

## Complement Factor H Variant Increases the Risk of Age-Related Macular Degeneration

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**Age-related macular degeneration (AMD) is a leading cause of visual impairment and blindness in the elderly whose etiology remains largely unknown. Previous studies identified chromosome 1q32 as harboring a susceptibility locus for AMD. Here we used single nucleotide polymorphisms (SNPs) to interrogate this region and identified a strongly associated haplotype in two independent data sets. DNA resequencing of the complement factor H gene (*CFH*) within this haplotype revealed a common coding variant, Y402H, that significantly increases the risk for AMD with odds ratios between 2.45 and 5.57. This common variant likely explains ~43% of AMD in older adults.**

Age-related macular degeneration (AMD) causes progressive impairment of central vision and is the leading cause of irreversible vision loss in older Americans (1). The most severe form of AMD involves neovascular/exudative (wet) and/or atrophic (dry) changes to the macula. Although the etiology of AMD remains largely unknown, implicated risk factors include age, ethnicity, smoking, hypertension, obesity and diet (2). Familial aggregation (3), twin studies (4), and segregation analysis (5) suggest that there is also a significant genetic contribution to the disease. The candidate gene approach, which focuses on testing biologically relevant candidates, has implicated variants in the *ABCA4*, *FBLN6*, and *APOE* genes as risk factors for AMD. Replication of the *ABCA4* and *FBLN6* findings has been difficult, and *in toto* these variants explain a small proportion of AMD (6–8). The alternative genomic approach uses a combination of genetic linkage and association to identify novel genes involved in AMD. We participated in a recent collaborative genome-wide linkage screen (9) in which chromosome 1q32 was identified as a likely region for an AMD risk gene, a location also supported by other studies (10, 11).

To identify the responsible gene on chromosome 1q32, we initially genotyped 44 SNPs (12) across the 24 megabases (Mb) incorporating this linkage region. We examined two independent data sets: the first contained 182 families (111 multiplex and 71 discordant sibpairs) and the second contained 495 AMD cases and 185 controls. Each SNP was tested for association independently in both data sets. Two SNPs (rs2019724 and rs6428379) in moderate linkage disequilibrium with each other ( $r^2=0.61$ ) generated highly significant associations with AMD in both the family-based data set (rs2019724,  $P=0.0001$ ; rs6428379,  $P=0.0007$ ) and in the case-control data set (rs2019724,  $P<0.0001$ ; rs6428379,  $P<0.0001$ ). These SNPs lie approximately 263 kilobases (Kb) apart.

To completely define the extent of linkage disequilibrium, an additional 17 SNPs were genotyped across approximately 655 Kb flanked by rs1538687 and rs1537319 and encompassing the 263 Kb region. Two linkage disequilibrium blocks of 11 Kb and 74 Kb were identified and were separated by 176 Kb (Fig. 1). The 11 Kb block contained rs2019724 and the 74 Kb block contained rs6428379. Association analysis of the 17 SNPs identified multiple additional SNPs giving highly significant associations in one or both of the family-based and case-control data sets (Fig. 2). In the case-control data set, a five SNP haplotype (GAGGT, defined by SNPs rs1831281, rs3753395, rs1853883, rs10494745, and rs6428279, respectively) comprised 46% of the case and 33% of the control chromosomes ( $P=0.0003$ ). This same haplotype was also significantly over-transmitted to affected individuals in the family-based data set ( $P=0.00003$ ). The convergence of the most significant associations to this same haplotype in the two independent data sets strongly suggests that this region contains a commonly inherited variant in an AMD risk gene.

The associated GAGGT haplotype spans approximately 261 Kb. It contains the Complement Factor H gene (*CFH*, OMIM #:134370, Accession #:NM\_000186) and the five Factor H-related genes *CFHLI-5*, and lies within the Regulator of Complement Activation (RCA) gene cluster. The most consistent association results (Fig. 2) from both the family-based and case-control data sets converge within the *CFH* gene implicating *CFH* as the AMD susceptibility gene. The biological role of Complement Factor H as a component of the innate immune system that modulates inflammation through regulation of complement (reviewed in (13)) enhances its attractiveness as a candidate AMD susceptibility gene. Inflammation has been repeatedly implicated in AMD pathology. C-reactive protein levels are elevated in advanced disease (14), anti-retinal autoantibodies have been detected in AMD patients (15), macrophages are localized near neovascular lesions (16), and the hallmark drusen deposits contain many complement-related proteins (17).

