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Genetic Risk, Lifestyle, and Age-Related Macular Degeneration in Europe

The EYE-RISK Consortium

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Purpose: Age-related macular degeneration (AMD) is a common multifactorial disease in the elderly with a prominent genetic basis. Many risk variants have been identified, but the interpretation remains 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 European Eye Epidemiology Consortium.

Participants: Seventeen thousand one hundred seventy-four individuals 45 years of age or older participating in 6 population-based cohort studies, 2 clinic-based studies, and 1 case-control study.

Methods: Age-related macular degeneration was diagnosed and graded based on fundus photographs. Data on genetics, lifestyle, and diet were harmonized. Minor allele frequencies and population-attributable fraction (PAF) were calculated. A total genetic risk score (GRS) and pathway-specific risk scores (complement, lipid, extra-cellular matrix, other) were constructed based on the dosage of SNPs and conditional β values; a lifestyle score was constructed based on smoking and diet.

Main Outcome Measures: Intermediate and late AMD.

Results: The risk variants with the largest difference between late AMD patients and control participants and the highest PAFs were located in *ARMS2* (rs3750846) and *CHF* (rs570618 and rs10922109). Combining all genetic variants, the total genetic risk score ranged from −3.50 to 4.63 and increased with AMD severity. Of the late AMD patients, 1581 of 1777 (89%) showed a positive total GRS. The complement pathway and *ARMS2* were by far the most prominent genetic pathways contributing to late AMD (positive GRS, 90% of patients with late disease), but risk in 3 pathways was most frequent (35% of patients with late disease). Lifestyle was a strong determinant of the outcome in each genetic risk category; unfavorable lifestyle increased the risk of late AMD at least 2-fold.

Conclusions: Genetic risk variants contribute to late AMD in most patients. However, lifestyle factors have a strong influence on the outcome of genetic risk and should be a strong focus in patient management. Genetic risks in *ARMS2* and the complement pathway are present in most late AMD patients but are mostly combined with risks in other pathways. *Ophthalmology 2020;■:1−11*

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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 in up to 4.8 million Europeans and up to 18.6 million persons worldwide, a blinding stage of AMD will develop by 2040.[1,2] Age-related macular degeneration is classified into 2 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 epithelium.[3] Only the wet form can be treated with anti−vascular endothelial growth factor agents, but visual decline is remains inevitable in the long term.[4]

Age-related macular degeneration is a complex genetic disease that is influenced strongly by a combination of environmental and genetic factors. In particular, smoking and diet are known to increase the risk of AMD considerably. The genetic cause is well established: 52 common, known AMD-associated variants and more than 100 rare

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variants have been reported.[5,6] These variants explain most of the disease causes and helped to pinpoint several pathogenic pathways. Of these, the complement cascade seemed to be most important, but the first attempts to target this pathway in intervention trials have achieved limited success.[7,8] This raises the question of 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.[9] 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 produced data on AMD genotype and phenotype available for analysis and were enrolled as a pooled dataset in the current study. The cohort descriptions of the included studies are listed in the Appendix (available at www.aaojournal.org). The Combined Ophthalmic Research Rotterdam Biobank, Muenster Aging and Retina Study (MARS), and the European Genetic Database were clinic-based studies, and the remaining studies were population based (the Rotterdam Study I, II, and III; Alienor-3C [Antioxidants, Essential Lipids, Nutrition and Ocular Diseases−3 cities]; Montrachet-3C [Maculopathy Optic Nerve nuTRition neurovAsCular and HEarT diseases−3 cities]; and the Coimbra Eye Study). Persons 45 years of age and older were included in the analyses; various analyses included only control participants 75 years of age or older. All studies were performed in accordance with the tenets of the Declaration of Helsinki for research involving human subjects and good epidemiologic practice guidelines, and written informed consent was obtained 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. Age-related macular degeneration features were graded locally by clinicians or experienced graders; classifications were grouped into 3 severity groups. Control participants did not display AMD, aside from only small drusen or only pigment irregularities; persons with early or intermediate AMD showed soft indistinct (large) drusen, reticular drusen, or both, with or without pigmentary irregularities, and further were considered to have intermediate AMD; persons with late AMD had GA or CNV; and persons with both end stages were diagnosed as having CNV. Lifestyle factors including smoking and dietary habits were assessed by questionnaire.

Genetic Analyses and Risk Scores

Age-related macular degeneration genetic risk variants were ascertained from the EYE-RISK and E3 database.[5,9] Studies used various platforms to determine the 52 known risk variants, such as whole-exome sequencing, exome chip (Illumina HumanExome BeadChip), genomic single-nucleotide polymorphism (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 with next-generation sequencing; for the EYE-RISK genotype assay,[10] see cohort descriptions. If variants had been determined by multiple methods that included direct genotyping, we used data from the latter method. When no direct genotyping was available, genotypes were dosages derived from Haplotype Reference Consortium imputation or 1000G. Three (rs71507014, rs67538026, and rs142450006) of the 52 known AMD risk variants could not be included in our analysis because genotypes were not available for multiple cohorts.

