Biochemical Analysis of a Common Human Polymorphism Associated with Age-Related Macular Degeneration†

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ABSTRACT: Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in developed countries. A large number of human genetic studies have associated a common variant (Y402H) of complement factor H (CFH) with a highly significant increase in AMD risk. CFH is a modular protein with 20 homologous short consensus repeats (SCRs). The Y402H variant is located in SCR7 of both CFH and factor H-like protein 1 (FHL-1), a splice variant of CFH (containing SCR1−7) with unique biochemical properties. Because SCR7 is known to bind to heparin, C-reactive protein (CRP), and M protein from *Streptococcus pyogenes*, it has been hypothesized that the AMD-associated polymorphism may affect interactions with these CFH ligands. In this study, we tested this hypothesis in the context of full-length CFH (SCR1−20) and FHL-1. We systematically analyzed the interactions of the Y402 and H402 variants of CFH and FHL-1 with heparin, CRP, and several bacterial ligands: M6 protein of *Streptococcus pyogenes*, PspC of *Streptococcus pneumoniea*, and BbCRASP-1 of *Borrelia burgdorferi*. In comparing the Y and H variants of CFH and FHL-1, we found no significant difference in their protein secretion, cofactor activity, or interactions with heparin, BbCRASP-1, or PspC, but a significant difference in binding to CRP and M6 protein. This study reveals the fundamental properties of a common polymorphism of CFH and lays the groundwork for elucidating the role of CFH in AMD pathogenesis.

Age-related macular degeneration (AMD[1]) is the leading cause of irreversible blindness in Western societies. The aging of the population will only increase the incidence of this disease. It was discovered in 2005 that a common polymorphism in the complement factor H (CFH) gene is associated with the development of AMD in about 50% of patients (*1*−*13*). Specifically, people with histidine at amino acid residue 402 (the H variant) of CFH are more likely to develop AMD than people with tyrosine at this position (the Y variant).

CFH is an abundant serum protein that functions as an inhibitor of the alternative pathway of the complement system, which is part of the innate immune system (*14*, *15*). CFH inhibits the alternative pathway of complement activation by acting as the cofactor for factor I in the breakdown of C3b, by accelerating the decay of C3 convertase and by competing with factor B for binding to C3b (*14*, *15*). CFH is a 150 kDa protein composed of 20 short consensus repeats (SCR). Factor H-like protein 1 (FHL-1) is a 42 kDa plasma protein produced as an alternatively spliced form of the CFH gene. The FHL-1 protein is identical to SCR1−7 of the CFH protein but with four additional amino acid residues at the C-terminus. Although FHL-1 shares many properties with CFH including inhibiting the alternative pathway of the complement system, it has biochemical properties distinct from those of CFH (*16*).

In addition to AMD, CFH is associated with other genetic diseases. In the case of hypocomplementemic glomerulonephritis, the secretion of CFH is blocked due to CFH mutations in SCR9 and SCR16, but FHL-1 secretion is normal (*17*). A large number of point mutations in CFH are associated with hemolytic uremic syndrome (HUS) (*18*). They are largely clustered in the C-terminal SCR16−20 of CFH and therefore are not located in FHL-1 (Figure 1) (*18*). In contrast, the polymorphism associated with AMD affects both CFH and FHL-1. At the transcriptional level, CFH and FHL-1 transcripts are differentially expressed and regulated, even though they are encoded by the same gene and have the same promoter (*19*). It has been proposed that post-transcriptional events are likely responsible for the differential regulation of their transcripts (*19*). Although CFH concentra-

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[1] Abbreviations: AMD, age-related macular degeneration; CFH, complement factor H; FHL-1, factor H-like protein 1; SCR, short consensus repeat; CRP, C-reactive protein.

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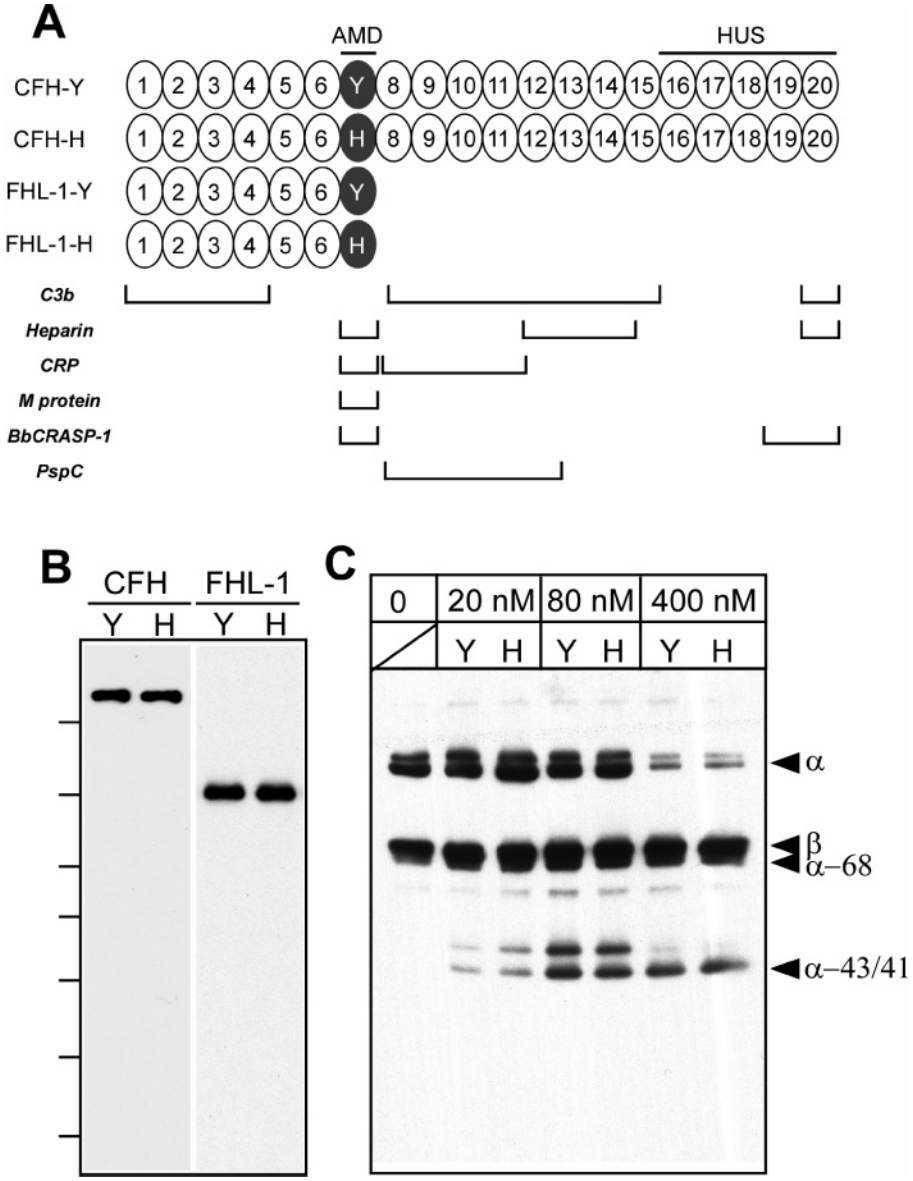

FIGURE 1: A. Schematic diagram of the modular structures of CFH (top two drawings) and its related protein FHL-1 (lower two drawings). The nomenclature used in this paper is shown on the left of the diagrams. Each SCR is shown as a circle. The polymorphism associated with AMD is located in SCR7, while point mutations associated with HUS are enriched in SCR16−20 (*18*). Binding sites for CFH ligands are indicated below the diagram. B. Western blot comparing the expression levels of Y and H variants of AP-CFH and AP-FHL-1 (all with a N-terminal Myc tag) using anti-Myc antibody. Molecular weight standards from top to bottom: 182, 115, 82, 64, 48, 37, 25 kD. C. The Y and H variants of FHL-1 show similar cofactor activity for factor I in the breakdown of C3b. Increasing concentrations of the Y or H variant of CFH were incubated with C3b and factor I. The cleavage products of C3b α chain are α-68 and α-43/41. Detection of cleaved products of human C3b using anti-C3c antibody was performed similarly as described (*48*).

tion is much higher than FHL-1 in human blood, FHL-1 is expressed in the regions of the eye involved in AMD pathogenesis (*1*) and may also be relevant in AMD pathogenesis.

Human cells recruit CFH, a major fluid-phase inhibitor of the alternative complement pathway, to protect themselves from complement attack (*20*). A major mechanism by which CFH recognizes a host cell is by binding to membrane-associated polyanions on the cell surface (*21, 22*). Since CFH binds strongly to heparin, heparin is widely used as a tool to study the interaction of CFH with polyanions. CFH has several heparin binding sites located in different SCRs (*18, 23*). It was found that SCR7 contributes to heparin binding in the context of SCR1−9 (*24*). However, SCR7 has no contribution to full-length CFH's heparin binding because CFH minus SCR7 still has >99% of heparin binding activity (*24*). Consistently, a monoclonal antibody recognizing the C-terminal domain of CFH completely abrogates CFH's heparin binding (*25*). A model of compact conformation of CFH has been proposed to explain the dominant role of C-terminal domain in CFH's heparin binding (*26*). If the Y402H polymorphism of CFH contributes to AMD pathogenesis, change in heparin binding is unlikely to be the cause because SCR7, where the polymorphism is located, has no contribution to heparin binding in the context of full-length CFH. In contrast, HUS, a disease associated with a heparin binding defect in CFH, has a very different mutation spectrum, which shows clustering of mutations in SCR20 (*18*). Although SCR7 makes no significant contribution to the heparin binding of CFH (SCR1−20), it does make a significant contribution to heparin binding in the context of SCR1−7 (*27*). This suggests that the AMD-associated

mutation located in SCR7 may affect the heparin binding of FHL-1.

