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Complement Factor D in Age-Related Macular Degeneration

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PURPOSE. To examine the role of complement factor D (CFD) in age-related macular degeneration (AMD) by analysis of genetic association, copy number variation, and plasma CFD concentrations.

METHODS. Single nucleotide polymorphisms (SNPs) in the CFD gene were genotyped and the results analyzed by binary logistic regression. CFD gene copy number was analyzed by gene copy number assay. Plasma CFD was measured by an enzyme-linked immunosorbent assay.

RESULTS. Genetic association was found between CFD gene SNP rs3826945 and AMD (odds ratio 1.44; P = 0.028) in a small discovery case-control series (462 cases and 325 controls) and replicated in a combined cohorts meta-analysis of 4765 cases and 2693 controls, with an odds ratio of 1.11 (P = 0.032), with the association almost confined to females. Copy number variation in the CFD gene was identified in 13 out of 640 samples examined, but there was no difference in frequency between AMD cases (1.3%) and controls (2.7%). Plasma CFD concentration was measured in 751 AMD cases and 474 controls and found to be elevated in AMD cases (P = 0.00025). The odds ratio for those in the highest versus lowest quartile for plasma CFD was 1.81. The difference in plasma CFD was again almost confined to females.

CONCLUSIONS. CFD regulates activation of the alternative complement pathway, which is implicated in AMD pathogenesis. The authors found evidence for genetic association between a CFD gene SNP and AMD and a significant increase in plasma CFD concentration in AMD cases compared with controls, consistent with a role for CFD in AMD pathogenesis. (Invest Ophthalmol Vis Sci. 2011;52:8828–8834) DOI:10.1167/iovs.11-7933

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tries. It is associated with the progressive deposition of extracellular material (drusen or basal deposits) between the basal surface of the macular RPE and Bruch’s membrane. This is thought to be associated with immune attack, leading to dysfunction and eventual death of macular RPE cells (geographic atrophy; GA). In 5%–10% of affected subjects, choroidal neovascularization (CNV) can lead to hemorrhage and exudation within the macula, causing catastrophic loss of vision. The major risk factors in AMD include age, smoking, and genetic influences. The latter include genetic variation in genes influencing the alternative complement pathway such as CFH, C2/BF, C3, and F1 (reviewed in Refs. 16, 18). Other genes influencing the alternative complement pathway prompted us to examine the role of genetic variation influencing CFD, including copy number variation and plasma concentrations, particularly because CFD has an important role in activation of this pathway.

**Materials and Methods**

**Study Cohorts**

The clinical and demographic features of the six case-control series are summarized in Table 1.

**Genotyping**

Initially, only three SNPs were identified within the CFD gene (rs1683564, rs3826945, rs1683563) one of which (rs16853564) was not successfully genotyped. DNA was genotyped in two UK case-control series (UK1, UK2) for two intronic SNPs, rs3826945 and rs1683563, using genotyping technology (TaQMan; Applied Biosystems) for RNAseP in a duplex real-time polymerase chain reaction. The CFD probe was FAM-labeled and the endogenous control was VIC-labeled. Ten-microliter reactions were set up in 384-well plates (TaQMan Universal PCR Master Mix, No AmpEraseUNG; Applied Biosystems) with 7.5 ng DNA, 1 μM of each primer, and 0.2 μM of probe. The thermal cycling reactions (95°C for 10 minutes, followed by 40 cycles at 92°C for 15 seconds and 60°C for 1 minute) were run and analyzed (7900HT Sequence Detection System; Applied Biosystems) with genetic analysis software (Genotyper SDS system, version 2.2; Applied Biosystems). As controls, each plate contained multiple blank wells without DNA. Similarly, for the USA 2 (University of Pennsylvania) and Dutch-German (Nijmegen) case-control series, assays (TaQMan; Applied Biosystems) were also used to genotype rs3826945 and rs1683563. In the USA 3 series, SNPs rs3826945 and rs1683563 were genotyped using a different assay (Sequenom iPLEX; Sequenom, San Diego, CA), as described.

