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Genetic risk, lifestyle, and AMD in Europe. The EYE-RISK consortium
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Abstract:	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 (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).
	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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Erasmus MC
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Ophthalmology
Editor

Rotterdam, March 21st, 2020

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

1 Genetic risk, lifestyle, and AMD in Europe. The EYE-RISK consortium

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32 **ABSTRACT**

33 **Purpose:** Age-related macular degeneration(AMD) is a common multifactorial disease in elderly with a

34 prominent genetic basis. Many risk variants have been identified, but the interpretation is still

35 challenging. We investigated the genetic distribution of AMD-associated risk variants in a large European

36 consortium, calculated attributable, and pathway-specific genetic risks, and assessed the influence of

37 lifestyle on genetic outcomes.

38 **Design:** Pooled analysis of cross-sectional data from the E3 consortium.

39 **Participants:** 17.174 individuals aged 45+ participating in 6 population-based cohort studies, 2 clinic
40 based studies, and 1 case-control study.

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

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

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

60

61 Word count: 350/350

73 Anneke den Hollander is a consultant for Ionis Pharmaceuticals.
74 Marius Ueffing is a consultant for Roche
75
76 **ABBREVIATIONS**

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

82 **Keywords**: Age-related macular degeneration (AMD), genetics, population, pathways, Europe
83
84 **Précis:** Age-related macular degeneration is driven by complement and ARMS2, but caused in most by
85 multiple genetic pathways. Someone's genetic effect can be severely reduced by healthy lifestyle.

86 This article contains additional online-only material. The following should appear online-only: Figure 3, 4
87 and 7, Tables 1-3 and cohort descriptions.
88
89 **INTRODUCTION**
90
91 Age-related macular degeneration (AMD) is a progressive degenerative disease of the retina and the
92 most important cause of blindness in the Western world. Projections show that up to 4.8 million
93 Europeans and up to 18.6 million persons worldwide will develop a blinding stage of AMD by 2040[1, 2].
94 AMD is classified into two end stages; a more common "wet" form characterized by choroidal
95 neovascularization (CNV), and a "dry" form characterized by geographic atrophy (GA) of the retinal
96 pigment epithelium[3]. Only the wet form can be treated with anti-vascular endothelial growth factor, but
97 visual decline is still inevitable at long-term[4].
98 AMD is a complex genetic disease, strongly influenced by a combination of environmental and genetic
99 factors. Smoking, diet, and cardiovascular determinants are known to increase the risk of AMD
100 considerably. The genetic etiology is well–established: 52 common known AMD-associated variants and
101 >100 rare variants have been reported[5, 6]. These variants explain the majority of the disease etiology,
102 and helped pinpoint several pathogenic pathways. Of these, the complement cascade appeared to be
103 most important, but the first attempts to target this pathway in intervention trials have had limited
104 success[7, 8]. This raises the question whether disease pathways are specific to groups of individuals. If this
105 is the case, intervention trials may be more successful by stratifying patients based on the major disease
106 pathway driving their disease.
107 In this study, we aimed to investigate the contribution of genetic variants to AMD risk in Europe using
108 data from the large European Eye Epidemiology (E3) consortium. We aimed to determine the

109   contribution of each disease pathway in AMD, and investigated whether lifestyle changes can reduce

110   the risk of late AMD, in particular in individuals with a high genetic risk of AMD.

111

112   **METHODS**

113

114   **Study population:**

115   The E3 consortium is a European collaboration of studies with epidemiologic data on common eye

116   disorders; a detailed description on the consortium can be found elsewhere[9]. All data on AMD were

117   harmonized and collected in the EYE-RISK database (version 6.0). Nine studies from France, Germany,

118   the Netherlands, and Portugal had data on AMD genotype and phenotype available for analysis, and

119   were enrolled in the current study. The cohort descriptions of the included studies are available at

120   External link http://www.aaojournal.org. CORRBI, MARS, and EUGENDA were clinic-based studies, the

121   remaining were population-based (RSI, RSII & RSIII, Alienor-3C, Montrachet-3C and CES (Coimbra Eye

122   Study)). Persons aged 45 years and older were included in the analyses; various analyses only included

123   controls aged 75 years or older. All studies were performed in accordance with the Declaration of

124   Helsinki for research involving human subjects and the good epidemiological practice guideline, and had

125   written informed consent from all participants.

126   **Clinical examination:**

127   The phenotype of AMD was determined on fundus photographs centered on the macula; individuals

128   received the diagnosis of the worst eye. AMD features were graded locally by clinicians or experienced

129   graders; classifications were grouped into three severity classes. Controls did not display AMD, aside

130   from only small drusen or only pigment irregularities; persons with early or intermediate AMD had soft

131   indistinct (large) drusen and/or reticular drusen, with or without pigmentary irregularities, and were

132   further referred to as intermediate AMD. Persons with late AMD had GA, or CNV. Persons with both end

133   stages were diagnosed as CNV.

134   Lifestyle factors including smoking and dietary habits were assessed by questionnaire.

135

136   **Genetic analyses and risk scores**

137   AMD genetic risk variants were ascertained from the EYE-RISK/E3 database[5, 9]. Studies had used various

138   platforms to determine the 52 known risk variants, such as whole exome sequencing, exome chip

139   (Illumina HumanExome BeadChip), genomic SNP arrays (Illumina 550K (duo) chip or Illumina 610 quad),

140   or Taqman assays, and a custom-made AMD genotyping platform using single molecule molecular

141 inversion probes (smMIPs) with next generation sequencing; the EYE-RISK genotype assay[10], see cohort

142 descriptions. If variants had been determined by multiple methods which included direct genotyping, we

143 used data from the latter method. When no direct genotyping was available, genotypes were dosages

144 derived from Haplotype Reference Consortium (HRC) imputation or 1000G. Three (rs71507014,

145 rs67538026, rs142450006) of the 52 known AMD risk variants could not be included in our analysis since

146 genotypes were not available for multiple cohorts.

