



Evaluating a Causal Relationship between Complement Factor I Protein Level and Advanced Age-Related Macular Degeneration Using Mendelian Randomization

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Purpose: Risk of advanced age-related macular degeneration (AAMD) is associated with rare genetic variants in the gene encoding *complement factor I* (CFI), which is associated with lower circulating CFI protein levels, but the nature of the relationship is unclear. Can genetic factors be used to infer whether low circulating CFI is associated with AAMD risk?

Design: Two-sample inverse variance-weighted Mendelian randomization (MR) was used to evaluate evidence for a relationship between CFI levels and AAMD risk, comparing CFI levels from genetically predefined subsets in AAMD and control cohorts.

Participants: We derived genetic instruments for systemic CFI level in 3301 healthy INTERVAL study participants. To evaluate a genetic causal odds ratio (OR) for the effect of CFI levels on AAMD risk, results from an AAMD genome-wide association study from the International AMD Genomics Consortium, were combined with CFI levels from SCOPE and SIGHT AAMD patients.

Methods: Published genetic and proteomic data was combined with data from cohorts of patients with geographic atrophy (GA) in a series of MR analyses.

Main Outcome Measures: Establishing a causal relationship for CFI on AAMD.

Results: One common CFI variant, rs7439493, was associated strongly with low CFI level, explaining 4.8% of phenotypic variance. Using rs7439493, MR estimates for AAMD odds increased per standard deviation (SD) CFI decrease in were 1.47 (95% confidence interval [CI], 1.30–1.65; $P = 2.1 \times 10^{-10}$). MR using rare variant (rs141853578 encoding p.Gly119Arg) indicated 1-SD decrease in CFI led to increased AAMD OR of 1.79 (95% CI, 1.46–2.19; $P = 1.9 \times 10^{-8}$). The rare variant rs141853578 explained a further 1.7% of phenotypic variance. To benchmark the effect of low CFI levels on AAMD ORs using a CFI-specific proteomic assay, we estimated the effect using CFI levels from 24 rs141853578 positive GA patients; each 1-SD reduction (3.5 $\mu\text{g/mL}$) in CFI associated with a 1.67-fold increased odds of AAMD (95% CI, 1.40–2.00; $P = 1.85 \times 10^{-8}$).

Conclusions: Concordance in MR calculations provide good genetic evidence for a potentially causal role of lower CFI level increasing AAMD risk. *Ophthalmology Science* 2022;2:100146 © 2022 Published by Elsevier Inc. on behalf of the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).



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Age-related macular degeneration (AMD) is the leading cause of irreversible central vision loss among elderly Western populations.^{1–3} Age-related macular degeneration is a progressive retinal disease in which the early stages are characterized by drusen and pigmentary changes, causing mild visual impairment. Many patients progress to advanced AMD (AAMD), which has 2 subtypes: exudative AMD, involving angiogenesis in the choroid and macular neovascularization (MNV), and nonexudative AMD, involving

degeneration or geographic atrophy (GA) of the retinal pigment epithelium.⁴ The prevalence of AAMD in European individuals 65 to 69 years of age is approximately 0.5%, rising to 9.8% in those 85 years of age or older.⁵

Antiangiogenic agents are effective at controlling MNV⁶; however, treated patients still have residual visual disability because of varying levels of retinal tissue disruption and atrophy, and monthly intravitreal injections are invasive

and costly, with increased risk of intraocular infection.⁷ Antioxidant and mineral supplementation can reduce the progression of nonexudative AMD to exudative AMD⁸; however, to date, no effective treatment exists for nonexudative AMD.⁹

The natural history of AMD has been studied extensively,^{4,10} and despite much effort invested in the identification of different biomarkers to identify high-risk individuals to guide screening, monitoring, and treatment options and outcomes, these remain confined to research settings and have not yet moved into clinical practice.¹¹ Advances in retinal imaging techniques have improved diagnosis and guide disease management, and efforts continue to refine disease characterization and selection of anatomic features that may be used as end points in future clinical trials to improve chances of success.¹²

Understanding the cause of nonexudative AMD has been limited partly because of lack of relevant animal disease models.¹³ Age-related macular degeneration development is influenced by advancing age, lifestyle factors like smoking and a high body mass index, and a positive family history of the disease.^{14,15} Compared with other complex traits, AMD has a strong genetic influence: heritability is estimated to be approximately 46% for disease development and for 71% disease severity.¹⁶ Study of human genetic variation can help to improve our biological understanding of causal pathways for disease, and this in turn has the potential to translate into improved clinical diagnostics and drug target selection.¹⁷ For AMD, several hypotheses for disease development and progression have focused on the underlying pathogenic pathways related to genetic predisposition.^{18,19}