Genetic risk scores (GRS) were calculated for the 17 174 individuals for whom the 5 major risk variants (*CFH* rs10922109, *CFH* rs570618, *C2* rs429608, *C3* rs2230199, and *ARMS2* rs3750846) were available. Complete genotype data on minor risk alleles were available in 62.3% persons; 85.1% individuals had 47 of 49 variants. Genetic risk scores were calculated by multiplying the conditional β value of the AMD risk variant[5] with the allele dosage. Subsequently, all calculations were summed. Pathway-specific GRSs were constructed in the same manner. For the complement GRSs, we included all risk variants in the *CFH*, *CFI*, *C9*, *C2*, *TMEM97*/*VTN*, and *C3* genes. For the lipid GRSs, variants in *ABCA1*, *LIPC*, *CETP*, and *APOE* were included. For the extracellular matrix (ECM) GRS, variants in *COL4A3*, *ADAMTS9-AS2*, *COL8A1*, *VEGFA*, and *SYN3*/*TIMP3* were included. The remaining variants were included in "other" GRSs. The function of *ARMS2* mostly was considered unsettled. However, because recent evidence suggests a role in the complement pathway,[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 and 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 1, that is, 120 g of vegetables per day, 120 g of fruit per day, and 100 g of fish per day. β coefficients for associations with late AMD were calculated by multivariate logistic regression, were multiplied by determinant values, and were summed to create a lifestyle risk score. Lifestyle risk scores were stratified into tertiles as an unfavorable, intermediate, or favorable lifestyle.

Statistical Analysis

The population-attributable fraction (PAF) was calculated for each variant using the formula of Miettinen[12]: $PAF = Pc \times ((OR - 1) / OR)$, where OR is the odds ratio and Pc is the proportion of exposed patients among the patients. The pooled dataset formed the basis for all analysis. We calculated the discriminative accuracy between late AMD patients and control participants for our model of genetic factors using 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 (control participants or patients). The dataset was fully balanced between control participants and patients; 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 0 but rather, imputed to the mean. Covariates were selected based on error expectation minimization.

Where appropriate, comparisons were made with the 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 analysis of variance. Graphical outputs were constructed with GraphPad Prism 5 version 7.00 for Windows software (GraphPad Software, La Jolla, CA). Histograms and a receiver operator characteristic curve were constructed with SPSS Statistics for Windows version 25.0 (IBM Corp).

Results

We identified a total of 17 174 individuals 45 years of age or older with data on genetics and AMD: 13 324 persons without AMD, 2073 persons with intermediate AMD, and 1777 persons with late AMD. Of the persons with late AMD, 309 demonstrated GA, and 1468 demonstrated CNV. Age ranged from 45 to 101 years old, with a mean of 68.7 years (standard deviation [SD], 10.4), the proportion of women was 58.5%, the proportion of current smoker was 16.8% (n = 2888), and the proportion of former smokers was 39.5% (n = 6786). For risk calculations, we aimed to ensure a true phenotype of no AMD and therefore included only control participants 75 years of age or older (n = 3167) in these analyses. The proportion of women in this subset (control participants 75 years of age or older and patients with intermediate or late AMD) was 61.3%, the proportion of current smokers was 9% (n = 630), and the proportion of former smokers was 36.2% (n = 2541).

Single Variants

First, we focused on frequency distributions of the 49 single-risk variants in the 3 phenotype groups and ranked variants according to frequency differences between late and no AMD (Fig 1A). Single-nucleotide polymorphisms from the complement pathway and *ARMS2* showed the largest difference in frequency between patients and control participants (rs10922109, rs61818925, and rs570618 [CFH]; rs429608 [C2]; rs2230199 [C3]; and rs3750846 [ARMS2]). Among the first 10 variants, 5 variants showed a lower frequency among patients, corresponding to a protective effect on AMD. Next, we calculated the PAF for each single variant. The *ARMS2* variant rs3750846 was associated with a large PAF (0.3) for late AMD, whereas variants in *CFH* exhibited both the largest PAF (0.33 for rs570618) and the largest inverse PAF (−0.37 for rs10922109; Fig 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 (0.024) AMD. Only 4 late AMD patients (0.2% [4/1777]) did not carry any of the 5 major risk SNPs, compared with 33 control participants (1% [33/3167]).