147 Genetic risk scores (GRS) were calculated for the 17,174 individuals for whom the five major risk

148 variants (*CFH* rs10922109, *CFH* rs570618, *C2* rs429608, *C3* rs2230199, *ARMS2* rs3750846) were

149 available. Complete genotype data on minor risk alleles were available in 62.3% persons; 85.1%

150 individuals had 47/49 variants. GRS were calculated by multiplying the conditional beta of the AMD risk

151 variant[5] with the allele dosage. Subsequently, all calculations were summed. Pathway-specific GRS were

152 constructed in the same manner. For the complement GRS, we included all risk variants in the *CFH, CFI,*

153 *C9, C2, TMEM97/VTN* and *C3* genes. For the lipid GRS, variants in *ABCA1, LIPC, CETP, APOE* were

154 included. For the extra-cellular matrix (ECM) GRS, variants in *COL4A3, ADAMTS9-AS2, COL8A1, VEGFA*

155 and *SYN3/TIMP3* were included. The remaining variants were included in 'other' GRS. The function of

156 *ARMS2* was mostly considered unsettled. However, as recent evidence suggests a role in the

157 complement pathway[11], we analyzed this gene as a stand-alone pathway GRS as well as part of the

158 complement pathway GRS.

159

160 **Lifestyle score**

161 Four well-established AMD lifestyle determinants (smoking status, servings of vegetables, fruit and fish

162 per day) were assessed by questionnaire. Smoking status was categorized as no, former, or current

163 smoker. Dietary intakes were analyzed in medium servings per day with a maximum of one, i.e., 120

164 grams of vegetables per day; 120 grams of fruit per day; 100 grams of fish per day. B-coefficients for

165 associations with late AMD were calculated by multivariable logistic regression, and were subsequently

166 multiplied by determinant values and summed to create a lifestyle risk score (LRS). LRS were stratified

167 into tertiles as unfavorable, intermediate or favorable lifestyle.

168

169 **Statistical analysis**

170 The population attributable fraction (PAF) was calculated for each variant using the formula of Miettinen

171 *et al*.[12] PAF = Pc * ((OR−1)/OR); where OR is the odds ratio, and Pc is the proportion of exposed cases

172 among the cases. We calculated the discriminative accuracy between late AMD cases and controls for

173   our model of genetic factors using the Saddle Point Signature software version 2.8.3 (Saddle Point

174   Science Ltd., Worcester Park, United Kingdom) in a batch multivariate regression analysis. Results were

175   cross-validated by the leave one out principle. Prediction performance at each iteration was quantified

176   by counting errors of persons assigned to the wrong category (controls or cases). The dataset was fully

177   balanced between controls and cases; the regression equations corresponded to a pseudo dataset, in

178   which the outcome classes were equal in size but the other statistical features were identical to the true

179   dataset. Missing values were not set to zero but imputed to the mean. Covariates were selected based

180   on error expectation minimization.

181   Where appropriate, comparisons were made with Pearson chi-square test, Jonckheere-Terpstra test for

182   ordered alternatives, or independent sample t-test. Interaction of genetic and lifestyle risk was assessed

183   by a univariate ANOVA. Graphical outputs were constructed with GraphPad Prism 5 (GraphPad Prism

184   version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com”).

185   Histograms and a receiver operator characteristic curve were constructed with SPSS (IBM Corp.

186   Released 2012 IBM SPSS Statistics for Windows, Version 25.0 Amonk, NY: IBM Corp).

187

188   **RESULTS**

189

190   We identified a total of 17,174 individuals aged 45 years and older with data on genetics and AMD;

191   13,324 persons without AMD, 2,073 with intermediate AMD and 1,777 individuals with late AMD. Of the

192   persons with late AMD; 309 had developed GA and 1,468 CNV. Age ranged from 45 to 101 years old with

193   a mean of 68.7 years (SD 10.4), the proportion of women was 58.5%, current smoking 16.8% (n=2,888),

194   former smoking 39.5% (n=6,786). To ensure a true phenotype of no AMD, we performed risk analyses

195   using participants aged 75 years and over as controls (n=3,167). The proportion of women in this subset

196   (controls 75+ and intermediate and late AMD cases) was 61.3%, current smoking 9% (n=630) and former

197   smoking 36.2% (n=2,541).

198

199   **Single variants**

200   First, we focused on frequency distributions of the 49 single risk variants in the three phenotype groups,

201   and ranked variants according to frequency differences between late and no AMD (**Figure 1a**). SNPs

202   from the complement pathway and *ARMS2* showed the largest difference in frequency between cases

203   and controls (rs10922109, rs61818925 and rs570618 (*CFH*), rs429608 (*C2*), rs2230199 (*C3*), rs3750846

204   (*ARMS2*)). Among the first ten variants, five variants had a lower frequency in cases, corresponding to a

205   protective effect on AMD. Next, we calculated the population attributable fraction (PAF) for each single

206   variant. *ARMS2* variant rs3750846 was associated with a high PAF (0.3) for late AMD, while variants in

207   *CFH* exhibited both the largest PAF (0.33) (rs570618) and the largest inverse PAF (-0.37) (rs10922109)

208   (**Figure 1b**). A similar pattern with smaller PAFs was observed for intermediate AMD. Only variant

209   rs11080055 in *TMEM97/VTN*, showed a higher PAF for intermediate (0.063) than for late AMD (0.024).

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

211   (1% or 33/3167) of controls.

212

213   **Genetic risk score for AMD**

214   We subsequently combined all genetic variants in a GRS and assessed its distribution. In the population-

215   based cohort studies (*n*= 13,194), the score ranged from -3.50 to 4.63 (mean 0.40, standard deviation

216   (SD) 1.24) and had a normal distribution (**Figure 2a**). With respect to the distribution per phenotype, the

217   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

218   (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

219   lowest GRS value was similar for all phenotypes, the entire distribution showed a significant increase

220   with increasing AMD severity (Jonckheere-Terpstra test for ordered alternatives; p-value <0.0001).

221   When stratifying late AMD into GA and CNV, slightly higher scores were noted for CNV (**Figure 2c**): GA

222   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,

223   independent sample t-test p-value=0.01). We estimated the discriminative accuracy of a score based on

224   the 49 AMD-associated genetic variants (Supplementary **Figure 3** and **4** available at External link

225   http://www.aaojournal.org) for identification of late AMD; the area under the curve (AUC) was 0.838.

