Unveiling Statins and Genetics in Age-Related Macular Degeneration: The Coimbra Eye Study—Report 9

Patrícia Barreto,[1–3] Cláudia Farinha,[1–5] Rita Coimbra,[1,6] Maria Luz Cachulo,[1,4,5] Joana Barbosa Melo,[2,3,7,8] Yara Lechanteur,[9] Carel B. Hoyng,[9] José Cunha-Vaz,[1] and Rufino Silva[1–5]

[1]Association for Innovation and Biomedical Research on Light and Image (AIBILI), Coimbra, Portugal
[2]Faculty of Medicine, Coimbra Institute for Clinical and Biomedical Research (iCBR), University of Coimbra, Coimbra, Portugal
[3]Centre for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal
[4]Ophthalmology Department, Coimbra Hospital and University Center (CHUC), Coimbra, Portugal
[5]Faculty of Medicine, Clinical Academic Center of Coimbra (CACC), University of Coimbra, Portugal
[6]Department of Mathematics, University of Aveiro, Aveiro, Portugal
[7]Cytogenetics and Genomics Laboratory, Clinical Academic Center of Coimbra (CACC), Faculty of Medicine, University of Coimbra, Portugal
[8]Faculty of Medicine, Center of Investigation in Environment, Genetics and Oncobiology (CIMAGO), University of Coimbra, Coimbra, Portugal
[9]Department of Ophthalmology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands

Correspondence: Patrícia Barreto, Association for Innovation and Biomedical Research on Light and Image (AIBILI), Edifício Prof. Doutor José Cunha-Vaz, Azinhaga de Santa Comba, Celas, Coimbra 3000-548, Portugal; pbarreto@aibili.pt.

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Purpose. To assess the association of age-related macular degeneration (AMD) progression and statins, connected with AMD genetic risk, and if there is an interplay between statins and genetics.

Methods. In this analysis, 682 subjects made two visits (6.5-year follow-up) of the Coimbra Eye Study. Subjects who started taking statins at any time point between the two visits were considered. Progressors were defined as not having AMD at baseline and having any AMD at follow-up. Genetic risk scores (GRSs) were calculated individually with 52 independent variants associated with AMD. Time to progression was estimated using unadjusted Kaplan–Meier curves. An extended Cox model was used for the association between statins and GRS with the risk for AMD progression. Multiplicative and additive interactions were assessed.

Results. Median survival time was 7.50 years for subjects not taking statins and 7.62 for subjects taking statins ($P < 0.001$). Statin intake reduced the risk for progression to AMD in 48%, adjusting for age, sex, body mass index, smoking, and diabetes (model 1) and GRS (model 2). The combined effects of not taking statins and having high GRS increased the progression risk fourfold compared to taking statins and having low GRS (hazard ratio [HR] = 4.25; 95% confidence interval [CI], 1.62–11.16; $P = 0.003$). For subjects not taking statins, an increased risk of progression was found for those subjects with high GRS compared to subjects with low GRS (HR = 1.80; 95% CI, 1.13–2.85; $P = 0.013$). No statistically significant multiplicative or additive interactions were found.

Conclusions. Statins seem to be protective against AMD progression, and genetics may play a role in treatment response.

Keywords: age-related macular degeneration, statins, genetics, progression

Age-related macular degeneration (AMD) is the main cause of irreversible vision loss in developed countries among people 55 years of age or older.[1] With an estimated prevalence of 8%,[2] it is expected that the number of people affected by this disease can be as high as 288 million by 2040.[3] AMD is a multifactorial disease. The genetic background has been established in a large genome-wide association study (GWAS),[4] and several single-nucleotide polymorphisms (SNPs) are associated with AMD. Other non-genetic factors, such as age, smoking habits, and unhealthy diet, have also been associated with AMD.[5,6]

Statins, the widely used systemic hypolipemic drugs, have been studied as a possible therapeutic option for AMD. Their use in the protection against the onset or progression of AMD relies on different assumptions. The lipid metabolism pathway, a pathophysiological mechanism in AMD, stands as an important therapeutic target but is still an unexplored strategy. Drusen, the main characteristic of the disease, are

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lipid-rich deposits, and high serum cholesterol levels have been associated with AMD in several studies,[7] a finding that substantiates the importance of this pathway. Also, cholesterol and phospholipids are present in the outer segments of photoreceptors, and retinal pigment epithelium (RPE) cells actively participate in the homeostasis and secretion of these lipids.[8,9] Despite this biologically plausible importance, results from different studies have been so far inconclusive[10–13]; therefore, the benefit of use of statins in AMD remains unproved.

Pharmacogenetics is an important step forward in precision and personalized medicine. To the best of our knowledge, only one study has addressed the question of how statins and genetics may interact to affect AMD onset and progression.[14] Hence, with this study (Coimbra Eye Study [CES]), our aim was to assess whether the risk for AMD progression is associated with the intake of statins and connected with genetic risk, and, if so, if there is an interplay between statins and genetics that may alter the risk for AMD progression.