A wide range of pathogenic bacteria also have cell surface proteins that recruit CFH and FHL-1 to protect the bacteria from complement attack (*14*, *28*). These include the YadA protein of *Yershinia enterocolitica* (*29*), the M protein of group A streptococci (*30*, *31*), the Por1A protein of *Neisseria gonorrheae* (*32*), the BbCRASP proteins of *Borrelia burgdorferi* (*33*), and the PspC protein of *Streptococcus pneumoniae* (*34*, *35*). Since SCR7, where residue 402 is located, is known to be involved in binding to several bacteria proteins (*27*, *28*), the Y402H polymorphism may affect the binding of CFH or FHL-1 to bacterial ligands. A change in binding affinity for pathogenic bacteria can have functional consequences. For example, if the H variant has a reduced ability to bind to certain pathogenic bacteria, these bacteria may be more prone to complement attack. This beneficial function would be analogous to the mutation in hemoglobin that causes sickle cell anemia but protects against malaria.

CRP is an acute phase protein that functions in both host defense and inflammation (*36*−*38*). Circulating CRP concentration rises rapidly in response to infection, tissue injury, and inflammation. CFH has been shown to be recruited to CRP bound to apoptotic cells and to prevent the assembly of terminal complement components and thus has an anti-inflammatory effect on the clearance of apoptotic cells (*39*). SCR7 of CFH, where Y402H is located, is known to be involved in binding of CFH to CRP (*27*, *40*). It was hypothesized that the AMD-associated polymorphism Y402H might affect the binding of CFH to CRP, heparin, and bacterial ligands (*1*−*4*). However, this hypothesis has never been tested in the context of full-length CFH and FHL-1 proteins, although the interaction between SCR6−8 of CFH and CFH ligands has been examined (*41*, *42*). In this study, we performed a systematic analysis of the interactions of CFH and FHL-1 variants with heparin, CRP, and several bacterial ligands.

MATERIALS AND METHODS

A System To Study the Interaction of CFH and FHL-1 with Their Ligands. We used alkaline phosphatase (AP) fusions to quantitate the interaction between CFH and its ligands. The AP system is an effective method to study the interaction between secreted eukaryotic proteins and their binding molecules (*43*−*46*). We cloned the cDNAs for the Y and H variants of FHL-1 from human retina/RPE cDNA and the Y and H variants of CFH from human embryonic brain cDNA. SCR7 alone was cloned using PCR. All clones were completely sequenced to make sure that there are no spurious mutations. The Y and H variants of CFH or FHL-1 differ only at residue 402. We tagged CFH, FHL-1, and SCR7 with AP at the N-terminus. A Myc tag was inserted immediately after the AP tag. To produce these AP fusion proteins, HEK293 cells were transfected with the plasmids using Fugene 6 (Roche). After overnight incubation, the cells were washed once with PBS and changed to serum free medium (SFM). After another 24 h of incubation, the SFM containing the secreted AP fusion proteins was harvested and concentrated 10-fold using Amicon Ultra (Millipore). The concentrations of AP fusion proteins were quantitated as described (*46*).

Cofactor Activity Assay for CFH/FHL-1. Cofactor activity of CFH/FHL-1 in factor I-mediated cleavage of C3b was assayed similarly to published protocols (*47*, *48*). Briefly, 0.5 μg of human C3b, 0.25 μg of human factor I and various amounts of CFH/FHL-1 proteins were incubated at 37 °C for 30 min in buffer (20 mM HEPES, pH 7.5, 140 mM NaCl, and 2 mM $CaCl_2$) in a final volume of 20 μL. The reactions were stopped by mixing with SDS−PAGE loading buffer and boiling. C3b fragments were detected by Western blot using anti-human C3c antibody (DakoCytomation).

Production of Heparin-BSA. Plates used for solid-phase binding assays such as the Maxi-sorb plate (Nunc) are designed for high affinity protein binding with very low polysaccharide binding ability. Therefore, heparin needs to be linked to a protein like BSA for this assay (*27*). We synthesized and purified heparin-BSA according to an established protocol (*49*). Briefly, 14 mg of heparin, 1 mg of BSA, and 0.5 mg of sodium cyanoborohydride were sequentially dissolved in 0.6 mL of 0.2 M potassium phosphate buffer, pH 8.0, and the mixture was incubated at 40 °C for 24 h. The buffer in this mixture was then exchanged to phosphate buffered saline (PBS) by centrifugation on a Microcon YM-10 membrane (Millipore). Heparin-BSA was precipitated by adding 1.5 volumes of 95% ethanol saturated with sodium acetate, and the mixture was stirred at room temperature for 30 min. The precipitate was pelleted at 11000*g* for 10 min and was dissolved in the original volume of PBS containing 4 M guanidine-HCl. Guanidine was then removed by centrifugation on Microcon YM-10 membrane 4 times with PBS washes.

Production of Bacterial Ligands for CFH. Bacterial ligands PspC and BbCRASP-1 were cloned by PCR from genomic DNAs of *S. pneumoniae* (strain TIGR4) and *B. burgdorferi* (strain B31), respectively (American Type Culture Collection). The proteins were produced as fusion proteins with maltose binding protein (MBP) using pMAL-cR1 vector (New England Biolabs) in BL21 cells and purified using Amylose resin according to the manufacture's protocol (New England Biolabs). MBP alone has insignificant binding to CFH and FHL-1 compared with MBP-PspC fusion and MBP-BbCRASP-1 fusion (data not shown). M6 protein of *Streptococcus pyogenes* was produced and purified as described (*50*).

Solid-Phase Binding Assays. Solid-phase binding assays were performed similarly to an established method for AP fusion proteins (*45*, *46*). BSA, a natural protein in the blood, was chosen as the blocking reagent because it has insignificant and the lowest binding activity to CFH and FHL-1 compared to other commonly used blocking reagents such as casein, milk, gelatin, and SuperBlock (Pierce) (Figure 2D and data not shown). Briefly, 1 μg of CFH/FHL-1 ligand in 100 μL of 50 mM sodium bicarbonate, pH 8.0 is bound per well to a Maxisorp plate (Nunc) at 4 °C overnight. After removal of unbound ligand, the wells are blocked with 200 μL of 2% BSA in PBS per well for 2 h at room temperature. After 3 washes with 200 μL of HBS/Ca (20 mM HEPES, pH 7.0, 140 mM NaCl, and 2 mM $CaCl_2$), AP-CFH, AP-FHL-1 or AP-SCR7 protein diluted in 100 μL of 2 mg/mL BSA in HBS/Ca is added to each well and is incubated in the well for 1 h at room temperature. After 3 washes of the wells with 200 μL of HBS/Ca, 200 μL of AP substrate, pNPP

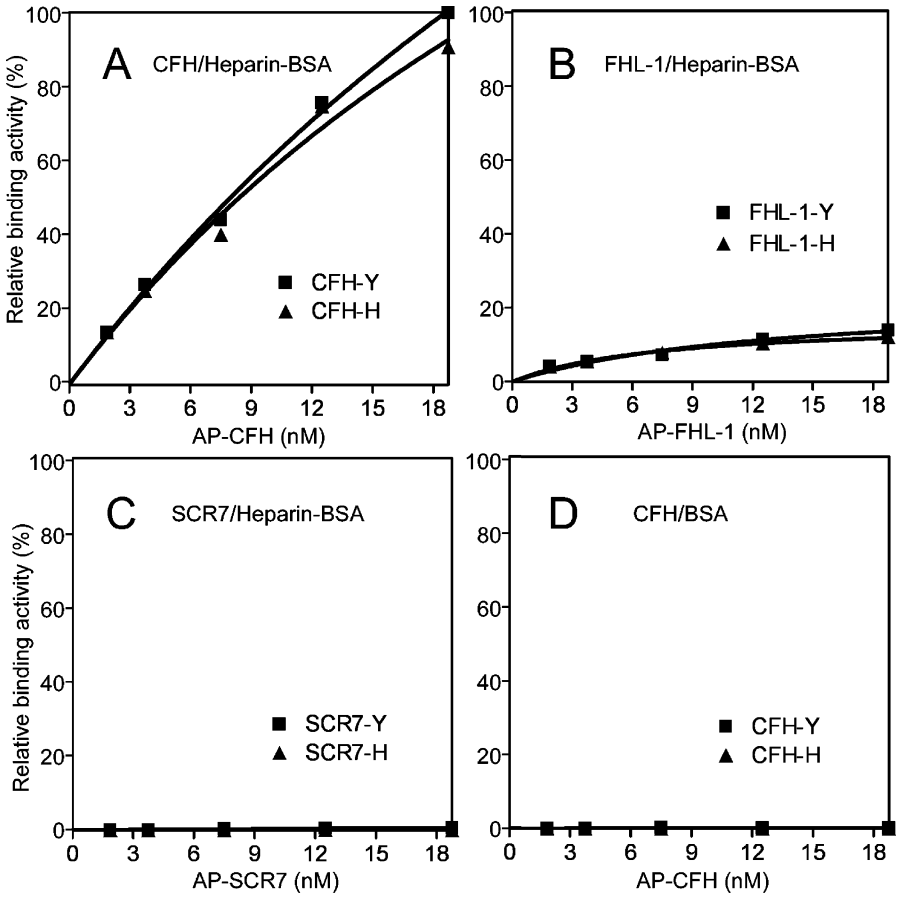

FIGURE 2: Binding to heparin-BSA or BSA. A. Binding of AP-CFH-Y, AP-CFH-H to heparin-BSA. B. Binding of AP-FHL-1-Y, AP-FHL-1-H to heparin-BSA. C. Binding of AP-SCR7-Y and AP-SCR7-H to heparin-BSA. D. Binding of AP-CFH-Y and AP-CFH-H to BSA. All experiments in A, B, C, and D were done under identical conditions. The highest binding activity (AP-CFH-Y to heparin-BSA) is defined as 100%.