226   We identified a minimal set of variants by using the leave one out principle, and found an almost

227   identical AUC (0.837) when including 27 AMD-associated variants (score is available in the

228   Supplementary material at External link http://www.aaojournal.org).

229

230   **Genetic risk scores per pathway**

231   Next, we constructed pathway-specific GRS; for the complement, lipids, extra-cellular matrix, age-

232   related maculopathy susceptibility 2 (*ARMS2*) and 'Other'. The complement pathway score ranged from

233   -3.15 to 3.64 in the population-based studies, and 55% of participants scored above 0 for this pathway.

234   The ARMS2 score ranged from 0 to 2.15 as only one risk variant determines this score. The lipid pathway

235   had GRS ranging from -1.44 to 0.49, the ECM pathway from -0.92 to 1.46, and 36% and 33%,

236   respectively, had a score higher than zero. The pathway 'Other' ranged from -1.06 to 1.45; 61% had a

237   positive score.

238   The distribution of all pathway GRS in our total study population showed a positive shift with increasing

239   AMD severity (Jonckheere-Terpstra test for ordered alternatives, p-value<0.0001, supplementary **Table**

240   **1** available at External link http://www.aaojournal.org and **Figure 5**), but the complement and ARMS2

241   GRS demonstrated the largest increase for late AMD, especially when combined (shift of mean GRS from

242   0.39 to 1.59).

243

244   **Frequency of positive GRS**

245   We studied the proportion of individuals with a positive (>0) GRS for each of the pathways, as this

246   indicates more genetic risk than protection from that particular pathway. Positive GRS for all pathways

247   were most frequent in late AMD (**Figure 6**). Positive GRS the for complement and 'other' pathways were

248   most prevalent in all phenotypes. The largest increase per phenotype severity was found for the

249   complement and ARMS2; the proportion of persons with positive GRS in the complement pathway rose

250   from 51% in controls to 77% (26% increase) in late AMD cases and ARMS2 rose from 35% in controls to

251   65% (30% increase) in late AMD cases (Pearson Chi-Square 2-sided test, p-value <0.0001 for both). Not

252   one pathway GRS was above zero in all late AMD cases, but 90% had a positive GRS for the combination

253   of complement and ARMS2. Upon closer inspection of the remaining 10% (n=152), these late AMD cases

254   did carry risk alleles in these two pathways but had a high frequency of protective variants which

255   resulted in a GRS below zero (supplementary **Table 2** available at External link

256   http://www.aaojournal.org). Subsequently, we examined the risk SNPs in greater detail by investigating

257   the proportion of persons with at least one risk allele per pathway (supplementary **Figure 7**, available at

258   External link http://www.aaojournal.org). 99% of persons with late AMD had a risk SNP in either the

259   complement or 'Other' pathway, but this was also the case for controls. For ARMS2, lipid and ECM

260   pathway this was less frequent.

261   Thus, it was possible to develop intermediate or late AMD without a risk SNP in a pathway, but this was

262   true for only few individuals; 0.7% (12/1777) of late AMD for the complement pathway, and 1.5%

263   (26/1777) of late AMD for the 'Other' pathway. For ARMS2, the lipids pathway and ECM pathway these

264   fractions were higher (34.8%, 6.1%, 19.6%), respectively. When combining complement and ARMS2,

265   only 5 (0.3%) late cases had no risk allele in this pathway.

266   Next, we calculated the distribution of pathways with a GRS above zero (see **Figure 8**). The majority of

267   participants had two to four pathways with a GRS above zero (85%). A small proportion (7%) of

268   individuals had a GRS in only one pathway above zero, and an even smaller proportion (1%; n=23) of

269   individuals had a GRS below or equal to zero for all pathways.

270

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

302 that were individually associated with AMD[5], and did not evaluate the contribution of rare variants to

303 AMD. This may alter the risk of AMD for individual cases considerably as is shown by de Breuk *et al[10]*.

304 The studies providing most of the cases were case-control studies without follow-up data, and we were

305 therefore restricted to cross-sectional analyses.

306

307 A positive GRS indicated more causative genetic risk than protection by genetic variants. As this was

308 present in (2546/4044) 63% of the population, we conclude that genetic susceptibility to AMD is highly

309 prevalent. Among cases with late AMD, the proportion of a positive GRS rose to (1581/1777) 89%. We

310 investigated this in greater detail, and found that the five major risk alleles were absent in only 66 (1%)

311 persons, indicating that 99% of the study population carried at least one major risk allele. Major risk

312 alleles were absent in only 0.2% (4/1777) of those with late AMD, hence, it appears that they are

313 virtually essential for the development of late AMD. Our AUC 0.837 for the discriminative accuracy of

314 genetic risk variants to identify late AMD cases from and controls was slightly better than in earlier

315 studies[13, 14].

316

317 Considering individual pathways, 19/52 common AMD risk variants are in the complement pathway[5].

318 Previous studies already reported that common variants in the complement pathway explain 57% of the

319 heritable risk of AMD[15], and our study underscored the high attribution of this pathway to the overall

320 GRS. Comparing the risk of the most important *CFH* SNP (rs570618 in high LD 0.991 with rs1061170,

321 Y402H) to an Asian population, we and others observed a slightly higher OR of late AMD in Europeans

322 (2.47 vs 2.09)[16] but very different allele frequencies (MAF 0.34 vs 0.049) [17]. With respect to function, the

323 complement pathway is part of the innate immune system, and numerous studies have shown that

324 imbalance of this cascade at the protein level is important for AMD pathogenesis. Genetically, this

325 system harbors strong causative as well as highly protective risk alleles (Figure 1), which mathematically

326 can add up to GRS zero. Whether this also reflects a neutral risk at the tissue level is unclear, because

327 persons with late AMD and a negative GRS for complement still carried risk-increasing alleles in this

328 pathway. Nevertheless, the risk-reducing effect of these protective alleles are of high biological interest,

329 and investigation into the functional consequences may provide leads for future therapy.

