Complement Factor H Polymorphism and Age-Related Macular Degeneration
Albert O. Edwards et al.
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haplotypes in the controls ($P < 0.0001$). The frequency of sequence variants within the CFH coding region on the associated haplotype was significantly reduced in cases compared to controls (12% versus 18%, $P = 0.002$). When the overrepresented T1277C variant was removed from the analysis, this difference became more pronounced (3% versus 16%, $P < 0.00001$). Thus, T1277C is the primary DNA sequence variant differentiating between the case and control haplotypes.

Complete genotyping of T1277C in the family-based and case-control data sets revealed a significant overtransmission in the families ($P = 0.019$) (12) and a highly significant overrepresentation in the cases compared to controls ($P = 0.00006$). The odds ratio for AMD was 2.45 [95% confidence interval (CI): 1.41 to 4.25] for carriers of one C allele and 3.33 (95% CI: 1.79 to 6.20) for carriers of two C alleles. When the analysis was restricted to only neovascular AMD, these odds ratios increased to 3.45 (95% CI: 1.72 to 6.92) and 5.57 (95% CI: 2.52 to 12.27), respectively. This apparent dose effect for risk associated with the C allele was highly significant ($P < 0.00001$). There was no apparent allelic or genotypic effect of T1277C on age at AMD diagnosis (mean age at diagnosis: TT, 76.5 years; TC, 77.5 years; CC, 75.5 years). The population attributable risk percent for carrying at least one C allele was 43% (95% CI: 23 to 68%).

The Y402H variant is predicted to have functional consequences consistent with AMD pathology. Residue 402 is located within binding sites for heparin (18) and C-reactive protein (CRP) (19). Binding to either of these partners increases the affinity of CFH for the complement protein C3b (20, 21), augmenting its ability to down-regulate complement's effect. The observed colocalization of CFH, CRP, and proteoglycans in the superficial layer of the arterial intima suggests that CFH may protect the host arterial wall from excess complement activation (22). We hypothesize that allele-specific changes in the activities of the binding sites for heparin and CRP would alter CFH's ability to suppress complement-related damage to arterial walls and might ultimately lead to vessel injury and subsequent neovascular/exudative changes such as those seen in neovascular AMD. Our data support this hypothesis, because the risk associated with the C allele is more pronounced when the analyses are restricted to neovascular AMD. Given the known functional interactions of genes within the RCA gene cluster (13), variants within these genes could interact with or modify the effect of the T1277C variant.

Plasma levels of CFH are known to decrease with smoking (23), a known risk factor for AMD (2). This confluence of genetic and environmental risk factors suggests an integrated etiological model of AMD involving chronic inflammation. Identification of the increased risk of AMD associated with the T1277C variant should enhance our ability to develop presymptomatic tests for AMD, possibly allowing earlier detection and better treatment of this debilitating disorder.

References and Notes

11. M. de la Paz, M. Klein, J. Caldwell, R. Domurath, K. Haynes, V. Mitchell, M. Shaw, and J. Galloway for participant ascertainment; R. Abramson, J. Benton, W. Lambert, B. Love, T. Skelly, E. Teggeln, M. Allen, C. Haynes, R. Chung, and J. Bunch for valuable technical assistance; J. M. Vance and M. Summar for critical reading of the manuscript; and D. J. M. Gass for patient ascertainment and clinical expertise. We also thank the following clinics and clinicians for referring individuals to the study: Southern Retina, LLC (C. Harris); Vitreo-Retinal Surgeons (M. Duan and C. Devine); Georgia Retina, P.C.; and The Retina Group of Washington. Supported by grants EY12178 (to M.A.P.-V. and J.L.H.) and EY015216 (to S.S.) from the NIH/National Eye Institute, grant AG11268 from the NIH/National Institute on Aging (to H. Cohen), and grant M01 RR-00095 from the NIH/National Center for Research Resources (to Vanderbilt University).

Supporting Online Material

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Materials and Methods
Table S1
References
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Complement Factor H Polymorphism and Age-Related Macular Degeneration

Albert O. Edwards, Robert Ritter III, Kenneth J. Abel, Alisa Manning, Carolien Panhuysen, Lindsay A. Farre

Age-related macular degeneration (AMD) is a common, late-onset, and complex trait with multiple risk factors. Concentrating on a region harboring a locus for AMD on 1q25-31, the ARM1 locus, we tested single-nucleotide polymorphisms for association with AMD in two independent case-control populations. Significant association ($P = 4.95 \times 10^{-10}$) was identified within the regulation of complement activation locus and was centered over a tyrosine-402 → histidine-402 protein polymorphism in the gene encoding complement factor H. Possession of at least one histidine at amino acid position 402 increased the risk of AMD 2.7-fold and may account for 50% of the attributable risk of AMD.
Fig. 1. The regulation of complement activation (RCA) locus located within chromosome 1q31.3 includes the gene for complement factor H (CFH), five related genes derived from CFH through ancestral duplications, and the gene for factor 13B (F13B). A megabase (Mb) scale of this region is provided at the top of the figure. SNPs genotyped across the RCA locus are shown along the bottom of the figure. The negative natural logarithm of the significance of allele association to AMD for each SNP is given in the graph (10). The 0.05 significance level is shown by the dotted line. Values greater than 15 on the y axis correspond to P values less than $10^{-7}$.

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Table 1. Association between the Try402 → His402 polymorphism (rs1061170) in CFH and AMD. The C allele codes for histidine. The genotype association compares CC with CT and TT.

of chromosome 1 (fig. S1) from subsequently reported small family studies, we focused our efforts on the ARMD1 locus (3, 4, 6, 8, 9).