Methods

Coimbra Eye Study

The CES is an epidemiological study composed of three studies: Epidemiological AMD Study (NCT01298674), AMD Incidence Study (NCT02748824), and Life Style and Food Habits Questionnaire in the Portuguese Population Aged 55 or More (NCT01715870). The main objectives of the studies were to determine AMD prevalence and the 6.5-year AMD incidence, as well as risk factors, of two cohorts: Lousã (inland) and Mira (coastal). The CES took place in the primary health care units of both towns. Details on the CES can be found elsewhere.[15–17] Participants signed informed consents, and the procedures of each of the studies complied with the tenets of the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice Guidelines. The three studies obtained the Association for Innovation and Biomedical Research on Light and Image (AIBILI) Ethics Committee approval.

Procedures

This manuscript refers to the data acquired in the Epidemiological AMD Study and the AMD Incidence Study in the coastal town of Mira. Participants went through different ophthalmological exams to allow for image grading. In summary, the following exams were performed: best-corrected visual acuity with Early Treatment Diabetic Retinopathy Study (ETDRS) charts, color fundus photography (Topcon TRC-NW8 fundus camera; Topcon Corporation, Tokyo, Japan), spectral-domain optical coherence tomography, fundus autofluorescence, and near-infrared reflectance imaging (SPECTRALIS HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). Furthermore, medical histories were recorded based on a standardized questionnaire. Participants were called upon to participate in the study by letter and by phone call. Blood samples were collected from participants who consented to genetic analysis.

AMD Grading and Definition of Progressors

Participants were graded according to the presence of AMD. Staging was performed in both eyes. The single eye was considered for differential grading if only one eye presented AMD. In case of bilateral disease, the worse eye was considered for AMD staging but both eyes were graded. Grading was performed at CORC (Coimbra Ophthalmology Reading Centre, AIBILI, Coimbra, Portugal), by certified ophthalmologists. The classification used to stage AMD was the Rotterdam Classification.[18,19] Briefly, in this grading system, stage 0 indicates no AMD features or only drusen $\leq$ 63 mm; stage 1a indicates soft, distinct drusen; stage 1b indicates pigmentary irregularities; stage 2a indicates soft, indistinct, or reticular drusen; stage 2b indicates soft, distinct drusen with pigmentary irregularities; stage 3 indicates soft, indistinct, or reticular drusen with pigmentary irregularities; and, finally, stage 4 indicates atrophic or neovascular macular degeneration. Progressors were subjects who progressed from grade 0 or 1 (as not having AMD at baseline) to grades 2, 3, or 4 at the 6.5-year follow-up. Participants with AMD at baseline (in the Epidemiological AMD Study) were excluded from this analysis, because they were not considered at risk for progression to AMD.

Genotyping of Participants and Genetic Risk Score

Participants were genotyped within the scope of the EYE-RISK project from the European Eye Epidemiology Consortium.[20] Sixty-nine SNPs associated with the disease by the International AMD Genomics Consortium (IAMDGC)[4] were successfully genotyped by targeted next-generation sequencing. The genetic risk score (GRS) was calculated individually for each participant as a weighted sum of the 52 independent variants identified by the GWAS of the IAMDGC.[4] At each locus, the number of risk alleles was multiplied by the associated effect size for each allele (measured on the log-odds scale).[4] No data imputation was performed. Subjects missing at least one of the major risk variants genotyped (*CFH* rs570618, *CFH* rs10922109, *C2/CFB/SKIV2L* rs429608, *ARMS2/HTRA1* rs3750846, or *C3* rs2230199) were not considered for the GRS analysis.

In addition to the overall GRS, we calculated pathway-specific GRSs based on the methodology of the EYE-RISK Consortium,[6] selecting the pathways with sets of genes that could potentially interact with statins to have an impact on AMD progression. To calculate the complement-specific GRSs, all risk variants in the *CFH*, *CFI*, *C9*, *C2*, *TMEM97/VTN*, and *C3* genes were included. For the lipid-specific pathway GRS, variants in *ABCA1*, *LIPC*, *CETP*, and *APOE* were included. For GRS *ARMS2*, only the *ARMS2* gene was considered.

Medication Assessment

The study nurse invited participants to the study by phone call and asked them to bring a list of any medications they were taking at the time of the study visit. In case of doubt or forgetfulness, the medication was checked in the patient's file of the primary health care unit. Each medication was managed by Anatomical Therapeutic Chemical (ATC) codes. The ATC code considered for this analysis was C10AA, HMG CoA reductase inhibitors. Statins were divided into strength categories (low, medium, and high), according to Chou et al.[21] Low-strength statins include 20- to 40-mg fluvastatin, 20-mg lovastatin, 10- to 20-mg pravastatin, and 10-mg simvastatin. Medium-strength statins include 10- to 20-mg atorvastatin, 80-mg fluvastatin (or 40 mg, 2×/day), 40- to 80-mg lovastatin, 1- to 4-mg pitavastatin, 40- to 80-mg pravastatin,

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5- to 10-mg rosuvastatin, and 20- to 40-mg simvastatin. High-strength statins include 40- to 80-mg atorvastatin and 20- to 40-mg rosuvastatin. We excluded from the analysis participants with no information on the start date of statin intake, as well as those participants who were already taking statins before the baseline visit. That is, we considered only subjects who started taking statins at any time point between the two visits.