330

331 The rs3750846 (or its proxy rs10490924, A69S) variant in the *ARMS2* locus carried the highest risk of late

332 AMD, and the second highest attribution to overall AMD occurrence in our study (Figure 1). In East Asia,

333 this allele is twice as common (MAF 0.40 in East Asia vs 0.19 in Europeans), but the risk of late AMD for

334   carriers appears comparable (OR 2.94 in India vs OR 3.06 in Europe)[18, 19]. The function of *ARMS2* is

335   subject of ongoing research. Recently, Micklisch *et al*. showed *in vitro* that ARMS2 functions as a surface

336   complement regulator by binding to the cell membrane of apoptotic and necrotic cells, and

337   subsequently binds properdin and activates complement[11]. This provides evidence that ARMS2 can be

338   an initiator of complement. We considered two different scenarios for the pathway of *ARMS2*: a

339   function in the complement pathway and as a stand-alone. When regarded as a complement gene, the

340   vast majority (90%) of late AMD had an increased genetic risk in this pathway, making complement the

341   main driver of late AMD. As a stand-alone, *ARMS2* also provided a significant contribution, as it was

342   present in two thirds of late AMD.

343   Variants in the lipid and ECM pathway had smaller effects and attribution to overall late AMD. Variants

344   in genes with other functions ('other' pathway) also had smaller effects, but the 16 variants combined

345   were rather frequent and predisposed considerably to late AMD.

346

347   We further investigated the impact of the most important lifestyle factors, smoking and diet, in relation

348   to genetic risk. As expected, persons with AMD had lower intake of vegetables, fish, and fruit, and

349   higher rates of smoking (Supplemental Table 3)[20-26]. Together, we showed that a more unfavorable

350   lifestyle almost doubled the risk of late AMD. This occurred in all genetic risk strata but the OR increase

351   was most prominent in those at high genetic risk. These findings confirm previous reports from the

352   Rotterdam Study[27, 28], which demonstrated interaction between single nutrients and risk variants in *CFH*

353   and *ARMS2*, and AREDS, which showed a protective role of diet in those with a high GRS[29]. The current

354   study analyzed a more comprehensive set of risk variants, and found that a healthy diet and non-

355   smoking was also beneficial in persons with low genetic risk. Oxidative stress is the most recognized

356   molecular effect of smoking in the pathogenesis of AMD[30], and antioxidants the most important

357   contribution of a healthy diet. Oxidative stress with abundant reactive oxygen species, peroxidation of

358   lipids, proteins, RNA, and DNA in the retina can lead to cytotoxic effects and inflammation, enhancing

359   the development of AMD[31]. Unfortunately, a healthy diet consisting of sufficient fruits, vegetables, and

360   fatty fish is consumed by only a minority of elderly[28], and smoking is still twice as high among those with

361   late AMD (Supplement Table 3). Adding non genetic variables to the model improved 10-year prediction

362   for incident late AMD to AUC 0.92 in EYE-RISK[32], highlighting the accuracy of personalized predictions.

363   Genetic testing for AMD as suggested by de Breuk *et al*.[10] has not yet become part of clinical routine and

364   many ophthalmologists still do not implement lifestyle advice in the management of AMD patients. This

365   may deny their patients the only opportunity for prevention[33] as our findings demonstrate the value of

366   genetic testing for AMD in conjunction with the promotion of lifestyle change in patient counseling.

367

368   In conclusion, this large European consortium showed that genetic risk of AMD is highly prevalent in the

369   population at large, and that risk variants in the complement pathway are by far the lead drivers of late

370   AMD. Nevertheless, late AMD is mostly a result of multiple genetic pathways, and lifestyle. Our

371   frequency and risk estimates can lay the foundation for future intervention studies which are tailored to

372   pathways.

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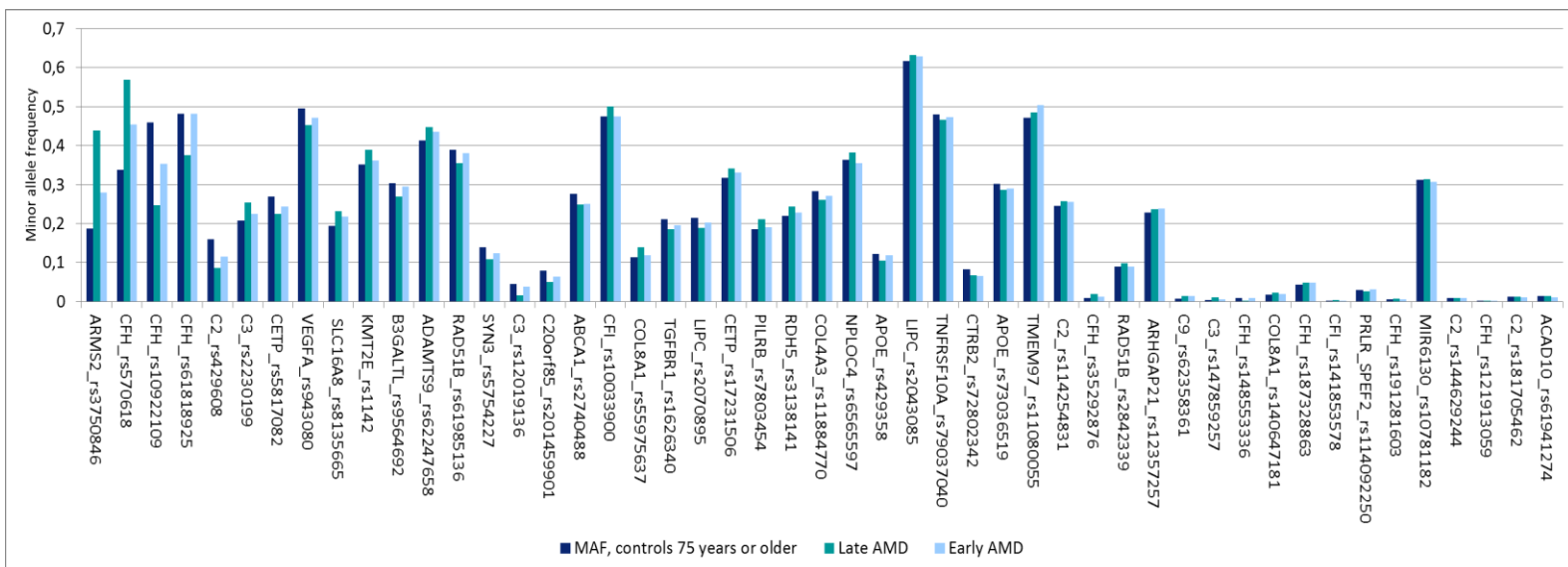
Figure