Genetic Risk Score for Age-Related Macular Degeneration

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; SD, 1.24) and showed a normal distribution (Fig 2A). With respect to the distribution per phenotype, the GRS in control participants ranged from −3.03 to 3.94 (mean, 0.26; SD, 1.16), that in intermediate AMD patients ranged from −3.11 to 4.71 (mean, 0.83; SD, 1.33), and that in late AMD patients ranged from −3.00 to 6.23 (mean, 1.64; SD, 1.32; Fig 2B). Although the lowest GRS value was similar for all phenotypes, the entire distribution showed a significant increase with increasing AMD severity ($P < 0.0001$, Jonckheere-Terpstra test for ordered alternatives). When stratifying late AMD into GA and CNV, slightly higher scores were noted for CNV (Fig 2C) that for GA ranged from −2.72 to 4.87 (mean, 1.46; SD, 1.41) and that for CNV ranged from −3.00 to 6.23 (mean, 1.67; SD, 1.30; $P = 0.01$, independent-sample *t* test). We estimated the discriminative accuracy of a score based on the 49 AMD-associated genetic variants (Figs S3 and S4, available at www.aaojournal.org) for identification of late AMD; the area under the receiver operating characteristic curve was 0.838. We identified a minimal set of variants by using the leave-one-out principle and found an almost identical area under the receiver operating characteristic curve (0.837) when including 27 AMD-associated variants (Genetic Risk Score for Age-Related Macular Degeneration and the Supplemental Appendix, available at www.aaojournal.org).

Genetic Risk Scores per Pathway

Next, we constructed pathway-specific GRSs for the complement, lipids, ECM, *ARMS2*, and other pathways. The complement pathway score ranged from −3.15 to 3.64 in the population-based studies, and 55% of participants scored more than 0 for this pathway. The *ARMS2* score ranged from 0 to 2.15 because only 1 risk variant determines this score. The lipid pathway showed a GRS ranging from −1.44 to 0.49, and the ECM pathway showed a GRS ranging from −0.92 to 1.46, and 36% and 33%, respectively, showed a score of more than 0. The pathway other ranged from −1.06 to 1.45, and 61% showed a positive score.

The distribution of all pathway GRSs in our total study population showed a positive shift with increasing AMD severity ($P < 0.0001$, Jonckheere-Terpstra test for ordered alternatives; Table S1, available at www.aaojournal.org; Fig 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 Genetic Risk Score

We studied the proportion of individuals with a positive (>0) GRS for each of the pathways, because this indicates more genetic risk than protection from that particular pathway. Positive GRSs for all pathways were most frequent in late AMD (Fig 6). Positive GRSs for the complement and other pathways were most prevalent in all phenotypes. The largest increase per phenotype severity was found for the complement and *ARMS2* pathways; the proportion of persons with positive GRSs in