Statistical Analysis

Categorical variables are summarized with frequencies and percentages, and numerical variables are presented as median and interquartile range (IQR), due to the non-normal distribution of the variables. Normal distribution was tested using the Shapiro–Wilk test and visually verified with histogram plots. Differences in variables of interest between groups (progressors and non-progressors) were analyzed using the Mann–Whitney U test for continuous variables not normally distributed and a Pearson's χ^2 test (or Fisher's exact test, when appropriate) for categorical variables.

To test the association between exposure to statin use and AMD progression, we used a time-to-event analysis (or survival analysis). The outcome was defined as the time to progression to occur. Subjects with no progression at the follow-up visit were treated as censoring events in this analysis. First, survival time, or time to progression, was estimated using unadjusted Kaplan–Meier curves and was compared between subjects taking statins and those not taking statins using a log-rank test, taking into account clustering in eyes of the same subjects.

Statin intake was not constant over time because the participants started taking these drugs some time from the baseline visit date on, at different time points. To test for the association between statins and the risk of AMD progression, we used two models. Model 1, an extended Cox model with statin intake as a time-dependent variable (i.e., the time period, in years, between the start and the stop dates of medication use were included), tested if there was an association with statin intake and risk of progression, adjusting for potential confounding variables known to influence AMD, including age, gender, body mass index (BMI), smoking status (smokers/ex-smokers vs. non-smokers), and diabetes assessed at the baseline. Because we found statistical significance, we designed model 2 similarly to model 1 but included the GRS in the model, in addition to the covariates included in model 1. A high GRS was considered equal or superior to the median GRS of the participants.

In order to assess if the use of statins and the GRS per pathway were associated with AMD progression, we used an extended Cox model adjusted to the same covariates of interest for each pathway separately. Because the grading of both eyes was potentially available, we used the individual eye as the unit of analysis. To take into account the correlation between the two eyes for the same participant, the standard errors of the results are based on an infinitesimal jackknife estimate. Model results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs), and the significance level was set to 0.05. The proportional hazards assumption was tested graphically and using the Schoenfeld residual method. No variables violated this assumption.

To evaluate the interactions between the two risk factors—that is, whether one risk factor strengthens the association of another factor with risk for progression to AMD—we used both multiplicative and additive interactions, as suggested by Knol et al.[22,23] Overall, these models were intended to analyze if the magnitude of the effects of two factors together differs from the multiplication or addition of the two factors separately, respectively. For the multiplicative scale, we evaluated whether the interaction between the two factors (statin intake and GRS) is multiplicatively associated with increased risk for progression to AMD—that is, if the risk among patients with a high GRS and statin intake is higher than the product of the individual risks due to each condition. The additive interaction between statin intake and the GRS was estimated based on the relative excess risk due to interaction (RERI), attributable proportion (AP), and the synergy index (SI). The RERI represents the portion of the overall risk effect that is due to interaction, and it is calculated as $RERI_{interaction} = HR_{statins\&GRS} - HR_{statins} - HR_{GRS} + 1$, where $HR_{statins}$ represents the risk for AMD progression conferred by taking statins only, HR_{GRS} represents the risk for AMD conferred by the GRS (low or high), and $HR_{statins\&GRS}$ is the risk conferred by both statin intake and GRS together. The AP, computed as $AP = RERI/HR_{statins\&GRS}$, is the part of the effect due to interaction in those subjects exposed to both factors (i.e., statin intake and low or high GRS). Finally, the SI is the ratio between the combined effect and the individual effects: $SI = (HR_{statins\&GRS} - 1)/(HR_{statins} + HR_{GRS} - 2)$. Also, as additive measures were developed for risk factors rather than for protective factors, we recoded the statin protective factor to guarantee that the stratum with the lowest risk was the single reference category.[22,23] To calculate the CIs of these additive measures, the delta method was used.[24]

Results

Figure 1 shows the flow chart of this analysis. From the original pool of 948 subjects in total, we excluded 266 for different reasons: 73 did not know treatment duration at the 6.5-year visit, 153 had started statins before the epidemiologic study (baseline visit), 36 were not at risk for progression, and four were of black ancestry with a different genetic background.

Overall, 682 subjects were eligible for the analysis, 305 (44.7%) of whom were males; the subjects had an overall median age of 64.7 years (IQR, 60.0–70.2) at baseline. The median follow-up time was 6.5 years (IQR, 5.9–6.8), and 122 subjects (17.9%) progressed to AMD (worse eye–based classification). Between the baseline and the follow-up visit, 169 subjects (24.8%) began taking statins. For these participants, the median of the proportion of years covered by the medication over the follow-up period (the drug exposure time) was 0.48 (IQR, 0.31–0.71). This means the time of statin intake during the follow-up period was 48% of the 6.5 years.