In the USA 1 case-control series (University of Michigan), CFD SNPs rs1683563 and rs3826945 were genotyped as follows. Primers were designed and used to polymerase chain reaction (PCR) amplify the amplicons for dye-termination Sanger sequencing. The primers for the rs1683563 were: forward primer 5′AGTGGCCCTCTCGCACAG and reverse primer 5′AAATCTCTCGTCTGCTGACTGA. Primers for rs3826945 were: forward primer 5′CACCGTGTAAGCCCTCT and reverse primer 5′TG-GAAAGCAGGAATGAGGT. Standard PCR conditions were followed with the use of polymerase (TaKaRa Ex Taq; Takara Bio Inc., Shiga, Japan) with 1 μL of DNA (approximately 100 ng/μL), and PCR conditions: melting temperature 94°C for 2 minutes; 35 cycles at 94°C for 30 seconds; annealing at 60°C for 30 seconds; extension at 72°C for 30 seconds; followed by extension at 72°C for 7 minutes. PCR products were run on 1% ethidium bromide agarose gels and viewed on a gel documentation system (Kodak 440; Kodak, Rochester, NY). Aliquots of the PCR products were submitted for sequencing at the University of Michigan core facility.

**Copy Number Variation Analysis**

Copy number variation at the CFD locus was assessed using a copy number assay (Hs01536182_cn; TaQMan; Applied Biosystems), run simultaneously with a copy number reference assay (TaQMan; Applied Biosystems) for RNAseP in a duplex real-time polymerase chain reaction. The CFD probe was FAM-labeled and the endogenous control was VIC-labeled. Ten-microlitter reactions were set up in 384-well plates (TaQMan Universal PCR Master Mix, No AmpEraseUNG; Applied Biosystems) with 10 ng genomic DNA, 1 μM of each primer, and 0.2 μM of probe. The thermal cycling reactions (95°C for 10 minutes, followed by 40 cycles at 92°C for 15 seconds and 60°C for 1 minute) were run on a sequence detection system (7900HT Sequence Detection System; Applied Biosystems).

**Table 1.** Cohort Characteristics

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Status</th>
<th>n</th>
<th>%</th>
<th>Grading</th>
<th>%</th>
<th>Mean Age (y)</th>
<th>SD</th>
<th>Sex</th>
<th>Male</th>
<th>%</th>
<th>Female</th>
<th>%</th>
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<tr>
<td>Scottish</td>
<td>Control</td>
<td>547</td>
<td>41.0</td>
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<td></td>
<td>78.0</td>
<td>8.5</td>
<td></td>
<td>152</td>
<td>43.8</td>
<td>199</td>
<td>57.3</td>
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<tr>
<td></td>
<td>Case</td>
<td>499</td>
<td>59.0</td>
<td>Late AMD</td>
<td>48.3</td>
<td>77.9</td>
<td>9.2</td>
<td></td>
<td>190</td>
<td>38.1</td>
<td>315</td>
<td>63.1</td>
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<td></td>
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<td>846</td>
<td></td>
<td></td>
<td></td>
<td>78.7</td>
<td>7.2</td>
<td></td>
<td>377</td>
<td>42.3</td>
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<td></td>
<td></td>
<td>75.6</td>
<td>7.7</td>
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<td>171</td>
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<td>232</td>
<td>55.1</td>
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<td></td>
<td>Case</td>
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<td>67.9</td>
<td>Late AMD</td>
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<td>78.7</td>
<td>7.2</td>
<td></td>
<td>377</td>
<td>42.3</td>
<td>472</td>
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<td>177</td>
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<td>143</td>
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<td>54.0</td>
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<tr>
<td></td>
<td>Case</td>
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<td>66.2</td>
<td>Late AMD</td>
<td>85.9</td>
<td>79.3</td>
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<td></td>
<td>222</td>
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<td></td>
<td></td>
<td>919</td>
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<td>80.0</td>
<td>8.5</td>
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<td>75.6</td>
<td>7.9</td>
<td></td>
<td>177</td>
<td>46.8</td>
<td>203</td>
<td>53.7</td>
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<tr>
<td></td>
<td>Case</td>
<td>520</td>
<td>57.9</td>
<td>Late AMD</td>
<td>100.0</td>
<td>80.0</td>
<td>8.5</td>
<td></td>
<td>206</td>
<td>39.6</td>
<td>314</td>
<td>60.4</td>
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<td>898</td>
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<td></td>
<td></td>
<td>75.4</td>
<td>6.1</td>
<td></td>
<td>394</td>
<td>44.4</td>
<td>501</td>
<td>56.5</td>
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<tr>
<td>USA 3</td>
<td>Control</td>
<td>887</td>
<td>40.1</td>
<td></td>
<td></td>
<td>75.4</td>
<td>6.1</td>
<td></td>
<td>394</td>
<td>44.4</td>
<td>501</td>
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</tr>
<tr>
<td></td>
<td>Case</td>
<td>1326</td>
<td>59.9</td>
<td>Late AMD</td>
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<td>80.7</td>
<td>6.3</td>
<td></td>
<td>583</td>
<td>44.0</td>
<td>758</td>
<td>57.2</td>
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<td></td>
<td>2213</td>
<td></td>
<td></td>
<td></td>
<td>80.7</td>
<td>6.3</td>
<td></td>
<td>583</td>
<td>44.0</td>
<td>758</td>
<td>57.2</td>
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<tr>
<td>Dutch-German</td>
<td>Control</td>
<td>562</td>
<td>31.9</td>
<td></td>
<td></td>
<td>72.7</td>
<td>6.6</td>
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<td>245</td>
<td>43.6</td>
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<td>55.9</td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>1201</td>
<td>68.1</td>
<td>Late AMD</td>
<td>91.3</td>
<td>75.9</td>
<td>8.2</td>
<td></td>
<td>456</td>
<td>38.0</td>
<td>744</td>
<td>61.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1763</td>
<td></td>
<td></td>
<td></td>
<td>75.9</td>
<td>8.2</td>
<td></td>
<td>456</td>
<td>38.0</td>
<td>744</td>
<td>61.9</td>
</tr>
</tbody>
</table>

