Age-related macular degeneration (AMD) is a common multifactorial disease in elderly with a prominent genetic basis. Many risk variants have been identified, but the interpretation is still challenging. We investigated the genetic distribution of AMD-associated risk variants in a large European consortium, calculated attributable, and pathway-specific genetic risks, and assessed the influence of lifestyle on genetic outcomes.

Pooled analysis of cross-sectional data from the E3 consortium. 17,174 individuals aged 45+ participating in 6 population-based cohort studies, 2 clinic based studies, and 1 case-control study.

AMD was diagnosed and graded based on fundus photographs. Data on genetics, lifestyle, and diet were harmonized and completed where necessary. Minor allele frequencies and population attributable fraction (PAF) were calculated per single nucleotide polymorphism (SNP). A total genetic risk score and pathway-specific risk scores (complement, lipid, extra-cellular matrix, other) were constructed based on the dosage of SNPs and conditional beta’s; a lifestyle score was constructed based on smoking and dietary intake.

The risk variants with the largest difference between late AMD cases and controls, and the highest PAFs were located in ARMS2 (rs3750846) and CHF (rs576018 and rs10922109). Both risk increasing and protective variants had the highest PAFs. Combining all genetic variants, the total genetic risk score ranged from -3.50 to 4.63, was normally distributed and increased with AMD severity. Of the late AMD cases,
1581/1777 (89%) had a positive total GRS. The complement pathway and ARMS2 were by far the most prominent genetic pathways contributing to late AMD (positive GRS in 90% of late cases), but risk in three pathways was most frequent (35%). Lifestyle was a strong determinant of the outcome in each genetic risk category, unfavorable lifestyle increasing the risk of late AMD at least twofold (OR 35.03 for unfavorable and high genetic risk). Genetic risk variants contribute to late AMD in the majority of cases. However, lifestyle factors have a strong influence on the outcome of genetic risk, and should be a major focus in patient management. Genetic risks in ARMS2 and the complement pathway are present in the majority of late AMD, but are mostly combined with risks in other pathways.

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Dear Editor,

We are pleased to submit two manuscripts, entitled ‘Genetic risk, lifestyle, and AMD in Europe: The EYE-RISK consortium’ and ‘Development of a genotype assay for age-related Macular degeneration: The EYE-RISK consortium’ for your consideration for back-to-back publication in *Ophthalmology*.

Age-related macular degeneration (AMD) is a complex disease, influenced by genetics and environmental factors. Many genetic variants associated with AMD have been identified, but their interpretation and implementation has not been clearly demonstrated. The genetic variants pinpointed several disease pathways that drive the etiology of AMD.

In our first manuscript ‘Genetic risk, lifestyle, and AMD in Europe: The EYE-RISK consortium’ we studied the distribution of genetic risk and the contribution of the disease pathways in AMD in a large European database. We show that AMD is mainly driven by the complement pathway and ARMS2, but in the majority of individuals AMD risk is attributed to multiple disease pathways, signifying the complex etiology of AMD. We demonstrate that a healthy lifestyle reduces the risk for AMD in each genetic risk category; in the highest genetic risk group a healthy lifestyle decreased the odds ratio from 35 to 15.

In our second manuscript, ‘Development of a genotype assay for age-related macular degeneration: The EYE-RISK consortium’ we describe the design of a cost-effective genetic test based on single molecule molecular inversion probes and next generation sequencing. Using this platform we demonstrate that genetic risk scores can be easily constructed and interpreted by clinicians. We show that carriers of rare variants in the complement genes are at high risk for late AMD, which is important for new treatments that are currently under development. We also demonstrate that potential misdiagnoses with inherited macular dystrophies that mimic AMD can be avoided by targeting specific variants in dystrophy genes.

We believe that our comprehensive epidemiologic analyses of the genetics of AMD in a large, European population underlines the importance of genetic testing for lifestyle counseling of those people with a high risk for developing AMD, for selecting patients for clinical trials, and to prevent of misdiagnosis of macular dystrophies. Both manuscripts connect seamlessly in terms of genetic drivers for AMD and how to interpret and apply genetic testing for AMD in clinical practice. We hope that you share our excitement in our findings, and are willing to consider both of our manuscripts for back-to-back publication in *Ophthalmology*.

Sincerely, on behalf of all authors,

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Précis: Age-related macular degeneration is driven by complement and ARMS2, but caused in most by multiple genetic pathways. Someone’s genetic effect can be severely reduced by healthy lifestyle.
Genetic risk, lifestyle, and AMD in Europe. The EYE-RISK consortium

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ABSTRACT

Purpose: Age-related macular degeneration (AMD) is a common multifactorial disease in elderly with a prominent genetic basis. Many risk variants have been identified, but the interpretation is still challenging. We investigated the genetic distribution of AMD-associated risk variants in a large European consortium, calculated attributable, and pathway-specific genetic risks, and assessed the influence of lifestyle on genetic outcomes.

