Evaluating a causal relationship between Complement Factor I protein level and advanced age-related macular degeneration using Mendelian Randomisation

Amy V. Jones PhD¹, Stuart MacGregor PhD², Xikun Han PhD²,³, James Francis PhD¹, Claire Harris PhD¹

¹,⁴ SCOPE Study group, David Kavanagh MD, PhD⁴,⁵, Andrew Lotery MD, FRCOphth⁶,⁷, Nadia Waheed MD, MPH¹,⁸

¹ Gyroscope Therapeutics Ltd, London, UK;

² Statistical Genetics, QIMR Berghofer Medical Research Institute, Brisbane, Australia;

³ School of Medicine, University of Queensland, Brisbane, Australia;

⁴ Clinical & Translational Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK;

⁵ National Renal Complement Therapeutics Centre, Royal Victoria Infirmary, Newcastle upon Tyne, UK

⁶ Clinical Neurosciences, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK

⁷ Southampton Eye Unit, University Hospital Southampton NHS Foundation Trust, Southampton, UK

⁸ Department of Ophthalmology, Tufts University School of Medicine, Boston MA, United States

¹SCOPE Study group investigators are listed in the Acknowledgements

*Corresponding author. Email: nadiakwaheed@gmail.com

New England Eye Center 260 Tremont St. Boston, MA 02116

Ph: 617-636-4600 Fax: 617-636-4866

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<table>
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<th>Abbreviations</th>
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<td>AAV</td>
<td>Adeno-Associated Virus</td>
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<tr>
<td>AMD</td>
<td>Age-related macular degeneration</td>
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<td>AAMD</td>
<td>Advanced Age-related macular degeneration</td>
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<td>AP</td>
<td>Alternative pathway</td>
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<td>BCVA</td>
<td>Best corrected visual acuity</td>
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<td>BP</td>
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<td>CFI</td>
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<td>Chr</td>
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<td>CI</td>
<td>Confidence interval</td>
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<td>GA</td>
<td>Geographic atrophy</td>
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<td>GWAS</td>
<td>Genome-wide association study</td>
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<td>IAMDGC</td>
<td>International AMD Genomics Consortium</td>
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<td>IVT</td>
<td>Intravitreal</td>
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<td>IVW</td>
<td>Inverse-variance weighted</td>
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<td>LD</td>
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<td>MAF</td>
<td>Minor allele frequency</td>
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<td>MNV</td>
<td>Macular neovascularisation</td>
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<td>MR</td>
<td>Mendelian randomisation</td>
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<td>OR</td>
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<td>PQTL</td>
<td>protein quantitative trait loci</td>
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<td>RPE</td>
<td>Retinal pigment epithelium</td>
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<td>SD</td>
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Abstract (max 350)

Importance
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.

Objective
Can genetic factors be used to infer whether low circulating CFI is associated with AAMD risk?

Design
Two-sample inverse variance weighted Mendelian Randomisation (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.

Setting
Published genetic and proteomic data was combined with data from cohorts of Geographic Atrophy (GA) patients in a series of MR analyses.
Participants

We derived genetic instruments for systemic CFI level in 3,301 healthy European participants in the INTERVAL study. To evaluate a genetic causal odds ratio (OR) for the effect of CFI levels on AAMD risk, we used results from a genome-wide association study of 12,711 AAMD cases and 14,590 European controls from the International AMD Genomics Consortium (IAMDGC), and CFI levels from patients entered into the research studies SCOPE and SIGHT.

Results

We identified one common CFI variant rs7439493 which was strongly associated with low CFI level, explaining 4.8% of phenotypic variance. Using rs7439493 our MR analysis estimated that AAMD odds increased per standard deviation (SD) decrease in CFI level; OR 1.47 (95% confidence interval (CI) 1.30-1.65, \( P=2.1\times10^{-10} \)). We identified one rare variant (rs141853578 encoding p.Gly119Arg) which was genome-wide significantly associated with CFI levels after imputation; based on this, a 1 SD decrease in CFI leads to increased AAMD odds of 1.79 (95% CI 1.46-2.19, \( P=1.9\times10^{-8} \)). The rare variant rs141853578 explained a further 1.7% of phenotypic variance. To benchmark the effect of low CFI levels on AAMD odds using a CFI-specific proteomic assay, we estimated the effect using CFI levels from 24 rs141853578 positive GA patients; each 1 SD (3.5µg/mL) reduction in CFI was associated with 1.67 fold increased odds of AAMD (95% CI 1.40-2.00, \( P=1.85\times10^{-8} \)).