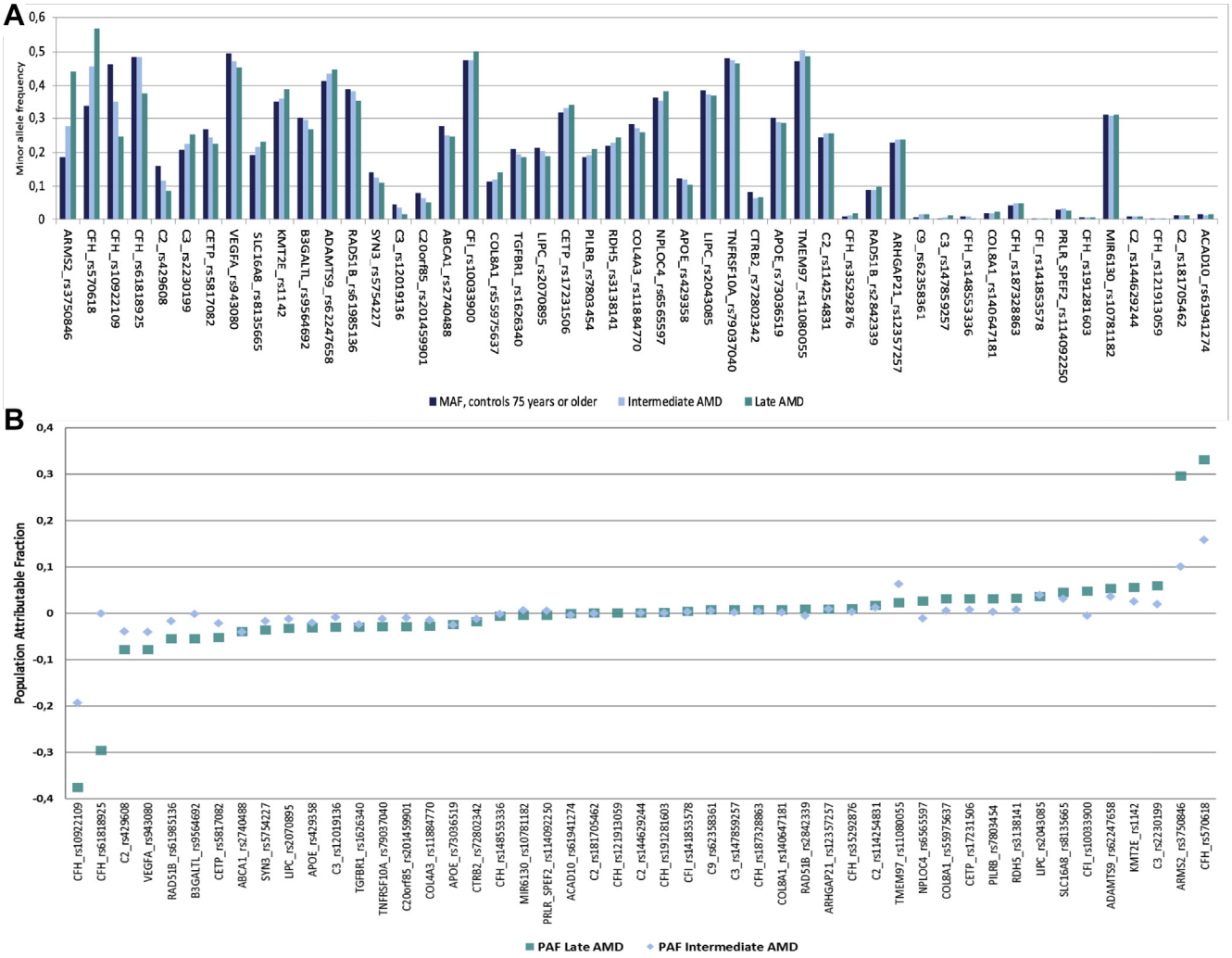

Figure 1. **A**, Bar graph showing the minor allele frequency (MAF) of patients and control participants for 49 genetic variants associated with age-related macular degeneration (AMD). The variants are ranked according to the difference in allele frequencies between late AMD patients and control participants, with the most discriminative variants on the left side of the graph. **B**, Bar graph showing the 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 because it was too rare to make meaningful calculations.

the complement pathway rose from 51% in control participants to 77% (a 26% increase) in late AMD patients, and that for the *ARMS2* pathway rose from 35% in control participants to 65% (a 30% increase) in late AMD patients ($P < 0.0001$ for both, Pearson chi-square 2-sided test). Not one pathway GRS was more than 0 in all late AMD patients, but 90% showed a positive GRS for the combination of complement and *ARMS2* pathways. On closer inspection of the remaining 10% (n = 152), these late AMD patients did carry risk alleles in these 2 pathways but showed a high frequency of protective variants that resulted in a GRS of less than 0 (Table S2, available at www.aaojournal.org). Subsequently, we examined the risk SNPs in greater detail by investigating the proportion of persons with at least 1 risk allele per pathway (Fig S7, available at www.aaojournal.org). Ninety-nine percent of persons with late AMD showed a risk SNP in either the complement or other pathway, but this was also the case for control participants. For the *ARMS2*, lipid, and ECM pathways, this was less frequent.

The next question we addressed for each pathway was this: Can late AMD develop without a risk variant in this pathway? For some pathways, this was rare: 0.7% (12/1777) of late AMD patients for the complement pathway and 1.5% (26/1777) of late AMD patients for the other pathway. For the *ARMS2*, lipids, and ECM pathways, these fractions were higher (34.8%, 6.1%, and 19.6%, respectively). When combining the complement and *ARMS2* pathways, only 5 late AMD patients (0.3%) showed no risk allele in this pathway.

Next, we calculated the distribution of pathways with a GRS of more than 0 (Fig 8). Most participants showed 2 to 4