Design: Pooled analysis of cross-sectional data from the E3 consortium.
**Participants:** 17,174 individuals aged 45+ participating in 6 population-based cohort studies, 2 clinic-based studies, and 1 case-control study.

**Methods:** AMD was diagnosed and graded based on fundus photographs. Data on genetics, lifestyle, and diet were harmonized and completed where necessary. Minor allele frequencies and population attributable fraction (PAF) were calculated per single nucleotide polymorphism (SNP). A total genetic risk score and pathway-specific risk scores (complement, lipid, extra-cellular matrix, other) were constructed based on the dosage of SNPs and conditional beta’s; a lifestyle score was constructed based on smoking and dietary intake.

**Results:** The risk variants with the largest difference between late AMD cases and controls, and the highest PAFs were located in ARMS2 (rs3750846) and CHF (rs570618 and rs10922109). Both risk increasing and protective variants had the highest PAFs. Combining all genetic variants, the total genetic risk score ranged from -3.50 to 4.63, was normally distributed and increased with AMD severity. Of the late AMD cases, 1581/1777 (89%) had a positive total GRS. The complement pathway and ARMS2 were by far the most prominent genetic pathways contributing to late AMD (positive GRS in 90% of late cases), but risk in three pathways was most frequent (35%). Lifestyle was a strong determinant of the outcome in each genetic risk category, unfavorable lifestyle increasing the risk of late AMD at least twofold (OR 35.03 for unfavorable and high genetic risk).

**Conclusions:** Genetic risk variants contribute to late AMD in the majority of cases. However, lifestyle factors have a strong influence on the outcome of genetic risk, and should be a major focus in patient management. Genetic risks in ARMS2 and the complement pathway are present in the majority of late AMD, but are mostly combined with risks in other pathways.

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Catherine Creuzot-Garcher reports grants and personal fees from Allergan, Bayer, Bausch and Lomb, Novartis, Théa and Horus, outside the submitted work.
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ABBREVIATIONS

AMD = Age-related macular degeneration; AREDS= Age Related Eye Disease Study; CORRBI = Combined Ophthalmic Research Rotterdam Biobank; EUGENDA = European Genetic Database; GA = Geographic Atrophy; GWAS= Genome wide association study; HRC = Haplotype Reference Consortium; OR = Odds Ratio; RPE = retinal pigment epithelium; RS= Rotterdam Study; SNP = Single Nucleotide Polymorphism; WARGMS= Wisconsin Age Related maculopathy Grading System.

Keywords: Age-related macular degeneration (AMD), genetics, population, pathways, Europe

Précis: Age-related macular degeneration is driven by complement and ARMS2, but caused in most by multiple genetic pathways. Someone’s genetic effect can be severely reduced by healthy lifestyle.

This article contains additional online-only material. The following should appear online-only: Figure 3, 4 and 7, Tables 1-3 and cohort descriptions.

INTRODUCTION

Age-related macular degeneration (AMD) is a progressive degenerative disease of the retina and the most important cause of blindness in the Western world. Projections show that up to 4.8 million Europeans and up to 18.6 million persons worldwide will develop a blinding stage of AMD by 20401, 2. AMD is classified into two end stages; a more common “wet” form characterized by choroidal neovascularization (CNV), and a “dry” form characterized by geographic atrophy (GA) of the retinal pigment epithelium3. Only the wet form can be treated with anti-vascular endothelial growth factor, but visual decline is still inevitable at long-term4.

AMD is a complex genetic disease, strongly influenced by a combination of environmental and genetic factors. Smoking, diet, and cardiovascular determinants are known to increase the risk of AMD considerably. The genetic etiology is well-established: 52 common known AMD-associated variants and >100 rare variants have been reported5, 6. These variants explain the majority of the disease etiology, and helped pinpoint several pathogenic pathways. Of these, the complement cascade appeared to be most important, but the first attempts to target this pathway in intervention trials have had limited success7, 8. This raises the question whether disease pathways are specific to groups of individuals. If this is the case, intervention trials may be more successful by stratifying patients based on the major disease pathway driving their disease.

In this study, we aimed to investigate the contribution of genetic variants to AMD risk in Europe using data from the large European Eye Epidemiology (E3) consortium. We aimed to determine the
contribution of each disease pathway in AMD, and investigated whether lifestyle changes can reduce the risk of late AMD, in particular in individuals with a high genetic risk of AMD.

METHODS

Study population:
The E3 consortium is a European collaboration of studies with epidemiologic data on common eye disorders; a detailed description on the consortium can be found elsewhere. All data on AMD were harmonized and collected in the EYE-RISK database (version 6.0). Nine studies from France, Germany, the Netherlands, and Portugal had data on AMD genotype and phenotype available for analysis, and were enrolled in the current study. The cohort descriptions of the included studies are available at External link http://www.aaojournal.org. CORRBI, MARS, and EUGENDA were clinic-based studies, the remaining were population-based (RSI, RSII & RSIII, Alienor-3C, Montrachet-3C and CES (Coimbra Eye Study)). Persons aged 45 years and older were included in the analyses; various analyses only included controls aged 75 years or older. All studies were performed in accordance with the Declaration of Helsinki for research involving human subjects and the good epidemiological practice guideline, and had written informed consent from all participants.

