Age-Related Macular Degeneration and Extramacular Drusen: Genetic Associations in the Coimbra Eye Study

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Received: January 22, 2024
Accepted: May 4, 2024
Published: May 22, 2024

Citation: Farinha C, Barreto P, Coimbra R, et al. Age-related macular degeneration and extramacular drusen: Genetic associations in the coimbra eye study. *Invest Ophthalmol Vis Sci.* 2024;65(5):35. https://doi.org/10.1167/iovs.65.5.35

Purpose. To explore the association between the genetics of age-related macular degeneration (AMD) and extramacular drusen (EMD) in patients with and without AMD.

Methods. We included 1753 eyes (912 subjects) with phenotypic characterization regarding AMD and EMD. Genetic sequencing and the genetic risk score (GRS) for AMD were performed according to the EYE-RISK consortium methodology. To test for differences in the GRS from EMD cases, AMD cases, and controls, a clustered Wilcoxon rank-sum test was used. The association of AMD, EMD, and the GRS was evaluated using logistic regression models adjusted for age and sex. Individual associations of common risk variants for AMD with EMD were explored.

Results. EMD were found in 755 eyes: 252 (14.4%) with AMD and 503 (28.7%) without. In total, 122 eyes (7.0%) had only AMD, and 876 (50.0%) were controls. EMD were strongly associated with AMD (odds ratio [OR], 3.333; 95% confidence interval [CI], 2.356–4.623; $P < 0.001$). The GRS was associated with an increased risk of AMD (OR, 1.416; 95% CI, 1.218–1.646; $P < 0.001$) but not with EMD. Individually, the common risk variants *ARMS2* rs10490924 ($P = 0.042$), *C3* rs2230199 ($P = 0.042$), and *CETP* rs5817082 ($P = 0.042$) were associated with EMD, after adjustment for AMD, sex, and age.

Conclusions. We found a strong association between EMD and AMD, suggesting a common pathogenesis. The GRS for AMD was not associated with EMD, but a partially overlapping genetic basis was suggested when assessing individual risk variants. We propose that EMD per se do not represent an increase in the global genetic risk for AMD.

Keywords: age-related macular degeneration, extramacular drusen, coimbra eye study, genotype–phenotype associations, genetic risk score

Age-related macular degeneration (AMD) is the leading cause of blindness in older population in industrialized countries, and its prevalence is estimated to significantly increase in the next decades.[1,2] Thus, significant efforts are being made to develop strategies to halt disease progression and in predicting individual risk. These have been difficult to achieve because of its complex and multifactorial nature, influenced by demographic, environmental, and genetic factors, which ultimately translates into significant interindividual variability regarding phenotype and rate of progression to late-stage disease.[3–7]

Many genetic risk variants have been identified as associated with AMD, but a large risk effect has been consistently reported for common genetic variants located at the *CFH* and *ARMS2/HTRA1* loci.[6,8,9] In fact, 52 variants at 34 genomic regions were found to be independently associated in a pivotal genome-wide association study (GWAS).[5] These have been used to calculate the genetic risk score (GRS) for AMD in a personalized way.[5,6,9] Plus, in this GWAS, a significant burden of rare variants was observed in the *CFH* and *CFI* genes, and others confirmed that rare variants conferred a high risk of disease and were associated with a more severe phenotype, including widespread extramacular drusen deposition.[5,10–14]

In AMD, most genotype–phenotype associations performed to date rely only on the phenotypic analysis of the macular area, with few studies considering extramacular features such as drusen and pigmentary changes.[15,16] Some, however, found that these features are frequently seen in eyes with AMD and are more prevalent with increasing drusen burden in the macula, but their relevance for the development and severity of AMD is not fully understood.[16–19] In addition, in individuals with intermediate AMD, drusen outside the macula do not seem to grant additional risk to previously identified risk factors in progression to late AMD. Genetic associations

between these peripheral changes and genotypes known to be associated with AMD were explored but with conflicting results.[15,20,21] In addition, to date, the GRS calculated for AMD has not been assessed in individuals with extramacular drusen (EMD), to the best of our knowledge.

