaagttcc

<210> SEQ ID NO 81
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 81
gcatcagcaacatccttc

<210> SEQ ID NO 82
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 82
gcactgactctggagt

<210> SEQ ID NO 83
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 83
ttcacagcaagaagttcc
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 83
atgaacottg aacacag

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<400> SEQUENCE: 84
cggatactta tttctgc

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<400> SEQUENCE: 88
gtgaacgctt ggtatttcg

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ctggtatgaa cattttgag

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tgctotctct totctcg

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cattgttagt gtccttagg

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acatgctagg attcagag

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<400> SEQUENCE: 94
ctttttctta ttcttccce

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tcacacattg ctgttac

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tgtaacagca gatggtg

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<400> SEQUENCE: 97
cccacaaga gaacaaag

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<400> SEQUENCE: 98
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<400> SEQUENCE: 99
atgpcattca tagtcc

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<400> SEQUENCE: 100
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(gt) zaatcag accaacc

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<210> SEQ ID NO 103
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caagaaagag aatgcgaac

agattacagg caatggg

ttgattgtt aagatgc

ttgagaggtt caggaatgg

tgaaactct ctatgg

tgaaactct ccatgg
ttatatagtg gaggagag

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tggaaatgtt gaggc

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<400> SEQUENCE: 112

agttgcttg atctctactc

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ttggagcagt cacttctg

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<400> SEQUENCE: 114

ttatgccacc ctcacac

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atatcact gaccaacac

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gtctatgaga atacaagcc

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<400> SEQUENCE: 117
gaatctgagg tygagg

<210> SEQ ID NO 118
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<400> SEQUENCE: 118
cctttgatt ttcattc

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<400> SEQUENCE: 119
agaactccat ttccc

<210> SEQ ID NO 120
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<400> SEQUENCE: 120
cacaccccc ccagatag

<210> SEQ ID NO 121
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<212> TYPE: DNA
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<400> SEQUENCE: 121
gcctaacctt cacactg

<210> SEQ ID NO 122
<211> LENGTH: 21
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<400> SEQUENCE: 122
gtctagtag gcctctgtatt g

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<400> SEQUENCE: 123

egtaacctc ttcctcatac 20

<210> SEQ ID NO 124
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<400> SEQUENCE: 124

cctcctgtta aatctccac 19

<210> SEQ ID NO 125
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<212> TYPE: DNA
<213> ORGANISM: Artificial
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<400> SEQUENCE: 125

caggcacca tacttatgc 19

<210> SEQ ID NO 126
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 126

taaagtttg cggaac 16

<210> SEQ ID NO 127
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 127

ggctcatacc attttg 16

<210> SEQ ID NO 128
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<212> TYPE: DNA
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<400> SEQUENCE: 128

ttacaaatg gatggag 17

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<400> SEQUENCE: 129

aagtgcggg attacaggcg 20
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<212> TYPE: DNA
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<400> SEQUENCE: 130
catactcaaa tgacacactag g                                  21

<210> SEQ ID NO 131
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 131
ttaacccctg ctaactcc                                  19

<210> SEQ ID NO 132
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 132
taatgggaa cttgaccy                                  17

<210> SEQ ID NO 133
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 133
accttattc ttgtgttcg                                  20

<210> SEQ ID NO 134
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 134
gtggtgccg tggcc                                   15

<210> SEQ ID NO 135
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<212> TYPE: DNA
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<400> SEQUENCE: 135
gacttttct cagccac                                  17

<210> SEQ ID NO 136
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<212> TYPE: DNA
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<400> SEQUENCE: 136

tctccacat tgcgaac

17

<210> SEQ ID NO: 137
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
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<400> SEQUENCE: 137

aacttttagtt ttcagg

17

<210> SEQ ID NO: 138
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 138

gttttgatgtgttttgag

20

<210> SEQ ID NO: 139
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 139

atgtgtctcg caatgtg

17

<210> SEQ ID NO: 140
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 140

tggaggtgca gtgaagattg

20

<210> SEQ ID NO: 141
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 141

gctaatgatg cttttcacag ga

22

<210> SEQ ID NO: 142
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 142

cctgtgacgt ttaggcatt ttt

22

<210> SEQ ID NO: 143
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 143

tagcctgaa ttatactactggcc

<210> SEQ ID NO 144
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 144

gctttgcat gttaatttc ccctt

<210> SEQ ID NO 145
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 145

aactatgatg gaataatta aatotgg

<210> SEQ ID NO 146
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 146

tgcatactct gtctatcttc

<210> SEQ ID NO 147
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 147

gtttctcatt aaatatcatt aaagtctc

<210> SEQ ID NO 148
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 148

tttctcctca tttatacttg ag

<210> SEQ ID NO 149
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 149

cggtctttct aaggaatatc agca
<210> SEQ ID NO 150
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 150

cctgatggaa acaacatttc tg

<210> SEQ ID NO 151
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<213> ORGANISM: Artificial
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<400> SEQUENCE: 151