Clinical examination:
The phenotype of AMD was determined on fundus photographs centered on the macula; individuals received the diagnosis of the worst eye. AMD features were graded locally by clinicians or experienced graders; classifications were grouped into three severity classes. Controls did not display AMD, aside from only small drusen or only pigment irregularities; persons with early or intermediate AMD had soft indistinct (large) drusen and/or reticular drusen, with or without pigmentary irregularities, and were further referred to as intermediate AMD. Persons with late AMD had GA, or CNV. Persons with both end stages were diagnosed as CNV. Lifestyle factors including smoking and dietary habits were assessed by questionnaire.

Genetic analyses and risk scores
AMD genetic risk variants were ascertained from the EYE-RISK/E3 database. Studies had used various platforms to determine the 52 known risk variants, such as whole exome sequencing, exome chip (Illumina HumanExome BeadChip), genomic SNP arrays (Illumina 550K (duo) chip or Illumina 610 quad), or Taqman assays, and a custom-made AMD genotyping platform using single molecule molecular
inversion probes (smMIPs) with next generation sequencing; the EYE-RISK genotype assay\textsuperscript{10}, see cohort descriptions. If variants had been determined by multiple methods which included direct genotyping, we used data from the latter method. When no direct genotyping was available, genotypes were dosages derived from Haplotype Reference Consortium (HRC) imputation or 1000G. Three (rs71507014, rs67538026, rs142450006) of the 52 known AMD risk variants could not be included in our analysis since genotypes were not available for multiple cohorts.

Genetic risk scores (GRS) were calculated for the 17,174 individuals for whom the five major risk variants (\textit{CFH} rs10922109, \textit{CFH} rs570618, \textit{C2} rs429608, \textit{C3} rs2230199, \textit{ARMS2} rs3750846) were available. Complete genotype data on minor risk alleles were available in 62.3\% persons; 85.1\% individuals had 47/49 variants. GRS were calculated by multiplying the conditional beta of the AMD risk variant\textsuperscript{5} with the allele dosage. Subsequently, all calculations were summed. Pathway-specific GRS were constructed in the same manner. For the complement GRS, we included all risk variants in the \textit{CFH}, \textit{CFI}, \textit{C9}, \textit{C2}, \textit{TMEM97}/\textit{VTN} and \textit{C3} genes. For the lipid GRS, variants in \textit{ABCA1}, \textit{LIPC}, \textit{CETP}, \textit{APOE} were included. For the extra-cellular matrix (ECM) GRS, variants in \textit{COL4A3}, \textit{ADAMTS9-AS2}, \textit{COL8A1}, \textit{VEGFA} and \textit{SYN3/TIMP3} were included. The remaining variants were included in ‘other’ GRS. The function of \textit{ARMS2} was mostly considered unsettled. However, as recent evidence suggests a role in the complement pathway\textsuperscript{11}, we analyzed this gene as a stand-alone pathway GRS as well as part of the complement pathway GRS.

**Lifestyle score**

Four well-established AMD lifestyle determinants (smoking status, servings of vegetables, fruit and fish per day) were assessed by questionnaire. Smoking status was categorized as no, former, or current smoker. Dietary intakes were analyzed in medium servings per day with a maximum of one, i.e., 120 grams of vegetables per day; 120 grams of fruit per day; 100 grams of fish per day. B-coefficients for associations with late AMD were calculated by multivariable logistic regression, and were subsequently multiplied by determinant values and summed to create a lifestyle risk score (LRS). LRS were stratified into tertiles as unfavorable, intermediate or favorable lifestyle.

**Statistical analysis**

The population attributable fraction (PAF) was calculated for each variant using the formula of Miettinen \textit{et al.}\textsuperscript{12} $\text{PAF} = \text{Pc} * ((\text{OR}-1)/\text{OR})$; where OR is the odds ratio, and Pc is the proportion of exposed cases among the cases. We calculated the discriminative accuracy between late AMD cases and controls for
our model of genetic factors using the Saddle Point Signature software version 2.8.3 (Saddle Point Science Ltd., Worcester Park, United Kingdom) in a batch multivariate regression analysis. Results were cross-validated by the leave one out principle. Prediction performance at each iteration was quantified by counting errors of persons assigned to the wrong category (controls or cases). The dataset was fully balanced between controls and cases; the regression equations corresponded to a pseudo dataset, in which the outcome classes were equal in size but the other statistical features were identical to the true dataset. Missing values were not set to zero but imputed to the mean. Covariates were selected based on error expectation minimization.

Where appropriate, comparisons were made with Pearson chi-square test, Jonckheere-Terpstra test for ordered alternatives, or independent sample t-test. Interaction of genetic and lifestyle risk was assessed by a univariate ANOVA. Graphical outputs were constructed with GraphPad Prism 5 (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com”). Histograms and a receiver operator characteristic curve were constructed with SPSS (IBM Corp. Released 2012 IBM SPSS Statistics for Windows, Version 25.0 Amonk, NY: IBM Corp).