The Coimbra Eye Study (CES) is a two-visit epidemiologic population-based study on the prevalence and incidence of AMD in a Portuguese population (NCT01298674, NCT02748824), and we have previously reported on the genetics influencing AMD risk.[14,22–26] The present work aims to expand our genotype–phenotype analysis by evaluating the association between the sequenced risk variants, the GRS, and the presence of extramacular drusen in subjects with and without AMD.

Materials and Methods

Study Population and Data Collection

From 2009 to 2013, a study to estimate the prevalence of AMD in the central region of Portugal was conducted (Epidemiological Coimbra Eye Study, NCT01298674). This study covered two geographically different Portuguese populations aged ≥55 years, one from Lousã, an inland town, and another from Mira, a coastal town.[22,23] The AMD Incidence study (NCT02748824), a single-center population-based study, was conducted 6.5 years later and included only participants from Mira.[24] The study population from the Incidence study was extensively characterized from a demographic, clinical, and imagiological perspective with multimodal imaging (MMI) comprehending color fundus photography (CFP), spectral domain optical coherence tomography (SD-OCT), and near infrared (NIR) and autofluorescence (FAF) imaging. Consenting participants in this Mira cohort also had blood collected for genetic analysis. A detailed characterization of the population is reported elsewhere.[24,25] Signed informed consent was obtained for all participants. The study adhered to the tenets of the Declaration of Helsinki (2008) and of the International Conference on Harmonization—Good Clinical Practice Guideline. The Association for Innovation and Biomedical Research on Light and Image (AIBILI) Ethics Committee issued a favorable opinion for the conduction of the study.

Ophthalmic Examination

All participants underwent a complete bilateral ophthalmologic examination, including best-corrected visual acuity (BCVA) evaluated with Early Treatment Diabetic Retinopathy Study (ETDRS) charts. Color fundus photographs were obtained after pharmacological mydriasis. Fields 1M (centered on the optic disc), 2 (centered on the macula), and 3M (temporal to the macula), acquired at 45° for both eyes, were recorded using a digital Topcon fundus camera (TRC-NW8; Topcon Corp., Tokyo, Japan). Fundus reflex photographs were taken to document media opacities. SD-OCT, NIR, and FAF exams (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) were also acquired for grading.[24,25] SD-OCT acquisitions consisted of one EDI Macular Volume Scan (20° × 20°, 49 B-scans, 16 frames per scan), one radial scan centered in the fovea (20° × 20°, 24 B-scans, 10 frames per scan), and two high-resolution EDI Line Scans (30°, acquired at 0° and 90°, with ≥20 frames each), with signal strength ≥25. Both FAF (488 nm) and NIR images were acquired for field 2 at 30° (high resolution with ≥15 frames each).[24,25]

Assessment of CFP Images and Extramacular Grading

All CFP images from subjects who had successful genetic analysis carried out in the incidence study cohort were included in this analysis for reassessment to evaluate the presence of extramacular features outside the ETDRS grid. All three fields acquired for each eye from each participant were analyzed. Prior to grading, these images were enhanced using color balance software, an algorithm developed to automatically standardize the brightness, contrast, and color balance of digital color fundus photographs in AMD grading to achieve maximum detail.[27] Extramacular features were defined as changes outside the 6-mm diameter ETDRS macular grid, this is, in the posterior pole nasal to the optic disc, temporal to the macula, and near and/or above the vascular arcades. Extramacular drusen were defined as an accumulation of extracellular material between the Bruch's membrane and retinal pigment epithelium (RPE). They were graded according to presence (>10 small drusen only or >5 drusen with at least one intermediate drusen in the three fields combined), location (nasal to the disc, temporal to the macula, near/above the arcades), type (hard, soft, soft-confluent, cuticular, crystalline, calcified), and size (large, ≥125 µm; intermediate, <125 µm and ≥63 µm; and small, <63 µm). Extramacular pigmentary changes were defined as hyperpigmentary or hypopigmentary (<250 µm and without visible choroidal vessels), and their presence/absence as well as the location was noted. Subretinal drusenoid deposits (SDDs) were defined as multiple yellowish-white lesions arranged in a reticular network pattern, and their location inside and/or outside the macula was registered. Finally, cases of atrophy, choroidal neovascularization (CNV), fibrosis, or retinal/subretinal hemorrhage outside the macula were also recorded. All grading was performed by certified retina specialist graders experienced in AMD grading at the Coimbra Ophthalmology Reading Centre (CORC, AIBILI).

