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 < 0.0001). 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 < 0.0001). There was no apparent allelic or genotypic effect of T1277C variants with other AMD loci.

Age-related macular degeneration (AMD) is a common, late-onset, and complex trait with multiple risk factors. Concentrating on a region harboring a SNP within the CFH gene (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 retinal pigment epithelium suggests that CFH may protect the host arterial wall from excess 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 etiologic 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
12. Materials and methods are available as supporting material on Science Online.
24. We thank all of the study participants and their relatives; M. de la Paz, M. Klein, J. Caldwell, R. Domrath, K. Haynes, V. Mitchell, M. Shaw, and J. Galloway for participant ascertainment; R. Abramson, J. Benton, W. Lambert, B. Love, T. Skelly, E. Tegnell, 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 EY12118 (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 MO1 RR-00095 from the NIH/National Center for Research Resources (to Vanderbilt University).

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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. Farrer

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 ARMD1 locus, we tested single-nucleotide polymorphisms for association with AMD in two independent case-control populations. Significant association (P = 4.95 × 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.

AMD is a leading cause of blindness in older individuals (1). It is a late-onset, complex trait with hereditary, lifestyle, and medical risk factors (2). The condition typically presents in the fifth decade of life with small yellow deposits external to the outer retina and retinal pigment epithelium (RPE) called drusen. Large numbers of drusen and clinical features of damage to the RPE markedly increase the risk of complications (atrophy of the RPE and abnormal neovascularization of the outer retina), leading to severe vision loss (1).

Although the primary pathogenic mechanisms of AMD were previously unknown, there is strong evidence that genetics plays a role (3–9). The first locus for AMD (ARMD1) was reported in a single extended family linked to chromosome 1q25.3-31.3 (5). Because there was strong evidence for linkage to this 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).

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 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 $10^{-7}$.

**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 $10^{-7}$.

### 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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Allele distribution</th>
<th>Genotype distribution</th>
<th>Genotype association ($P$ value)</th>
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<tr>
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<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
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<tr>
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<td></td>
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</tbody>
</table>

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portion of CFHL4, a third 50-kb block contained SNPs in the distal portion of CFHL4 and in CFHL2, and a fourth 146-kb block contained SNPs in F13B, ASPM, and FRBZ1 (Fig. 2). The SNPs most significantly associated with AMD were in CFH or within 221 kb downstream of CFH (Fig. 1 and table S3).

The association between AMD and haplotypes comprising two to five contiguous SNPs was evaluated by using the Haplo.stat software and a sliding window approach (12, 13). Additional comparisons were made by using all possible haplotypes formed by pairwise combinations, including at least one nscSNP within the RCA locus. The analysis did not reveal any SNP combination showing greater association with AMD than the individual SNPs. All of the AMD high-risk haplotypes, including a CFH SNP from haplotype block 1 and a non–CFH SNP from other regions of the RCA locus (Fig. 1), contained the AMD risk allele from the CFH SNP but not necessarily the AMD risk allele from the non–CFH SNP. These analyses provide further evidence that the multiple signals in the RCA locus are related to a single haplotype and therefore likely caused by a single genetic effect.

To verify these findings, we genotyped 14 SNPs in the RCA locus in the replication sample. Association with AMD was observed with the seven markers that were significant in the discovery sample, including rs1061170, but not with the seven markers yielding negative results in the discovery sample (table S4). Notably, the genotype frequencies for rs1061170 among cases enriched for a positive family history of AMD were nearly identical to the frequencies among cases without this characteristic (Table 1), suggesting that this CFH protein polymorphism is a risk factor for AMD more generally. Taking into account data from the entire sample, a conservative estimation of the relative risk for AMD conferred by having at least one C allele (i.e., having either the CC or CT genotypes) was 2.7 (95% confidence interval of 1.9 to 3.9).

Complement activation has been implicated in the pathogenesis of a number of complex traits, including AMD, and can arise through the classical, lectin, or alternative pathways (14). All three pathways lead to the generation of a C3 convertase enzyme and subsequent activation of the immune response, the terminal pathway pore-like membrane attack complex (C5b-9), and cell lysis. The alternative complement pathway is spontaneously activated, and CFH is an essential inhibitor preventing uncontrolled complement activation (15). Components of the terminal complement pathway and other markers of inflammation are deposited in drusen and the choroid of eyes with AMD (16, 17). Abnormal regulation of the alternative pathway of complement activation by CFH is consistent with these observations.

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 Try<sup>402</sup> → His<sup>402</sup> 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 Tyr<sup>402</sup> → His<sup>402</sup> substitution could affect the level of inflammation in the outer retina, thereby contributing to AMD. Although our results are consistent with the Tyr<sup>402</sup> → His<sup>402</sup> 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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28. We thank the McDermott Sequencing Center at UT Southwest for genotyping assistance and T. Hyatt for technical advice and assistance. Supported by the National Eye Institute (EY014467), a center grant from the Foundation Fighting Blindness, and Research to Prevent Blindness.

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