(Sigma), is added to each well. After 1 h of incubation at 37 °C in the dark, the plate is read at 405 nM in a microplate reader (Opsys MR, Dynex Technologies). The concentration dependent binding assays for all ligands (shown in Figures 2−4) were performed more than 3 times with independent protein preparations of CFH, FHL-1, and SCR7 variants. Side-by-side comparison of all ligands performed at one concentration (20 nM) of CFH and FHL-1 variants is shown in Figure 6. The color reaction for CRP binding (Figure 3) was performed for 2 h due to low signals. CRP from 3 commercial sources [CRP purified from human serum (Calbiochem), purified recombinant human CRP (Calbiochem), and CRP purified from human pleural fluid (Chemicon)] generated similar results. Modified CRP was prepared using urea as described (*51*).

Protein Overlay Assay for CFH/FHL-1 Binding. Protein overlay assay for the binding of CFH and FHL-1 to their ligands was performed using a standard protocol of Western blot, but with AP-CFH and AP-FHL-1 as the probing reagents. AP activity of AP-CFH or AP-FHL-1 bound on the blot was assayed using Lumi-Phos WB chemiluminescent substrate (Pierce).

PepSpot Analysis of CRP. Fifty-four overlapping 14-mer peptides covering the whole human CRP protein were synthesized on cellulose-βalanine-membrane (JPT Peptide Technologies GmbH, Berlin, Germany). PepSpot analysis to map the major epitopes of CRP recognized by FHL-1 was performed as described (*52*, *53*).

Production and Purification of Anti-Y and Anti-H Specific Antibodies for CFH. The technique we used to produce Y and H specific antibodies for CFH is similar to that for producing phospho-specific antibodies. For the immunogen, we selected a CFH peptide that includes residue 402. The "CFH-Y peptide" (YLENGYNQNYGRKF) has Y at the position corresponding to residue 402, and the "CFH-H peptide" (YLENGYNQNHGRKF) has H at this position. After conjugating each peptide to keyhole limpet hemocyanin (KLH), the peptide−KLH conjugate is used to immunize rabbits. Peptide synthesis and immunization were done commercially (Genemed Synthesis). After the fourth bleed, the rabbits were terminated. We selected the rabbit sera with the strongest immune response to CFH based on Western blot analysis of all crude sera, and we used these sera for further purification. We used a sequential purification and depletion procedure to select for Y and H specific antibodies. The affinity columns were prepared by conjugating CFH-Y and CFH-H peptides to Affigel (Bio-Rad). For Y-specific antibodies, the serum from rabbit immunized with CFH-Y peptide was first purified on an Affigel−CFH-Y column to select for antibodies that specifically recognize the CFH-Y peptide. Even for a single peptide, there are many potential epitopes for antibody recognition that are shared by the CFH-Y and CFH-H peptides. Antibodies bound to Affigel−CFH-Y column are eluted off in low pH, neutralized, and passed through the Affigel−CFH-H column to deplete antibodies that recognize the CFH-H peptide. The flowthrough

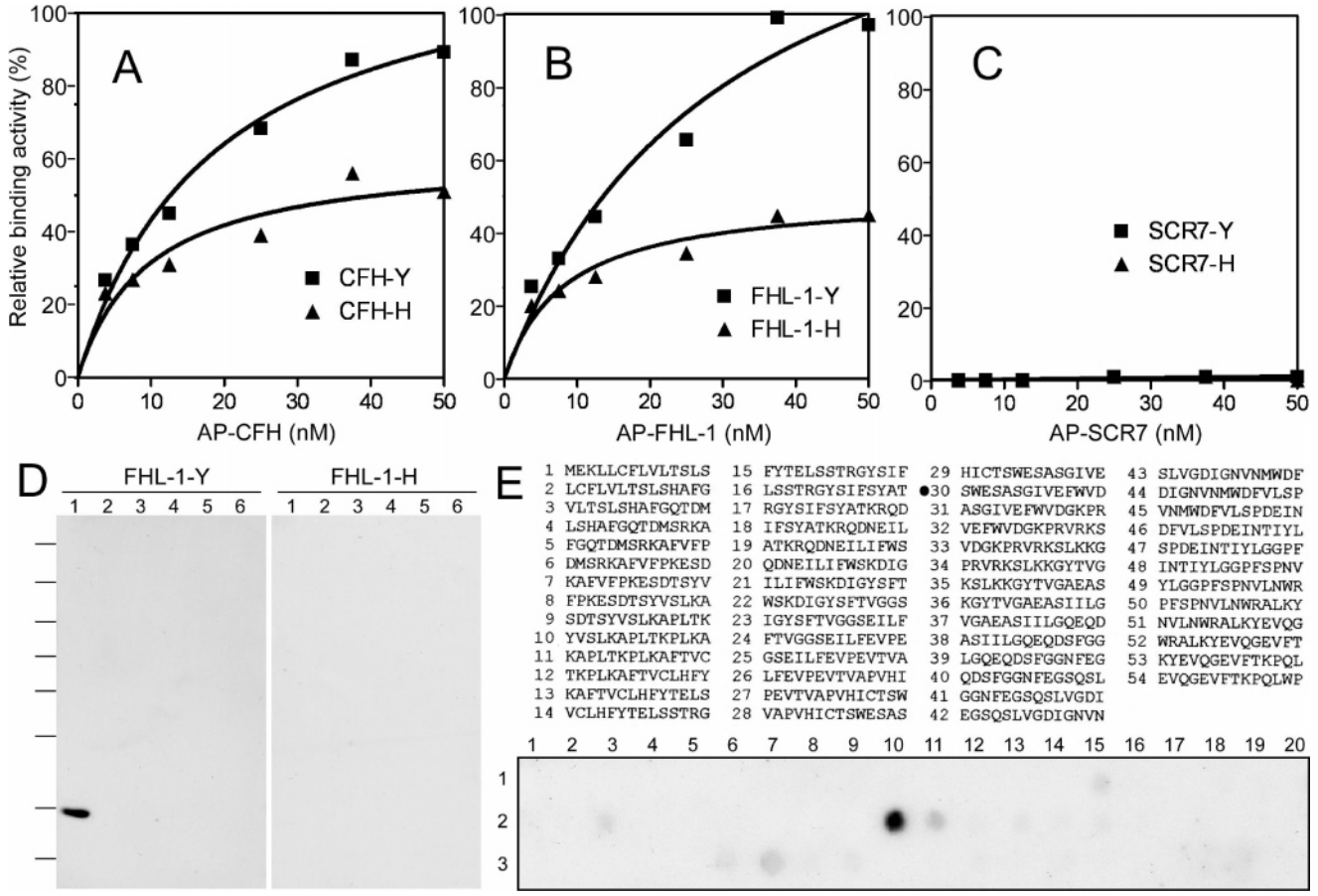

FIGURE 3: A−C. Solid-phase binding assays for the binding of AP-tagged CFH (A), FHL-1 (B), and SCR7 (C) to CRP. All experiments in A, B, and C were done under identical conditions. The highest binding activity is defined as 100%. D. Binding of AP-tagged FHL-1-Y (left) or FHL-1-H (right) to CRP in a protein overlay assay with 0.5 mg of each protein per lane. Lane 1, CRP; lane 2, BSA; lane 3, lactalbumin; lane 4, ovalbumin; lane 5, prealbumin; lane 6, human retinol binding protein. Molecular weight standards from top to bottom: 182, 115, 82, 64, 48, 37, 25, 20 kD. E. Localization of FHL-1 binding site in CRP. PepSpot analysis of AP-FHL-1-Y binding to overlapping peptide fragements from CRP.

from the second column contains antibodies that recognize CFH-Y but not CFH-H. A similar procedure was performed to purify H-specific antibodies.

Detection of CFH-Y and CFH-H in Human Sera Using Western Blot Analysis. Human sera from individual donors were purchased from a commercial source (Innovative Research). Since we did not obtain any identifiable private information about the blood donors, this study is not considered human subject research. Purified Y-specific and H-specific antibodies for CFH were used to identify CFH-Y and CFH-H in human sera using Western blot analysis. Due to the strong cross-reactivity of anti-rabbit IgG antibody to human IgG in serum, we used HRP-conjugated anti-rabbit IgG antibody cross absorbed against human IgG (Southern-Biotech) as the secondary antibody for Western blot analysis of human sera.