AMD Definitions and Classification

The Rotterdam staging system was used to classify AMD severity of all eyes in the CES.[23,24,28] Early AMD was defined by the presence of large (≥125 µm), soft, indistinct or reticular drusen only, or of soft distinct (≥63 µm), indistinct (≥125 µm), or reticular drusen with pigmentary abnormalities, within the macula (Rotterdam stages 2 and 3). Late AMD was defined by the existence of neovascular AMD (nAMD), and/or geographic atrophy (GA). GA was present when complete retinal pigment epithelium and outer retina atrophy (cRORA) with at least 250 µm was present in SD-OCT scans. All graded AMD features were confirmed by reviewing the corresponding SD-OCT scans and NIR and FAF images.[25]

Based on the described grading for AMD and EMD, subjects were distributed by four groups: patients with AMD without EMD, patients with AMD and EMD, subjects having EMD but not AMD, and controls (neither AMD nor EMD).

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Genetic Sequencing Procedures

Genomic DNA samples obtained from individuals who participated in the AMD Incidence Study were genotyped according to standard procedures in the context of a collaboration with E3—The European Eye Epidemiology Consortium and the EYE-RISK Consortium. The EYE-RISK genotype assay is designed to genotype 87 single-nucleotide polymorphisms (SNPs), including the 52 independently associated SNPs identified by the International AMD Genomics Consortium (IAMDGC).[5,9] Sequencing was performed by combining genomic capture using single-molecule molecular inversion probes and next-generation sequencing. Filtering of variants to ensure quality of the data was carried out by the EYE-RISK Consortium. Variants with fewer than 40 reads coverage were changed to missing values. Cross-validation with other genotyping platforms was performed by EYE-RISK, and for the variants that passed these quality steps, only variants in Hardy–Weinberg equilibrium (HWE) were maintained. To ensure high quality of the data set, samples with more than 10% missing calls and variants with more than 10% missing calls were removed from the data set. After quality control, 69 SNPs were successfully genotyped in our cohort by the EYE-RISK in the context of that collaboration as previously reported.[9]

Genetic Risk Score Assessment

Regarding the genetic risk score, the 52 independent variants identified by the IAMDGC were selected, although only 42 were successfully genotyped.[5,9] The 10 variants not successfully genotyped were subsequentially genotyped by KASP genotype assays by the EYE-RISK consortium to ensure a complete data set of the 52 AMD-associated variants.[9] For each participant, the GRS was generated according to the following formula: $GRS = \sum_{i=1}^{52}(G_i\beta_i)$, where G_i represents the genotype of variant i coded as 0, 1, or 2 based on the number of minor alleles and β_i represents the effect size of variant, based on the GWAS of the IAMDGC fully conditioned analysis.[5] No data imputation was performed. Only subjects with all major risk variants genotyped (*CFH* rs570618, *CFH* rs10922109, *C2/CFB/SKIV2L* rs429608, *ARMS2/HTRA1* rs3750846, and *C3* rs2230199) were considered for GRS computation. In the absence of at least one major risk variant, the GRS was considered null.[9] Pathway-specific GRS were also calculated by the same method. The genes included in each pathway were as follows: (1) complement, *CFH*, *CFI*, *C9*, *C2*, *TMEM97/VTN*, and *C3*; (2) lipid, *ABCA1*, *LIPC*, *CETP*, and *APOE*; (3) extracellular matrix (ECM), *COL4A3*, *ADAMTS9-AS2*, *COL8A1*, *VEGFA*, and *SYN3/TIMP3*; and (4) *ARMS2*, considered separately.[6]