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.
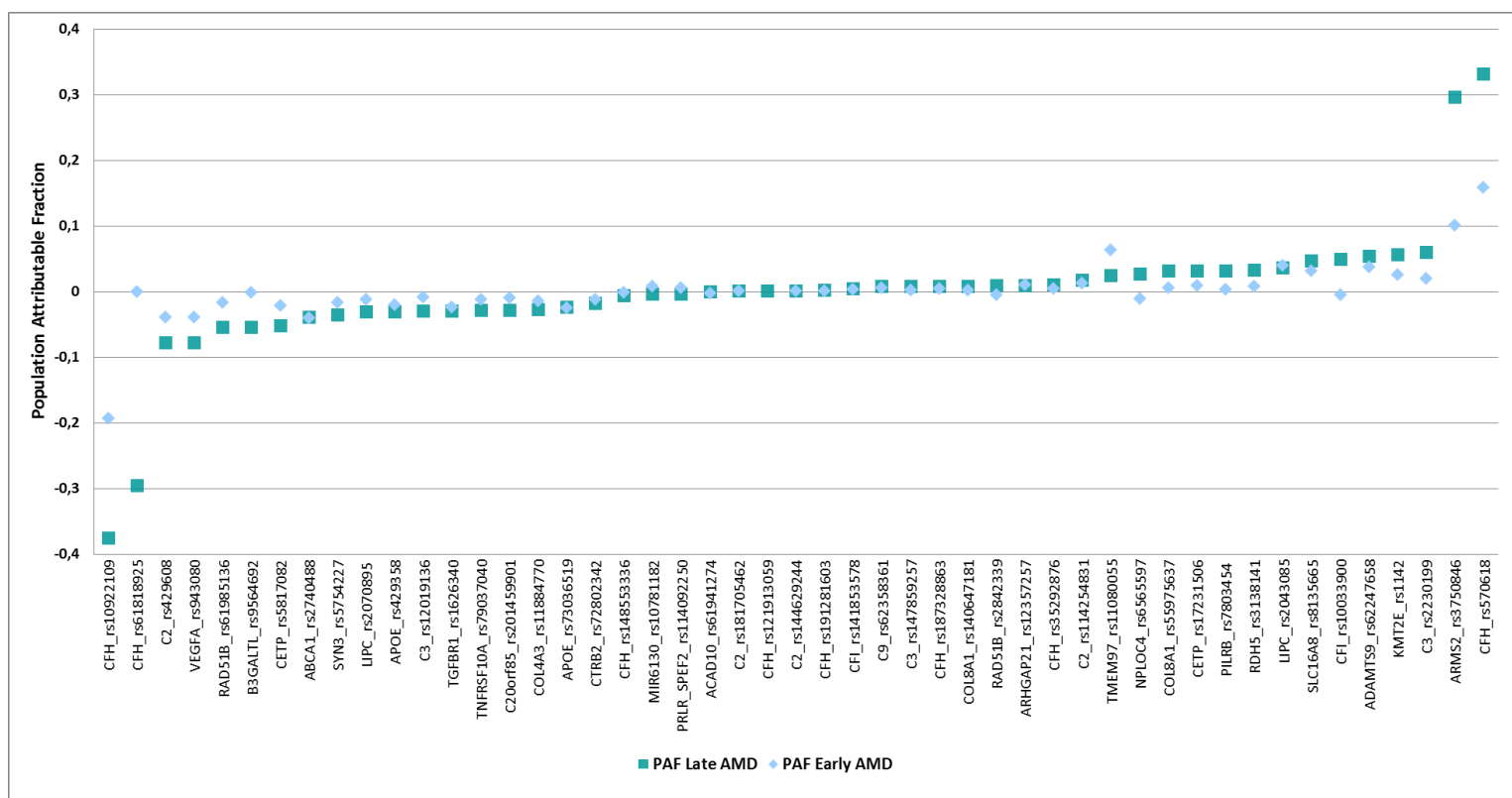