Conclusion and relevance

Excellent concordance in direction and effect size derived from rare and common variant calculations provide good genetic evidence for a potentially causal role of lower CFI level increasing AAMD risk.
Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible central vision loss among elderly Western populations. AMD 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 two subtypes; exudative AMD involves angiogenesis in the choroid and macular neovascularisation (MNV) and non-exudative AMD involves degeneration or geographic atrophy (GA) of the retinal pigment epithelium (RPE). The prevalence of AAMD in European individuals aged 65 to 69 years is approximately 0.5%, rising to 9.8% in those 85 years or older.

Anti-angiogenic agents are effective at controlling MNV, however treated patients still have residual visual disability due to varying levels of retinal tissue disruption and atrophy, and monthly intravitreal (IVT) injections are invasive and costly, with increased risk of intra-ocular infection. Antioxidant and mineral supplementation can reduce the progression of non-exudative AMD to exudative AMD, however to date there is no effective treatment for non-exudative AMD.

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

Understanding the aetiology of non-exudative AMD has been limited partly due to lack of relevant animal disease models. AMD development is influenced by advancing age, lifestyle factors...
smoking and a high body mass index, and a positive family history of disease\textsuperscript{14,15}. Compared to other complex traits there is a strong genetic influence on AMD, where heritability is estimated to be approximately 46% for disease development, and for 71% disease severity\textsuperscript{16}. Study of human genetic variation can help 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\textsuperscript{17}. For AMD, several hypotheses for disease development and progression have focused on the underlying pathogenic pathways related to genetic predisposition\textsuperscript{18,19}.

Genome-wide association studies (GWAS) have identified common genetic variants that increase risk of AAMD, with the two strongest risk factors mapping to the region surrounding $CFH$ on chromosome 1q32, and the $ARMS2/HTRA1$ region on chromosome 10q26. $CFH$ codes for the complement Factor H (CFH) protein, implicating the complement system (CS) in AAMD disease pathogenesis\textsuperscript{20,21}. Less is understood about how the association at $ARMS2/HTRA1$ contributes functionally to disease\textsuperscript{22,23}, but carriers of this risk factor are reported to have more severe disease and a MNV-like phenotype compared to carriers of $CFH$ risk alleles\textsuperscript{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 $CFI$, $CFB$, and $C3$ genes\textsuperscript{19,26}. Additionally, AAMD GWAS 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 organisation and assembly and angiogenesis pathways\textsuperscript{19}.

Detailed analyses of underlying biological pathways identified by GWAS can help 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\textsuperscript{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 RPE cells at the site of disease\textsuperscript{29–31}. The CS is
a critical feature of the innate immune response, comprising of 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.

IVT injection of different inhibitors against various complement proteins has been trialled in AMD with limited success, for example, lampalizumab (anti-complement factor D; Genentech Inc., San Francisco, CA), LFG316 (anti-C5 antibody; Novartis AG, Basel Switzerland), eculizumab (anti-C5 antibody; Alexion Pharmaceuticals Inc., Boston, MA), and CLG561 (anti-properdin; Novartis AG, Basel Switzerland). A phase 2/3 trial of an IVT pegylated RNA aptamer that inhibits complement factor C5 (avacincaptad pegol, Iveric Bio, New York, NY) did meet its primary endpoint of slowing GA lesion area growth, and is undergoing further evaluation. Another strategy overcoming the requirement for regular IVT injections, is using Adeno-Associated Virus (AAV) gene therapy to deliver the human complement Factor I (CFI) gene to the retinal pigment epithelium to drive expression of endogenous Complement Factor I (CFI) protein to slow atrophic disease. The rationale for ocular CFI supplementation is to enable normalisation of complement control at the level of the retina and 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 to 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. Rare CFI variants have been described in familial AMD and sporadic AMD cases carrying rare CFI variants are more likely to report a positive family history and a younger age of symptom onset. The CFI protein is produced systemically by the liver and locally in the eye by the retinal pigment epithelium (RPE) tissues. CFI is a critical inhibitor of the AP and is a key regulator of all three complement activating pathways including the AP, by irreversibly cleaving C3b or C4b and halting further complement activation. CFI-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.
Normal variation in systemic CFI protein level is influenced by age, immunological processes, and genetic background. Approximately 4-7% AAMD patients carry a rare genetic variant in CFI, and a reported 36% have low CFI protein levels in their blood serum. Low CFI protein level is considered the functional consequence of some underlying rare CFI variant genotype, which ultimately fail to produce a secreted CFI protein.