Table 1 compares the baseline characteristics and use of concomitant medication between non-progressors (n = 560) and progressors (n = 122). Smoking (6.1% vs. 11.5%; P = 0.034), oral antidiabetic medication (17.5% vs. 9.0%; P = 0.020), use of statins (26.8% vs. 15.6%; P = 0.009), and exposure to statins by strength (P = 0.009) were significantly associated with AMD progression. Post hoc test results found that only medium- and high-strength statins were significantly associated with AMD progression when compared to not taking statins (P = 0.011). In the group of non-progressors, the most used statin was atorvastatin (50.7%), followed by simvastatin (28.7%). In the group of progressors,

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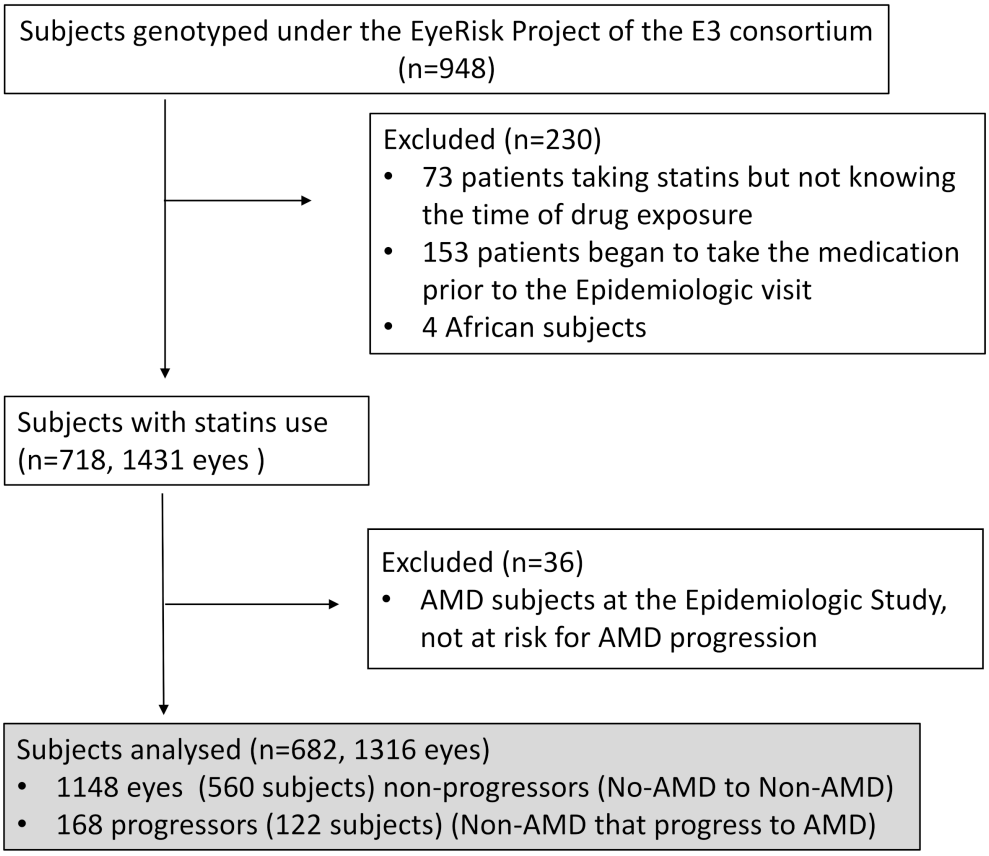

Figure 1. Flowchart of the study participants in this analysis.

Table 1. Baseline Characteristics and Use of Concomitant Medication During the Period Between the Baseline and the Follow-Up Visits

Characteristic	Non-Progressors (n = 560)	Progressors (n = 122)	P
Gender, n (%)			0.910*
Female	309 (55.2)	68 (55.7)	
Male	251 (44.8)	54 (44.3)	
Age at baseline (y), median (IQR)	64.1 (59.8–69.4)	68.1 (63.0–74.0)	**<0.001**†
Smoking, n (%)			**0.034***
Non-smoker	526 (93.9)	108 (88.5)	
Smoker/ex-smoker	34 (6.1)	14 (11.5)	
BMI, median (IQR)	27.8 (25.4–30.1)	26.6 (24.5–28.9)	**0.003**†
Diabetes, n (%)	67 (12.0)	14 (11.5)	0.880*
Years of follow-up, median (IQR)	6.5 (6.0–6.8)	6.3 (5.7–6.8)	**0.017**†
On insulin, n (%)	14 (2.5)	3 (2.5)	>0.999*
On oral antidiabetic medication, n (%)	98 (17.5)	11 (9.0)	**0.020***
On antihypertensive medication, n (%)	249 (44.5)	55 (45.1)	0.901*
Exposure to statins, n (%)	150 (26.8)	19 (15.6)	**0.009***
Exposure by statin, n (%)			0.630*
Atorvastatin	76 (50.7)	7 (36.8)	
Fluvastatin	1 (0.7)	0 (0.0)	
Pitavastatin	7 (4.7)	1 (5.3)	
Pravastatin	8 (5.3)	2 (10.5)	
Rosuvastatin	15 (10.0)	2 (10.5)	
Simvastatin	43 (28.7)	7 (36.8)	
Exposure to statins by strength, n (%)			**0.009***,‡
High/medium	138 (24.6)	17 (13.9)	
Low	12 (2.1)	2 (1.6)	
None	410 (73.2)	103 (84.4)	

BMI, body mass index; IQR, interquartile range.

Bold type indicates significance. Table shows baseline characteristics and use of concomitant medication of progressors to AMD and non-progressors during the 6.5-year period of follow up.

* Pearson's χ^2 test and Fisher's exact test.