Assay of C3b Deposition Using Human Sera. To specifically study alternative pathway activation, we diluted sera from 402Y homozygotes (Y/Y), 402Y/H heterozygotes (Y/H), and 402H homozygotes (H/H) in GVB-Mg-EGTA buffer (*54*, *55*). Complement fixation reactions were performed at 37 °C for 30 min. M6 protein was coated onto 96-well plates similar to a solid-phase binding assay. Since zymosan (Sigma) is insoluble and cannot be coated onto a 96-well plate, changes of solutions were done by low-speed centrifugation of zymosan. C3b deposition was detected using anti-human C3c antibody (DakoCytomation) as described (*56*) except that AP-conjugated secondary antibody (Calbiochem) was used. AP activity was measured using pNPP (Sigma) similarly to the solid-phase binding assay as described above.

RESULTS

Protein Secretion and Cofactor Activity. We cloned and produced the Y and H variants of CFH (SCR1−20), FHL-1 (SCR1−7), and SCR7 as alkaline phosphatase (AP) fusion proteins and used these proteins for systematic functional analysis of the Y402H polymorphism. We found no difference in the protein production and secretion of the Y and H variants of CFH and FHL-1 using Western blot analysis (Figure 1B). Quantitation of AP activity of secreted AP-CFH and FHL-1 fusion proteins also did not reveal any significant difference between Y and H variants of these proteins. However, FHL-1 is produced more efficiently than CFH, consistent with the fact that FHL-1 has a much smaller molecular weight than CFH (they are driven by the same promoter for expression) (data not shown). In contrast, two CFH mutations associated with CFH deficiency and hypocomplementemic glomerulonephritis block CFH secretion (*17*), and this suggests that the Y402H polymorphism does not cause misfolding of CFH to block its secretion. The Y and H variants of CFH and FHL-1 have similar activities in

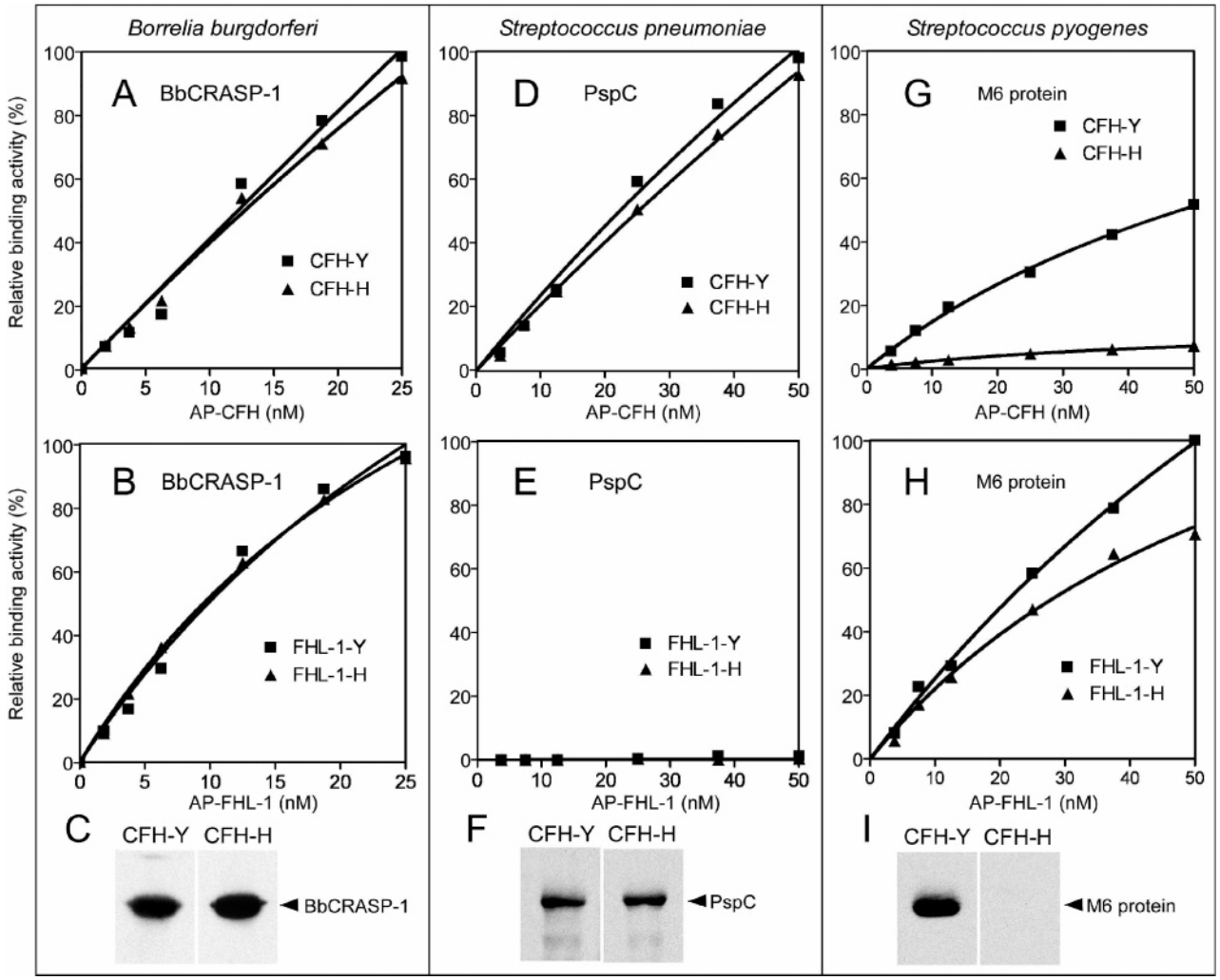

FIGURE 4: Interaction of CFH and FHL-1 with bacterial ligands of CFH. Solid-phase binding assays for each ligand were done under identical conditions. The highest binding activity is defined as 100%. For protein overlay assays, 0.5 mg of bacterial ligand was loaded per lane. A and B. Solid-phase binding assay for the binding of AP-CFH-Y, AP-CFH-H, AP-FHL-1-Y, and AP-FHL-1-H, to BbCRASP-1 of *B. burgdorferi*. C. Protein overlay assay for the binding of CFH-Y and CFH-H to BbCRASP-1. D and E. Solid-phase binding assay for the binding of AP-CFH-Y, AP-CFH-H, AP-FHL-1-Y, and AP-FHL-1-H to PspC of *S. pneumoniae*. F. Protein overlay assay for the binding of CFH-Y and CFH-H to PspC. G and H. Solid-phase binding assay for the binding of AP-CFH-Y, AP-CFH-H, AP-FHL-1-Y, and AP-FHL-1-H to M6 protein of *S. pyogenes*. I. Protein overlay assay for the binding of CFH-Y and CFH-H to M6 protein.

acting as a cofactor for factor I in the breakdown of C3b (results for FHL-1 are shown in Figure 1C). This is consistent with the fact that SCR1−4 but not SCR7 is essential for the cofactor activity. These results suggest that the AMD-associated Y402H polymorphism has no significant effect on the production, secretion, or cofactor activity of CFH or FHL-1.

Interaction of CFH and FHL-1 with Heparin. Consistent with earlier studies on full-length CFH that found no contribution of SCR7 in CFH's heparin binding (*24*, *25*), our experiments showed that there is no significant difference between the Y and H variants of CFH in binding to heparin (Figure 2A). This is also true for FHL-1 (Figure 2B). We also found that SCR7 alone is not sufficient for heparin binding (Figure 2C).

Interaction of CFH and FHL-1 with CRP. We performed solid-phase binding assays of CRP to the Y and H variants of CFH, FHL-1, and SCR7 (Figure 3). The Y variants of CFH and FHL-1 bound to CRP more strongly than the H variants did (Figure 3A and 3B). SCR7 alone was also not sufficient for CRP binding (Figure 3C). We further analyzed the binding of AP-FHL-1 to CRP using protein overlay assay, which has been widely used to study the interaction between CFH and its ligands (*30*, *52*, *53*). In this assay, the Y variant interacts strongly with CRP, while the H variant almost completely fails to interact (Figure 3D). Likely explanations for the more dramatic difference between Y and H variants in the protein overlay assay are the nonlinearity of the signal for protein overlay assay and the denaturing conditions used to run the assay. The interaction of FHL-1 with CRP is specific because control proteins bovine serum albumin, lactalbumin, ovalbumin, prealbumin, and retinol binding protein did not give a signal (Figure 3D). Using the protein overlay assay, we mapped the major epitope of CRP recognized by FHL-1-Y using PepSpots analysis, which has been used as an unbiased approach to map epitopes in CFH ligands (*52*, *53*). The PepSpots membrane contains 54 overlapping 14-mer peptides covering the human CRP protein. We identified the major linear epitope in CRP recognized by FHL-1-Y as peptide 30 (SWESASGIVEF-WVD), while most other peptides showed little or no binding signal (Figure 3E).