Grading was performed by an ophthalmologist and refers to the worst eye. Controls were also examined. Late AMD includes cases of GA and neovascular AMD (CNV). Grading was based on the Clinical Age-Related Maculopathy Grading System (CARMs). All cohorts were made up of Caucasian individuals. The numbers shown in the main text refer to the number of individuals that were successfully genotyped for CFD SNPs.
Applied Biosystems). Samples were analyzed in triplicate, and each plate contained multiple ‘no template’ control wells without DNA. The relative copy number of CFD, normalized to the known copy number of the RNaseP reference sequence, was calculated using commercially available software (Copycaller v1.0; Applied Biosystems).

**Plasma Samples**

Blood samples were collected in dipotassium EDTA-coated tubes. Plasma was separated from blood cells by centrifugation within 3 hours of collection and was frozen in aliquots at −80°C until use.

**Measurement of CFD in Plasma by ELISA**

Plasma CFD was measured using an ELISA development kit (DuoSet) for human complement factor D (R&D Systems, Minneapolis, MN), optimized for use in plasma. The kit was used as described by the manufacturer; plasma samples were diluted 1 in 4000 for analysis. A standard curve ranging from 0 to 2.5 ng/mL was included on each plate, alongside three internal control plasma samples used to assess interassay variability. The interassay coefficient of variation was below 10%. CFD was measured without prior knowledge of disease status for each sample. AMD cases and control samples were included on each plate assayed and all samples were measured in duplicate.

**Statistical Analysis**

Genotyping, copy number variation, and CFD ELISA data were maintained using statistical analysis and data management software (SPSS version 17; SPSS Inc., Chicago, IL). SNP genotyping was assessed for deviation from Hardy-Weinberg equilibrium in AMD cases and controls using a χ² test. The association of SNPs with AMD was tested using logistic regression analysis to model the probability of disease occurrence, including known risk factors—age, sex, and smoking history—as variables along with SNP genotype in the analyses. Age and smoking history (ever/never) were considered as categorical variables. A Sex and smoking history (ever/never) were considered as categorical variables.

**RESULTS**

**Genetic Association between CFD Variants and AMD**

Initially we examined the association of AMD with SNPs within the CFD gene. Only three common CFD SNPs (rs3826945, rs1683564, and rs1683563) with a minor allele frequency >10% in the CEPH Caucasian reference population were identified in HapMap. One of these, rs16853564, was not successfully genotyped. Other SNPs had lower minor allele frequencies and so were unlikely to provide adequate power. The Tagger-pairwise Tagging algorithm (http://hapmap.ncbi.nlm.nih.gov/) identified rs3826945 and rs1683564 as tagging SNPs. Rs3826945 and rs1683563 are in high linkage disequilibrium (LD) (D’ = 1), but not complete LD (r² = 0.314), so both SNPs remain informative. A dominant genotypic model and binary logistic regression analysis was used, including sex, smoking, and SNP as categorical variables and exact age as a continuous covariate.