Figure 1b Population attributable fraction of 49 AMD-associated genetic variants for intermediate (light blue) and late (green) AMD. CFH_rs121913059 is not included for intermediate AMD since it was too rare to make useful calculations.
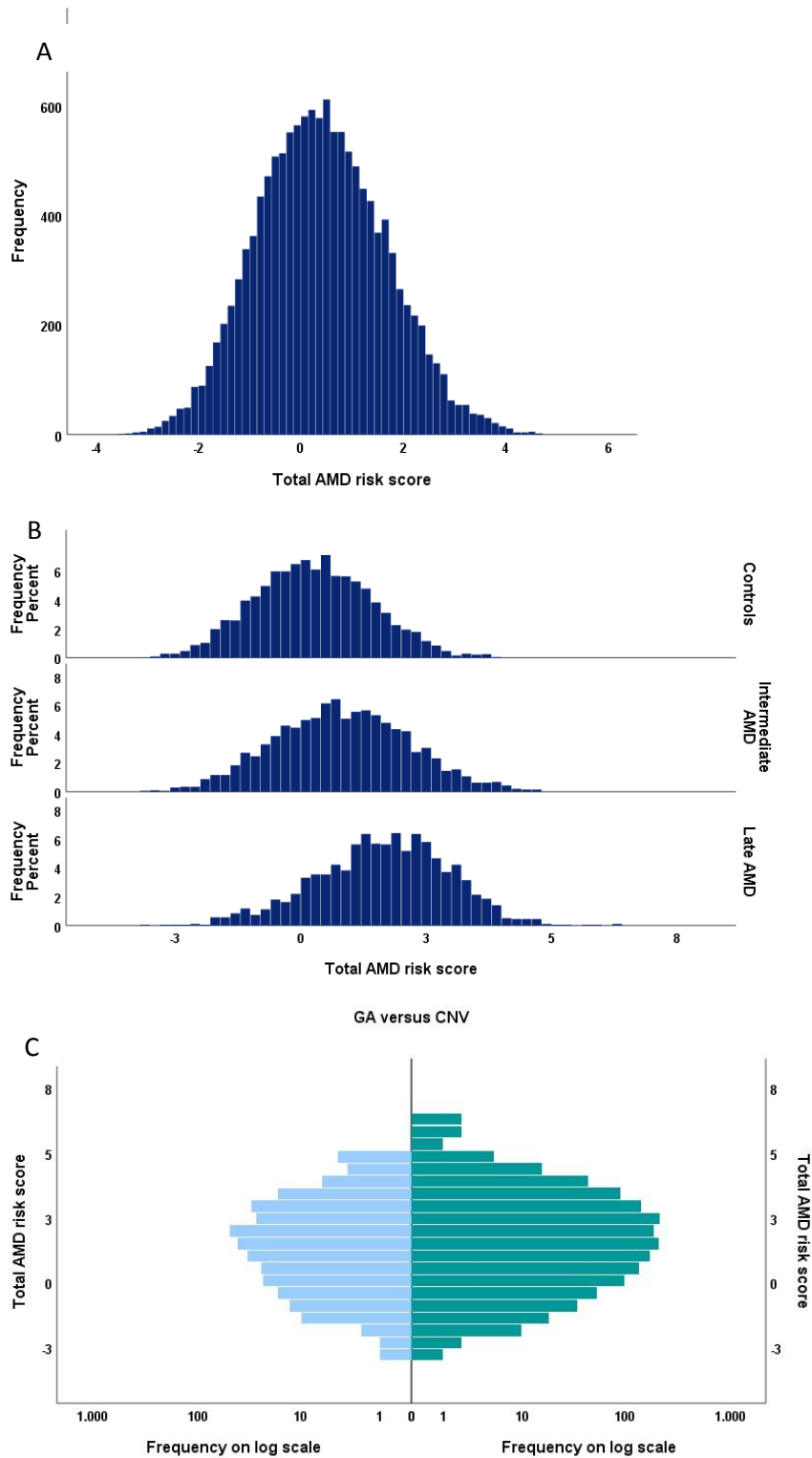
Figure 2. A. Distribution of the total AMD GRS (genetic risk score) in the European population. **B.** Distributions of the total AMD GRS, top panel showing the controls (aged ≥75 years), middle panel intermediate AMD and bottom panel late AMD. **C.** Distributions of the total AMD GRS, left panel (light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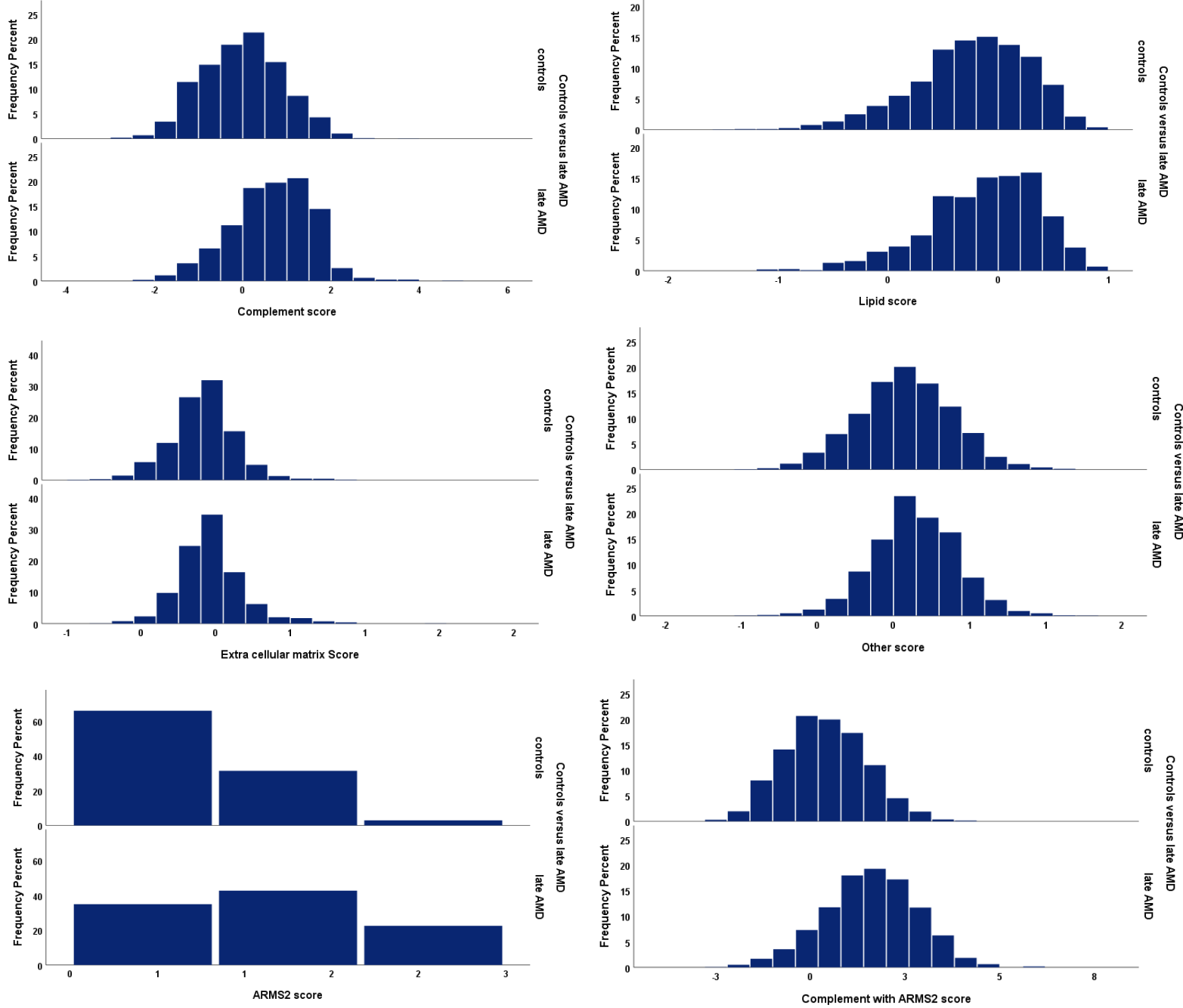
Figure 5 Distributions of the genetic risk scores for the complement, lipids, extra-cellular matrix, ARMS2 and the other pathway and complement with ARMS2 combined in controls and late AMD cases.
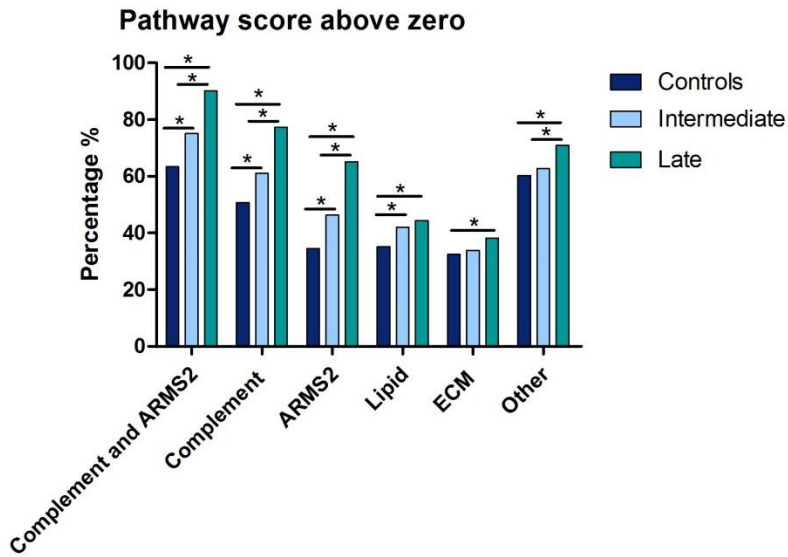
Figure 6. Percentage of individuals with a GRS above zero for each of the pathways. Dark blue = the controls 75 years and older, light blue = intermediate AMD cases, green = late AMD cases. The asterisk (*) indicated statistical differences in a Pearson Chi-Square test (2-sided) with p-value <0.0001,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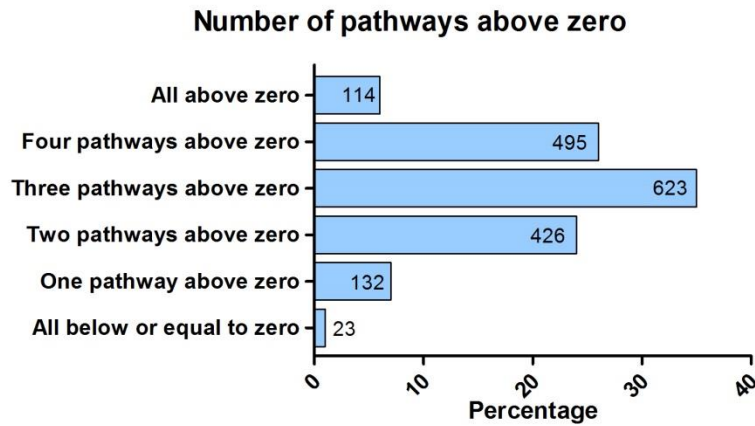