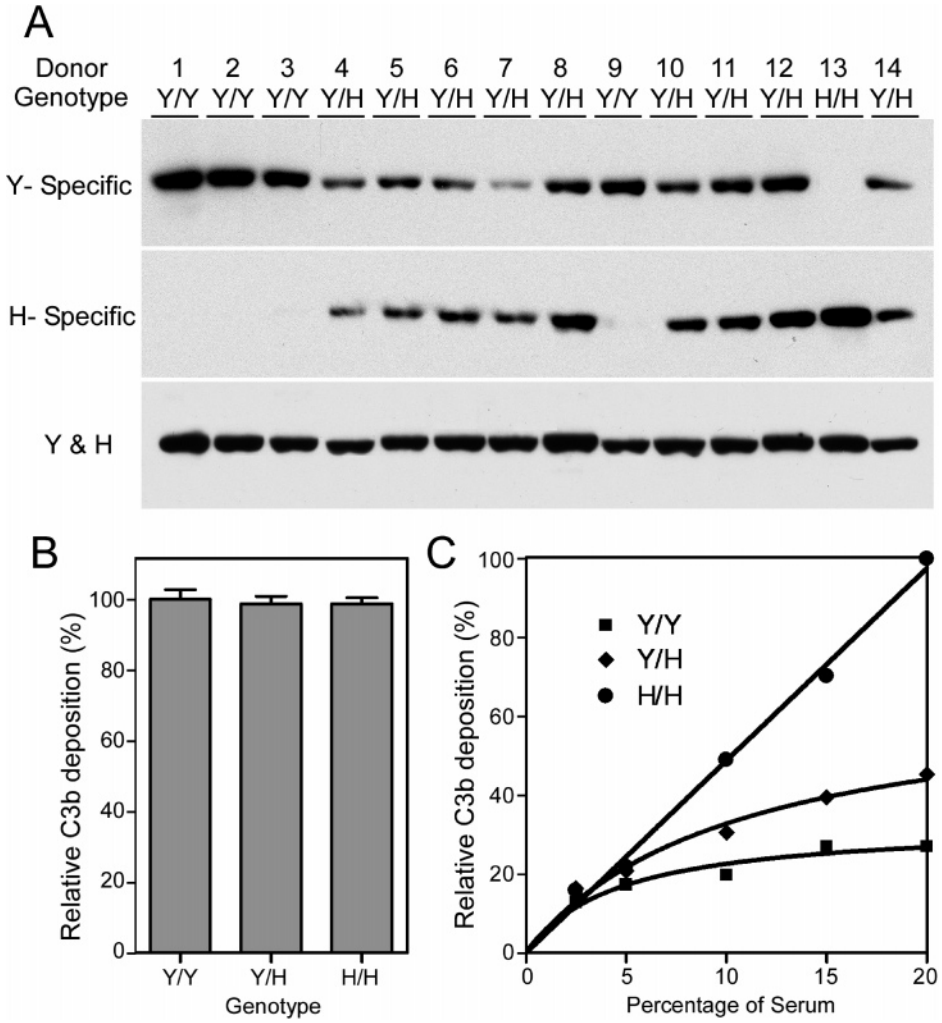

FIGURE 5: A. Genotyping of human sera using Y-specific antibody and H-specific antibody for CFH. Detecting CFH in sera from 14 Caucasian donors using the Y specific antibody (top gel), H specific antibody (middle gel), and an antibody that recognizes both Y and H variants of CFH (lower gel). Since CFH concentration is much higher than FHL-1 concentration in human serum, CFH signals were used in genotyping of the sera. The genotype of each donor is indicated on the top. B and C. Comparison of the activation of the alternative pathway of the complement system using sera from Y/Y, Y/H, and H/H donors. The highest level of C3b deposition is defined as 100%. There is no significant difference in C3b deposition on zymosan using sera from donors with Y/Y, Y/H and H/H genotypes (B). Sera from donors with Y/Y, Y/H, and H/H genotypes show dramatic differences in C3b deposition on M6 protein (C).

Interactions of CFH and FHL-1 with Ligands from Pathogens. We studied the interactions of CFH and FHL-1 variants with three bacterial ligands of CFH: PspC of *S. pneumoniae* (*34*), M6 protein of *S. pyogenes* (*30*, *57*), and BbCRASP-1 of *B. burgdorferi* (*52*). PspC was chosen in our study as a control because the PspC binding region of CFH has been localized to SCR8−11 but not SCR7 (*58*). Therefore, CFH-Y and CFH-H are predicted to have no difference in binding to PspC, and FHL-1 should not bind to PspC. M6 and BbCRASP-1 were chosen because SCR7 is known to be involved in binding to both proteins (*28*). We produced and purified PspC, M6 protein, and BbCRASP-1 and performed both solid-phase binding assays and protein overlay assays on these CFH ligands (Figure 4). We found no significant difference between the Y and H variants of either CFH or FHL-1 in binding to BbCRASP-1 (Figure 4A-4C). Consistent with the known properties of PspC, our experiments showed no difference in the binding of the Y and H variants of CFH to PspC and insignificant binding of FHL-1 to PspC (Figure 4D−4F). However, we found dramatically weaker binding of CFH-H than CFH-Y in binding to M6 protein (Figure 4G, 4I). The difference between the binding of the Y and H variants of FHL-1 to M6 is much smaller (Figure 4H).

The large difference in the binding of Y and H variants of CFH to M6 protein predicts that sera containing Y and H variants of CFH will differ in C3b deposition on the M6 protein. We tested this hypothesis using human sera from individual donors that have been genotyped using Y-specific or H-specific antibodies for CFH that we produced and purified (Figure 5A). There is significant variation in CFH levels between sera from different donors. However, the difference in CFH level does not correlate with the Y and H genotypes, consistent with our earlier experiment that the Y402H polymorphism does not affect CFH protein expression (Figure 1). For the complement activation assay, we used human sera with similar CFH levels as judged by the Western blot signals. To compare C3b deposition of human sera, we diluted sera from 402Y homozygotes (Y/Y), 402Y/H heterozygotes (Y/H), and 402H homozygotes (H/H) in GVB-Mg-EGTA buffer (*54*, *55*) to study alternative pathway activation. We found no significant difference in Y/Y, Y/H, and H/H sera in C3b deposition on zymosan, a well-known activator of the alternative pathway of the complement

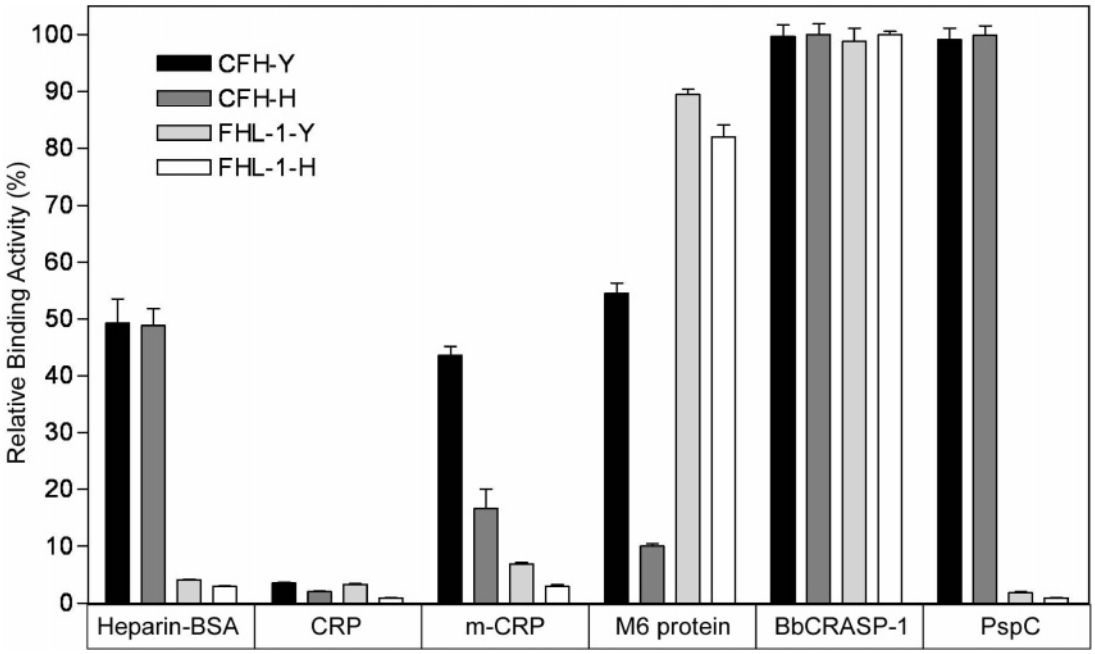

FIGURE 6: Comparison of the binding of 20 nM of CFH-Y, CFH-H, FHL-1-Y, and FHL-1-H to heparin-BSA, CRP, modified CRP (m-CRP), C3b, M6 protein, PspC, and BbCRASP-1 (1 μg of each ligand). The binding for all ligands was done under identical conditions for side-by-side comparison. The highest binding activity (CFH for BbCRASP-1) is defined as 100%.

system (Figure 5B). In contrast, we found dramatic differences in C3b deposition on M6 protein between Y/Y, Y/H, and H/H sera. Consistent with the stronger binding of the Y variant of CFH to M6 protein, Y/Y serum has significantly less C3b deposition than Y/H serum, which has significant less C3b deposition than H/H serum (Figure 5C). This experiment demonstrates an inverse correlation between the binding activities of CFH of different genotypes and the level of activation of the alternative complement pathway.