Statistical Analysis

Descriptive statistics was used to describe all the variables assessed. Categorical variables were summarized using frequencies and percentages and numerical variables with mean and standard deviation. To evaluate the agreement between two eyes of the same subject, an intereye agreement analysis was done, using Cohen's κ, for the graded variables. Given the diversity of levels of correlation provided, a moderate agreement coefficient between eyes was assumed. To test the association between EMD characteristics and AMD, for patients with EMD, and to take into account intereye observations, a logistic regression model with generalized estimating equations (GEEs) was used. False discovery rate (FDR) was used to adjust for multiple comparison.

To test for differences in the GRS between four groups (patients with AMD but without EMD, patients with AMD and EMD, individuals with EMD but without AMD, and controls), while controlling for correlation between eyes of the same patient, a clustered Wilcoxon rank-sum test was used and pairwise comparisons between categories were calculated, adjusting for multiple comparisons using FDR.

Associations of the GRS with EMD and AMD were assessed using logistic regression models while adjusting for age and sex. GEEs were used to consider intereye correlations. For this purpose, odds ratio (ORs) at 95% confidence interval (CI) were computed for each analysis. An exchangeable working correlation structure was used. To check the quality of the models, the area under the curve (AUC) for each model was calculated. Association of the GRS by pathway with EMD and adjusted by AMD was calculated using the same model as above.

To test the association of common risk variants for AMD with EMD, we explored each of the common variants that were significantly associated in our cohort and reported previously elsewhere, plus the five major risk variants, on a total of 14 SNPs (*C2/CFB/SKIV2L* rs429608, *CFH* rs1410996, *CFH* rs10922109, *ARMS2* rs10490924, *ARMS2/HTRA1* rs3750846, *CNN2* rs10422209, *CFB* rs641153, *CETP* rs5817082, *SLC16A8* rs8135665, *CFH* rs35292876, *RDBP_CFB* rs760070, *TGFBR1* rs1626340, *CFH* rs570618, *C3* rs2230199).[5,9,26]

All statistical analyses were performed using R Core Team (R Project for Statistical Computing, Vienna, Austria) and Stata Stata (16.1, StataCorp LLC; College Station, TX, USA) and P values less than 0.05 were considered statistically significant.

Results

Considering the 1617 eligible subjects who participated in the AMD Incidence study, 948 subjects (1896 eyes) were genotyped. A total of 50 eyes were excluded from the analysis because of absent or low-quality CFP images for accurate grading or other ophthalmic conditions hampering correct grading. The remaining 1846 eyes (939 subjects) were successfully graded for both AMD and extramacular features. To avoid including controls (Rotterdam stages 0 and 1) that could develop AMD, participants classified as stage 1 were removed from the analysis if their age was below 70 years, with a final analyzed group of 1753 eyes (912 subjects) (Fig. 1). The mean age of the 912 subjects was 72.5 ± 6.8 years and 530 (58.1%) were women.

Of the 1753 eyes included, 122 eyes (7.0%) had AMD without EMD, 252 eyes (14.4%) had AMD and EMD, 503 eyes (28.7%) had EMD without AMD, and 876 (50.0%) were controls. General characteristics, namely, type, size, location, and distribution of EMD in the total sample, are given in Table 1, along with a comparison between those having EMD with AMD and without AMD. EMD characteristics were more severe when associated with AMD, with more large soft and confluent drusen and more widespread distribution in the fundus, compared to EMD as a standalone feature (Table 1, Fig. 2). The characteristics of all other extramacular features analyzed per eye are described in detail in Supplementary Table S1.