A Scottish case-control series (UK 1) was examined initially, consisting of 462 genotyped AMD cases, 52% of whom had early AMD, and 48% had late AMD (GA or CNV) and 325 examined controls. The rs1683563 SNP was not significantly associated with AMD (P = 0.07). The rs3826945 SNP however showed evidence of association with AMD, particularly in females, who showed an odds ratio of 1.70 (95% CI, 1.11–2.63; P = 0.016), although the combined sexes also showed a significant odds ratio of 1.44 (95% CI, 1.04–2.00; P = 0.028) (Table 2).

**Table 2. SNP Association Results for CFD SNP rs3826945**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Cases (n)</th>
<th>Controls (n)</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK1</td>
<td>287</td>
<td>182</td>
<td>1.7</td>
<td>1.11–2.63</td>
<td>0.016</td>
</tr>
<tr>
<td>UK2, USA 1, USA 2</td>
<td>1133</td>
<td>576</td>
<td>1.36</td>
<td>1.11–1.68</td>
<td>0.004</td>
</tr>
<tr>
<td>USA 3, Dutch-German</td>
<td>1409</td>
<td>736</td>
<td>1.05</td>
<td>0.85–1.24</td>
<td>0.801</td>
</tr>
<tr>
<td>Combined cohorts</td>
<td>2833</td>
<td>1497</td>
<td>1.18</td>
<td>1.04–1.34</td>
<td>0.012</td>
</tr>
</tbody>
</table>

A dominant model was used to assess the association of the minor allele of rs3826945 to AMD, controlling for age, sex, and smoking in a total of six cohorts, comprising the discovery cohort, and two further replication groups. UK 1, UK 2, USA 1, and USA 2 cohorts were also genotyped for rs1683563, which was not significantly associated with AMD when included as a covariate in the analysis of these cohorts.
In view of the low power to reliably detect such a small effect, we sought to replicate this result by genotyping both SNPs in a larger case-control set, consisting of an additional UK series (UK 2) (773 genotyped cases—all with severe AMD—and 362 examined controls) and two north American series (USA 1, USA 2). USA 1 (University of Michigan) included 580 genotyped cases (86% severe AMD) and 296 examined controls while the USA 2 (University of Pennsylvania) series included 512 genotyped cases (100% severe AMD) and 371 examined controls. The rs1683563 SNP was not significantly associated with AMD (controls. The rs1683563 SNP was not significantly associated while the USA 2 (University of Pennsylvania) series included (USA 1, USA 2). USA 1 (University of Michigan) included 580 and 362 examined controls) and two north American series effect, we sought to replicate this result by genotyping both showed a significant association in the jmegen]). The result of meta-analysis series (UK 1–2, and Dutch-German [Nijegen]). The result of meta-analysis of association series (0.32), so is an unlikely explanation for the apparent differences between cohorts (Table 1).

We combined all the above results into a single meta-analysis (4765 cases and 2693 controls), which showed a significant association overall (OR, 1.11; CI, 1.01–1.23; \(P = 0.032\)) between AMD and rs3826945. The odds ratio was significant in females (OR, 1.18; 95% CI, 1.04–1.34 in females; \(P = 0.012\)) but not in males (OR, 1.04; 95% CI, 0.90–1.21; \(P = 0.57\)). Similar results were obtained using an allelic (additive) model (data not shown). The results of the meta-analysis are summarized as a Forest plot in Figure 1 and Table 2. Interactions between smoking and CFD SNP rs3826945, or between body mass index and rs3826945, did not influence the association with AMD in cohorts where these data were available.

The minor allele of rs3826945 was not significantly associated with either GA or CNV in any of the series analyzed independently. In the combined series, there was a borderline significant association between the minor allele and CNV (OR = 0.043; OR, 1.154; 95% CI, 1.001–1.325), but not with GA (OR = 0.359; OR, 1.089; 95% CI, 0.908–1.306). This analysis included 1681 controls, 660 GA cases, and 1687 CNV cases. Splitting the combined series by sex, there was no significant association observed in males (755 controls, 266 GA cases, and 659 CNV cases), and there was only suggestively significant association in females (926 controls, 394 GA cases, and 1028 CNV cases). For GA females, the minor allele \(P\) value was 0.098 (OR, 1.223; 95% CI, 0.963–1.552); for CNV females, the \(P\) value was 0.056 (OR, 1.196; 95% CI, 0.995–1.437). The GA group is however much smaller than the CNV group, reducing the power to detect effects of CFD on these clinical subgroups. Plasma CFD is therefore significantly elevated in both neovascular and atrophic AMD, although it is more significant in neovascular AMD (but again, there are more cases).