Comparison of the Binding of CFH/FHL-1 to Heparin, CRP, M6 Protein, PspC, and BbCRASP-1. Few previous studies did side-by-side comparison of multiple CFH ligands in binding to CFH and FHL-1. This comparison may provide a unique perspective on the abilities of these ligands to recruit CFH and FHL-1. We performed a side-by-side comparison of the binding of CFH and FHL-1 to heparin, CRP, M6 protein, PspC, and BbCRASP-1 (Figure 6). We found that bacterial ligands generally bind much more strongly to CFH/FHL-1 than the host ligands heparin and CRP. This finding is consistent with the fact that these bacteria are highly successful human pathogens in evading complement attack. Surprisingly, the binding to CRP is significantly weaker than all other ligands tested. Similar results were obtained for purified human CRP from 3 different sources (CRP purified from serum, CRP from pleural fluid, and recombinant CRP). This is consistent with a recent study that found much weaker binding of CFH to native CRP than to modified CRP (*51*). When we tested modified CRP in our assay, we also found significantly stronger binding of CFH (but not FHL-1) to modified CRP.

DISCUSSION

Since the Y and H variants of CFH and FHL-1 have never been systematically analyzed at the functional level in the context of full-length CFH and FHL-1, we compared several fundamental properties of these proteins in this study. We found no difference in protein secretion and cofactor activity between the Y and H variants of either protein (Figure 1). Consistent with earlier studies on full-length CFH that found no contribution of SCR7 in CFH's heparin binding (*24*, *25*), we found no significant difference between CFH-Y and CFH-H in binding to heparin (Figure 2 and Figure 6). In contrast, a recent study using CFH fragment SCR6−8 expressed in bacteria found significant difference in the binding of Y and H forms of SCR6−8 to heparin (*41*). Our experiments are different in many ways from this study. First, this study used a fragment of CFH (SCR6−8), while we studied the full-length forms of CFH and FHL-1. Second, this study used a bacterial expression system for the CFH fragment, while we used a mammalian expression system. Since CFH is a secreted mammalian protein with many disulfide bonds, a bacterial expression system may be less effective in producing SCRs with the correct disulfide bonds. In addition, our results are consistent with previous publications (*24*−*26*) on full-length CFH's interaction with heparin.

Consistent with the fact that SCR7 is not involved in binding to PspC (*58*), we found no significant difference between CFH-Y and CFH-H in binding to PspC (Figure 4 and Figure 6). Of the two bacterial ligands (BbCRASP-1 and M protein) that CFH bind through SCR7, CFH-H substantially loses the ability to bind to M6 protein of *S. pyogenes*, but binds to BbCRASP-1 similarly to CFH-Y (Figure 4 and Figure 6). These findings demonstrated that the Y402H polymorphism is not neutral and has potential implications in the evolutionary origin of this polymorphism. We also found that the Y variants of CFH and FHL-1 preferentially bind to CRP and modified CRP (Figure 3 and Figure 6).

FHL-1's binding to most substrates is significantly different from that of CFH. These differences can be largely explained by the fact that FHL-1 is composed of SCR1−7 while CFH is composed of SCR1−20. For example, the

stronger binding of CFH than FHL-1 in binding to heparin can be explained by the presence of additional heparin binding sites on CFH and the loss of binding of FHL-1 to PspC can be explained by the absence of a PspC binding site in SCR1−7 (*58*). However, a previous study found that FHL-1, but not CFH, can mediate RGD-dependent adhesion to CC164 cells (*59*). This result is puzzling given the fact that both proteins contain SCR4, where the RGD motif is located. It was proposed that the RGD sequence may be hidden in CFH due to the interactions between SCRs and that the head and tail domains of CFH may fold back to form a loop (*26*, *60*, *61*). The ability of SCR7 alone, where the polymorphism Y402H is located, to bind to CFH ligands has never been studied. We found that SCR7 alone has little binding activity to heparin, CRP, or bacterial ligands of CFH. This finding is consistent with the cooperative nature of SCRs of CFH in molecular interactions. The distinct properties of CFH, FHL-1, and SCR7 suggest that results from studies of truncated forms of CFH may not be extrapolated to full length CFH.

S. pyogenes is a major human pathogen that was responsible for as many as 50% of postpartum deaths before World War II (*62*−*64*). It is a major cause of puerperal fever and scarlet fever (*65*). Therefore, it could have exerted a significant selective pressure on human populations in the past. The major virulence factor of *S. pyogenes* is M protein, which is responsible for its anti-phagocytosis activity (*66*). CFH binds to M protein through SCR7 (*27*, *31*, *40*, *67*). The large decrease in the binding of the H variants of CFH and FHL-1 to M protein may have implications in the evolution of the Y402H variant associated with AMD. The weaker binding of the H variants of CFH to M protein may allow a better defense against *S. pyogenes* because M protein cannot effectively recruit CFH to avoid complement attack. This is consistent with a previous hypothesis that pathogen exposure may select for CFH variants with weaker binding to the pathogen and hence reduced complement inhibition and stronger protection against infection (*1*). This beneficial function of a disease-associated mutation is analogous to the E6V mutation of the hemoglobin β gene, which causes sickle cell anemia but protects against malaria, and the ΔF508 mutation in CFTR, which causes cystic fibrosis but protects against *Salmonella typhi* (*68*, *69*).

Do the Y and H variants of CFH and/or FHL-1 affect AMD pathogenesis through their differential binding to CRP? CRP can be considered a primitive form of antibody. CRP binds to pathogenic bacteria and apoptotic cells by recognizing phosphorylcholine on these cells' surfaces (*70*−*73*). It has been hypothesized that CRP recruits CFH to apoptotic cells and bacteria to activate the early complement cascade without activating the terminal complement complex and therefore it promotes phagocytosis of apoptotic cells and reduces inflammation (*39*). Therefore, a decreased binding of the H variant of CFH may lead to a decreased ability of CFH to suppress inflammation. Further studies are necessary to elucidate the detailed role of CRP and CFH interaction in AMD pathogenesis.

Unlike early onset macular degenerations, there is no single genetic (or environmental) factor that is sufficient to cause AMD (*74*). Genetic analysis of AMD pathways is complicated by multiple genetic and environmental factors contributing to AMD pathogenesis. For the chromosome 1q locus contributing to AMD development (*75*), Y402H of CFH is not the only polymorphism associated with AMD, although it is the nonsynonymous polymorphism in this locus most significantly associated with AMD and is highly prevalent in many human populations affected by AMD. There are other polymorphisms in CFH associated with AMD (*1*, *76*) and a deletion of CFHR1 and CFHR3 that protects against AMD (*77*, *78*). Mechanistic analysis of the functional consequences of these AMD-associated genetic changes will provide further clues to the AMD pathogenic pathway involving CFH.

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REFERENCES

1. Hageman, G. S., Anderson, D. H., Johnson, L. V., Hancox, L. S., Taiber, A. J., Hardisty, L. I., Hageman, J. L., Stockman, H. A., Borchardt, J. D., Gehrs, K. M., Smith, R. J., Silvestri, G., Russell, S. R., Klaver, C. C., Barbazetto, I., Chang, S., Yannuzzi, L. A., Barile, G. R., Merriam, J. C., Smith, R. T., Olsh, A. K., Bergeron, J., Zernant, J., Merriam, J. E., Gold, B., Dean, M., and Allikmets, R. (2005) A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration, *Proc. Natl. Acad. Sci. U.S.A. 102*, 7227−7232.
2. Klein, R. J., Zeiss, C., Chew, E. Y., Tsai, J. Y., Sackler, R. S., Haynes, C., Henning, A. K., Sangiovanni, J. P., Mane, S. M., Mayne, S. T., Bracken, M. B., Ferris, F. L., Ott, J., Barnstable, C., and Hoh, J. (2005) Complement factor H polymorphism in age-related macular degeneration, *Science 308*, 385−389.
3. Edwards, A. O., Ritter, R., 3rd, Abel, K. J., Manning, A., Panhuysen, C., and Farrer, L. A. (2005) Complement factor H polymorphism and age-related macular degeneration, *Science 308*, 421−424.
4. Haines, J. L., Hauser, M. A., Schmidt, S., Scott, W. K., Olson, L. M., Gallins, P., Spencer, K. L., Kwan, S. Y., Noureddine, M., Gilbert, J. R., Schnetz-Boutaud, N., Agarwal, A., Postel, E. A., and Pericak-Vance, M. A. (2005) Complement factor H variant increases the risk of age-related macular degeneration, *Science 308*, 419−421.
5. Conley, Y. P., Thalamuthu, A., Jakobsdottir, J., Weeks, D. E., Mah, T., Ferrell, R. E., and Gorin, M. B. (2005) Candidate gene analysis suggests a role for fatty acid biosynthesis and regulation of the complement system in the etiology of age-related maculopathy, *Hum. Mol. Genet. 14*, 1991−2002.
6. Zareparsi, S., Branham, K. E., Li, M., Shah, S., Klein, R. J., Ott, J., Hoh, J., Abecasis, G. R., and Swaroop, A. (2005) Strong Association of the Y402H Variant in Complement Factor H at 1q32 with Susceptibility to Age-Related Macular Degeneration, *Am. J. Hum. Genet. 77*, 149−153.
7. Magnusson, K. P., Duan, S., Sigurdsson, H., Petursson, H., Yang, Z., Zhao, Y., Bernstein, P. S., Ge, J., Jonasson, F., Stefansson, E., Helgadottir, G., Zabriskie, N. A., Jonsson, T., Bjornsson, A., Thorlacius, T., Jonsson, P. V., Thorleifsson, G., Kong, A., Stefansson, H., Zhang, K., Stefansson, K., and Gulcher, J. R. (2005) CFH Y402H Confers Similar Risk of Soft Drusen and Both Forms of Advanced AMD, *PLoS Med. 3*, e5.
8. Seddon, J. M., George, S., Rosner, B., and Klein, M. L. (2006) CFH Gene Variant, Y402H, and Smoking, Body Mass Index, Environmental Associations with Advanced Age-Related Macular Degeneration, *Hum. Hered. 61*, 157−165.
9. Sepp, T., Khan, J. C., Thurlby, D. A., Shahid, H., Clayton, D. G., Moore, A. T., Bird, A. C., and Yates, J. R. (2006) Complement factor H variant Y402H is a major risk determinant for geographic atrophy and choroidal neovascularization in smokers and nonsmokers, *Invest. Ophthalmol. Vis. Sci. 47*, 536−540.