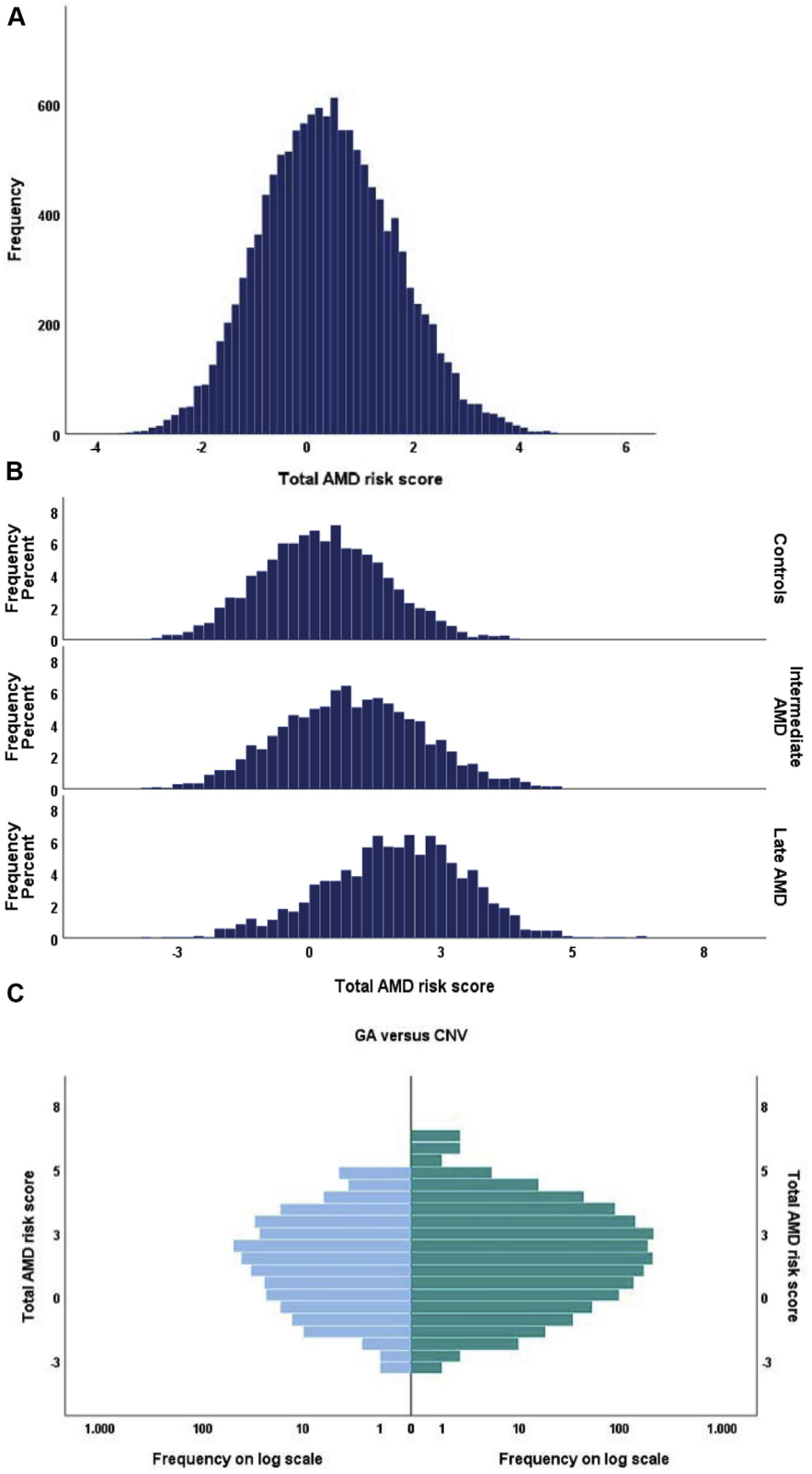

Figure 2. **A**, Bar graph showing the distribution of the total age-related macular degeneration (AMD) genetic risk score (GRS) in the European population. **B**, Bar graphs showing distributions of the total AMD GRS: (top panel) control participants (age, ≥75 years), (middle panel) intermediate AMD, and (bottom panel) late AMD. **C**, Bar graphs showing the distributions of the total AMD GRS: (left panel; light blue) frequency of geographic atrophy (GA) for each total AMD GRS and (right panel; green) frequency of choroidal neovascularization (CNV) for each total AMD GRS, both on a log scale.