**Copy Number Variation in the CFD Gene**

Copy number variation in the CFD gene was measured using a copy number assay (TaqMan; Applied Biosystems), in a duplex real-time polymerase chain reaction, as described in Materials and Methods. The relative copy number of CFD was normalized to the known copy number of the RNaseP reference sequence and calculated using software (Copycaller; Applied Biosystems) using a conservative \(C_\text{T}\) threshold value of 32 across all plates. Copy number was measured in 311 AMD cases and 329 controls from the UK 1 (Scottish AMD) case-control series. Copy number variation in the CFD gene was identified in 4 out of 311 AMD cases (two with a single copy and two with three copies) and 9 out of 329 controls (one with zero copies, five with a single copy, and three with three copies). There was no significant difference in CFD duplication/deletion frequency between cases and controls (1.3% of AMD cases and 2.7% of controls; \(P = 0.51\) using Fisher’s exact test). The results are summarized in Figure 2.

**Plasma CFD Concentration in AMD Cases and Controls**

An enzyme-linked immunosorbent assay (ELISA) was developed to measure plasma CFD concentrations, as described in Materials and Methods. The plasma CFD concentration was measured in 751 AMD cases and 474 controls from the UK 1 and UK 2 case-control series and found to be elevated in AMD cases compared with controls; median plasma CFD concentration was 2.31 ± 0.043 (SEM) \(\mu\)g mL\(^{-1}\) in cases and 2.08 ± 0.046 \(\mu\)g mL\(^{-1}\) in controls (\(P = 0.00025\); Fig. 3). When samples were broken down by sex, plasma CFD was significantly greater in AMD females than control females (\(P = 0.0004\)) but there was no significant difference between AMD males and control males (\(P = 0.135\)). The odds ratio for those of both sexes in the highest versus lowest quartile of plasma CFD was 1.81 (95% CI, 1.27–2.57; \(P = 0.001\)), while for the sexes.

### Figure 1.

Forest plot summarizing the results of the meta-analysis of association studies between CFD SNP rs3826945 and AMD in three United States AMD case-control series (USA 1–3) and three European case-control series (UK 1–2, and Dutch-German [Nijmegen]). The result of meta-analysis showed a significant association in the combined sexes (OR, 1.11; \(P = 0.032\)) and in combined females (OR, 1.18; \(P = 0.012\)) but not in males.
separately, the corresponding odds ratios were 2.15 (95% CI, 1.35–3.44; \( P = 0.001 \)) in females and 1.42 (95% CI, 0.82–2.47; \( P = 0.215 \)) in males.

There was no association between genotype at rs3826945 and plasma CFD concentration (\( P = 0.309 \)). However, plasma CFD was found to be elevated in individuals with BMI \( \geq 30 \) who had a median plasma concentration of 2.11 ± 0.076 (SEM) \( \mu \text{g} \text{ mL}^{-1} \), compared with BMI \( < 25 \) (median value 1.77 ± 0.064 (SEM) \( \mu \text{g} \text{ mL}^{-1} \); \( P = 3 \times 10^{-6} \)). When BMI was included as a covariate in regression analysis for 272 controls and 402 AMD cases for whom BMI data were available, the odds ratio for the highest versus lowest quartile of plasma CFD was 2.03 (95% CI, 1.26–3.27; \( P = 0.004 \)).

**DISCUSSION**

Complement factor D is a unique member of the alternative complement pathway both in terms of its unusual mode of activation and its tissue expression profile.\(^1\)\(^,\)\(^2\) Adipose tissue is the major source of plasma CFD, which is thought to be constitutively secreted at a high rate but rapidly catabolized in tissues such as the kidney.\(^1\)\(^,\)\(^4\) The fractional catabolic rate of plasma CFD has been estimated to be 60% per hour,\(^2\)\(^2\) contributing to its low serum concentrations. White adipose tissue is increasingly regarded as an endocrine organ, secreting a variety of hormones, such as leptin, as well as immune and inflammatory mediators, such as CFD and tumor necrosis factor-\( \alpha \).\(^{23,24} \)

Adipose tissue contains several different cell types, the most abundant being adipocytes, but bone-marrow derived macrophages are also recruited in substantial numbers, possibly by chemokines such as monocyte chemoattractant protein-1 (MCP-1) that are expressed by adipocytes. The liver is the major source of most complement components found in plasma, with the notable exceptions of CFD and C1q.\(^{25} \) However, adipocytes are also able to synthesize many complement components, particularly those involved in the initial steps of complement activation via both classical and alternative pathways (e.g., factor B, C2, C3, C4, C1Q, C1R, C1S) but they...
Complement Factor D in AMD

The possibility that AMD therapy could account for the observed changes in plasma CFD was considered but we do not have information on who was currently undergoing anti-VEGF therapy at the time of sampling. Changes in VEGF can result from activation of the alternative complement pathway but we are not aware that the reverse can occur, so there is no theoretical reason why such treatment would influence our results.