10. Rivera, A., Fisher, S. A., Fritsche, L. G., Keilhauer, C. N., Lichtner, P., Meitinger, T., and Weber, B. H. (2005) Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk, *Hum. Mol. Genet. 14*, 3227−3236.
11. Souied, E. H., Leveziel, N., Richard, F., Dragon-Durey, M. A., Coscas, G., Soubrane, G., Benlian, P., and Fremeaux-Bacchi, V. (2005) Y402H complement factor H polymorphism associated with exudative age-related macular degeneration in the French population, *Mol. Vis. 11*, 1135−1140.
12. Simonelli, F., Frisso, G., Testa, F., di Fiore, R., Vitale, D. F., Manitto, M. P., Brancato, R., Rinaldi, E., and Sacchetti, L. (2006) Polymorphism p.402Y>H in the complement factor H protein is a risk factor for age-related macular degeneration in an Italian population, *Br. J. Ophthalmol*.
13. Baird, P. N., Islam, F. M., Richardson, A. J., Cain, M., Hunt, N., and Guymer, R. (2006) Analysis of the Y402H variant of the complement factor H gene in age-related macular degeneration, *Invest. Ophthalmol. Vis. Sci. 47*, 4194−4198.
14. Pangburn, M. K. (2000) Host recognition and target differentiation by factor H, a regulator of the alternative pathway of complement, *Immunopharmacology 49*, 149−157.
15. Zipfel, P. F. (2001) Complement factor H: physiology and pathophysiology, *Semin. Thromb. Hemost. 27*, 191−199.
16. Zipfel, P. F., and Skerka, C. (1999) FHL-1/reconectin: a human complement and immune regulator with cell-adhesive function, *Immunol. Today 20*, 135−140.
17. Ault, B. H., Schmidt, B. Z., Fowler, N. L., Kashtan, C. E., Ahmed, A. E., Vogt, B. A., and Colten, H. R. (1997) Human factor H deficiency. Mutations in framework cysteine residues and block in H protein secretion and intracellular catabolism, *J. Biol. Chem. 272*, 25168−25175.
18. Rodriguez de Cordoba, S., Esparza-Gordillo, J., Goicoechea de Jorge, E., Lopez-Trascasa, M., and Sanchez-Corral, P. (2004) The human complement factor H: functional roles, genetic variations and disease associations, *Mol. Immunol. 41*, 355−367.
19. Friese, M. A., Hellwage, J., Jokiranta, T. S., Meri, S., Peter, H. H., Eibel, H., and Zipfel, P. F. (1999) FHL-1/reconectin and factor H: two human complement regulators which are encoded by the same gene are differently expressed and regulated, *Mol. Immunol. 36*, 809−818.
20. Jozsi, M., Manuelian, T., Heinen, S., Oppermann, M., and Zipfel, P. F. (2004) Attachment of the soluble complement regulator factor H to cell and tissue surfaces: relevance for pathology, *Histol. Histopathol. 19*, 251−258.
21. Fearon, D. T. (1978) Regulation by membrane sialic acid of beta1H-dependent decay-dissociation of amplification C3 convertase of the alternative complement pathway, *Proc. Natl. Acad. Sci. U.S.A. 75*, 1971−1975.
22. Pangburn, M. K., and Muller-Eberhard, H. J. (1978) Complement C3 convertase: cell surface restriction of beta1H control and generation of restriction on neuraminidase-treated cells, *Proc. Natl. Acad. Sci. U.S.A. 75*, 2416−2420.
23. Ormsby, R. J., Jokiranta, T. S., Duthy, T. G., Griggs, K. M., Sadlon, T. A., Giannakis, E., and Gordon, D. L. (2006) Localization of the third heparin-binding site in the human complement regulator factor H1, *Mol. Immunol. 43*, 1624−1632.
24. Blackmore, T. K., Sadlon, T. A., Ward, H. M., Lublin, D. M., and Gordon, D. L. (1996) Identification of a heparin binding domain in the seventh short consensus repeat of complement factor H, *J. Immunol. 157*, 5422−5427.
25. Prodinger, W. M., Hellwage, J., Spruth, M., Dierich, M. P., and Zipfel, P. F. (1998) The C-terminus of factor H: monoclonal antibodies inhibit heparin binding and identify epitopes common to factor H and factor H-related proteins, *Biochem. J. 331* (Part 1), 41−47.
26. Oppermann, M., Manuelian, T., Jozsi, M., Brandt, E., Jokiranta, T. S., Heinen, S., Meri, S., Skerka, C., Gotze, O., and Zipfel, P. F. (2006) The C-terminus of complement regulator Factor H mediates target recognition: evidence for a compact conformation of the native protein, *Clin. Exp. Immunol. 144*, 342−352.
27. Giannakis, E., Jokiranta, T. S., Male, D. A., Ranganathan, S., Ormsby, R. J., Fischetti, V. A., Mold, C., and Gordon, D. L. (2003) A common site within factor H SCR 7 responsible for binding heparin, C-reactive protein and streptococcal M protein, *Eur. J. Immunol. 33*, 962−969.
28. Zipfel, P. F., Skerka, C., Hellwage, J., Jokiranta, S. T., Meri, S., Brade, V., Kraiczy, P., Noris, M., and Remuzzi, G. (2002) Factor H family proteins: on complement, microbes and human diseases, *Biochem. Soc. Trans. 30*, 971−978.
29. China, B., Sory, M. P., N'Guyen, B. T., De Bruyere, M., and Cornelis, G. R. (1993) Role of the YadA protein in prevention of opsonization of Yersinia enterocolitica by C3b molecules, *Infect. Immun. 61*, 3129−3136.
30. Fischetti, V. A., Horstmann, R. D., and Pancholi, V. (1995) Location of the complement factor H binding site on streptococcal M6 protein, *Infect. Immun. 63*, 149−153.
31. Kotarsky, H., Hellwage, J., Johnsson, E., Skerka, C., Svensson, H. G., Lindahl, G., Sjobring, U., and Zipfel, P. F. (1998) Identification of a domain in human factor H and factor H-like protein-1 required for the interaction with streptococcal M proteins, *J. Immunol. 160*, 3349−3354.
32. Ram, S., McQuillen, D. P., Gulati, S., Elkins, C., Pangburn, M. K., and Rice, P. A. (1998) Binding of complement factor H to loop 5 of porin protein 1A: a molecular mechanism of serum resistance of nonsialylated Neisseria gonorrhoeae, *J. Exp. Med. 188*, 671−680.
33. Kraiczy, P., Skerka, C., Brade, V., and Zipfel, P. F. (2001) Further characterization of complement regulator-acquiring surface proteins of Borrelia burgdorferi, *Infect. Immun. 69*, 7800−7809.
34. Dave, S., Brooks-Walter, A., Pangburn, M. K., and McDaniel, L. S. (2001) PspC, a pneumococcal surface protein, binds human factor H, *Infect. Immun. 69*, 3435−3437.
35. Quin, L. R., Carmicle, S., Dave, S., Pangburn, M. K., Evenhuis, J. P., and McDaniel, L. S. (2005) In vivo binding of complement regulator factor H by Streptococcus pneumoniae, *J. Infect. Dis. 192*, 1996−2003.
36. Mold, C., Gewurz, H., and Du Clos, T. W. (1999) Regulation of complement activation by C-reactive protein, *Immunopharmacology 42*, 23−30.
37. Marnell, L., Mold, C., and Du Clos, T. W. (2005) C-reactive protein: ligands, receptors and role in inflammation, *Clin. Immunol. 117*, 104−111.
38. Agrawal, A. (2005) CRP after 2004, *Mol. Immunol. 42*, 927−930.
39. Gershov, D., Kim, S., Brot, N., and Elkon, K. B. (2000) C-Reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity, *J. Exp. Med. 192*, 1353−1364.
40. Giannakis, E., Male, D. A., Ormsby, R. J., Mold, C., Jokiranta, T. S., Ranganathan, S., and Gordon, D. L. (2001) Multiple ligand binding sites on domain seven of human complement factor H, *Int. Immunopharmacol. 1*, 433−443.
41. Clark, S. J., Higman, V. A., Mulloy, B., Perkins, S. J., Lea, S. M., Sim, R. B., and Day, A. J. (2006) His-384 Allotypic Variant of Factor H Associated with Age-related Macular Degeneration Has Different Heparin Binding Properties from the Non-disease-associated Form, *J. Biol. Chem. 281*, 24713−24720.
42. Sjoberg, A. P., Trouw, L. A., Clark, S. J., Sjolander, J., Heinegard, D., Sim, R. B., Day, A. J., and Blom, A. M. (2007) The factor H variant associated with age-related macular degeneration (H384) and the non-disease associated form bind differentially to C-reactive protein, fibromodulin, DNA and necrotic cells, *J. Biol. Chem. 282*, 10894−10900.
43. Cheng, H. J., and Flanagan, J. G. (1994) Identification and cloning of ELF-1, a developmentally expressed ligand for the Mek4 and Sek receptor tyrosine kinases, *Cell 79*, 157−168.
44. Flanagan, J. G., and Cheng, H. J. (2000) Alkaline phosphatase fusion proteins for molecular characterization and cloning of receptors and their ligands, *Methods Enzymol. 327*, 198−210.
45. Hsieh, J. C., Rattner, A., Smallwood, P. M., and Nathans, J. (1999) Biochemical characterization of Wnt-frizzled interactions using a soluble, biologically active vertebrate Wnt protein, *Proc. Natl. Acad. Sci. U.S.A. 96*, 3546−3551.
46. Xu, Q., Wang, Y., Dabdoub, A., Smallwood, P. M., Williams, J., Woods, C., Kelley, M. W., Jiang, L., Tasman, W., Zhang, K., and Nathans, J. (2004) Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair, *Cell 116*, 883−895.
47. Alsenz, J., Lambris, J. D., Schulz, T. F., and Dierich, M. P. (1984) Localization of the complement-component-C3b-binding site and the cofactor activity for factor I in the 38kDa tryptic fragment of factor H, *Biochem. J. 224*, 389−398.