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Levels of intraocular CFI are positively correlated with systemic CFI levels in healthy individuals and those with AMD. Lower ocular CFI level is hypothesised 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.

Mendelian randomisation (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). MR 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 causally associated with disease. As genetic instruments are randomly distributed at conception, the genetically predicted CFI levels are unlikely to be related to confounders of AMD risks or consequentially influenced by AMD disease status through reverse causality.

In this study, we investigated whether common and rare genetic factors play a part in driving variation in circulating CFI. These data were then used to infer the causal relationship between CFI on AAMD risk; showing a causal relationship would further validate CFI as an important prognostic biomarker and have therapeutic implications for disease prevention and clinical development of treatments for AAMD.
Materials and 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 study considering both directly genotyped common variants and a selected set of previously reported rare variants which were assessed via genotype imputation (Supplementary Figure 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. To benchmark the effect of low CFI levels on AAMD odds, we used CFI-specific proteomic measurements from GA patients carrying a rare CFI variant rs141853578, who have taken part in two natural history studies, SCOPE and SIGHT.

Genomic and proteomic biomarker datasets

We used genetic and proteomics data from Sun et. al. (2018), which links germline genotypes to plasma protein levels, allowing determination of particular genetic markers acting in an allele-specific manner. Following quality control, data was available for use in protein quantitative trait loci (pQTL) analysis from 3,283 proteins, including CFI.

We also used blood serum CFI and genetic data from 24 pseudo-anonymised GA patients positive for rs141853578, of which 14 were recruited into SCOPE (study number NCT03894020; https://clinicaltrials.gov/), and a further 10 from the SIGHT study. Serum CFI levels were compared to 329 CFI rare variant negative GA patients from SIGHT. GA 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 (BCVA), 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 (Oregon, USA). A customised capture panel was designed using Agilent SureSelect Target Enrichment kit to amplify the CFI coding region. Paired-end reads were sequenced using Illumina Miseq V2 platform, using acceptance thresholds of >30X coverage over >98% target region. Sequencing reads were aligned to human reference genome (NCBI build GRCh37v3) using NextGENe software v2.4.2.3 and genotypes were called using Genetics Assistant v1.4.7 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 stored at -80°C. Serum CFI protein concentrations were measured using a validated sandwich enzyme-linked immunosorbent assay (Hycult Biotech, The Netherlands), by Eurofins BioPharma Service. Serum CFI levels were compared to those generated in 125 normal controls (BioIVT, UK).

Ethics Statement

For the SCOPE and SIGHT studies, written consent was obtained from all subjects in accordance with the Declaration of Helsinki after explanation of the nature and implications of the study, and all methods were carried out in accordance with the relevant guidelines and regulations of local or country-specific Ethics Committees.

AAMD data

AMD GWAS data were used to characterize the relationship between genetic markers and AAMD risk. The dataset comprised 12,711 AAMD cases and 14,590 controls of European descent from the IAMDGC 19. Data was handled as described previously 62 and the resultant GWAS summary statistics were taken forward for analysis.
Statistical analysis

For the proteomics dataset, Sun et al. provided pQTL 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 SNPs represent per SD change in protein abundances.

To allow assessment of genetic polymorphisms not presented in the original manuscript, we performed genome-wide imputation on individual level genotyping array data. Although the original manuscript presented some imputed single nucleotide polymorphisms (SNPs), many rare SNPs of interest were filtered out (SNPs with minor allele count <8, Minor allele frequency (MAF) ~ 0.1%) and not presented. In particular, we were interested in several rare variants recently reported in the literature (rs141853578, rs1017242313, rs752163277, rs587779635). We imputed these variants in Michigan Imputation Server based on ~600K high quality SNPs with MAF > 0.01 which overlapped with ~800K UK Biobank array SNPs. The association between the imputed CFI rare variants and CFI level was assessed in a linear regression model in R.

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). A 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 analysis, we selected lead independent genome-wide significant (GWS) 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 (IVW) regression method was used as our primary analysis. The MR analysis was conducted in R packages MendelianRandomization and TwoSampleMR. Because in some scenarios there are relatively few genetic markers which exceed the genome-wide significance threshold in a GWAS, we conducted 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 which 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 (The R Foundation).