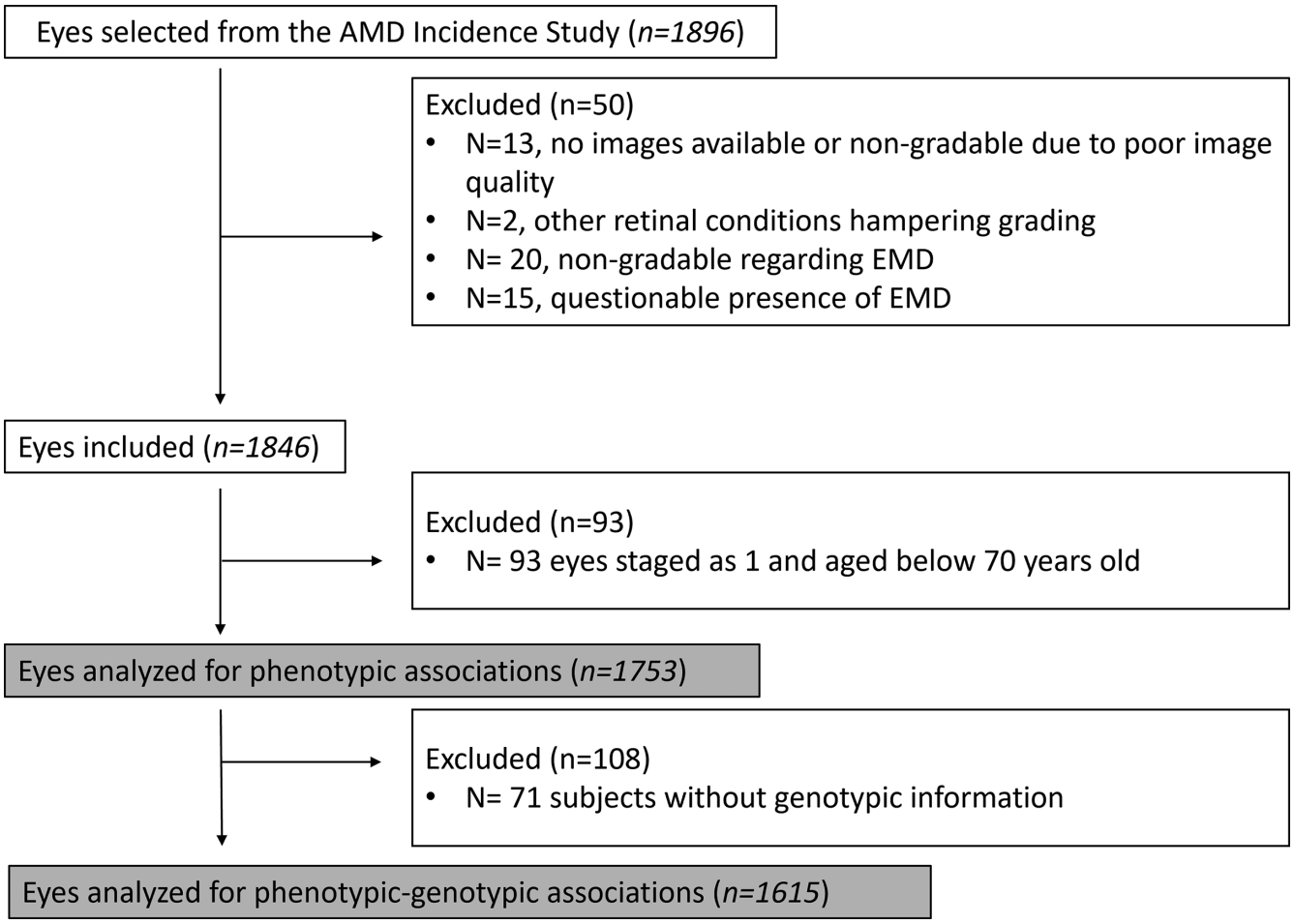

Figure 1. Flowchart of the participants and included subjects in the analysis.