RESULTS
We identified a total of 17,174 individuals aged 45 years and older with data on genetics and AMD; 13,324 persons without AMD, 2,073 with intermediate AMD and 1,777 individuals with late AMD. Of the persons with late AMD; 309 had developed GA and 1,468 CNV. Age ranged from 45 to 101 years old with a mean of 68.7 years (SD 10.4), the proportion of women was 58.5%, current smoking 16.8% (n=2,888), former smoking 39.5% (n=6,786). To ensure a true phenotype of no AMD, we performed risk analyses using participants aged 75 years and over as controls (n=3,167). The proportion of women in this subset (controls 75+ and intermediate and late AMD cases) was 61.3%, current smoking 9% (n=630) and former smoking 36.2% (n=2,541).

Single variants
First, we focused on frequency distributions of the 49 single risk variants in the three phenotype groups, and ranked variants according to frequency differences between late and no AMD (Figure 1a). SNPs from the complement pathway and ARMS2 showed the largest difference in frequency between cases and controls (rs10922109, rs61818925 and rs570618 (CFH), rs429608 (C2), rs2230199 (C3), rs3750846 (ARMS2)). Among the first ten variants, five variants had a lower frequency in cases, corresponding to a protective effect on AMD. Next, we calculated the population attributable fraction (PAF) for each single
variant. *ARMS2* variant rs3750846 was associated with a high PAF (0.3) for late AMD, while variants in *CFH* exhibited both the largest PAF (0.33) (rs570618) and the largest inverse PAF (-0.37) (rs10922109) (*Figure 1b*). A similar pattern with smaller PAFs was observed for intermediate AMD. Only variant rs11080055 in *TMEM97/VTN*, showed a higher PAF for intermediate (0.063) than for late AMD (0.024).

Only four (0.2% or 4/1777) late AMD cases did not carry any of the five major risk SNPs, compared to 33 (1% or 33/3167) of controls.

**Genetic risk score for AMD**

We subsequently combined all genetic variants in a GRS and assessed its distribution. In the population-based cohort studies (n = 13,194), the score ranged from -3.50 to 4.63 (mean 0.40, standard deviation (SD) 1.24) and had a normal distribution (*Figure 2a*). With respect to the distribution per phenotype, the GRS in controls ranged from -3.03 to 3.94 (mean 0.26, SD 1.16), in intermediate AMD from -3.11 to 4.71 (mean 0.83, SD 1.33), and in late AMD from -3.00 to 6.23 (mean 1.64, SD 1.32) (*Figure 2b*). Although the lowest GRS value was similar for all phenotypes, the entire distribution showed a significant increase with increasing AMD severity (Jonckheere-Terpstra test for ordered alternatives; p-value <0.0001).

When stratifying late AMD into GA and CNV, slightly higher scores were noted for CNV (*Figure 2c*): GA ranged from -2.72 to 4.87 (mean 1.46, SD 1.41) and CNV ranged from -3.00 to 6.23 (mean 1.67, SD 1.30, independent sample t-test p-value=0.01). We estimated the discriminative accuracy of a score based on the 49 AMD-associated genetic variants (Supplementary *Figure 3* and *4* available at External link http://www.aaojournal.org) for identification of late AMD; the area under the curve (AUC) was 0.838.

We identified a minimal set of variants by using the leave one out principle, and found an almost identical AUC (0.837) when including 27 AMD-associated variants (score is available in the Supplementary material at External link http://www.aaojournal.org).

**Genetic risk scores per pathway**

Next, we constructed pathway-specific GRS; for the complement, lipids, extra-cellular matrix, age-related maculopathy susceptibility 2 (*ARMS2*) and ‘Other’. The complement pathway score ranged from -3.15 to 3.64 in the population-based studies, and 55% of participants scored above 0 for this pathway. The *ARMS2* score ranged from 0 to 2.15 as only one risk variant determines this score. The lipid pathway had GRS ranging from -1.44 to 0.49, the ECM pathway from -0.92 to 1.46, and 36% and 33%, respectively, had a score higher than zero. The pathway ‘Other’ ranged from -1.06 to 1.45; 61% had a positive score.
The distribution of all pathway GRS in our total study population showed a positive shift with increasing AMD severity (Jonckheere-Terpstra test for ordered alternatives, p-value<0.0001, supplementary Table 1 available at External link http://www.aaojournal.org and Figure 5), but the complement and ARMS2 GRS demonstrated the largest increase for late AMD, especially when combined (shift of mean GRS from 0.39 to 1.59).