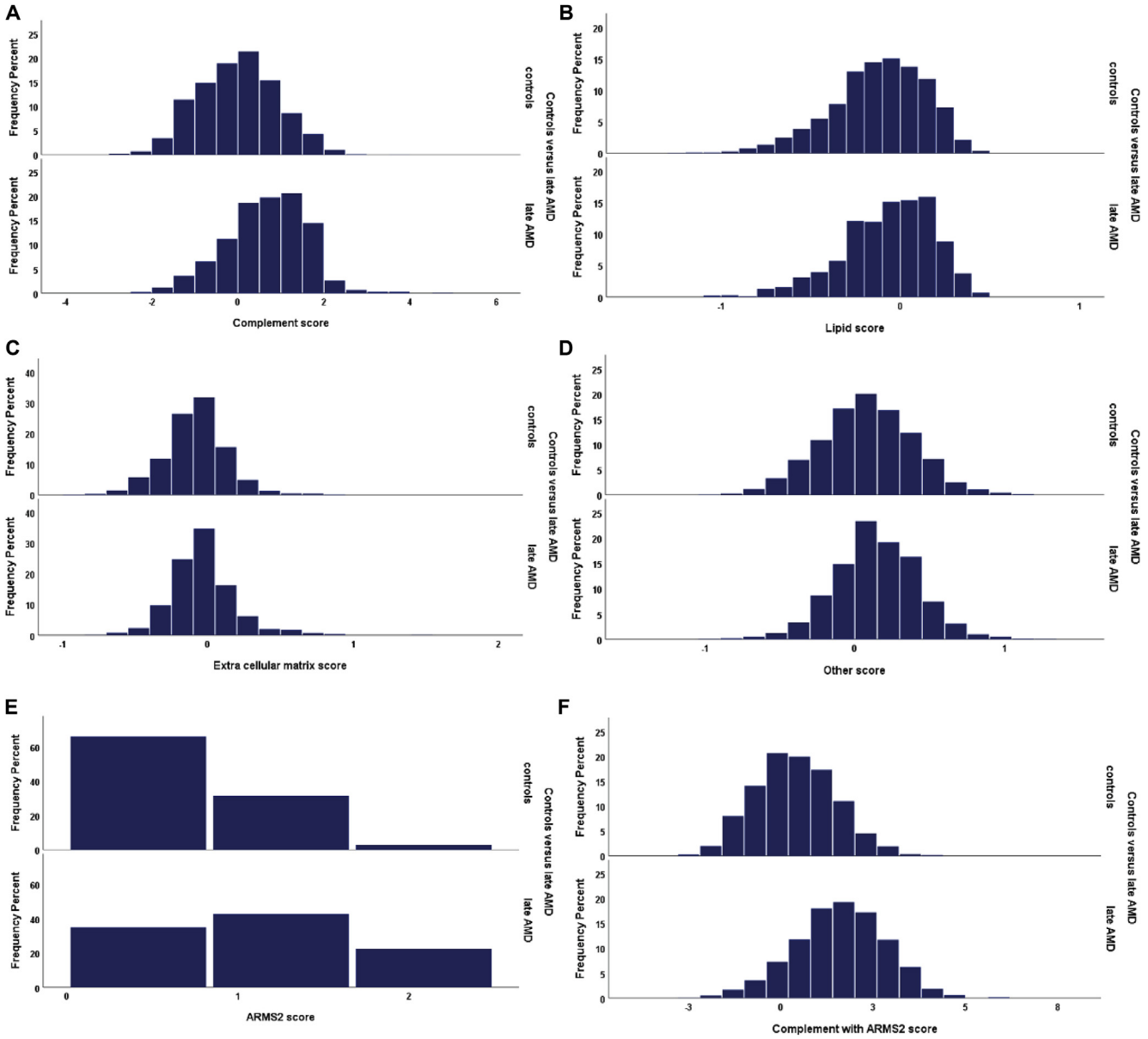

Figure 5. Bar graphs showing the distributions of the genetic risk scores for the (**A**) complement, (**B**) lipids, (**C**) extracellular matrix, (**D**) *ARMS2*, and (**E**) other pathways and (**F**) the complement with *ARMS2* combined pathways in control participants and late age-related macular degeneration (AMD) patients.

pathways with a GRS of more than 0 (85%). A small proportion (7%) of individuals showed a GRS in only 1 pathway of more than 0, and an even smaller proportion (1% [n = 23]) of individuals showed a GRS of 0 or less for all pathways.

Combining Genetics with Lifestyle

Data on lifestyle factors were available for a subset of the study population (n = 3525). In these participants, we investigated the AMD lifestyle factors of smoking and dietary intake of vegetables, fruit, and fish. Patients more often were current smokers (OR, 1.39) and consumed fewer vegetables (OR, 0.40), less fruit (OR, 0.35), and less fish (OR, 0.17; $P < 0.0001$ for all; Table S3, available at 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 to 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 (Fig 9).

Discussion

This study provides a comprehensive interpretation of AMD genetic risk in the European population. The risk allele most

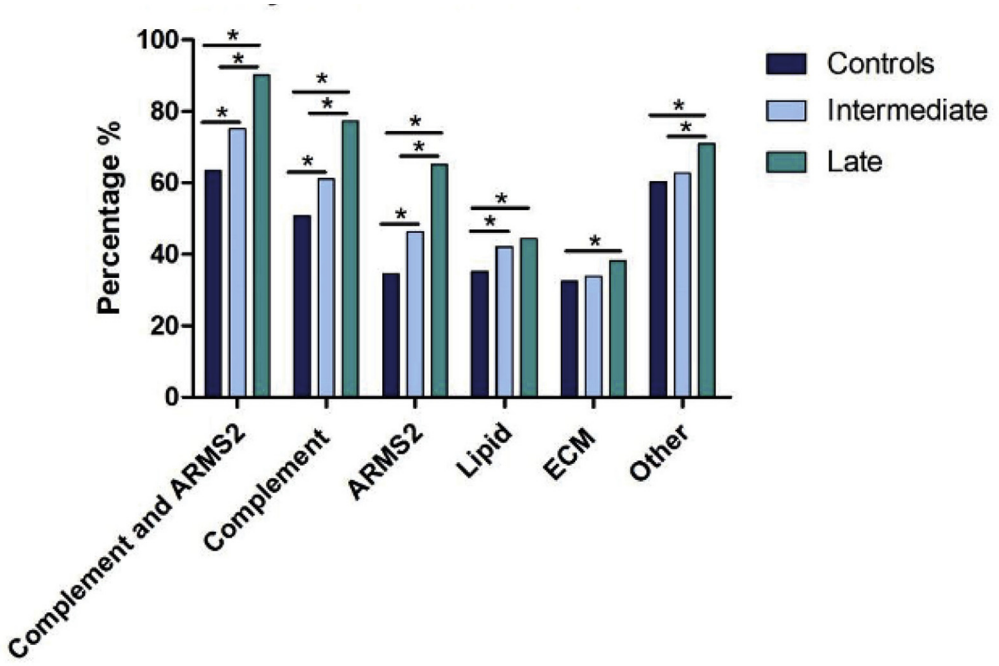

Figure 6. Bar graph showing the percentage of individuals with a positive genetic risk score for each of the pathways. Dark blue, control participants 75 years of age or older; light blue, intermediate age-related macular degeneration (AMD) patients; green, late AMD patients. The asterisk (*) indicates statistical differences in a Pearson chi-square test (2-sided) with $P < 0.0001$; $P = 0.0028$ with Bonferroni correction for multiple testing. ECM = extracellular matrix.

discriminative between late AMD patients and control participants was located in *ARMS2*, closely followed by a risk-increasing and a protective allele in *CFH*. We observed a normal distribution of AMD-associated GRS, with variants increasing disease risk, but also a significant number offering protection against AMD. Patients with late AMD showed higher GRSs than control participants. Mathematically, we showed that the genetic contribution of the complement pathway and *ARMS2* to late AMD was at least 90%. However, most patients carried genetic risk in multiple pathways, signifying the complex cause of AMD. All persons benefitted from a healthy lifestyle, but those with a high GRS showed the strongest risk reduction. This highlights the possibilities of counteracting predicted disease outcomes with lifestyle choices.