We screened for potential risk-associated sequence variants in the coding region of *CFH* by sequencing 24 cases with severe neovascular disease and 24 controls with no evidence of AMD. To maximize the likelihood of identifying the risk-associated allele, all sequenced cases and controls were homozygous for the GAGGT haplotype. Five novel and six known sequence variants were detected (Table 1). Only one variant (rs1061170, sequence: T1277C, protein: Y402H) was present significantly more often in cases than controls, occurring on 45/48 haplotypes in the cases and on 22/48 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% vs. 18%,  $P = 0.002$ ). When the over-represented T1277C variant was removed from the analysis, this difference became more pronounced (3% vs. 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 over-transmission in the families ( $P = 0.019$ ) (12) and a highly significant over-representation in the cases compared to controls ( $P = 0.00006$ ). The odds ratio for AMD was 2.45 (95% CI: 1.41-4.25) for carriers of one C allele and 3.33 (95% CI: 1.79-6.20) for carriers of two C alleles. When the analysis was restricted to only neovascular AMD, these odds ratios increased to 3.45 (95% CI: 1.72-6.92) and 5.57 (95% CI: 2.52-12.27), 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 on age at AMD diagnosis (mean age at diagnosis: TT: 76.5yrs; TC 77.5yrs; CC 75.5 yrs). The population attributable risk

percent for carrying at least one C allele was 43% (95% CI: 23–68%).

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 co-localization 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 since 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.

Interestingly, 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

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### Supporting Online Material

[www.sciencemag.org/cgi/content/full/1110359/DC1](http://www.sciencemag.org/cgi/content/full/1110359/DC1)

Materials and Methods

Table S1

References

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**Fig. 1.** Haploview plot defining haplotype block structure of AMD associated region. The relative physical position of each SNP is given in the upper diagram, and the pairwise linkage disequilibrium ( $D'$ ) between all SNPs is given below each SNP combination. Dark red shaded squares indicated  $D'$  values  $>0.80$ .  $D'=1.0$  when no number is given.

**Fig. 2.** Plot of family-based and case-control  $P$  values for all SNPs within the AMD-associated haplotype. The genomic region spanning each gene is indicated in green.  $-\log_{10}$  of the nominal  $P$  values are plotted for each SNP. Results for both the family-based and case-control data sets converge within the *CFH* gene.

**Table 1.** *CFH* sequence variants identified in neovascular AMD cases and normal controls. All individuals were homozygous for the AMD-associated GAGGT haplotype. The 24 affected individuals selected for sequencing had severe neovascular disease (grade 5) (12) with diagnosis before age 74 (mean age at diagnosis: 65.8 yrs). The 24 control individuals selected for sequencing had no evidence of AMD (grade 1) with age at exam after age 64 (mean age at exam: 69.8 yrs). The six previously identified SNPs are labeled using standard nomenclature. The five novel variants are labeled given their base pair location on chromosome 1, Ensembl build 35. Five SNPs create non-synonymous amino acid changes within *CFH* and five SNPs create synonymous changes. Exon 1 is not translated.

Location	SNP ID	effect	Minor Allele Frequency (%)	
			AMD	Controls
exon 1	rs3753394	n/a	18	24
exon 2	rs800292	V62I	0	6
exon 6	193,380,486 A/G	R232R	0	2
exon 7	rs1061147	A307A	10	38
exon 8	193,390,164 C/T	H332Y	0	5
exon 9	rs1061170	Y402H	94	46
exon 11	193,414,604 A/G	A473A	0	31
exon 12	193,416,415 A/G	T519A	0	2
exon 14	rs3753396	Q672Q	0	23
exon 18	193,438,299 C/T	H878H	6	2
exon 19	HGVbase 000779895	E936D	0	23