Genome-wide association studies (GWASs) have identified common genetic variants that increase risk of AAMD, with the two strongest risk factors mapping to the region surrounding *complement factor H (CFH)* on chromosome 1q32, and the age-related maculopathy susceptibility 2 (*ARMS2*)/ *high-temperature requirement A serine peptidase 1 (HTRA1)* region on chromosome 10q26. *CFH* codes for the CFH protein, implicating the complement system (CS) in AAMD disease pathogenesis.^{20,21} Less is understood about how the association at *ARMS2/HTRA1* contributes functionally to disease,^{22,23} but carriers of this risk factor are reported to have more severe disease and a MNV-like phenotype compared with carriers of *CFH* risk alleles.^{24,25} Other AAMD GWAS risk factors also mapped to genes coding for proteins in the alternative pathway (AP) of the CS; these include the *CFI*, complement factor B (*CFB*), and complement component (*C3*) genes.^{19,26} Additionally, AAMD GWASs also identified genetic risk factors mapping to genes in other biological pathways, such as the high-density lipoprotein cholesterol, collagen synthesis, receptor-mediated endocytosis, extracellular matrix organization and assembly, and angiogenesis pathways.¹⁹

Detailed analyses of underlying biological pathways identified by GWAS can help to identify important regulators or modifiers that may be targeted therapeutically. The CS is a promising target pathway for intervention indicated by genetic evidence, immunohistochemistry, and protein biomarker studies. The prolonged overactivity of the CS is a

main driver of AMD development and progression,^{27,28} and the link to disease is supported by the observations of a number of CS proteins, activators, and regulatory proteins being identified as molecular constituents of drusen, the hallmark extracellular deposits associated with AMD that are found alongside retinal pigment epithelium cells at the site of disease.^{29–31} The CS is a critical feature of the innate immune response, comprising complex pathways of multiple cascading proteins with tightly controlled enzymatic functions, and it is this complexity that makes it challenging to determine the optimal position to intervene.³²

Intravitreal injection of different inhibitors against various complement proteins has been trialed in AMD with limited success^{33–35}; for example, lampalizumab (anti-complement factor D; Genentech, Inc),^{33,36} LFG316 (anti-C5 antibody; Novartis AG), eculizumab (anti-C5 antibody; Alexion Pharmaceuticals, Inc), and CLG561 (antiproperdin; Novartis AG). A phase 2/3 trial of an intravitreal pegylated RNA aptamer that inhibits complement factor C5 (avacincaptad pegol; Iveric Bio) did meet its primary end point of slowing GA lesion area growth³⁵ and is undergoing further evaluation. Another strategy overcoming the requirement for regular intravitreal injections is using adeno-associated virus gene therapy to deliver the human *complement factor I (CFI)* gene to the retinal pigment epithelium to drive expression of endogenous CFI protein to slow atrophic disease.³⁷ The rationale for ocular CFI supplementation is to enable normalization of complement control at the level of the retina and to restore homeostasis to slow the disease process and retinal degeneration. However, it remains unclear what the most appropriate target patient population is and what role CFI plays as a biomarker in clinical development of new therapies. Compared with the common *CFI* AAMD-associated risk variant identified by GWAS, individuals carrying rare genetic variants in *CFI* are at an even greater risk of developing AAMD.^{19,21,26,38–41} Rare *CFI* variants have been described in familial AMD, and sporadic patients with AMD carrying rare *CFI* variants are more likely to report a positive family history and a younger age at symptom onset.^{42,43} The CFI protein is produced systemically by the liver⁴⁴ and locally in the eye by the retinal pigment epithelium tissues.⁴⁵ Complement factor I is a critical inhibitor of the AP and is a key regulator of all 3 complement activating pathways including the AP, by irreversibly cleaving C3b or C4b and halting further complement activation.⁴⁶ Complement factor I-mediated cleavage of C3b represents a critical step in regulation of the AP, which is under a finely tuned positive feedback loop controlling complement deposition.^{47,48}

Normal variation in systemic CFI protein level is influenced by age,⁴⁹ immunologic processes,⁵⁰ and genetic background. Approximately 4% to 7% of patients with AAMD carry a rare genetic variant in *CFI*,^{51,52} and a reported 36% have low CFI protein levels in the blood serum.⁵¹ Low CFI protein level is considered the functional consequence of some underlying rare *CFI* variant genotype, which ultimately fails to produce a secreted CFI protein.^{40,51,53–55}

Table 1. Common Genetic Instrument Variable for Circulating Complement Factor I level