Frequency of positive GRS
We studied the proportion of individuals with a positive (>0) GRS for each of the pathways, as this indicates more genetic risk than protection from that particular pathway. Positive GRS for all pathways were most frequent in late AMD (Figure 6). Positive GRS for complement and ‘other’ pathways were most prevalent in all phenotypes. The largest increase per phenotype severity was found for the complement and ARMS2; the proportion of persons with positive GRS in the complement pathway rose from 51% in controls to 77% (26% increase) in late AMD cases and ARMS2 rose from 35% in controls to 65% (30% increase) in late AMD cases (Pearson Chi-Square 2-sided test, p-value <0.0001 for both). Not one pathway GRS was above zero in all late AMD cases, but 90% had a positive GRS for the combination of complement and ARMS2. Upon closer inspection of the remaining 10% (n=152), these late AMD cases did carry risk alleles in these two pathways but had a high frequency of protective variants which resulted in a GRS below zero (supplementary Table 2 available at External link http://www.aaojournal.org). Subsequently, we examined the risk SNPs in greater detail by investigating the proportion of persons with at least one risk allele per pathway (supplementary Figure 7, available at External link http://www.aaojournal.org). 99% of persons with late AMD had a risk SNP in either the complement or ‘Other’ pathway, but this was also the case for controls. For ARMS2, lipid and ECM pathway this was less frequent. Thus, it was possible to develop intermediate or late AMD without a risk SNP in a pathway, but this was true for only few individuals; 0.7% (12/1777) of late AMD for the complement pathway, and 1.5% (26/1777) of late AMD for the ‘Other’ pathway. For ARMS2, the lipids pathway and ECM pathway these fractions were higher (34.8%, 6.1%, 19.6%), respectively. When combining complement and ARMS2, only 5 (0.3%) late cases had no risk allele in this pathway.

Next, we calculated the distribution of pathways with a GRS above zero (see Figure 8). The majority of participants had two to four pathways with a GRS above zero (85%). A small proportion (7%) of individuals had a GRS in only one pathway above zero, and an even smaller proportion (1%; n=23) of individuals had a GRS below or equal to zero for all pathways.
Combining genetics with lifestyle

Data on lifestyle factors were available for a subset of the study population (n=3,525). In these subjects, we investigated the AMD lifestyle factors smoking, and dietary intake of vegetables, fruit and fish. Cases were more often current smokers (OR 1.39), consumed less vegetables (OR 0.40), less fruit (0.35) and less fish (OR 0.17, all with a p-value<0.0001, supplementary Table 3 available at External link http://www.aaojournal.org). We composed a lifestyle score based on these variables, and stratified the score into tertiles: favorable, intermediate, and unfavorable lifestyle. For each GRS category (also tertiles) we observed that, the more unfavorable the lifestyle, the higher the risk of late AMD. Lifestyle increased the risk 2-2.3 times depending on the genetic risk. In the highest genetic risk group, the OR increased from 14.9 to 35.0 in individuals with an unfavorable lifestyle (Figure 9).

DISCUSSION

This study provides a comprehensive interpretation of AMD genetic risk in the European population. The risk allele most discriminative between late AMD cases and controls was located in ARMS2, closely followed by a risk-increasing and a protective allele in CFH. We observed a normal distribution of AMD associated genetic risk score, with variants increasing disease risk but also a significant number offering protection against AMD. Individuals with late AMD had higher GRS than controls. Mathematically, we showed that the genetic contribution of the complement pathway and ARMS2 to late AMD was at least 90%. However, most cases carried genetic risk in multiple pathways, signifying the complex etiology of AMD. People from every genetic risk strata benefitted from a healthy lifestyle, with the strongest effect in individuals with a high GRS, highlighting the importance of prevention by diet and cessation of smoking.

Our results need to be seen in light of the strengths and limitations of this study. An important strength was the very large number of Europeans included in this study. From the E3 consortium, we included nine studies with genetic data, i.e., population studies from the Netherlands, France, and Portugal, as well as case-control studies from the Netherlands and Germany. Data were harmonized and entered into a single database, which allowed us to perform in depth analyses on combinations of phenotype, genotype, and lifestyle. Grouping genes into pathways and calculating pathway-specific genetic susceptibility enabled us to study molecular drivers and personalized risks. A limitation of our study was the incompleteness of data on several determinants in some studies. We focused on 49 genetic variants...
that were individually associated with AMD\textsuperscript{5}, and did not evaluate the contribution of rare variants to
AMD. This may alter the risk of AMD for individual cases considerably as is shown by de Breuk \textit{et al}\textsuperscript{10}.
The studies providing most of the cases were case-control studies without follow-up data, and we were
therefore restricted to cross-sectional analyses.

A positive GRS indicated more causative genetic risk than protection by genetic variants. As this was
present in (2546/4044) 63\% of the population, we conclude that genetic susceptibility to AMD is highly
prevalent. Among cases with late AMD, the proportion of a positive GRS rose to (1581/1777) 89\%. We
investigated this in greater detail, and found that the five major risk alleles were absent in only 66 (1\%)
persons, indicating that 99\% of the study population carried at least one major risk allele. Major risk
alleles were absent in only 0.2\% (4/1777) of those with late AMD, hence, it appears that they are
virtually essential for the development of late AMD. Our AUC 0.837 for the discriminative accuracy of
genetic risk variants to identify late AMD cases from and controls was slightly better than in earlier
studies\textsuperscript{13, 14}.