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 9 studies with genetic data, that is, population studies from The Netherlands, France, and

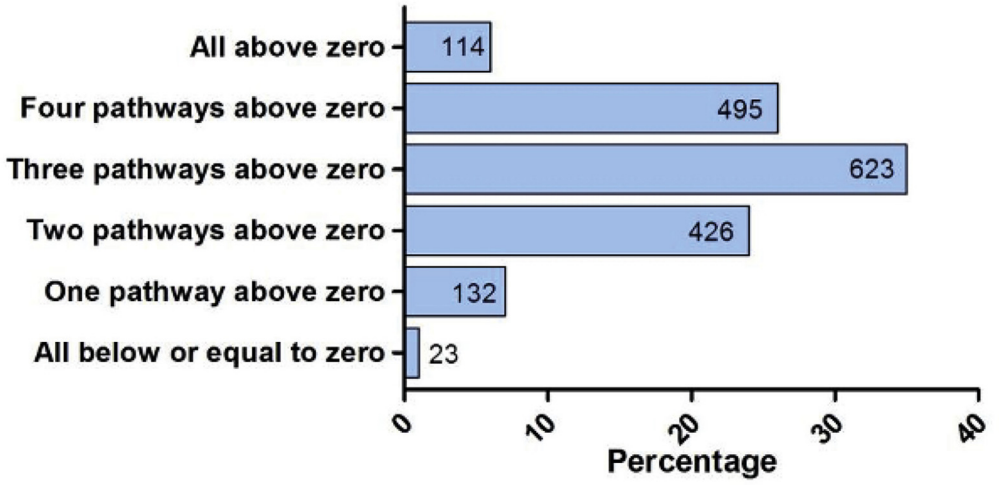

Figure 8. Bar graph showing the number of late age-related macular degeneration patients with positive pathway scores. Numbers inside the bars indicate the frequency.

Subgroup	Cases/Controls	Odds Ratio	CI 95%	*P*-value
Low genetic risk				
Favorable lifestyle	27/292	1 reference		
Intermediate lifestyle	46/250	1.99	1.30-3.30	0.007
Unfavorable lifestyle	37/198	2.02	1.19-3.43	0.009
Intermediate genetic risk				
Favorable lifestyle	51/207	2.67	1.62-4.39	<0.0001
Intermediate lifestyle	84/167	5.44	3.39-8.73	<0.0001
Unfavorable lifestyle	95/170	6.04	3.79-9.65	<0.0001
High genetic risk				
Favorable lifestyle	124/90	14.90	9.23-24.05	<0.0001
Intermediate lifestyle	198/84	25.94	15.94-40.77	<0.0001
Unfavorable lifestyle	230/71	35.03	21.77-56.37	<0.0001

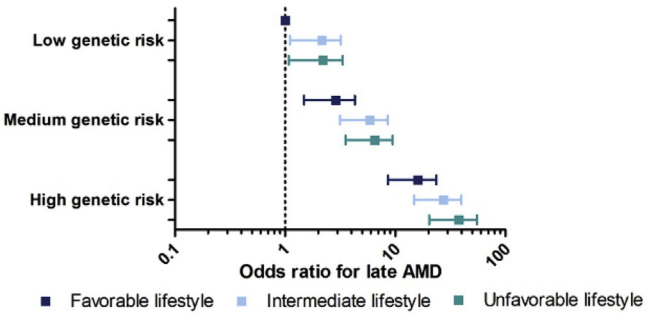

Figure 9. Table and graph showing the odds ratio of risk for late age-related macular degeneration (AMD) stratified by genetic risk score (GRS) and lifestyle risk. CI = confidence interval.

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 factors in the pooled dataset. 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 associated individually with AMD,[5] of which only few were rare. Hence, we cannot elaborate on risks provided by most of the currently known rare variants. The studies providing the greater part of patients were case-control studies without follow-up data, and therefore we were restricted to cross-sectional analyses.

A positive GRS indicated more causative genetic risk than protection by genetic variants. Because this was present in 63% of the population (2546/4044), we conclude that genetic susceptibility to AMD is highly prevalent. Among patients with late AMD, the proportion of a positive GRS rose to 89% (1581/1777). We investigated this in greater detail and found that the 5 major risk alleles were absent in only 66 persons (1%), indicating that 99% of the study population carried at least 1 major risk allele. By contrast, on average, 2.5 major risk alleles were present among late AMD patients and were absent in only 0.2% (4/1777). A set of 27 risk variants was enough to reach discriminative accuracy 0.84 for late AMD versus no AMD. Adding more variants did not improve this further, and the area under the receiver operating characteristic curve was in line with previous studies.[13,14] It should be emphasized that such high discrimination based solely on genetic variants is exceptional for a complex disorder, although this is still challenging at mean GRS levels.