Single-Nucleotide Polymorphism	Chromosome	Base Pair Position	Effect Allele	Noneffect Allele	β	P Value	Age-Related Macular Degeneration Type	Odds Ratio (95% Confidence Interval)	P Value
rs7439493	4	110656730	A	G	0.31	8.9×10^{-37}	Advanced	1.47 (1.30–1.65)	2.1×10^{-10}
							Intermediate	1.24 (1.07–1.44)	0.004
							GA	1.54 (1.26–1.88)	2.1×10^{-5}
							MNV	1.44 (1.26–1.64)	7.4×10^{-8}

GA = geographic atrophy; MNV = macular neovascularization.

The effect estimate was most pronounced in the advanced age-related macular degeneration subgroups compared with the intermediate age-related macular degeneration subgroup. Mendelian randomization estimates of odds ratios and 95% confidence intervals are based on a 1-standard deviation decrease in complement factor I protein levels based on the Wald ratio method.

Levels of intraocular CFI are correlated positively with systemic CFI levels in healthy individuals and those with AMD.⁵⁶ Lower ocular CFI level is hypothesized to contribute to uncontrolled C3b accumulation, resulting in an imbalance in the AP, which over time leads to deposition of complement at the site of disease, driving macular degeneration.^{27,28,57}

Mendelian randomization (MR) is a statistical approach that can be applied to investigate the causal relationships between risk factors and outcomes via the use of genetic instruments (in this context, genetic variants^{58,59}). Mendelian randomization uses genetic association data with the risk factor (systemic CFI protein levels) and genetic association data with disease outcome (risk of development of AAMD) to assess whether the risk factor is likely to be associated causally with disease. Because genetic instruments are distributed randomly at conception, the genetically predicted CFI levels are unlikely to be related to confounders of AMD risks or to be influenced consequentially by AMD disease status through reverse causality.⁵⁸

In this study, we investigated whether common and rare genetic factors play a part in driving variation in circulating CFI. These data then were used to infer the causal relationship between CFI on AAMD risk; showing a causal relationship would validate CFI further as an important prognostic biomarker and would have therapeutic implications for disease prevention and clinical development of treatments for AAMD.

Methods

Overview of Methods

We conducted a series of 2-sample MR analyses to evaluate the association between genetically predicted circulating CFI level and AAMD risk using genetic instruments identified in 3,301 healthy European participants in the INTERVAL study⁶⁰ considering both directly genotyped common variants and a selected set of previously reported rare variants that were assessed via genotype imputation (Supplemental Fig 1). To evaluate a genetic causal odds ratio (OR) for the effect of CFI levels on AAMD risk, we used inverse variance-weighted MR analysis, using results from an AAMD meta-GWAS.¹⁹ To benchmark the effect of low CFI levels on AAMD odds, we used CFI-specific proteomic

measurements from patients with GA carrying a rare *CFI* variant, rs141853578, who have taken part in two natural history studies, SCOPE (ClinicalTrials.gov identifier, NCT03894020) and SIGHT.⁶¹

Genomic and Proteomic Biomarker Datasets

We used genetic and proteomics data from Sun et al⁶⁰ that links germline genotypes to plasma protein levels, allowing determination of particular genetic markers acting in an allele-specific manner. After quality control, data were available for use in protein quantitative trait loci analysis from 3283 proteins, including CFI.

We also used blood serum CFI and genetic data from 24 pseudoanonymized patients with GA who harbored the rs141853578 variant, of whom 14 were recruited into SCOPE, and a further 10 recruited from the SIGHT study.⁶¹ Serum CFI levels were compared with 329 *CFI* rare variant-negative patients with GA from SIGHT. Geographic atrophy was determined in both studies using fundus autofluorescence, and patients were entered based on the presence of unilateral or bilateral GA and a reading performance of ≥ 40 letters by best-corrected visual acuity, but not MNV or diabetic retinopathy, as determined by a retinal specialist.

For SCOPE patients, saliva DNA was screened for *CFI* rare variants using targeted next-generation sequencing conducted by Molecular Vision Laboratory. A customized capture panel was designed using Agilent SureSelect Target Enrichment kit to amplify the *CFI* coding region. Paired-end reads were sequenced using Illumina Miseq version 2 platform, using acceptance thresholds of more than 30 times coverage over more than 98% of the target region. Sequencing reads were aligned to the human reference genome (National Center for Biotechnology Information build GRCh37 version 3) using NextGENe software version 2.4.2.3, and genotypes were called using the Genetics Assistant version 1.4.7 program. SIGHT patient blood DNA was screened for *CFI* variants using Sanger sequencing, described elsewhere.⁶¹

SCOPE and SIGHT patient serum samples were collected according to standard protocols and were stored at -80°C . Serum CFI protein concentrations were measured using a validated sandwich enzyme-linked immunosorbent assay (Hycult Biotech), by Eurofins BioPharma Service. Serum CFI levels were compared with those generated in 125 normal control participants (BioIVT, UK).