Table 1. Extramacular Drusen Characteristics, All Eyes, and by Presence/Absence of Associated AMD

EMD Characteristics	All EMD Eyes, *n* (%) *n* = 755	EMD Without AMD, *n* (%) *n* = 503	EMD With AMD, *n* (%) *n* = 252	*P* Value*
Drusen type				
Hard (<63 µm)	640 (84.8)	444 (88.3)	196 (77.8)	0.005
Soft distinct (≥63 µm)	525 (69.5)	322 (64.0)	203 (80.6)	0.020
Soft indistinct (≥63 µm)	385 (51.0)	203 (40.4)	182 (72.2)	<0.001
Soft confluent	157 (20.8)	76 (15.1)	81 (32.1)	0.236
Cuticular	4 (0.5)	2 (0.4)	2 (0.8)	NA
Crystalline/calcified	2 (0.3)	0 (0)	2 (0.8)	NA
Drusen size				
<63 µm	649 (86.0)	448 (89.1)	201 (79.8)	0.010
≥63 µm and <125 µm	636 (84.2)	402 (79.9)	234 (92.9)	0.002
≥125 µm	276 (36.6)	123 (24.5)	153 (60.7)	<0.001
Drusen location				
Nasal to the disc	575 (76.2)	364 (72.4)	211 (83.7)	0.001
Temporal to the macula	486 (64.4)	289 (57.5)	197 (78.2)	<0.001
Near/above superotemporal arcade	565 (74.8)	361 (71.8)	204 (81.0)	0.012
Near/above inferotemporal arcade	350 (46.4)	206 (41.0)	144 (57.1)	0.006

AMD, age-related macular degeneration; EMD, extramacular drusen.
* *P* values were obtained using a logistic regression with GEE, to take into account intereye correlation and FDR for adjustment for multiple comparisons.

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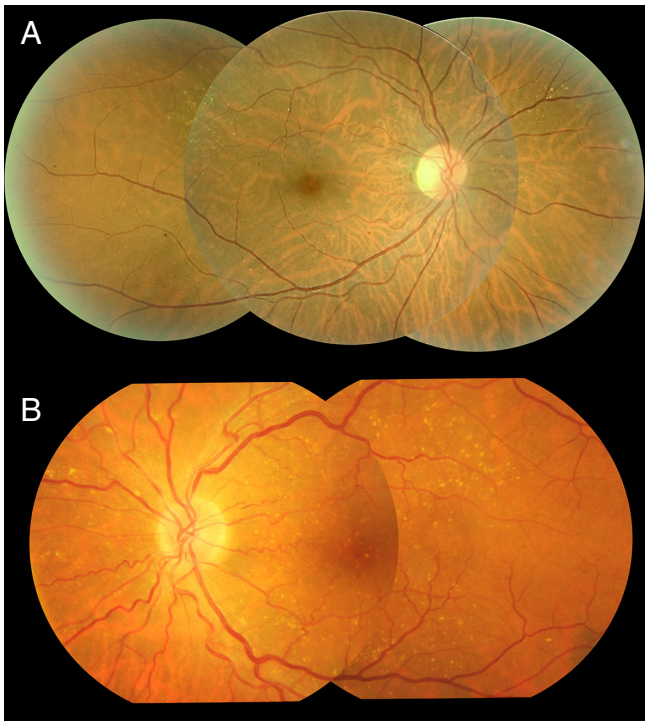

Figure 2. (**A**) A 71-year-old woman with only EMD, which are mainly located in the superior and nasal retina, and displaying mild features, despite a relatively high GRS for AMD of 3.21 (GRS by pathway = *ARMS2* 2.15, complement 1.30, lipids –0.27, ECM –0.08). (**B**) A 64-year-old man with AMD and EMD, displaying more severe features regarding the type, number, and distribution of EMD, and with a high GRS for AMD of 4.12 (GRS by pathway = *ARMS2* 2.15, complement 1.63, lipids 0.35, ECM –0.02).

Table 2. Frequencies (and Percentages) of EMD and AMD Eyes in the Genotypic–Phenotypic Sample in Analysis

AMD Stage	No EMD, *n* (%)	EMD, *n* (%)	Total	*P* Value*
No AMD (stages 0, 1)	809 (63.8%)	460 (36.2%)	1269	
AMD (stages 2, 3, 4)	113 (32.7%)	233 (67.3%)	346	
Total	922	693	1615	**<0.001**

P in Bold is for statistical significance.

* *P* values were obtained using a logistic regression with GEE, to take into account intereye correlation.