† Mann–Whitney U test.

‡ χ^2 test for trend in proportion. Using a logistic regression, a statistically significant association was found between high/medium statin class when compared to no statins in progression to AMD (P = 0.011).

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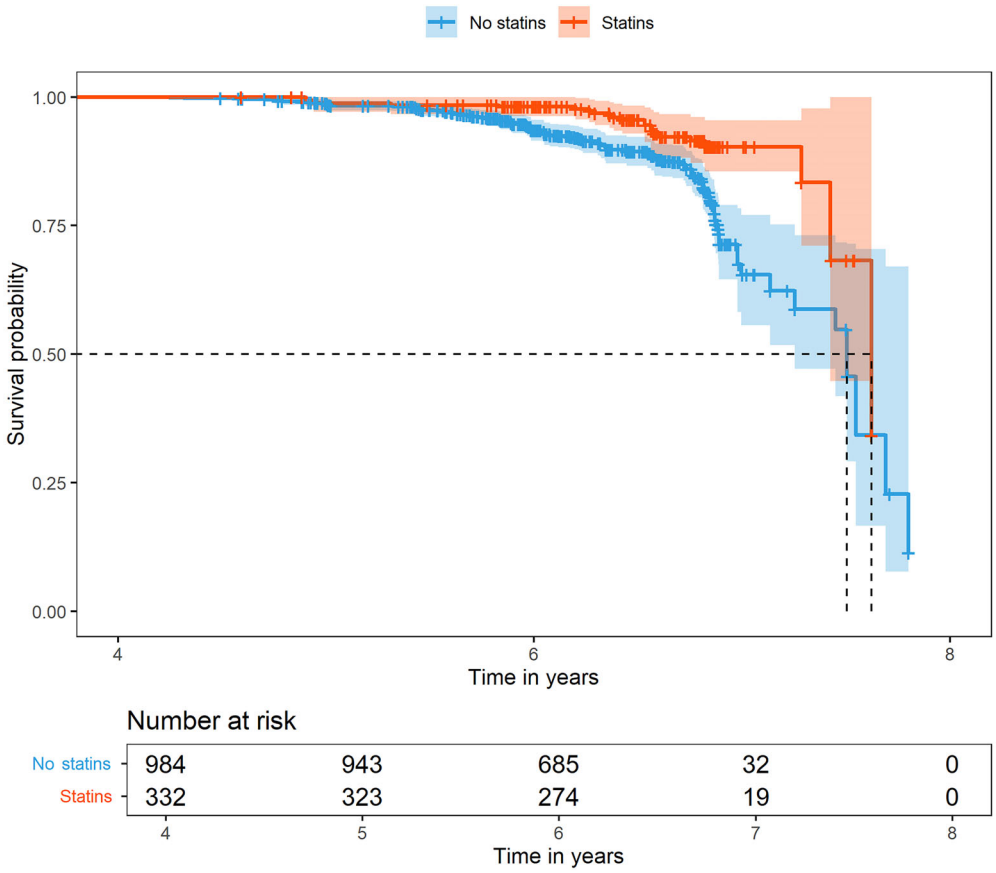

Figure 2. Kaplan–Meier curve for time to progression from no-AMD to AMD by statin intake. The *shaded areas* indicate 95% CIs, and the *dashed lines* indicate the median survival times for each group.

these statins were also the most prescribed, corresponding to 36.8% each.

Between non-progressors and progressors, there were significant differences in (1) age at baseline: 64.1 years (range, 59.8–69.4) versus 68.1 years (range, 63.0–74.0), respectively ($P < 0.001$); (2) BMI: 27.8 kg/m^2 (range, 25.4–30.1) versus 26.6 (range, 24.5–28.9), respectively ($P = 0.003$); and (3) years of follow-up: 6.5 years (range, 6.0–6.8) versus 6.3 years (5.7–6.8), respectively ($P = 0.017$). For patients who progressed, we analyzed the AMD progression stage versus the strength of statin intake. Regarding the number of progressors not taking statins, 77 progressed to early AMD, 13 to intermediate AMD, and 13 to late AMD. Very few subjects on low-strength statins progressed to early AMD (one subject) or intermediate AMD (one subject). For those on medium- or high-strength statins, 14 progressed to early AMD, two to intermediate AMD, and one to late AMD.

We compared the median time to progression between the two groups of participants taking and those not taking statins. The median survival time was 7.50 years for subjects not taking statins and 7.62 for the ones taking statins, and the differences between the two groups were statistically different ($P < 0.001$). The survival curves using the Kaplan–Meier method are shown in Figure 2.

To investigate if the conclusions were maintained regarding exposure (statin intake), after including other covariables of interest, we used an extended Cox regression model with statin intake as a time-dependent variable (Table 2). In model 1, participants taking statins had an estimated 48% lower risk of progression to AMD than participants not taking statins (HR = 0.52; 95% CI, 0.31–0.87; $P = 0.013$). Also found to be significantly associated with an increased risk for AMD progression were age (HR = 1.07; 95% CI, 1.04–1.10; $P < 0.001$) and smoking (HR = 1.73; 95% CI, 1.02–2.93; $P = 0.042$). Including the GRS in our model (model 2), we found that a high GRS increased the risk for progression almost by a factor of two (HR = 1.88; 95% CI, 1.22-2.89; $P = 0.004$) compared to subjects with a low GRS. Also, in model 2, statins were still associated with a 50% decreased risk of AMD progression (HR = 0.52; 95% CI, 0.30–0.91; $P = 0.023$).