aacagggcca gaasagtcca

<210> SEQ ID NO 152
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 152

tgttcatttt aastgccattt tg

<210> SEQ ID NO 153
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 153

agttttcga gttgccggaa

<210> SEQ ID NO 154
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 154

cctagaaacc caatggaat gtg

<210> SEQ ID NO 155
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 155

tgttcaagca aagtgaacca a

<210> SEQ ID NO 156
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 156

**tgagcaatt tatgttctct attt**

24

<210> SEQ ID NO 157
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 157

**atgctacctt gttttaccas tgg**

23

<210> SEQ ID NO 158
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 158

**tgastgtcttc tggtatccga gtt**

23

<210> SEQ ID NO 159
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 159

**aaaaacctgca ggaacaagtc**

20

<210> SEQ ID NO 160
<211> LENGTH: 26
<212> TYPE: DNA
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<400> SEQUENCE: 160

**tcttgaatgt ggaattaacctc agattg**

26

<210> SEQ ID NO 161
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 161

**tggttttccaa gaaattcatt tcca**

24

<210> SEQ ID NO 162
<211> LENGTH: 24
<212> TYPE: DNA
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<400> SEQUENCE: 162

**atgttaaatt aaaccttgccgca atga**

24

<210> SEQ ID NO 163
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<220> FEATURE:
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<400> SEQUENCE: 163

ttgctgaaat aagaattaga actttg

<210> SEQ ID NO 164
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 164
tgastaaaag aagaaatcct ttccca

<210> SEQ ID NO 165
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 165
atctaaaca catacatcat gttttaa

<210> SEQ ID NO 166
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 166
aaaaacata catcatgttt tcaaa

<210> SEQ ID NO 167
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 167
gatagcctc aacatttcca gtc

<210> SEQ ID NO 168
<211> LENGTH: 23
<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 168
gttggtttga ttoctatcat ttg

<210> SEQ ID NO 169
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<400> SEQUENCE: 169
ttgaaagt astaggtatg tgtgtc

26
25
27
26
23
23
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<220> FEATURE:
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<400> SEQUENCE: 170
tatgagaattacaagccaaaagttc 25

<210> SEQ ID NO 171
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 171
ttccttgtgtcttcgtgtaaaca 23

<210> SEQ ID NO 172
<211> LENGTH: 23
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 172
aacccctttgtaattcattcttca 23

<210> SEQ ID NO 173
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 173
tcasaagtgaaggaaatattga 22

<210> SEQ ID NO 174
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 174
aatttatgagttagtgaaccctggaat 26

<210> SEQ ID NO 175
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 175
ttcctactcaagatgtaaggttacc 26

<210> SEQ ID NO 176
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 176
acaaaatgcctaatatattttctcag 27

<210> SEQ ID NO 177
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 177
taatgttgtgggccagcc 18

<210> SEQ ID NO 178
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 178
casatgaacactaggttggacacc 23

<210> SEQ ID NO 179
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 179
atgtgttggtttggttcag 21

<210> SEQ ID NO 180
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 180
cctgtgtgtgcgttgcctta 20

<210> SEQ ID NO 181
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 181
ttcacgttgcttgtgaaactc 20

<210> SEQ ID NO 182
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 182
ttttttttttggaaaaatac 26

<210> SEQ ID NO 183
<211> LENGTH: 23
tcaatcataa agtgcacace ttt

<210> SEQ ID NO 184
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 184

cagtoocatt tcggattgtt cca

<210> SEQ ID NO 185
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 185

gtgaggata attggaaggg g

<210> SEQ ID NO 186
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 186

gtattcact gatgtagctc ttt

<210> SEQ ID NO 187
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 187

aattgccaga ggegcgttga a

<210> SEQ ID NO 188
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 188

tgtgtagct tttcaagtt ttc

<210> SEQ ID NO 189
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 189

aacctctc tcatgtggtc aattat
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<212> TYPE: DNA
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<400> SEQUENCE: 190

cacattaat tggttctgc aatga

<210> SEQ ID NO 191
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gaacattcatt acatccc

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tttttttttt tggtttt

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gaaatacgc aaaaagc

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aagaaaaagc gaaatat

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aagaaaaagc gaaatat

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17

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ttatatgta gatctaa

17

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ttatatgta tatctaa

17

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17

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17

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ataaaaatca atgtgtt

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atataattc agggtgtt  17

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ctcaccctga tatattct

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cagagaast ctcaggt

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gtgtgagac atgatga

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AAATCGAG GATT

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AAATCGAG CTGCTG

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AAGTTTAC ACGTAC

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AATGGTAAG TCTATC

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ccgggaat acgca

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agtaccttg catttt

ggataatac aaaaatc
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casactcttct cccact

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LENGTH: 16
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Primer
SEQUENCE: 296
cttaaaagaa aagcgc

SEQ ID NO 297
LENGTH: 16
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Primer
SEQUENCE: 297
ccttaaaagaa aagcca

SEQ ID NO 298
LENGTH: 16
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Primer
SEQUENCE: 298
ttgcatgtaatttc

SEQ ID NO 299
LENGTH: 16
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Primer
SEQUENCE: 299
ttgcatgtaatttt