Figure 8. Distribution of late AMD cases according to pathway scores above zero, numbers inside the bars indicate the frequency.

| 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
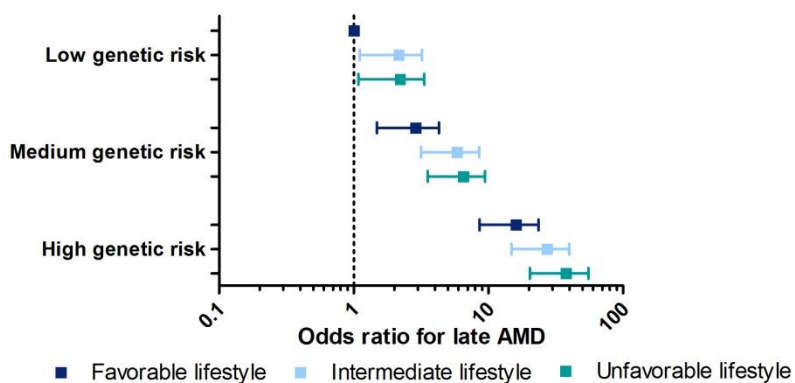
Figure 9. Odds ratio of risk for late AMD stratified by GRS and lifestyle risk. CI = Confidence interval.

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 + (-0.515653666814)*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)*C20orf85_rs201459901 + (-0.206712996496)*SYN3_rs5754227 + (0.128277021888)*SLC16A8_rs8135665 - (-0.180853557395).
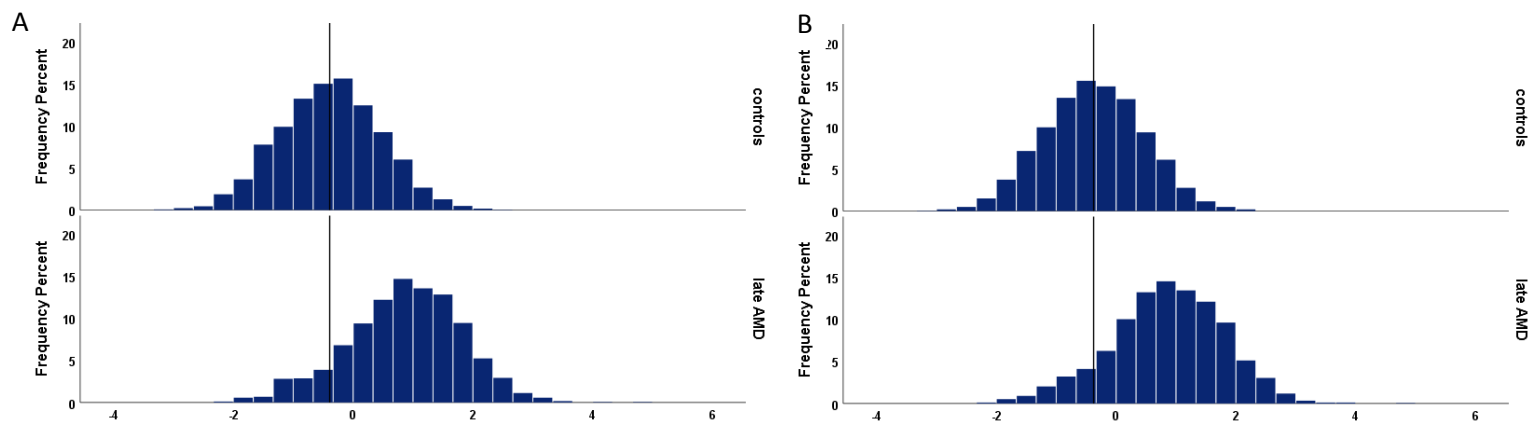