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.

The variance explained in CFI levels by genetic variants was computed using the formula $2f(1-f)\beta^2$, where f is the allele frequency of the variant and $\beta$ is the estimated effect on CFI levels (in SD units).

LD was determined using LDlink, using data from European populations. Variants were annotated with non-Finnish European MAF from GnomAD (v2.1.1), function using HaploReg (v4.1), clinical variant annotation (ClinVar) and prior trait associations using Open Targets Genetics Portal.

Results

Genetic determinants of normal variation in systemic CFI level
The systemic CFI GWAS focused on common variants and identified one GWS SNP, rs7439493, located near the CFI gene loci (Supplementary Figure 2). Rs7439493 explained 4.8% of the variance in circulating CFI levels, indicating it confers a relatively strong cis effect on CFI levels.

Investigating the genetic correlation between CFI and AAMD risk

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

Association between CFI and the risk of AAMD 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 negatively associated with risk of AAMD (Table 1), where a one unit SD decrease in CFI level leads to an odds ratio (OR) increase of 1.47 (47% increased risk) of AAMD (95% CI 1.30-1.65, P=2.1x10^{-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<5x10^{-6}). We applied MR Egger, Weighted mean and IVW methods, and their estimates were broadly consistent in effect size and direction to the estimate from the single SNP method (Figure 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 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 OR 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 CFI may be involved comparably in progression to all forms of AAMD.

Rare variant analysis for systemic CFI level on AAMD risk

We also conducted analysis based on a rare CFI variant rs141853578 encoding p.Gly119Arg, linked to AAMD risk \(^{19,42}\). We utilised serum CFI protein levels from 24 individuals with GA who carried a heterozygous p.Gly119Arg variant. CFI levels were compared to that measured in 125 normal controls. The mean CFI level in p.Gly119Arg-positive GA individuals was 9.735µg/mL (SD; 1.457), compared to the mean CFI level of 19.140µg/mL (SD; 3.5) observed in normal controls (Figure 2). The mean difference (9.404µg/mL) was then used to compute an estimate of the causal effect of serum CFI protein on AAMD risk. The rare variant MR estimate revealed a 1 unit SD (3.5µg/mL) reduction in CFI protein level leads to a 1.67 OR (67%) increased risk of AAMD (95% CI 1.40-2.00, \( P=1.85 \times 10^{-8} \); Table 3).

To verify this finding, we used the imputed genetic data for p.Gly119Arg in the Sun et. al. (2018) dataset \(^{60}\), 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 this SNP explains 1.7% of the variance in CFI levels in this population). MR analysis was then 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 odds 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 controls, 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 on AAMD risk by selecting first a common CFI variant, and secondly 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 developing AAMD of between 47% and 67%, respectively. Using the SD determined in serum CFI level from normal controls, we estimate that an 18.3% (3.5µg/mL) reduction in CFI levels from the mean causes an approximate 50% increased odds of AAMD (Supplementary Figure 3).

Our study confirms 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% 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 [19,38] ($r^2=0.38$), suggesting both variants tag the same functional effect on AAMD risk and variation in macular thickness [70] but have no impact on risk for 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 due to sample size limitations, and lack of testing individuals carrying *CFI* rare 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]. Since ocular CFI level studies involve small sample sizes that are not sufficiently powered to detect genetic associations, and given that intraocular CFI levels are strongly correlated with systemic CFI levels, we used systemic CFI levels in this study as a surrogate for local CFI levels in the eye. 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 suggests that levels of the master regulator of the alternative pathway, CFI, is under relatively tight local genetic control. A one-time administration approach using AAV to enable cellular transduction and induce sustained expression of CFI following subretinal injection is currently being tested in patients with GA in Phase 1 and 2 clinical trials (NCT03846193, NCT04437368 and NCT04566445).

Recent phase II studies suggesting *intraocular* inhibition of complement factor C3 cleavage using an IVT cyclic peptide-bound polyethylene glycol polymer (pegcetacoplan; Apellis Pharmaceuticals, Waltham, MA) may significantly slow GA growth [34] adding further weight to controlling complement dysregulation at site of disease being a viable approach [37]. This is despite lack of success reported in earlier phase 3 trials targeting complement Factor D using an IVT monoclonal antibody approach (lampalizumab, Genentech Inc., San Francisco, CA) [36]. Following detection of a positive signal using
retrospective subgroup analysis using a CFI genetic variant on the phase 2 data, 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, enhancing ocular CFI activity may be beneficial for treating AAMD and 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 used as a surrogate marker for predicting CFI levels.