Afterwards, we analyzed if GRS per pathway could have an impact on the association of statins with AMD progression. Results are presented in Table 3. The use of statins was associated with an approximately 50% decreased risk of progression for AMD, regardless of the pathway of GRS. Subjects with a high complement-specific GRS or *ARMS2*-specific GRS had an increased risk of progression to AMD (HR = 1.61; 95% CI, 1.05–2.46; $P = 0.027$ and HR = 1.60; 95% CI, 1.06–2.40; $P = 0.025$, respectively) when compared with subjects with a low GRS. We found no statistically significant differences between subjects with a high lipid-specific GRS when compared to subjects with a low lipid-specific GRS.

Next, we examined the multiplicative and additive interactions effects on the risk for AMD progression (Table 4).

Table 2. Extended Cox Regression Analysis, With Use of Statins as a Time-Dependent Exposure Without (Model 1) and With (Model 2) the GRS

	Model 1			Model 2		
Characteristic	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Use of statins						
No	1.00 (ref.)	—	—	1.00 (ref.)	—	—
Yes	0.52	0.31–0.87	**0.013**	0.52	0.30–0.91	**0.023**
Age at baseline	1.07	1.04–1.10	**<0.001**	1.07	1.04–1.10	**<0.001**
Sex						
Female	1.00 (ref.)	—	—	1.00 (ref.)	—	—
Male	0.92	0.61–1.38	0.682	0.90	0.59–1.39	0.639
BMI at baseline	0.97	0.92–1.01	0.142	0.97	0.93–1.02	0.232
Smoking at baseline						
Non-smokers	1.00 (ref.)	—	—	1.00 (ref.)	—	—
Smokers/ex-smokers	1.73	1.02–2.93	**0.042**	1.51	0.82–2.78	0.183
Diabetes at baseline						
No	1.00 (ref.)	—	—	1.00 (ref.)	—	—
Yes	1.06	0.59–1.91	0.850	0.86	0.45–1.66	0.659
GRS						
Low GRS	—	—	—	1.00 (ref.)	—	—
High GRS	—	—	—	1.88	1.22–2.89	**0.004**

CI, confidence interval; GRS, genetic risk score; HR, hazard ratio; ref, reference.

Bold type indicates significance. Model 1 is corrected for age, sex, BMI, smoking, and diabetes mellitus, and model 2 is corrected for age, sex, BMI, smoking, diabetes mellitus, and GRS.

Table 3. Extended Cox Regression Analysis, With Use of Statins as a Time-Dependent Exposure With GRS by Pathways

	GRS Complement		GRS *ARMS2*		GRS Lipids	
Characteristic	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Use of statins						
No	1.00 (ref.)	—	1.00 (ref.)	—	1.00 (ref.)	—
Yes	0.49 (0.28–0.86)	**0.012**	0.53 (0.31–0.91)	**0.022**	0.52 (0.30–0.90)	**0.019**
GRS						
Low GRS	1.00 (ref.)	—	1.00 (ref.)	—	1.00 (ref.)	—
High GRS	1.61 (1.05–2.46)	**0.027**	1.60 (1.06–2.40)	**0.025**	1.17 (0.79–1.75)	0.434

CI, confidence interval; GRS, genetic risk score; HR, hazard ratio; ref, reference.

Bold type indicates significance. The three analyses for each GRS-specific pathway are corrected for age, sex, BMI, smoking, and diabetes mellitus.

We assessed only interaction with the overall GRS because the lipid-specific pathway GRS, the most relevant for this analysis, was not statistically significant.

The combined effects of not taking statins and having a higher GRS increased the risk for progression four times compared to taking statins and having a low GRS (HR = 4.25; 95% CI, 1.62–11.16; $P = 0.003$). Stratifying the analysis by statin intake for the subjects not taking statins, an increased risk of progression was found for those subjects with a high GRS compared to the subjects with a low GRS

Table 4. Combined Effects and Interaction Between Statins Intake and GRS on AMD Progression Risk

	Low GRS		High GRS		GRS Within Strata of Statin Intake	
Characteristic	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Statins, yes	1.00	Ref.	2.44 (0.80–7.42)	0.116	2.91 (0.93–9.14)	0.068
Statins, no	2.36 (0.87–6.45)	0.093	4.25 (1.62–11.16)	**0.003**	1.80 (1.13–2.85)	**0.013**
Statin intake within strata of GRS	2.31 (0.86–6.20)	0.097	1.80 (0.88–3.66)	0.105	—	—
Scale of interaction	Metric				Value (95% CI); *P*	
Multiplicative	HR interaction				0.74 (0.22 to 2.45); 0.618	
Additive	$RERI_{interaction} = HR_{statins\&GRS} - HR_{statins} - HR_{GRS} + 1$				0.44 (–1.71 to 2.59); 0.686	
	$AP = RERI/HR_{statins\&GRS}$				0.10 (–0.42 to 0.63); 0.697	
	$SI = (HR_{statins\&GRS} - 1)/(HR_{statins} + HR_{GRS} - 2)$				1.16 (0.52 to 2.60); 0.722	

AMD, age-related macular degeneration; AP, attributable proportion; CI, confidence interval; GRS, genetic risk score; HR, hazard ratio; ref, reference; RERI, relative excess risk due to interaction; SI, synergy index.