Considering individual pathways, 19/52 common AMD risk variants are in the complement pathway\textsuperscript{5}.
Previous studies already reported that common variants in the complement pathway explain 57\% of the
heritable risk of AMD\textsuperscript{15}, and our study underscored the high attribution of this pathway to the overall
GRS. Comparing the risk of the most important \textit{CFH} SNP (rs570618 in high LD 0.991 with rs1061170,
Y402H) to an Asian population, we and others observed a slightly higher OR of late AMD in Europeans
(2.47 vs 2.09)\textsuperscript{16} but very different allele frequencies (MAF 0.34 vs 0.049)\textsuperscript{17}. With respect to function, the
complement pathway is part of the innate immune system, and numerous studies have shown that
imbalance of this cascade at the protein level is important for AMD pathogenesis. Genetically, this
system harbors strong causative as well as highly protective risk alleles (Figure 1), which mathematically
can add up to GRS zero. Whether this also reflects a neutral risk at the tissue level is unclear, because
persons with late AMD and a negative GRS for complement still carried risk-increasing alleles in this
pathway. Nevertheless, the risk-reducing effect of these protective alleles are of high biological interest,
and investigation into the functional consequences may provide leads for future therapy.

The rs3750846 (or its proxy rs10490924, A69S) variant in the \textit{ARMS2} locus carried the highest risk of late
AMD, and the second highest attribution to overall AMD occurrence in our study (Figure 1). In East Asia,
this allele is twice as common (MAF 0.40 in East Asia vs 0.19 in Europeans), but the risk of late AMD for
carriers appears comparable (OR 2.94 in India vs OR 3.06 in Europe). The function of ARMS2 is subject of ongoing research. Recently, Micklisch et al. showed in vitro that ARMS2 functions as a surface complement regulator by binding to the cell membrane of apoptotic and necrotic cells, and subsequently binds properdin and activates complement. This provides evidence that ARMS2 can be an initiator of complement. We considered two different scenarios for the pathway of ARMS2: a function in the complement pathway and as a stand-alone. When regarded as a complement gene, the vast majority (90%) of late AMD had an increased genetic risk in this pathway, making complement the main driver of late AMD. As a stand-alone, ARMS2 also provided a significant contribution, as it was present in two thirds of late AMD.

Variants in the lipid and ECM pathway had smaller effects and attribution to overall late AMD. Variants in genes with other functions ('other' pathway) also had smaller effects, but the 16 variants combined were rather frequent and predisposed considerably to late AMD.

We further investigated the impact of the most important lifestyle factors, smoking and diet, in relation to genetic risk. As expected, persons with AMD had lower intake of vegetables, fish, and fruit, and higher rates of smoking (Supplemental Table 3). Together, we showed that a more unfavorable lifestyle almost doubled the risk of late AMD. This occurred in all genetic risk strata but the OR increase was most prominent in those at high genetic risk. These findings confirm previous reports from the Rotterdam Study, which demonstrated interaction between single nutrients and risk variants in CFH and ARMS2, and AREDS, which showed a protective role of diet in those with a high GRS. The current study analyzed a more comprehensive set of risk variants, and found that a healthy diet and non-smoking was also beneficial in persons with low genetic risk. Oxidative stress is the most recognized molecular effect of smoking in the pathogenesis of AMD, and antioxidants the most important contribution of a healthy diet. Oxidative stress with abundant reactive oxygen species, peroxidation of lipids, proteins, RNA, and DNA in the retina can lead to cytotoxic effects and inflammation, enhancing the development of AMD. Unfortunately, a healthy diet consisting of sufficient fruits, vegetables, and fatty fish is consumed by only a minority of elderly, and smoking is still twice as high among those with late AMD (Supplement Table 3). Adding non-genetic variables to the model improved 10-year prediction for incident late AMD to AUC 0.92 in EYE-RISK, highlighting the accuracy of personalized predictions. Genetic testing for AMD as suggested by de Breuk et al. has not yet become part of clinical routine and many ophthalmologists still do not implement lifestyle advice in the management of AMD patients. This
may deny their patients the only opportunity for prevention as our findings demonstrate the value of genetic testing for AMD in conjunction with the promotion of lifestyle change in patient counseling.

In conclusion, this large European consortium showed that genetic risk of AMD is highly prevalent in the population at large, and that risk variants in the complement pathway are by far the lead drivers of late AMD. Nevertheless, late AMD is mostly a result of multiple genetic pathways, and lifestyle. Our frequency and risk estimates can lay the foundation for future intervention studies which are tailored to pathways.

REFERENCES


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The EYE-RISK Consortium
Figure 1a. Minor allele frequency of cases and controls for 49 AMD associated genetic variants. The variants are ranked according to the difference in allele frequencies between late AMD cases and controls, with the most discriminative variants on the left side of the graph.