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 pathological mechanism of AMD, where CFI levels can be used to identify high-risk individuals.

Figures and Tables

Figure 1. MR estimates of the causal effects of circulating CFI level on AAMD.
The x-axis is the estimated OR for AMD subtypes per SD 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 (1 SNP MR-IVW, solid line; MR-IVW; dashed line; MR-Egger, dotted line; Weighted mean, larger dashed line) and the line either side of the point estimates represent the 95% CI. CNV; choroidal/macular neovascularisation.

Figure 2. Serum CFI levels measured in normal controls (n=125), GA patients (n=329) and GA patients carrying a CFI p.Gly119Arg variant (n=24).

Serum CFI data is shown as individual results and the line represents the median. A Mann-Whitney test was used for statistical analysis.

Table 1. Common genetic instrument variable for circulating CFI level. The effect estimate was most pronounced in all the AAMD subgroups compared to the intermediate AMD subgroup.

Table 2. Rare genetic instrument variable for circulating CFI level based on SCOPE and SIGHT proteomic data. The effect estimate was most pronounced in the GA subgroup, compared to the MNV subgroup, and intermediate AMD subgroup.

Table 3. Rare genetic instrument variable for circulating CFI level based on Sun et. al. proteomic data. The effect estimate was most pronounced in the AAMD and GA subgroup, compared to the MNV and intermediate AMD subgroups.
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Gyroscope Therapeutics provided funding for the design and conduct of the SCOPE study, and approved the design, conduct, analysis and interpretation of the data; preparation of the manuscript; and decision to submit the manuscript for publication.

For the AMD datasets, all contributing sites and additional funding information are acknowledged in this publication: Fritsche et al. (2016) Nature Genetics 48 134–143, (doi : 10.1038/ng.3448). The International AMD Genomics consortium’s web page is: http://eaglep.case.edu/iamdgc_web/, and additional information is available on: http://csg.sph.umich.edu/abecasis/public/amd2015/.
For the INTERVAL dataset, all contributing sites and additional funding information are acknowledged in this publication: Sun et. al. (2018) Nature 2018;558(7708):73-79 (doi:10.1038/s41586-018-0175-2).

The SomaLogic plasma protein GWAS summary statistics web page containing instructions for access is: Proteins - Cardiovascular Epidemiology Unit (cam.ac.uk)

Drs Jones, Han and Prof. MacGregor had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

SCOPE Study group

Dr Catherine Creuzot-Garcher

CHU Dijon - Hopital Mitterrand

Cedex, France

Dr Salvatore Grisanti

University Hospital Schleswig-Holstein Campus

Luebeck, Germany

Dr Martin Spitzer

Universitaetsklinikum Hamburg-Eppendorf

Hamburg, Germany

Dr Reiner Schlingerman
Amsterdam UMC - Locatie AMC
Amsterdam, The Netherlands

Dr Tsveta Ivanova
Manchester Royal Eye Hospital
Manchester, UK

Prof. David Steele
Sunderland Eye Infirmary
Sunderland, UK

Dr Jared Nielsen
Wolfe Eye Clinic
West Des Moines, USA

Dr Raj Maturi
Midwest Eye Institute Northside
Indianapolis, USA

Dr Jeff Heier
Ophthalamic Consultants of Boston (OCB)

Boston, USA

Dr Tongalp Tezel

Columbia University Medical Center

New York, USA

Dr Ghassan Ghorayeb

West Virginia University

Morgantown, USA

Dr David Eichenbaum

Retina Vitreous Associates of Florida

St. Petersburg, USA
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Table 1. Common genetic instrument variable for circulating CFI level. The effect estimate was most pronounced in the AAMD subgroups compared to the intermediate AMD subgroup.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>BP</th>
<th>A1</th>
<th>A2</th>
<th>Beta</th>
<th>P</th>
<th>AMD</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7439493</td>
<td>4</td>
<td>110656730</td>
<td>A</td>
<td>G</td>
<td>0.31</td>
<td>8.9e-37</td>
<td>Advanced</td>
<td>1.47 (1.30-1.65)</td>
<td>2.1e-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intermediate</td>
<td>1.24 (1.07-1.44)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GA</td>
<td>1.54 (1.26-1.88)</td>
<td>2.1e-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MNV</td>
<td>1.44 (1.26-1.64)</td>
<td>7.4e-08</td>
</tr>
</tbody>
</table>