Bold type indicates significance.

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(HR = 1.80; 95% CI, 1.13–2.85; $P = 0.013$). No statistically significant multiplicative or additive interactions were found.

Discussion

We have found a 50% progression risk reduction with the use of statins, adjusting for age, smoking, BMI, diabetes, and genetic risk for AMD. The time to progression for participants not taking statins was shorter when compared to time to progression for participants taking statins. The joint effects of not taking statins along with having a high genetic risk for AMD increased the risk for progression fourfold. Additionally, in the group of participants who did not take statins, the risk for progression was significantly higher in those participants with a high GRS compared to those with a low GRS.

AMD is a complex and multifactorial disease. Evidence shows that patients do not respond equally to treatment, so different therapeutic targets should be identified for better disease management. In this context, the lipid metabolism pathway has long been subject of research since different pieces of evidence point to its involvement in AMD pathophysiology. Drusen, the deposits that are hallmarks of AMD, are rich in lipids. In fact, up to 40% of their volume can be made up of lipids.[25] Also, lipid homeostasis genes have been independently associated with AMD in a large GWAS,[4] and their downstream translation in metabolomics has corroborated the important role that glycerophospholipids play in AMD.[26,27]

Statins are used in atherosclerotic disease, with the therapeutic indication of lowering cholesterol levels through the inhibition of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. This mechanism of action is hinged on cardiovascular disease underlying pathophysiological and biochemical mechanisms, which has been confirmed by genetic studies showing that distinct SNPs are associated with decreased low-density lipoprotein (LDL) cholesterol levels,[28,29] as well as by Mendelian randomization studies that, in, fact, proved causality of high LDL levels being associated with coronary heart disease.[30–32] However, statins present pleiotropic effects, most likely because this inhibition prevents not only endogenous cholesterol but also the synthesis of mevalonate, a precursor of other compounds.[33] The anti-inflammatory effect would be an important pleiotropic role of statins to consider in AMD,[34] as parainflammation and inflammation, which lead to enhancement of complement system activation and the release of cytokines and chemokines, are well-defined pathophysiological mechanisms involved in the disease.[35] Statins induce a reduction of C-reactive protein, an unspecific lipid-independent inflammatory marker, which may have an underlying important role in disease progression.[36] However, it has been shown that, specifically in the eye, statins may also affect AMD pathophysiology. Wu and colleagues[9] have shown that statins, particularly lipophilic statins, can inhibit, via cellular cholesterol reduction, the synthesis and secretion of cholesteryl ester–rich apolipoprotein B100. This is a lipoprotein that can accumulate in Bruch's membrane prior to the onset of AMD; thus, decreasing cholesteryl ester rich apolipoprotein B100 reduces the development of drusen.[9] Other studies on cholesterol homeostasis have provided evidence that lipoprotein secretion by the RPE cells can be decreased by statins.[37,38] Moreover, single-cell RNA sequencing evidence greatly suggests that HMG-CoA expressed in RPE can be targeted by statins.[38] These data are strongly suggestive of statins operating directly in the eye.

A successful topical ophthalmological treatment with statins relies on penetration of the drug into the retina, but most available pharmacological data are based on oral formulations and are particularly related to diffusion through the blood–brain barrier, which depends on the lipophilicity of the statin.[9] Some studies on ophthalmological statin formulations have been successful in overcoming some obstacles related to working with such drugs, specifically insolubility, instability, and high molecular weight, as well as effective posterior pole delivery.[9,39–41] Clinical trials of these formulations for AMD would most likely provide crucial information on the role of statins in AMD.

Various non-interventional studies have revisited the role of statins in AMD. All of this important evidence has been gathered based on oral statin intake, which may be limiting to treatment success, as eye bioavailability may be decreased due to the blood–aqueous barrier.[9,41] However, the delivered results have been equivocal, mostly due to different study designs (e.g., population-based, case control, cohort) and different co-variable adjustments, which make comparisons challenging. Also, most analyses are retrospective, with different AMD classification systems and with different statin intakes being investigated, sometimes concomitantly along with other drugs.

Meta-analyses are of great interest, but most do not associate the use of statins with protection against AMD or its progression.[42–45] However, Chuo and colleagues[45] noted that lipid-lowering agents, among which statins are included, cannot be clinically disregarded because they convey appreciable effects. This has been proven by clinical trials reporting that statins have been associated with a reduction in the risk of progression.[14,46]

Our results comply with clinical trial evidence, particularly with the 57% reduction reported by Guymer and colleagues,[14] who used 40-mg simvastatin in a 3-year trial. These authors showed that patients with late AMD in the fellow eye at baseline did not benefit from treatment, adjusting for age, sex, and smoking, but those with bilateral intermediate AMD presented a 77% reduction in the risk of progression. In that study, various analyses of the effect of 40-mg simvastatin on the risk of progression of AMD were performed, and, despite not showing statistical significance in all, there was a tendency for simvastatin protection.[14] Vavvas and colleagues[46] reported vision gain associated with drusen deposit regression in a 12-month trial with 80-mg atorvastatin in high-risk patients, none of whom progressed to neovascular AMD. Similar results were shown by a large retrospective cohort study of an insurance database studying the outcome of statins on progression from non-exudative AMD to exudative AMD.[47] Patients on very high-dose lipophilic statin (80 mg atorvastatin) showed no progression from non-exudative AMD to exudative AMD.