The colocalization of CFD and its substrates C3 and factor B in choroidal tissue need not imply local complement activation, which requires their deposition on a surface in which they can evade control by CFH, CFI, DAF, MCP, and complement receptors. In AMD, most components of both the classical and alternative complement pathways and their regulators are deposited between RPE and Bruch’s membrane in drusen, the hallmark deposits of early-stage AMD. CFD deposition is not a feature of drusen, perhaps because of its very low plasma concentration but raised plasma CFD concentration in AMD compared with control subjects could reflect a high level of complement activation occurring in the choriocapillaris-Bruch’s membrane-RPE region, rather than a systemic disorder of complement activation.

This is the first report of genetic association between AMD and variation within the CFD gene. The association is confined to a single noncoding SNP, rs826945, within intron 4 of the CFD gene (chr19:813,912), which spans 3.9 kb on 19p13.3 and contains five exons. This variant is unlikely to directly influence CFD activity but is most likely to be in LD with a nearby functional variant(s). The region is one of high gene density, high recombination, and relatively low LD (http://hapmap.ncbi.nlm.nih.gov) perhaps explaining why the association does not appear to extend far outside the rs826945 region.

The association was confirmed in a large meta-analysis, including 4765 predominantly severe AMD cases and 2693 examined controls. The effect size was very small (odds ratio 1.11) and almost confined to females (Table 1, Fig. 1). The sex difference is unlikely to result from the smaller male sample size—if this were true, the overall P value for the SNP in combined males and females (P = 0.032) should be more significant than in females only (P = 0.012). The same is true of the plasma CFD measurements. Multinomial logistic regression was performed using a custom/stepwise approach to investigate interaction between rs826945 and sex. In agreement with the results obtained by regression analysis performed in females only, females appear to be more susceptible to AMD when they carry the risk allele of rs826945 with an odds ratio (OR) for the interaction of 1.304 (95% CI, 1.142–1.489; P = 8.73 × 10−5) which was increased from OR 1.236 (95% CI, 1.117–1.367) for females without accounting for genotype, but not significantly so. Sex differences in the heritability of complex traits are common so the specific nature of the CFD association is both biologically plausible and interesting in light of the sex-specific changes in plasma CFD, both involving an effect more or less confined to females. This could suggest that the CFD SNP association serves to indicate an as yet unidentified variant regulating plasma CFD levels in females that is in linkage disequilibrium with rs826945. No significant association was found between the CFD SNP (rs826945) and plasma CFD concentration but our study almost certainly lacked power to detect such an effect, given that almost 5000 cases were required to show the genetic association with AMD. If one or more CFD variant does influence plasma CFD directly (in females), then a causal effect on AMD risk, rather than raised plasma CFD being a consequence of AMD, has to be considered.

BMI does not explain the raised plasma CFD in AMD cases but if the proposed effect of CFD genotype on AMD is mediated by differences in plasma CFD (both of which are largely confined to females), it is possible that the observed variability in replication of the genetic association across case-control series may have been due to differences in BMI between populations because BMI data were only available in some series (UK 2, USA 1, Dutch-German). The lack of a statistically significant interaction between SNP and BMI argues against this, although the analysis may again have been underpowered.

Manipulation of plasma CFD concentration or inhibition of its activation in individuals at high genetic risk of AMD progression might be therapeutically useful regardless of whether the plasma CFD association is a cause or a consequence of the...
disease. Further work is therefore required not only to confirm the genetic association but also to identify candidate regulatory variants by re-sequencing and functional studies of SNP effects on CFD expression in adipocytes or macrophages.

Follow-up of our findings, particularly investigating the relationship between rs3826945 and putative regulatory variants in its vicinity, may have therapeutic implications because of the important role of CFD in regulation of the alternative complement pathway. The proposed genetic association between CFD gene variants and AMD requires further investigation in large meta-analyses and, if confirmed, adds to the evidence implicating this pathway in AMD pathogenesis.

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References