Considering individual pathways, 19 of 52 common AMD risk variants are in the complement pathway.[5] Previous studies have reported already that common variants in the complement pathway explain 57% of the heritable risk of AMD,[15] and our study underscores the high attribution of this pathway to the overall GRS. Comparing the risk of the most important *CFH* SNP (rs570618 in high linkage disequilibrium (LD) 0.991 with rs1061170, Y402H) with an Asian population, we and others observed only a slightly higher OR of late AMD in Europeans (2.47 vs. 2.09)[16] but very different allele frequencies (minor allele frequency, 0.34 vs. 0.049).[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 (Fig 1), which mathematically can add up to a GRS of 0. Whether this also reflects a neutral risk at the tissue level is unclear, because persons with late AMD and a negative GRS for the complement pathway 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 *ARMS2* locus carried the highest risk of late AMD and the second highest attribution to overall AMD occurrence in our study (Fig 1). In East Asia, this allele is twice as common (minor allele frequency, 0.40 in East Asians vs. 0.19 in Europeans), but the risk of late AMD for carriers seems comparable (OR, 2.94 in India vs. OR, 3.06 in Europe).[18,19] The function of *ARMS2* is the subject of ongoing research. Recently, Micklisch et al[11] showed in vitro that *ARMS2* functions as a surface complement regulator by binding to the cell membrane of apoptotic and necrotic cells and that it subsequently binds properdin and activates complement. This provides evidence that *ARMS2* can be an initiator of complement. We considered 2 different scenarios for the pathway of *ARMS2*: a function in the complement pathway and an independent function. When regarded as a complement gene, the vast majority (90%) of late AMD patients showed an increased genetic risk in this pathway, making complement the main driver of late AMD. As a stand-alone function, *ARMS2* also provided a significant contribution because it was present in two thirds of late AMD patients.

Variants in the lipid and ECM pathways showed smaller effects and attribution to overall late AMD. Variants in genes with other functions (the other pathway) also showed

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 relationship to genetic risk. As expected, persons with AMD showed a lower intake of vegetables, fish, and fruit and higher rates of smoking (Table S3).[20–26] Together, 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[27,28] and Age-Related Eye Disease Study, which demonstrated interaction between single nutrients and *CFH* and *ARMS2* and a protective role of diet in those with a high GRS.[29] The current study analyzed a more comprehensive set of risk variants and found that a healthy diet and not smoking also were beneficial in persons with low genetic risk. Oxidative stress is the most recognized molecular effect of smoking in the pathogenesis of AMD,[30] and antioxidants are the most important contribution to 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.[31] Unfortunately, a healthy diet consisting of sufficient fruits, vegetables, and fatty fish is consumed by only a minority of the elderly,[28] and smoking is still twice as high among those with late AMD (Table S3). This asks for more rigorous measures for prevention, and training of doctors in behavioral change techniques may be part of this.

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 choices. The frequency and risk estimates provided by this study can lay the foundation for future intervention studies that are tailored to pathways.

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HUMAN SUBJECTS: Human subjects were included in this study. The recruitment and research protocols were reviewed and approved by the local institutional review boards: the Alienor-3C study was approved by the Ethical Committee of Bordeaux (Comité de Protection des Personnes Sud-Ouest et Outre-Mer III); the Coimbra eye study was approved by the Association for Innovation and Biomedical Research on Light and Image (AIBILI) Ethics Committee; the CORRBI study was approved by the medical ethics review committee of the Erasmus Medical Center, Rotterdam, the Netherlands; the EUGENDA study was approved by the ethics committees in Cologne and Nijmegen; the MARS study was approved by the Institutional Review Board of the University of Muenster: the Montrachet-3C study was approved by the Ethical Committee of the University Hospital of Kremlin-Bicêtre; the Rotterdam Studies I, II, and III were approved by the Medical Ethics Committee of the Erasmus MC (registration number: MEC 02.1015); and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). Informed consent was obtained from all study participants in compliance with the Declaration of Helsinki.

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Abbreviations and Acronyms:
AMD = age-related macular degeneration; **CNV** = choroidal neovascularization; **ECM** = extracellular matrix; **E3** = European Eye Epidemiology; **GA** = geographic atrophy; **GRS** = genetic risk score; **GWAS** = genome-wide association study; **LD** = linkage disequilibrium; **MAF** = minor allele frequency; **OR** = odds ratio; **PAF** = population-attributable fraction; **RPE** = retinal pigment epithelium; **RS** = Rotterdam Study; **SD** = standard deviation; **SNP** = single nucleotide polymorphism; **WARGMS** = Wisconsin Age-Related Maculopathy Grading System.

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