Ethics Statement

For the SCOPE study, written consent was obtained from all participants in accordance with the tenets of the Declaration of Helsinki after explanation of the nature and implications of the study,

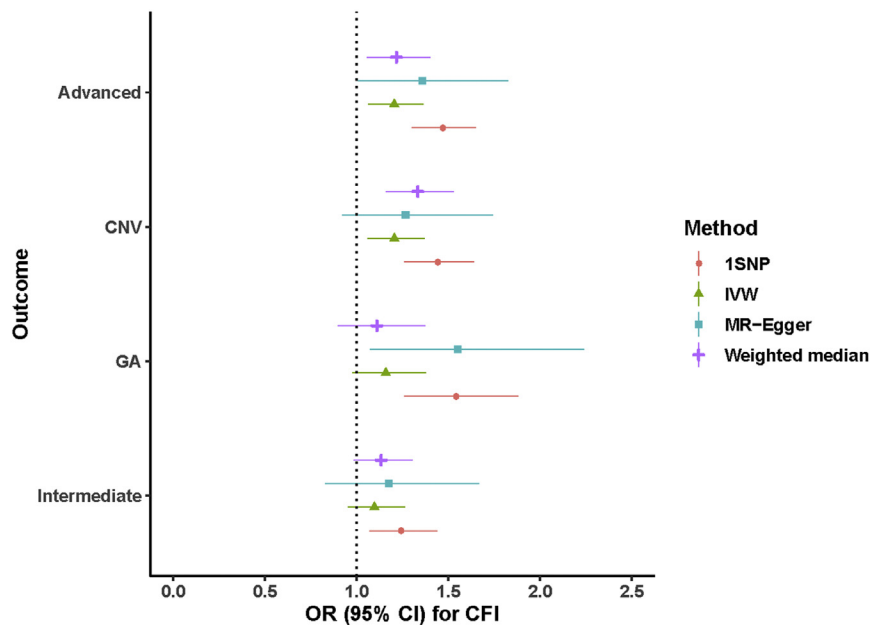


Figure 1. Forest plot showing the Mendelian randomization (MR) estimates of the causal effects of circulating complement factor I (CFI) level on advanced age-related macular degeneration (AMD). The x-axis is the estimated odds ratio (OR) for AMD subtypes per 1-standard deviation increase in genetically predicted CFI level for the common rs7439493 variant evaluated. The vertical dashed line is the reference at OR = 1. The y-axis lists the different AMD subtypes. Different MR methods are displayed with different line types: solid line, 1 single nucleotide polymorphism (SNP) MR inverse variance-weighted (IVW) analysis; dashed line, MR IVW analysis; dotted line, MR-Egger analysis; larger dashed line, weighted mean. The line on either side of the point estimates represent the 95% confidence interval (CI). CNV = choroidal neovascularization; GA = geographic atrophy.

and all methods were carried out in accordance with the relevant guidelines and regulations of local or country-specific ethics committees. For SCOPE, institutional review board (IRB)/ ethics committee/ regulatory authority approval was obtained on a local or country-specific basis; United Kingdom (Medicines and Healthcare products Regulatory Agency, London; North of Scotland Research Ethics Committee), United States of America (Oregon Health & Science University, Portland; The Johns Hopkins Medicine IRB, Baltimore; Wills Eye Hospital IRB, Philadelphia; Columbia University IRB, New York; Advarra), Australia (Therapeutic Goods Administration, Woden; Bellberry Human Research Ethics Committee, Eastwood), Poland (Komisja Bioetyczna przy Bydgoskiej Izbie Lekarskiej; Komisja Bioetyczna przy Okręgowej Izbie Lekarskiej w Łodzi; Komisja Bioetyczna Śląskiej Izby Lekarskiej w Katowicach; Office for Registration of Medicinal Products, Medical Devices and Biocidal Products, Warszawa), Germany (Ethikkommission der Medizinischen Fakultät der Eberhard Karls Universität und am Universitätsklinikum Tübingen), France (Comité de protection des Personnes Sud Méditerranée I - Marseille), The Netherlands (CMO Regio Arnhem-Nijmegen UMC St. Radboud), and Spain (CEIC-Hospital Clinico San Carlos, Madrid), SIGHT study IRB/ethics approval, registration and regulation were described previously.⁶¹

Advanced Age-Related Macular Degeneration Data

Age-related macular degeneration GWAS data were used to characterize the relationship between genetic markers and AAMD risk. The dataset comprised 12 711 patients with AAMD and 14 590 control participants of European descent from the International AMD Genomics Consortium (IAMGDC).¹⁹ Data were handled as described previously,⁶² and the resultant GWAS summary statistics were taken forward for analysis.