SEQ ID NO 300
LENGTH: 15
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Primer
SEQUENCE: 300
tgagggagtc aaga

SEQ ID NO 301
LENGTH: 16
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Primer
LOCATION: (16)...(16)
NAME/KEY: misc_feature
OTHER INFORMATION: n is a, c, g, or t
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tgagggagtc aagaag

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<223> OTHER INFORMATION: n is a, c, g, or t
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cattggccct tgtca

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ctttatgtat tagtc

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ctttatgtat tagata
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acctaataaa attczaa

16

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16

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16

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cctoatca acacca

16

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16

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<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 314
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<400> SEQUENCE: 322

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<210> SEQ ID NO 323
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<220> FEATURE:
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<400> SEQUENCE: 323

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<400> SEQUENCE: 324

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<400> SEQUENCE: 326

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<210> SEQ ID NO 334
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<400> SEQUENCE: 334
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<210> SEQ ID NO 335
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<211> LENGTH: 1231
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 337

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Leu Thr Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala
35   40
Ile Tyr Lys Cys Arg Pro Gly Tyr Ser Leu Gly Asn Val Ile Met
50   55
Val Cys Arg Lys Gly Trp Val Ala Leu Asn Pro Leu Arg Lys Cys
65   70   75
Gln Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe
85   90
Thr Leu Thr Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr
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Cys Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val
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705 710 715 720
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945 950 955 960
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1040 1045 1050
Val Gln Asn Ala Tyr Ile Val Ser Arg Gin Met Ser Lys Tyr Pro
What is claimed is:

1. A pharmaceutical composition for administration to a human patient comprising a Complement Factor II (CFH) polypeptide, wherein the Complement Factor II polypeptide (a) has at least 90% sequence identity to SEQ ID NO:2, comprises isoleucine at position 62, numbered relative to SEQ ID NO:2, comprises tyrosine at residue 402, numbered relative to SEQ ID NO:2, and binds complement component 3b (C3b), or (b) comprises a fragment of (a) that binds C3b and comprises isoleucine at residue 62 and tyrosine at residue 402, both numbered relative to SEQ ID NO:2, and a pharmaceutically acceptable excipient, wherein said composition is free of pathogens and suitable for administration to a human patient.

2. The composition of claim 1 wherein the complement factor II polypeptide has at least 95% sequence identity to SEQ ID NO:2, comprises isoleucine at position 62, numbered relative to SEQ ID NO:2, comprises tyrosine at residue 402, numbered relative to SEQ ID NO:2, and binds complement component 3b (C3b) or (b) comprises a fragment of (a) that binds C3b and comprises isoleucine at residue 62 and tyrosine at residue 402, both numbered relative to SEQ ID NO:2, and a pharmaceutically acceptable excipient, wherein said composition is free of pathogens and suitable for administration to a human patient.

3. The composition of claim 1, wherein the complement factor II polypeptide is a recombinant polypeptide.

4. The composition of claim 1, wherein the complement factor II polypeptide is purified from blood.

5. The composition of claim 1 wherein the complement factor II polypeptide has a sequence consisting of residues 19-1231 of SEQ ID NO:5.

6. A pharmaceutical composition for administration to a human patient comprising a complement factor II (CFH) polypeptide, wherein the complement factor II polypeptide is a naturally occurring human complement factor II polypeptide comprising residues 19-1231 of SEQ ID NO:5, or a naturally occurring splice variant thereof, or an allelic variant of said human CFH polypeptide or splice variant, with the proviso that residue 62, numbered relative to SEQ ID NO:5, is isoleucine, residue 402, numbered relative to SEQ ID NO:5, is tyrosine, and residue 1210, numbered relative to SEQ ID NO:5, is arginine, wherein the human CFH polypeptide binds complement component 3b (C3b).

7. The composition of claim 1 wherein residue 1210 of the complement factor II polypeptide, numbered relative to SEQ ID NO:2, is arginine.

8. The composition of claim 1 that is a lyophilized composition.
PATENT NO. : 8,497,350 B2
APPLICATION NO. : 12/479716
DATED : July 30, 2013
INVENTOR(S) : Gregory S. Hageman

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the specification, Col. 1, lines 10-11 of U.S. Patent No. 8,497,350 B2,

In the CROSS-REFERENCE TO RELATED APPLICATIONS Section, please delete

“This application is a continuation of application Ser. No. 11/354,599”

And insert

--This application is a divisional of application No. 11/354,559, now Pat. No. 7,745,389--

This certificate supersedes the Certificate of Correction issued October 1, 2013.

Signed and Sealed this
Twenty-sixth Day of November, 2013

[Signature]
Margaret A. Focarino
Commissioner for Patents of the United States Patent and Trademark Office
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

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**In the CROSS-REFERENCE TO RELATED APPLICATIONS Section**, please delete

“This application is a continuation of application Ser. No. 11/354,599”

And insert

--This application is a divisional of application No. 11/354,599, now Pat. No. 7,745,389--

Signed and Sealed this  
First Day of October, 2013  

Teresa Stanek Rea  
Deputy Director of the United States Patent and Trademark Office