Figure 1b Population attributable fraction of 49 AMD-associated genetic variants for intermediate (light blue) and late (green) AMD. CFH_rs121913059 is not included for intermediate AMD since it was too rare to make useful calculations.
Figure 2. A. Distribution of the total AMD GRS (genetic risk score) in the European population. B. Distributions of the total AMD GRS, top panel showing the controls (aged ≥75 years), middle panel intermediate AMD and bottom panel late AMD. C. Distributions of the total AMD GRS, left panel (light blue) showing the frequency of geographic atrophy (GA) for each total AMD GRS and the right panel (green) showing the frequency of choroidal neovascularization (CNV) for each total AMD GRS, both on log scale.
Figure 5 Distributions of the genetic risk scores for the complement, lipids, extra-cellular matrix, ARMS2 and the other pathway and complement with ARMS2 combined in controls and late AMD cases.
Figure 6. Percentage of individuals with a GRS above zero for each of the pathways. Dark blue = the controls 75 years and older, light blue = intermediate AMD cases, green = late AMD cases. The asterisk (*) indicated statistical differences in a Pearson Chi-Square test (2-sided) with p-value <0.0001, Bonferroni correction for multiple testing is p=0.0028.

Figure 8. Distribution of late AMD cases according to pathway scores above zero, numbers inside the bars indicate the frequency.
Figure 9. Odds ratio of risk for late AMD stratified by GRS and lifestyle risk. CI = Confidence interval.
SUPPLEMENTARY MATERIAL

Minimal predictive late AMD score
Score = (-0.258756093052) * CFH_rs187328863 + (-0.736522175552) * CFH_rs148553336 + (0.354770851719) * CFH_rs570618 + (-0.476857042305) * CFH_rs10922109 + (-0.263161372994) * CFH_rs61818925 + (0.114737794639) * ADAMTS9_rs62247658 + (0.189221377369) * COL8A1_rs55975637 + (0.126638072819) * CFI_rs10033900 + (1.636051550235) * CFI_rs141853578 + (0.435358693961) * C9_rs62358361 + (-0.51565366814) * C2_rs429608 + (-0.118654834114) * C2_rs943080 + (0.140597830637) * PILRB_rs7803454 + (-0.125229980190) * ABCA1_rs2740488 + (0.797585978388) * ARMS2_rs3750846 + (-0.105468741453) * B3GALTL_rs9564692 + (-0.107352793223) * RAD51B_rs61985136 + (-0.103183047303) * LIPC_rs2070895 + (-0.131118121329) * CETP_rs5817082 + (-0.214785423493) * CTRB2_rs72802342 + (-0.794460666804) * C3_rs12019136 + (0.833236756674) * C3_rs147859257 + (0.193178632924) * C3_rs2230199 + (-0.136216103628) * APOE_rs429358 + (-0.216309010648) * C2orf85_rs201459901 + (-0.206712996496) * SYN3_rs5754227 + (0.128277021888) * SLC16A8_rs8135665 - (-0.180853557395).

Figure 3. a Showing the distribution of the predictive score for controls and late AMD including 49 AMD associated variants. b. Distribution of the predictive score with the minimal set of 27 variants for controls and late AMD.
Figure 4. Receiver operator curve for predictive risk scores to differentiate between late AMD cases and controls. The blue line indicates the GRS including all 49 AMD-associated variants (AUC 0.838), the red line indicates the GRS for the minimal set of 27 AMD-associated genetic variants (AUC 0.837).

Table 1. Difference in the mean of each pathway score per AMD stage

<table>
<thead>
<tr>
<th></th>
<th>Complement</th>
<th>ARMS2</th>
<th>Lipid</th>
<th>ECM</th>
<th>Other</th>
<th>Complement+AMRS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls ≥75 years</td>
<td>-0.01</td>
<td>0.4</td>
<td>-0.12</td>
<td>-0.09</td>
<td>0.08</td>
<td>0.39</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.29</td>
<td>0.58</td>
<td>-0.09</td>
<td>-0.06</td>
<td>0.10</td>
<td>0.88</td>
</tr>
<tr>
<td>Late</td>
<td>0.65</td>
<td>0.94</td>
<td>-0.06</td>
<td>-0.03</td>
<td>0.14</td>
<td>1.59</td>
</tr>
<tr>
<td>p-value*</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Jonckheere-Terpstra test for ordered alternatives
Table 2 Frequency of SNPs in 152 late AMD cases with complement pathway score below 0 and no ARMS2 risk allele. Sorted by frequency.