Statistical Analysis

For the proteomics dataset, Sun et al⁶⁰ provided protein quantitative trait loci GWAS summary statistics. Methods of the protein GWAS were described previously. Briefly, the residuals of protein abundance levels from linear regression were rank-inverse normalized, meaning the effect sizes for single nucleotide polymorphisms (SNPs) represent per standard deviation (SD) change in protein abundances.

To allow assessment of genetic polymorphisms not presented in the original article, we performed genome-wide imputation on individual-level genotyping array data. Although the original manuscript presented some imputed SNPs, many rare SNPs of interest were filtered out (SNPs with minor allele count of < 8, minor allele frequency [MAF] of approximately 0.1%) and are not presented. In particular, we were interested in several rare variants recently reported in the literature (rs141853578, rs1017242313, rs752163277, and rs587779635).^{51,54} We imputed these variants in the Michigan Imputation Server based on approximately 600 K high-quality SNPs with MAF of more than 0.01 that overlapped with approximately 800 K UK Biobank array SNPs.⁶³ The association between the imputed *CFI* rare variants and CFI level was assessed in a linear regression model in R software (R Foundation for Statistical Computing, Vienna, Austria).

The linkage disequilibrium (LD) score regression method was used to estimate the genetic correlation between the biomarker traits and AAMD using GWAS summary statistics (sample size and genotyping limitations precluded this being undertaken in SIGHT and SCOPE data). An MR approach was used to investigate the potential causal relationship between biomarker traits and AAMD. In MR analysis, we obtained genetic instruments for biomarker traits from the above biomarker GWAS summary statistics. We focused in the first instance on individual genetic markers with large effects on the trait (this contrasts with the situation with LD score regression, where a large number of genetic markers of typically small effect are used). For our primary

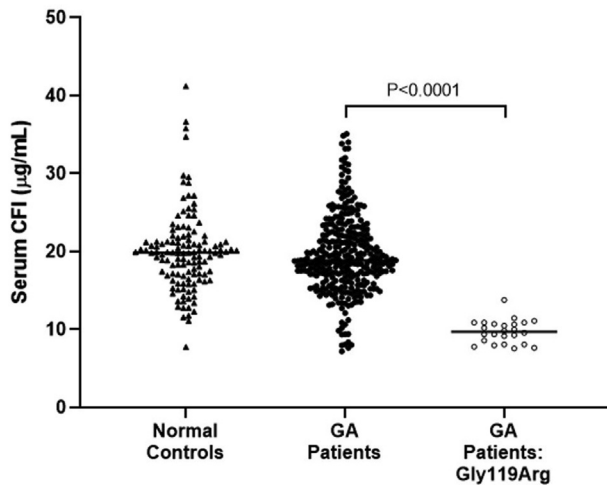


Figure 2. Scatterplot showing serum complement factor I (CFI) levels measured in normal control participants ($n = 125$), patients with geographic atrophy (GA; $n = 329$), and patients with GA carrying a *CFI* p.Gly119Arg variant ($n = 24$). Serum CFI data are shown as individual results, and the line represents the median. A Mann–Whitney U test was used for statistical analysis.

analysis, we selected lead independent genome-wide significant variants (significance set at 2-tailed $P < 5 \times 10^{-8}$ and LD between single nucleotide variants $r^2 < 0.001$).

The inverse-variance weighted regression method was used as our primary analysis. The MR analysis was conducted in R packages MendelianRandomization and TwoSampleMR.⁶⁴ Because in some scenarios relatively few genetic markers are available that exceed the genome-wide significance threshold in a GWAS, we conducted a secondary analysis considering more genetic markers. We used a less strict P value threshold to include more SNPs, with independent SNPs attaining $P < 5 \times 10^{-6}$ included in the model. Using this larger set of SNPs, we generated results that should be more reliable in the face of deviations from the MR assumptions using alternative MR estimators. These alternative estimators were the weighted median and MR-Egger regression methods. Analyses were performed with R software version 3.4.1.