Regarding the genetic analysis, out of the 912 subjects, we excluded 71 subjects whose genetic samples did not pass quality checks or had major variants missing. As a result, our final sample available for phenotypic–genotypic associations comprised 1615 eyes from 841 subjects (346 eyes with AMD diagnosis and 1266 without). Distribution of these eyes regarding EMD and AMD presence is depicted in Table 2.

GRS Comparison in Eyes With EMD, AMD, and Controls

The GRS was compared between four groups: (1) AMD without EMD, (2) AMD with EMD, (3) EMD without AMD, and (4) controls. Overall, $P = 0.0003$ was obtained using a clustered Wilcoxon rank-sum test. The pairwise comparisons between the categories were calculated, adjusting for multiple comparisons using the FDR. A statistical difference in the GRS was found between the categories: eyes with AMD with or without EMD and healthy controls ($P = 2.9e\text{-}5$ and $P = 0.00014$, respectively) and eyes with AMD with or without EMD and eyes with only EMD ($P = 0.00021$ and $P = 0.00011$, respectively). However, the GRS was not truly discriminative between groups, since there was substantial overlapping. Plus, the GRS of eyes with EMD but without AMD was not significantly different from controls, and in AMD eyes, there was no difference between those without EMD and those with EMD, despite a slight tendency for higher scores in the latter ($P = 0.93$ and $P = 0.66$, respectively) (Figs. 3 and 4).

Association of EMD With AMD

The results regarding the association between EMD and AMD are shown in Table 3 and indicate a strong association. In fact, eyes with EMD showed an approximately threefold increased risk for AMD compared to eyes without EMD ($P < 0.001$; OR, 3.333; 95% CI, 2.388–4.653). Age was also significantly associated with AMD ($P < 0.001$; OR, 1.068; 95% CI, 1.041–1.095), whereas sex was not associated.

Association of the GRS With AMD

In the logistic regression analyses with GEE, the GRS was strongly associated with AMD ($P < 0.001$; OR, 1.416; 95% CI, 1.218–1.646). EMD ($P < 0.001$; OR, 3.330; 95% CI, 2.376–4.666) and age ($P < 0.001$; OR, 1.068; 95% CI, 1.041–1.095) were also associated (Table 3).

Association of the GRS With EMD

Because of the strong association between EMD and AMD, the correlation between EMD and the GRS was adjusted for AMD presence. However, the global GRS showed no significant association with EMD when the presence of AMD was considered. Only AMD, age, and sex were significantly associated with EMD (Table 3). When analyzing the association of the GRS by pathway with EMD, considering AMD presence, and adjusted by sex and age, again no associations were found (Table 3).

Association of Common Risk Variants for AMD With EMD

When testing individual associations, we found that of 14 SNPs associated with AMD and included in the analysis, only the variants *ARMS2* rs10490924 ($P = 0.042$), *C3* rs2230199 ($P = 0.042$), and *CETP* rs5817082 ($P = 0.042$) were associated with EMD, after adjustment for the presence of AMD, sex, and age (Table 4).

Discussion

This is the first population-based cohort study to evaluate the relationship between the GRS calculated for AMD and the presence of extramacular drusen. We aimed to expand our genotype–phenotype cohort analysis and to investigate whether the extramacular phenotype shared a genetic basis with AMD and serve as a marker of increased susceptibility to the disease. We found that eyes with EMD have a higher risk for AMD versus eyes without EMD, and eyes with AMD also had a higher risk of having EMD. Interestingly, no