48. Hegasy, G. A., Willhoeft, U., Majno, S. A., Seeberger, H., Zipfel, P. F., and Hellwage, J. (2003) Pig complement regulator factor H: molecular cloning and functional characterization, *Immunogenetics 55*, 462−471.
49. Dimitrellos, V., Lamari, F. N., Militsopoulou, M., Kanakis, I., and Karamanos, N. K. (2003) Capillary electrophoresis and enzyme solid phase assay for examining the purity of a synthetic heparin proteoglycan-like conjugate and identifying binding to basic fibroblast growth factor, *Biomed. Chromatogr. 17*, 42−47.
50. Fischetti, V. A., Jones, K. F., Manjula, B. N., and Scott, J. R. (1984) Streptococcal M6 protein expressed in Escherichia coli. Localization, purification, and comparison with streptococcal-derived M protein, *J. Exp. Med. 159*, 1083−1095.
51. Biro, A., Rovo, Z., Papp, D., Cervenak, L., Varga, L., Fust, G., Thielens, N. M., Arlaud, G. J., and Prohaszka, Z. (2007) Studies on the interactions between C-reactive protein and complement proteins, *Immunology*.
52. Kraiczy, P., Hellwage, J., Skerka, C., Becker, H., Kirschfink, M., Simon, M. M., Brade, V., Zipfel, P. F., and Wallich, R. (2004) Complement resistance of Borrelia burgdorferi correlates with the expression of BbCRASP-1, a novel linear plasmid-encoded surface protein that interacts with human factor H and FHL-1 and is unrelated to Erp proteins, *J. Biol. Chem. 279*, 2421−2429.
53. Pandiripally, V., Wei, L., Skerka, C., Zipfel, P. F., and Cue, D. (2003) Recruitment of complement factor H-like protein 1 promotes intracellular invasion by group A streptococci, *Infect. Immun. 71*, 7119−7128.
54. Kazatchkine, M. D., Fearon, D. T., Silbert, J. E., and Austen, K. F. (1979) Surface-associated heparin inhibits zymosan-induced activation of the human alternative complement pathway by augmenting the regulatory action of the control proteins on particle-bound C3b, *J. Exp. Med. 150*, 1202−1215.
55. Forsgren, A., McLean, R. H., Michael, A. F., and Quie, P. G. (1975) Studies of the alternate pathway in chelated serum, *J. Lab. Clin. Med. 85*, 904−912.
56. Sjoberg, A., Onnerfjord, P., Morgelin, M., Heinegard, D., and Blom, A. M. (2005) The extracellular matrix and inflammation. Fibromodulin activates the classical pathway of complement by directly binding C1q, *J. Biol. Chem. 280*, 32301−32308.
57. Sharma, A. K., and Pangburn, M. K. (1997) Localization by site-directed mutagenesis of the site in human complement factor H that binds to Streptococcus pyogenes M protein, *Infect. Immun. 65*, 484−487.
58. Jarva, H., Janulczyk, R., Hellwage, J., Zipfel, P. F., Bjorck, L., and Meri, S. (2002) Streptococcus pneumoniae evades complement attack and opsonophagocytosis by expressing the pspC locus-encoded Hic protein that binds to short consensus repeats 8−11 of factor H, *J. Immunol. 168*, 1886−1894.
59. Hellwage, J., Kuhn, S., and Zipfel, P. F. (1997) The human complement regulatory factor-H-like protein 1, which represents a truncated form of factor H, displays cell-attachment activity, *Biochem. J. 326* (Part 2), 321−327.
60. DiScipio, R. G. (1992) Ultrastructures and interactions of complement factors H and I, *J. Immunol. 149*, 2592−2599.
61. Barlow, P. N., Steinkasserer, A., Norman, D. G., Kieffer, B., Wiles, A. P., Sim, R. B., and Campbell, I. D. (1993) Solution structure of a pair of complement modules by nuclear magnetic resonance, *J. Mol. Biol. 232*, 268−284.
62. Spencer, R. C. (1995) Invasive streptococci, *Eur. J. Clin. Microbiol. Infect. Dis. 14* (Suppl. 1), S26−S32.
63. Bridson, E. Y. (1996) Iatrogenic epidemics of puerperal fever in the 18th and 19th centuries, *Br. J. Biomed. Sci. 53*, 134−139.
64. Burnet, M., and White, D. O. (1972) *Natural History of Infectious Disease*, 4th ed., Cambridge University Press, New York.
65. Katz, A. R., and Morens, D. M. (1992) Severe streptococcal infections in historical perspective, *Clin. Infect. Dis. 14*, 298−307.
66. Fischetti, V. A. (1989) Streptococcal M protein: molecular design and biological behavior, *Clin. Microbiol. Rev. 2*, 285−314.
67. Blackmore, T. K., Fischetti, V. A., Sadlon, T. A., Ward, H. M., and Gordon, D. L. (1998) M protein of the group A Streptococcus binds to the seventh short consensus repeat of human complement factor H, *Infect. Immun. 66*, 1427−1431.
68. Nesse, R. M., and Williams, G. C. (1998) Evolution and the origins of disease, *Sci. Am. 279*, 86−93.
69. Pier, G. B., Grout, M., Zaidi, T., Meluleni, G., Mueschenborn, S. S., Banting, G., Ratcliff, R., Evans, M. J., and Colledge, W. H. (1998) Salmonella typhi uses CFTR to enter intestinal epithelial cells, *Nature 393*, 79−82.
70. Volanakis, J. E., and Wirtz, K. W. (1979) Interaction of C-reactive protein with artificial phosphatidylcholine bilayers, *Nature 281*, 155−157.
71. Chang, M. K., Binder, C. J., Torzewski, M., and Witztum, J. L. (2002) C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: Phosphorylcholine of oxidized phospholipids, *Proc. Natl. Acad. Sci. U.S.A. 99*, 13043−13048.
72. Hack, C. E., Wolbink, G. J., Schalkwijk, C., Speijer, H., Hermens, W. T., and van den Bosch, H. (1997) A role for secretory phospholipase A2 and C-reactive protein in the removal of injured cells, *Immunol. Today 18*, 111−115.
73. Thompson, D., Pepys, M. B., and Wood, S. P. (1999) The physiological structure of human C-reactive protein and its complex with phosphocholine, *Structure 7*, 169−177.
74. Rattner, A., and Nathans, J. (2006) Macular degeneration: recent advances and therapeutic opportunities, *Nat. Rev. Neurosci. 7*, 860−872.
75. Weeks, D. E., Conley, Y. P., Tsai, H. J., Mah, T. S., Rosenfeld, P. J., Paul, T. O., Eller, A. W., Morse, L. S., Dailey, J. P., Ferrell, R. E., and Gorin, M. B. (2001) Age-related maculopathy: an expanded genome-wide scan with evidence of susceptibility loci within the 1q31 and 17q25 regions, *Am. J. Ophthalmol. 132*, 682−692.
76. Li, M., Atmaca-Sonmez, P., Othman, M., Branham, K. E., Khanna, R., Wade, M. S., Li, Y., Liang, L., Zareparsi, S., Swaroop, A., and Abecasis, G. R. (2006) CFH haplotypes without the Y402H coding variant show strong association with susceptibility to age-related macular degeneration, *Nat. Genet. 38*, 1049−1054.
77. Hughes, A. E., Orr, N., Esfandiary, H., Diaz-Torres, M., Goodship, T., and Chakravarthy, U. (2006) A common CFH haplotype, with deletion of CFHR1 and CFHR3, is associated with lower risk of age-related macular degeneration, *Nat. Genet. 38*, 1173−1177.
78. Hageman, G. S., Hancox, L. S., Taiber, A. J., Gehrs, K. M., Anderson, D. H., Johnson, L. V., Radeke, M. J., Kavanagh, D., Richards, A., Atkinson, J., Meri, S., Bergeron, J., Zernant, J., Merriam, J., Gold, B., Allikmets, R., and Dean, M. (2006) Extended haplotyes in the complement factor H (CFH) and CFH-related (CFHR) family of genes protect against age-related macular degeneration: characterization , ethnic distribution and evolutionary implications, *Ann. Med. 38*, 592−604.

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