We performed an allele association study on a new case-control population that is highly discordant for clinical phenotypes. Cases were enrolled on the basis of ocular features (extensive drusen or pigmentary abnormalities of the macula) placing subjects at high risk for development of the complications of AMD or the presence of those complications in one or both eyes (10). Control subjects were from the same patient population and could have no more than four small hard drusen in the central retina (macula) and no known family history of AMD. A subset of 224 cases and 134 controls meeting these criteria were selected as a discovery sample for initial genotyping (table S1). The discovery sample was enriched for AMD cases showing familial clustering of AMD and high-risk, early AMD. A second, replication sample of 176 cases and 68 controls was ascertained at the same clinic following the same protocol (table S1).

Evaluation of the reported Glr5346 → Arg5346 variation in the fibulin 6 gene (FIBL6) and 23 single-nucleotide polymorphisms (SNPs) across this gene [supporting online material (SOM) text] gave no evidence for allelic or haplotype association in the discovery sample (11). To determine whether common coding sequence variation within the ARMD1 locus was associated with AMD, we searched publicly available databases for nonsynonymous coding SNPs (nscSNPs). We identified 24 nscSNPs with known minor allele frequencies of at least 10% across the 14-Mb ARMD1 locus. Genotyping of the discovery sample gave evidence for allele association with the majority, and the most significant of these, including the nscSNP rs1061170, concentrated in the CFH gene (table S3, GenBank accession no. NM_000186).

The genotype frequency data for rs1061170 revealed that the association with AMD was largely due to an excess of CC homozygotes in cases compared with controls (Table 1). A similar pattern of association was evident with seven adjacent SNPs in CFH (Fig. 1 and table S3). The strength of the evidence for association diminished markedly with SNPs located immediately proximal to CFH and distal to its derivatives CFHL1 to CFHL5, suggesting that the effect was due to one or more polymorphisms in the complement factor genes only (Fig. 1 and table S3).

Haplotype analyses of 34 SNPs spanning 418 kb revealed extensive linkage disequilibrium across the full length of the RCA locus (Fig. 2). The highest level of linkage disequilibrium was discernable among four haplotype blocks across the RCA locus. Thirteen contiguous SNPs in CFH (i.e., all but the first two CFH SNPs in Fig. 2) form a 64-kb haplotype block. A second 9-kb haplotype block contained SNPs in the proximal region of chromosome 1 (fig. S1) from subsequently reported small family studies, we focused our efforts on the ARMD1 locus (3, 4, 6, 8, 9).

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Fig. 2. Pairwise linkage disequilibrium (LD) among 38 SNPs in and surrounding the RCA locus, spanning the region including the 5′ upstream region of CFH (represented by the SNP rs3753394) and the proximal portion of FRBZ1 (represented by the SNP rs10733295), a distance of 516 kb. LD was measured by the D′ statistic with use of data from all subjects in the discovery sample (10). A D′ value of 1 indicates complete linkage disequilibrium between two markers, and a D′ value of 0 indicates complete linkage equilibrium. By using the Haploview version 3.0 software, we could divide the RCA locus into four haplotype blocks (29). D′ values for all or nearly all pairwise comparisons within a haplotype block are at least 0.8 (black squares). The haplotype block structure is similar to that obtained by the HapMap Project for this region in Caucasians, with the notable difference that blocks 2 and 3 in our structure compose one block in the HapMap report (10). The lone SNP (rs379370) between blocks 2 and 3 is a nonsNP in the CFH4 gene (table S2) showing moderate LD (gray squares), modest LD (light gray squares), or low LD (white squares) with the SNPs in block 3.

The tyrosine-to-histidine polymorphism (rs1061170) at amino acid 402 of CFH may be a primary pathogenic variation increasing the risk of developing AMD. CFH is composed of 20 repetitive units of 60 amino acids called short consensus repeats (SCRs). The Try402 → His402 polymorphism is located within SCR7, which contains the overlapping binding sites for heparin, C-reactive protein (CRP), and M protein (18). Serum amounts of CRP were elevated in AMD subjects compared with controls in one large prospective clinical trial (19). CRP activates the classic complement pathway but reduces deposition of C5b-9 through the direct binding of CFH (20). Risk factors for development of complications of AMD, including cigarette smoking, lack of exercise, hypertension, and obesity (2, 21), increased serum CRP or decreased serum CFH (22–25). Further, drusen with terminal complement deposition indistinguishable from AMD were observed in eyes from patients with a kidney disease (membranoproliferative glomerulonephritis type II) that can be caused by mutations in CFH (26, 27). In principle, altered binding of CFH to CRP or heparin on outer retinal surfaces caused by the Try402 → His402 substitution could affect the level of inflammation in the outer retina, thereby contributing to AMD. Although our results are consistent with the Try402 → His402 variant causing AMD, they do not rule out the existence of other coding or splice site variants within CFH that modulate risk of AMD.

More than 7 million individuals in the United States have retinal features placing them at high risk for developing vision loss from complications of AMD (28). The attributable fraction for the C allele derived from the total sample of subjects in this study is 50%, suggesting that persons either homo-
zygous or heterozygous for histidine at amino acid 402 of CFH may account for one-half of AMD cases. Given the rapid aging of the population, an estimated 3 million individuals will have atrophic and exudative complications of AMD by 2020 (28). Our findings suggest previously unknown avenues for developing preventative and therapeutic strategies for AMD.

References and Notes
10. Materials and methods are available as supporting material on Science Online.
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References
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