To examine the relationship between rare variation and complement protein levels, we used linear regression models. The results from the linear regressions were then taken forward for use in causal inference.⁶⁴ The variance explained in CFI levels by genetic variants was computed using the formula $2 \times f \times (1 - f) \times \beta^2$, where f is the allele frequency of the variant and β is the estimated effect on CFI levels (in SD units).

The LD was determined using LDlink, using data from European populations.⁶⁵ Variants were annotated with non-Finnish European MAF from GnomAD version 2.1.1,⁶⁶ function was annotated with using HaploReg version 4.1,⁶⁷ clinical variant was annotated with ClinVar,⁶⁸ and prior trait associations were annotated using Open Targets Genetics Portal.⁶⁹

Results

Genetic Determinants of Normal Variation in Systemic Complement Factor I Level

The systemic CFI GWAS focused on common variants and identified one genome-wide significant SNP, rs7439493,

located near the *CFI* gene loci (Supplementary Fig 2). Rs7439493 explained 4.8% of the variance in circulating CFI levels, indicating that it confers a relatively strong *cis* effect on CFI levels.

Investigating the Genetic Correlation between Complement Factor I and Advanced Age-Related Macular Degeneration Risk

The LD score regression first was used to explore the genome-wide genetic correlation between the CFI level and risk of different AMD subtypes (Supplemental Table 1); however, this failed to detect any significant correlations. This indicates that beyond the genomic region around *CFI* (where a strong association exists between *CFI* variants and AMD), no clear evidence exists that genes distant from the target gene are important determinants of CFI level.

Association Between Complement Factor I and the Risk of Advanced Age-Related Macular Degeneration from Mendelian Randomization Analysis

The lead *CFI* SNP rs7439493 was taken forward as an instrument variant for MR analysis using the single SNP Wald ratio method. The results show that CFI level was associated negatively with risk of AAMD (Table 1), where a 1-SD decrease in CFI level leads to an OR increase of 1.47 (47% increased risk) of AAMD (95% confidence interval [CI] 1.30–1.65; $P = 2.1 \times 10^{-10}$).

To test the sensitivity of this MR estimate to our modelling assumptions, we applied alternative MR methods using a less strict P value threshold for SNP selection ($P < 5 \times 10^{-6}$). We applied MR Egger, weighted mean, and inverse variance-weighted methods, and their estimates broadly were consistent in effect size and direction to the estimate from the single SNP method (Fig 1). The MR-Egger intercepts showed no evidence of directional pleiotropy effects (intercepts were approximately 0; $P > 0.05$). All three MR methods showed increased risk of AAMD from lower levels of CFI, showing that our results are robust with respect to the SNP instrument used.

We then evaluated the relationship between systemic CFI levels and different AMD subtypes. The effect estimate was consistent between GA and MNV (OR, 1.54 [95% CI, 1.26–1.88; $P = 2.1 \times 10^{-5}$] and 1.44 [95% CI, 1.26–1.64; $P = 7.8 \times 10^{-8}$], respectively), but was weaker in intermediate AMD (OR, 1.24; 95% CI, 1.07–1.44; $P = 0.004$). These findings suggest that CFI may be involved comparably in progression to all forms of AAMD.

Rare Variant Analysis for Systemic Complement Factor I Level on Advanced Age-Related Macular Degeneration Risk

We also conducted an analysis based on a rare *CFI* variant, rs141853578, encoding p.Gly119Arg linked to AAMD risk.^{19,42} We used serum CFI protein levels from 24 individuals with GA who carried a heterozygous p.Gly119Arg variant. The CFI levels were compared with those measured in 125 normal control participants.

Table 2. Rare Genetic Instrument Variable for Circulating Complement Factor I Level Based on SCOPE and SIGHT Proteomic Data

Single Nucleotide Polymorphism	Chromosome	Base Pair Position	Effect Allele	Noneffect Allele	β	P Value	Age-Related Macular Degeneration Type	Odds Ratio (95% Confidence Interval)		P Value
rs141853578	4	110685820	T	C	-9.41	1.5×10^{-25}	Advanced	1.67 (1.40–2.00)	1.85×10^{-8}	
							Intermediate	1.47 (1.19–1.82)	3.80×10^{-4}	
							GA	1.98 (1.59–2.46)	7.04×10^{-10}	
							MNV	1.53 (1.25–1.88)	3.73×10^{-5}	

GA = geographic atrophy; MNV = macular neovascularization.

The effect estimate was most pronounced in the GA subgroup compared with the MNV subgroup and intermediate age-related macular degeneration subgroup. Mendelian randomization estimates of odds ratios and 95% confidence intervals are based on 1-standard deviation (3.5 $\mu\text{g/ml}$) decrease in complement factor I protein levels based on the Wald ratio method.