Figure 3. a Showing the distribution of the predictive score for controls and late AMD including 49 AMD associated variants. **b**. Distribution of the predictive score with the minimal set of 27 variants for controls and late AMD.
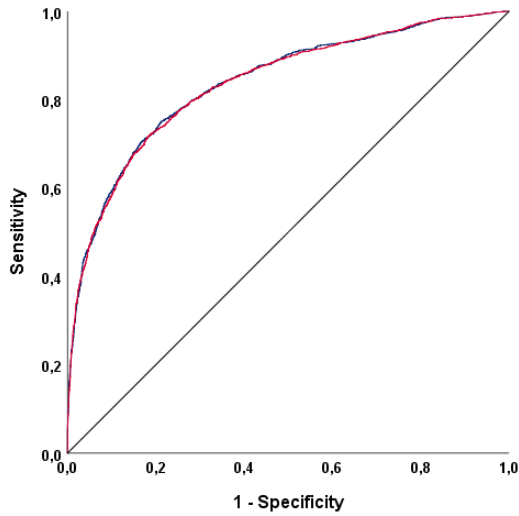
Figure 4. Receiver operator curve for predictive risk scores to differentiate between late AMD cases and controls. The blue line indicates the GRS including all 49 AMD-associated variants (AUC 0.838), the red line indicates the GRS for the minimal set of 27 AMD-associated genetic variants (AUC 0.837).

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

	Complement	ARMS2	Lipid	ECM	Other	Complement+AMRS2
Controls ≥75 years	-0.01	0.4	-0.12	-0.09	0.08	0.39
Intermediate	0.29	0.58	-0.09	-0.06	0.10	0.88
Late	0.65	0.94	-0.06	-0.03	0.14	1.59
p-value*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* 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.

SNP	%	Freq	OR	OR Fritsche et al	SNP	%	Freq	OR	OR Fritsche et al	SNP	%	Freq	OR	OR Fritsche et al
CFH_rs10922109	96	146	0.40	0.38	TNFRSF10A_rs79037040	37	56	0.94	0,9	CFH_rs187328863	3	4	1.20	2.27
LIPC_rs2043085	86	131	1.06	0.87	CFH_rs61818925	36	55	0.56	0,6	ACAD10_rs61941274	3	4	0.94	1.51
C2_rs943080	74	112	0.85	0.88	PILRB_rs7803454	36	54	1.18	1,13	CFH_rs191281603	2	3	1.41	1.07
CFI_rs10033900	73	111	1.11	1.15	C2_rs429608	34	52	0.52	0,57	COL8A1_rs140647181	2	3	1.53	1.59
TMEM97_rs11080055	73	111	1.05	0.91	KMT2E_rs1142	34	51	1.17	1,11	CFH_rs148553336	1	1	0.32	0.29
ADAMTS9_rs62247658	69	105	1.14	1.14	C2_rs114254831	32	48	1.07	1,13	C2_rs144629244	1	1	1.12	1.39
C3_rs2230199	69	105	1.31	1.43	LIPC_rs2070895	30	45	0.86	0,87	C2_rs181705462	1	1	1.03	1.55
RAD51B_rs61985136	65	99	0.87	0.9	SLC16A8_rs8135665	29	44	1.25	1,14	C3_rs147859257	1	1	2.82	2.86
NPLOC4_rs6565597	59	89	1.07	1.13	CFH_rs570618	29	44	2.40	2,38	C9_rs62358361	1	1	2.00	1.8
MIR6130_rs10781182	54	82	0.99	1.11	RDH5_rs3138141	29	44	1.15	1,16	CFH_rs35292876	0	0	2.11	2.42
CETP_rs17231506	49	74	1.10	1.16	SYN3_rs5754227	26	40	0.75	0,77	CFH_rs121913059	0	0	2.43	20.28
B3GALTL_rs9564692	47	71	0.83	0.89	COL8A1_rs55975637	24	37	1.28	1,15	CFI_rs141853578	0	0	57.92	3.64
TGFBR1_rs1626340	46	70	0.86	0.88	RAD51B_rs2842339	19	29	1.10	1,14	ARMS2_rs3750846	0	0	3.06	2.81
COL4A3_rs11884770	45	69	0.90	0.9	APOE_rs429358	18	28	0.77	0,7					
APOE_rs73036519	45	69	0.92	0.91	CTRB2_rs72802342	9	14	0.79	0,79					
ABCA1_rs2740488	44	67	0.86	0.9	PRLR_SPEF2_rs114092250	8	12	0.88	0,7					
ARHGAP21_rs12357257	40	61	1.04	1.11	C20orf85_rs201459901	6	9	0.64	0,76					
CETP_rs5817082	39	60	0.81	0.84	C3_rs12019136	5	8	0.34	0,71					

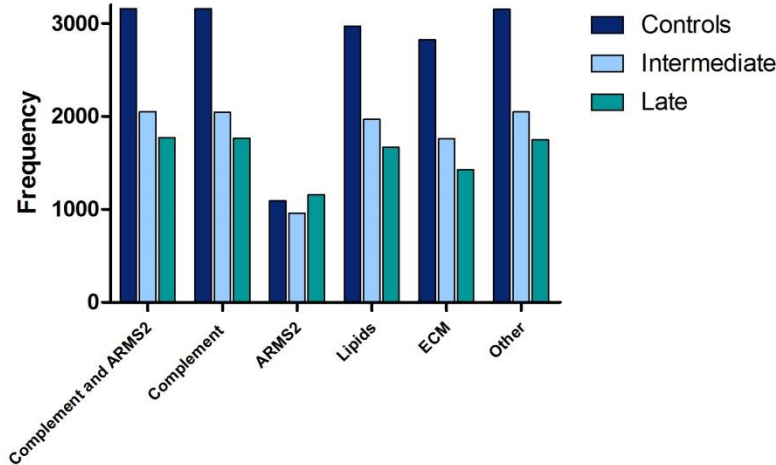**Number of people with a risk allele per pathway**

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.

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