<table>
<thead>
<tr>
<th>SNP</th>
<th>%</th>
<th>Freq</th>
<th>OR</th>
<th>SNP</th>
<th>%</th>
<th>Freq</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH_rs10922109</td>
<td>96</td>
<td>146</td>
<td>0.40</td>
<td>TNFRSF10A_rs170637</td>
<td>060</td>
<td>37</td>
<td>0.94</td>
</tr>
<tr>
<td>LIPC_rs2043085</td>
<td>86</td>
<td>131</td>
<td>1.06</td>
<td>CFH_rs16188925</td>
<td>365</td>
<td>55</td>
<td>0.56</td>
</tr>
<tr>
<td>C2_rs943080</td>
<td>74</td>
<td>112</td>
<td>0.85</td>
<td>PRLRB_rs7803454</td>
<td>364</td>
<td>54</td>
<td>1.18</td>
</tr>
<tr>
<td>CFI_rs100393000</td>
<td>73</td>
<td>111</td>
<td>1.11</td>
<td>C2_rs429608</td>
<td>344</td>
<td>52</td>
<td>0.52</td>
</tr>
<tr>
<td>TMEM97_rs11090055</td>
<td>73</td>
<td>111</td>
<td>1.05</td>
<td>KMT2E_rs114422</td>
<td>345</td>
<td>51</td>
<td>1.17</td>
</tr>
<tr>
<td>ADAMT58_rs462247658</td>
<td>69</td>
<td>105</td>
<td>1.14</td>
<td>C2_rs1144254831</td>
<td>324</td>
<td>48</td>
<td>1.07</td>
</tr>
<tr>
<td>C3_rs12301199</td>
<td>69</td>
<td>105</td>
<td>1.31</td>
<td>LIPC_rs2070895</td>
<td>304</td>
<td>45</td>
<td>0.86</td>
</tr>
<tr>
<td>RAD51B_rs61985136</td>
<td>65</td>
<td>99</td>
<td>0.87</td>
<td>SLC16A8_rs1835665</td>
<td>294</td>
<td>44</td>
<td>1.25</td>
</tr>
<tr>
<td>NPLOC4665655</td>
<td>34</td>
<td>52</td>
<td>1.28</td>
<td>RAD51B_rs284339</td>
<td>264</td>
<td>40</td>
<td>0.75</td>
</tr>
<tr>
<td>MR1307110078</td>
<td>47</td>
<td>71</td>
<td>0.83</td>
<td>COLBA1_rs55975637</td>
<td>244</td>
<td>37</td>
<td>1.28</td>
</tr>
<tr>
<td>CETP_rs17231506</td>
<td>47</td>
<td>71</td>
<td>0.99</td>
<td>SYN3_rs1854227</td>
<td>264</td>
<td>40</td>
<td>1.10</td>
</tr>
<tr>
<td>B3GALT1_rs9565462</td>
<td>46</td>
<td>70</td>
<td>0.86</td>
<td>RAD51B_rs284339</td>
<td>194</td>
<td>29</td>
<td>1.10</td>
</tr>
<tr>
<td>COL4A3_rs11388770</td>
<td>45</td>
<td>69</td>
<td>0.90</td>
<td>COLBA1_rs55975637</td>
<td>244</td>
<td>37</td>
<td>1.15</td>
</tr>
<tr>
<td>APOE_rs7030651</td>
<td>45</td>
<td>69</td>
<td>0.92</td>
<td>CFB82_rs72801342</td>
<td>944</td>
<td>14</td>
<td>0.79</td>
</tr>
<tr>
<td>ABCA1_rs194088</td>
<td>44</td>
<td>67</td>
<td>0.86</td>
<td>PRR16091280</td>
<td>884</td>
<td>12</td>
<td>0.88</td>
</tr>
<tr>
<td>ARHGAPI21123_57237</td>
<td>40</td>
<td>61</td>
<td>1.04</td>
<td>C20orf85_rs2014599</td>
<td>666</td>
<td>9</td>
<td>0.64</td>
</tr>
<tr>
<td>CETP_rs5178082</td>
<td>39</td>
<td>60</td>
<td>0.81</td>
<td>C3_rs12019136</td>
<td>555</td>
<td>5</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Figure 7. The number of people with a risk allele, per pathway. Dark blue = the controls 75 years and older, light blue = intermediate AMD cases, green = late AMD cases.
Table 3. Comparison of controls versus late AMD cases with a logistic regression corrected for age and sex, in EUGENDA, RSI & RSIII and Alienor.

<table>
<thead>
<tr>
<th></th>
<th>Controls ≥75</th>
<th>Late AMD</th>
<th>OR</th>
<th>CI 95%</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Never Smoked</strong></td>
<td>N=1029</td>
<td>N=435</td>
<td>1.39</td>
<td>1.23-1.57</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Former smoker</strong></td>
<td>N=757</td>
<td>N=533</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Current smoker</strong></td>
<td>N=185</td>
<td>N=152</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vegetables medium servings per day</strong></td>
<td>0.94 (SD 0.18)</td>
<td>0.89 (SD 0.25)</td>
<td>0.40</td>
<td>0.27-0.58</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N=1535</td>
<td>N=939</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fruit medium servings per day</strong></td>
<td>0.92 (SD 0.22)</td>
<td>0.84 (SD 0.32)</td>
<td>0.35</td>
<td>0.25-0.47</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N=1535</td>
<td>N=941</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fish medium servings per day</strong></td>
<td>0.24 (SD 0.23)</td>
<td>0.17 (SD 0.16)</td>
<td>0.17</td>
<td>0.11-0.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N=1534</td>
<td>N=938</td>
<td></td>
<td></td>
<td></td>
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</table>