In our study, in the survival analysis, subjects taking statins took longer to progress compared to those not taking statins, which strengthens the association that statins might present in protecting against AMD. Our results also suggest that the strength of statins may be associated with protection against AMD progression. In fact, the use of rosuvastatin and atorvastatin, statins with a higher potency used in secondary prevention, suggests a protective effect against AMD progression (Table 1). Our results were not altered by the inclusion of the GRS in the model. Statins maintained the 50% risk reduction for AMD progression, even considering

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the weight that genetic risk for AMD might have in the risk of progression.

We did not find statistically significant association between lipid-specific GRS and AMD progression, probably because the SNPs used in the calculation of this pathway-specific GRS are not associated with AMD progression in our cohort.[48]

We then tested for interactions between statin intake and overall GRS. To the best of our knowledge, no study has yet performed such an analysis comprehensively, with the assessment of combined effects and both multiplicative and additive interactions. Guymer and colleagues[14] assessed multiplicative interaction and stratification between treatment with simvastatin and three SNPs of two susceptibility AMD genes (*CFH* and *ApoE*) that have been associated with inflammation and lipid metabolism, respectively. A statistically significant interaction between simvastatin use and the risk genotype (CC) of the *CFH* Y402H was found, unlike our findings. We did not find a statistically significant multiplicative interaction, and we built upon these results, analyzing the additive interaction as well. Even though the three additive measures failed to show statistical significance, they were concordant among themselves, pointing toward a positive interaction—that is, a higher risk for AMD progression with the interaction between statins and GRS compared to the sum of the factors individually, as the RERI and the AP are both >0 and the SI is >1.

We must emphasize we analyzed the genetics for AMD in an aggregated manner, with a GRS that covers SNPs associated with AMD weighted by their effect size and not a SNP specifically known to be highly causal for the disease. In the stratified analysis by genotype, Guymer and colleagues[14] showed that, for the different genotypes of the *CFH* Y402H (CC vs. CT + TT), patients homozygous for the CC risk genotype benefited from simvastatin, with a 92% risk reduction of AMD progression, when compared with placebo. This is highly relevant, as it suggests that the treatment response is dependent on the genotype of each patient; thus, its benefit must be considered in a personalized way aimed at achieving personalized medicine. As Guymer et al.[14] pointed out, previous analyses might have reported unfavorable results due to a lack of pharmacogenetic assessment. This is, obviously, important in both outcomes of hypercholesterolemia treatment and AMD management. Our stratification analysis by intake of statins revealed that people who do not take statins have a statistically significant higher risk of AMD progression if they are carriers of a higher genetic risk for AMD compared to those who have a lower genetic risk. This is in agreement with our combined effects analysis, which found noteworthy significance. The joint effect of having a high genetic risk for AMD and not taking statins increases the risk for AMD progression fourfold when compared to having a low genetic risk for AMD and taking statins. Finally, we acknowledge that other non-genetic factors that were not considered in our analysis may contribute to the role of statins against AMD pathogenesis.

We must acknowledge that, out of the 682 subjects in this analysis, few of them progressed (122), and, of these, only 19 began taking statins between the two visits, which is a small sample. Additionally, we have an unbalanced sample, inherently due to an epidemiologic, population-based study. We also realize that we are assessing statins as a therapeutic family rather than a specific statin. Clinically, it would be preferable to study various statins separately, namely high-strength statins, and considering the best SNPs for a pharmacogenetic approach, in terms of pharmacokinetics and pharmacodynamics, to a better treatment response. Another limitation of this analysis is the fact that, within the 6.5 years of this Coimbra Eye Study, we do not know when subjects progressed. Also, we did not consider subjects' lipid profiles, as we had scarce information for them. Should they have been available, such information would be important to have, as hypercholesterolemia has been associated with AMD[49] and a low-fat diet is also an important protective factor for AMD.[50,51] These facts considered, it is quite interesting that a statistically significant association was established between statins and AMD risk of progression.

We built a time-dependent model, meaning that the treatment duration was considered when assessing the effect of statin intake. Our model was also controlled for most of the variables that could bias the results, and, additionally, we minimized the risk of bias of progression by considering as progressors only those subjects who had no AMD at baseline.

A well-designed, randomized clinical trial with a large sample and long follow-up period[43,45] to assess genetics, as well as the interaction of statins and genetics, and to compare topical ophthalmological formulations would be important in the study of statins and AMD. Considering their mechanisms of action that involve common pathways with AMD pathophysiology, concomitant medications indicated to treat other pathologies may be an option in the management of AMD. Such an approach would provide protection against the disease, and, ultimately, assessing the treatment response on a genetic-based approach could also serve as an important strategy in precision medicine.

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