Participants

We ascertained AMD patients and their affected and unaffected family members through two clinics in the Southeastern United States - Duke University Medical Center (DUMC) and Vanderbilt University Medical Center (VUMC). Unrelated controls of similar age and ethnic background were enrolled via (i) study advertisement in DUMC- and VUMC-affiliated newsletters; (ii) recruitment presentations by study coordinators at local retirement communities, who were likely to obtain health care at DUMC or VUMC, respectively; (iii) AMD-related seminars for the general public sponsored by DUMC or VUMC ophthalmology clinics. (iv) referrals from other clinics in the Duke and Vanderbilt Eye Centers of individuals without evidence of ocular disease. Spouses of AMD patients were also asked to participate as potential controls. Controls eligible for enrollment were offered a free comprehensive eye exam including fundus photography to ensure that the same methodology was used to assign AMD grades as for the AMD patients and their relatives ascertained in clinic. All cases and controls included in this study were Caucasian and at least 55 years of age. The study protocol was approved by the respective Institutional Review Boards (IRB) at DUMC and VUMC, and the research adhered to the tenets of the Declaration of Helsinki.
The family-based data set consisted of 111 multiplex families with at least two individuals with grade 3 or higher AMD in at least one eye. Seventy-three families had two affected individuals, 29 families had three affected individuals, and nine families had four or more affected individuals. Unaffected spouses and siblings were collected whenever possible. 71 additional families consisted of one affected individual and at least one unaffected sibling (discordant sibpairs).

Clinical Assessment

The assignment of AMD affection status was based on the clinical evaluation of stereoscopic color fundus photographs of the macula (EAP, AA), according to a 5-grade system described previously (S1). Grade 1 has no AMD features, grade 2 has only small non-extensive drusen, grade 3 has extensive intermediate and/or large drusen, grade 4 is geographic atrophy, and grade 5 is neovascular AMD. This system is a slight modification of the Age-Related Eye Disease Study (AREDS) grading system and uses example slides from the Wisconsin Grading System (S2) and the International Classification System (S3) as guides. Affection status was defined by the most severe grade in either eye. All questionnaire data and samples were collected after informed consent was obtained.

Molecular Analyses

Genomic DNA was extracted from whole blood by the Duke CHG or Vanderbilt CHGR DNA banking cores using the PureGene system (Gentra Systems, Minneapolis, MN) on an Autopure LS. Genotyping was performed using Taqman on the ABI Prism 7900HT,
and analyzed with the SDS software. SNP Assays-On-Demand or Assays-By-Design were obtained from Applied Biosystems Incorporated (Foster City, CA). The initial set of 44 SNPs was chosen to approximate a 500 Kb spacing between markers.

Exons of \textit{CFH} were PCR amplified from genomic DNA, sequenced using Big Dye v3.1 (ABI) on an ABI 3730 automated sequencer, and analyzed using Mutation Surveyor software (Softgenetics, State College, PA). T1277C falls within a genomic duplication and could not be genotyped using TaqMan assays. All individuals were sequenced using primers GGTTTCTTCTTGAAAATCACAGG and CCATTGGTAAAACAAGGTGACA to determine T1277C genotypes.

\textbf{Statistical Analyses}

Linkage disequilibrium and Hardy-Weinberg equilibrium calculations were done using Haploview version 3.0 using all case and control samples and one random individual from each of the families (\textit{S4}). Haplotype blocks were defined using the D' parameter and the default definitions within Haploview. Allele frequency differences were tested using a \(\chi^2\) test.

Single-locus and haplotype family-based association was tested using the Association in the Presence of Linkage (APL) method (\textit{S5}) that performs a correct TDT-style test of association in the presence of linkage, using nuclear families with at least one affected individual and any number of unaffected siblings or parents. Odds ratios were calculated using standard logistic regression models (SAS version 9.1, SAS Institute, Cary, NC). The outcome variable was AMD affection status and genotypes were coded according to a log-additive model. Dose-response was tested using the \(\chi^2\) test for trend.
Haplotype analysis in the case-control data set was tested using the “haplo.stats” program that uses a likelihood-based method to estimate haplotype frequencies (S6).

The 95% confidence interval for the population attributable risk percent (PAR%) for T1277C was calculated on the point estimate of the PAR% (43%), which was calculated from the combined frequency of genotypes CT and CC in controls and the unadjusted odds ratio (OR) of AMD for these genotypes relative to the TT reference group (S7). Calculation of the PAR% from case-control data assumes that the controls are representative of the general population and the disease is rare (< 5% population prevalence across all exposure levels). PAR% calculated from OR adjusted for age and sex was similar.

We note that the \( P \)-value of the T1277C association in the family-based data set is not as significant as the \( P \)-value for the two original SNPs. This results from the ascertainment bias toward severe disease in the family collection, which results in an oversampling of T1277C-CC homozygotes. Family-based tests of association depend on both transmission and association. Oversampling for homozygosity reduces the power of any family-based transmission disequilibrium test. Since the original SNPs have low linkage disequilibrium values with T1277C (\( r^2 = 0.00 \) and 0.14 for rs2019724 and rd6428379, respectively), they were not over-sampled for homozygosity to the extent of T1277C. In the case-control data set where the sampling bias is not as profound, the \( P \)-values for all three SNPs are similarly highly significant.
Haplotype Analysis

The five SNP haplotype block, defined by SNPs rs1831281, rs3753395, rs1853883, rs10494745, and rs6428279, identified five common haplotypes that capture over 95% of the haplotype variation (Table S1). The GAGGT haplotype is the most common in both the cases and controls, but is significantly more frequent in the cases.

SUPPORTING TABLE

Table S1. The haplotypes and their frequencies calculated from the case-control data.

The haplotype consists of SNPs rs1831281, rs3753395, rs1853883, rs10494745, and rs6428279, respectively.

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<th>Haplotype Frequency</th>
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SUPPORTING REFERENCES