MR estimates of OR and 95% CI based on 1 unit (SD) decrease in CFI protein levels based on Wald ratio method. SNP, single-nucleotide polymorphism; Chr, chromosome; BP, base pair position; A1, effect allele; A2, non-effect allele; OR, odds ratio; CI, confidence interval; SD, standard deviation, AAMD, advanced age-related macular degeneration; GA, geographic atrophy; MNV, macular neovascularization.
Table 2. Rare genetic instrument variable for circulating CFI level based on SCOPE and SIGHT proteomic data. The effect estimate was most pronounced in the GA subgroup, compared to the MNV subgroup, and intermediate AMD subgroup.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>BP</th>
<th>A1</th>
<th>A2</th>
<th>Beta</th>
<th>P</th>
<th>AMD</th>
<th>OR (95% CI)</th>
<th>P</th>
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<tr>
<td>rs141853578</td>
<td>4</td>
<td>110685820</td>
<td>T</td>
<td>C</td>
<td>-9.41</td>
<td>1.5e-25</td>
<td>Advanced</td>
<td>1.67 (1.40-2.00)</td>
<td>1.85e-08</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>Intermediate</td>
<td>1.47 (1.19-1.82)</td>
<td>3.80e-04</td>
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<td></td>
<td>GA</td>
<td>1.98 (1.59-2.46)</td>
<td>7.04e-10</td>
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<td></td>
<td>MNV</td>
<td>1.53 (1.25-1.88)</td>
<td>3.73e-05</td>
</tr>
</tbody>
</table>

MR estimates of OR and 95% CI based on 1 SD (3.5 µg/ml) decrease in CFI protein levels based on Wald ratio method. SNP, single-nucleotide polymorphism; Chr, chromosome; BP, base pair position; A1, effect allele; A2, non-effect allele; OR, odds ratio; CI, confidence interval; SD, standard deviation, AAMD, advanced age-related macular degeneration; GA, geographic atrophy; MNV, macular neovascularization.
Table 3. Rare genetic instrument variable for circulating CFI level based on Sun et. al. proteomic data. The effect estimate was most pronounced in the AAMD and GA subgroup, compared to the MNV and intermediate AMD subgroups.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>BP</th>
<th>A1</th>
<th>A2</th>
<th>Rsq</th>
<th>Beta</th>
<th>P</th>
<th>AMD</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs141853578</td>
<td>4</td>
<td>110685820</td>
<td>T</td>
<td>C</td>
<td>0.80</td>
<td>-2.37</td>
<td>5.58e-14</td>
<td>Advanced</td>
<td>1.79 (1.46-2.19)</td>
<td>1.85e-08</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Intermediate</td>
<td>1.55 (1.22-1.97)</td>
<td>3.80e-04</td>
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<td></td>
<td></td>
<td></td>
<td>GA</td>
<td>2.17 (1.69-2.77)</td>
<td>7.04e-10</td>
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<td></td>
<td></td>
<td>MNV</td>
<td>1.62 (1.29-2.04)</td>
<td>3.73e-05</td>
</tr>
</tbody>
</table>

MR estimates of OR and 95% CI based on 1 SD (3.5 μg/ml) decrease in CFI protein levels based on Wald ratio method. SNP, single-nucleotide polymorphism; Chr, chromosome; BP, base pair position; A1; ancestral allele, A2; effect allele, Rsq; imputation quality score, OR, odds ratio; CI, confidence interval; SD, standard deviation, AAMD, advanced age-related macular degeneration; GA, geographic atrophy; MNV, macular neovascularization.
Collaborators

SCOPE Study group

Dr Catherine Creuzot-Garcher

CHU Dijon - Hopital Mitterrand

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West Virginia University

Morgantown, USA

Dr David Eichenbaum

Retina Vitreous Associates of Florida

St. Petersburg, USA
Precis

A genetic and proteomic study that combines published datasets with data from GA patients in statistical analyses to provide evidence that low CFI protein can be considered causal for AAMD over a person’s lifetime.