The mean CFI level in p.Gly119Arg-positive patients with GA was 9.735 $\mu\text{g/ml}$ (SD, 1.457 $\mu\text{g/ml}$) compared with the mean CFI level of 19.140 $\mu\text{g/ml}$ (SD, 3.5 $\mu\text{g/ml}$) observed in normal control participants (Fig 2). The mean difference (9.404 $\mu\text{g/ml}$) then was used to compute an estimate of the causal effect of serum CFI protein on AAMD risk. The rare variant MR estimate revealed that a 1-SD (3.5 $\mu\text{g/ml}$) reduction in CFI protein level led to a 1.67 OR (67%) increased risk of AAMD (95% CI, 1.40–2.00; $P = 1.85 \times 10^{-8}$; Table 2).

To verify this finding, we used the imputed genetic data for p.Gly119Arg in the Sun et al⁶⁰ dataset, achieving a good imputation quality score (0.8). The frequency of the minor allele T was 0.15%, and the effect of the rare variant on CFI levels was statistically significant ($P = 5.58 \times 10^{-14}$, $\beta = 2.37$, implying that this SNP explains 1.7% of the variance in CFI levels in this population). Mendelian randomization analysis then was conducted using the imputed p.Gly119Arg variant and variant association data from the IAMDGC GWAS. The MR estimate for the causal effect of CFI on AAMD risk for a 1-SD decrease in CFI protein level was calculated to confer an increased OR of 1.79 (95% CI, 1.46–2.19; $P = 5.58 \times 10^{-14}$; Table 3). The effect estimate was similar in direction and magnitude to our previous findings using direct measurement of CFI levels in p.Gly119Arg variant carriers and control participants, building confidence in the accuracy of our prediction.

Discussion

In this study, we conducted comprehensive MR analysis using common and rare variation at *CFI* to demonstrate a causal relationship between lower (genetically predicted) CFI levels and increased risk of AAMD and different AMD subtypes. We separately estimated the causal effect of low serum CFI level on AAMD risk by selecting first a common *CFI* variant and second the rare p.Gly119Arg *CFI* variant to use as genetic instruments in a 2-sample MR analysis. The concordance in direction and effect size from both calculations provide good genetic evidence for a potentially causal role that lower CFI level increases AAMD risk. The MR estimates

from the common and rare variant calculations showed that a 1-SD decrease in CFI level led to increased odds of AAMD developing of between 47% and 67%, respectively. Using the SD determined in serum CFI level from normal control participants, we estimate that an 18.3% (3.5 $\mu\text{g/ml}$) reduction in CFI levels from the mean causes an approximate 50% increased odds of AAMD (Supplemental Fig 3).

Our study confirmed that a proportion of normal variation in CFI level is explained by genetic factors located at the *CFI* gene. The variant rs7439493 is common in the European population (MAF, 41.3%) and accounted for 4.8% of normal variation in CFI levels, representing a substantial contribution from a single region. In contrast, the rare p.Gly119Arg variant (annotated as a variant with “conflicting interpretations of pathogenicity”) conferred a greater magnitude of effect on CFI levels, but given the rarer allele frequency (MAF, 0.08%), this resulted in a smaller overall contribution to normal phenotypic CFI variance of 1.7%. When present on an AMD background, p.Gly119Arg accounted for 3.3% of variance in CFI level.

The p.Gly119Arg variant results in a protein secretion failure leading to lower CFI and increased complement activation *in vitro*.²⁶ The functional mechanism explaining why the common rs7439493 variant is linked to CFI levels is not known. Rs7439493 is in LD with the lead AAMD risk factor previously identified at *CFI*, rs10033900 ($r^2 = 0.38$),^{19,38} suggesting that both variants tag the same functional effect on AAMD risk and variation in macular thickness,⁷⁰ but have no impact on risk of other diseases.⁶⁹ Gene expression studies in healthy systemic tissues link rs7439493 to expression levels of *CFI*.^{71,72} A correlation was not detectable in retinal tissues, but this is likely because of sample size limitations and lack of testing individuals carrying rare *CFI* variants.^{51,73,74}

Systemic CFI levels have been shown to be associated with intraocular CFI levels in both normal eyes as well as in eyes with AMD.⁵⁶ Because ocular CFI level studies involve small sample sizes that are not sufficiently powered to detect genetic associations, and given that intraocular CFI levels are correlated strongly with systemic CFI levels, we used systemic CFI levels in this study as a surrogate for local CFI levels in the eye.

Table 3. Rare Genetic Instrument Variable for Circulating Complement Factor I Level Based on Sun et al⁶⁰ Proteomic Data

Single Nucleotide Polymorphism	Chromosome	Base Pair Position	Ancestral Allele	Effect Allele	Imputation Quality Score	β	P Value	Age-Related Macular Degeneration Type	Odds Ratio (95% Confidence Interval)		P Value
rs141853578	4	110685820	T	C	0.80	-2.37	5.58×10^{-14}	Advanced	1.79 (1.46–2.19)	1.85×10^{-8}	
								Intermediate	1.55 (1.22–1.97)	3.80×10^{-4}	
								GA	2.17 (1.69–2.77)	7.04×10^{-10}	
								MNV	1.62 (1.29–2.04)	3.73×10^{-5}	

GA = geographic atrophy; MNV = macular neovascularization.

The effect estimate was most pronounced in the advanced age-related macular degeneration and GA subgroup compared with the MNV and intermediate age-related macular degeneration subgroups. Mendelian randomization estimates of odds ratios and 95% confidence intervals are based on 1-standard deviation (3.5 $\mu\text{g/ml}$) decrease in complement factor I protein levels based on the Wald ratio method.

Evidence from genetics, immunohistochemistry, and biomarker studies confirm a critical role for complement dysregulation and low CFI at the site of disease being a main driver for macular degeneration.^{27,28,57} Our study suggested that levels of the master regulator of the alternative pathway, CFI, is under relatively tight local genetic control. A one-time administration approach using adeno-associated virus to enable cellular transduction and to induce sustained expression of *CFI* after subretinal injection currently is being tested in patients with GA in phase 1 and 2 clinical trials ([ClinicalTrials.gov](https://clinicaltrials.gov) identifiers, NCT03846193, NCT04437368, and NCT04566445).

Recent phase 2 studies suggesting intraocular inhibition of complement factor C3 cleavage using an intravitreal cyclic peptide-bound polyethylene glycol polymer (pegcetacoplan; Apellis Pharmaceuticals, Waltham, MA) may slow GA growth significantly,³⁴ adding further weight to controlling complement dysregulation at the site of disease being a viable approach.³⁷ This is despite a lack of success reported in earlier phase 3 trials targeting complement factor D using an intravitreal monoclonal antibody approach (lampalizumab; Genentech, Inc., San Francisco, CA).³⁶ After detection of a positive signal using retrospective subgroup analysis using a *CFI* genetic variant on the phase 2 data,³³ the lampalizumab phase 3 trial studies used the same *CFI* genetic variant as a biomarker to select a patient subpopulation for investigation, not the same core clinical trial design as the phase 2 study, which may be one reason for trial failure.

Our study adds support that CFI is an optimal target for therapeutic intervention in the CS pathway, that enhancing

ocular CFI activity may be beneficial for treating AAMD,³⁷ and that serum CFI level is a useful biomarker for understanding ocular CFI level. Moreover, CFI levels are driven by genetic determinants, and genetic stratification can be used to identify patients at a higher risk of developing AAMD, as well as being used as a surrogate marker for predicting CFI levels.

Study Limitations

Our approach used studies conducted in different populations of undefined ethnicity and combined proteomic datasets generated using different CFI assays. Our findings warrant replication in independent AAMD cohorts, correlating CFI levels with genetic and environmental factors.

Conclusions

Genetically predicted lower CFI levels were associated with increased risk of all AMD subtypes. The causal estimates derived using the rare and common *CFI* variants were strongly concordant, which provides confidence in a potential causal role for low levels on development of AMD. This furthers our understanding of the underlying pathologic mechanism of AMD, where CFI levels can be used to identify high-risk individuals.

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Author Contributions:

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Abbreviations and Acronyms:

AMD = age-related macular degeneration; **AAMD** = advanced age-related macular degeneration; **AP** = alternative pathway; **ARMS2** = age-related maculopathy susceptibility 2; **BP** = base pair; **CFB** = complement factor B; **CFH** = complement factor H; **CFI** = complement factor I; **CI** = confidence interval; **CS** = complement system; **C3** = complement component 3; **GA** = geographic atrophy; **GWAS** = genome-wide association study; **HTRA1** = high-temperature requirement A serine peptidase 1; **IAMDGC** = International AMD Genomics Consortium; **IRB** = institutional review board; **LD** = linkage disequilibrium; **MAF** = minor allele frequency; **MNV** = macular neovascularization;

MR = Mendelian randomization; **OR** = odds ratio; **PQTL** = protein quantitative trait loci; **SD** = standard deviation; **SNP** = single nucleotide polymorphism.

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