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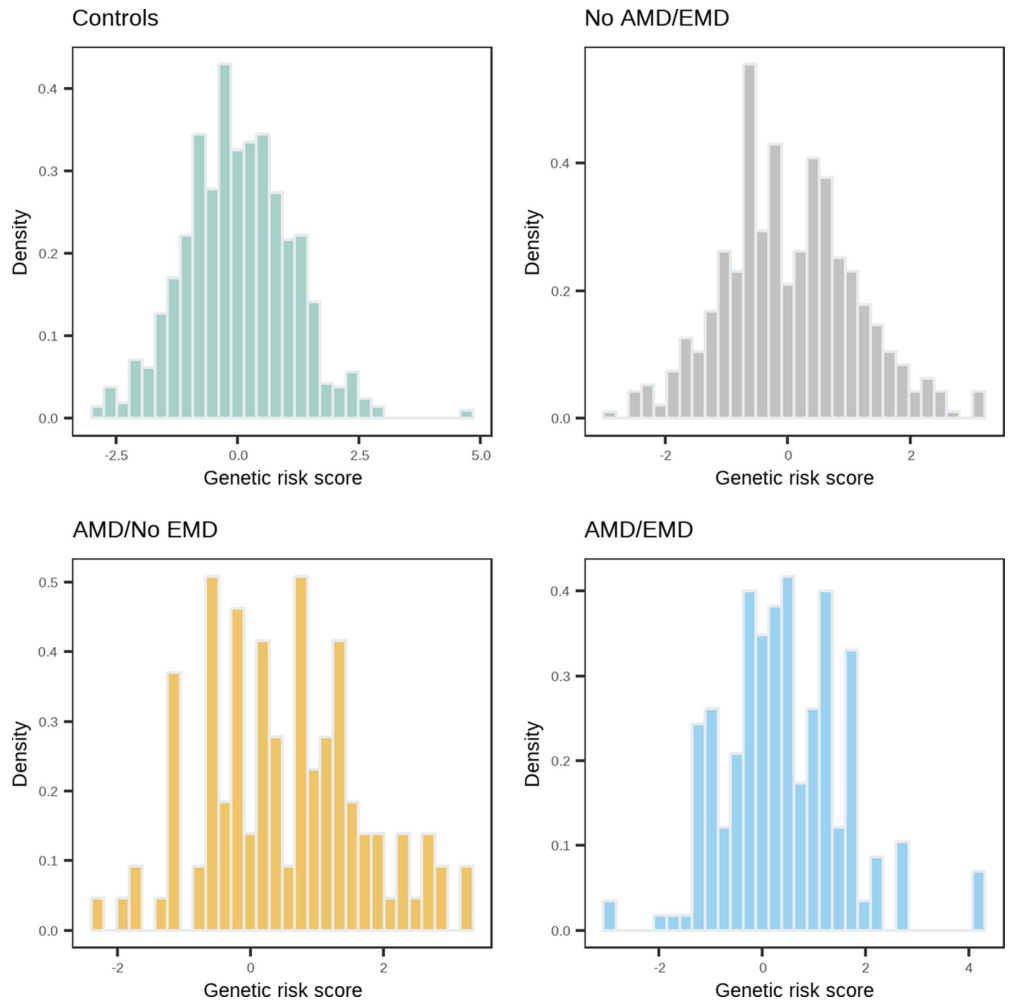

Figure 3. Distributions of the GRS for controls, eyes with EMD without AMD, eyes with AMD without EMD, and eyes with AMD and EMD.

association was found between EMD and the calculated GRS based on the risk alleles for AMD identified by Fritsche et al.[5] Despite this, three variants were independently associated with EMD and AMD. This suggests that genetic factors are not the same considering the global genetic risk, but there are overlapping risk variants linked to both conditions.

Current AMD staging systems do not include extramacular and peripheral changes. However, the recent introduction of ultra-widefield (UWF) imaging reignited the discussion on whether they represent risk factors for the development of the disease or if they are features of AMD itself as a disease involving the entire retina and not only located in the macular area.[21,29] Reticular pigmentary changes, for example, were found to be independently associated with a prolonged time to dark-adapt, a typical functional feature of AMD.[18] In our study, AMD eyes with EMD were more prevalent than eyes with AMD without EMD, and eyes with EMD showed an approximately threefold increased risk for AMD. Also, EMD appeared to have more severe characteristics in AMD eyes compared to non-AMD cases, namely, more extramacular large soft drusen and wider and more uniform distribution in all quadrants. These findings are in agreement with those from the AREDS2 report, which reported that EMD were present in 87% of eyes with intermediate AMD, and their prevalence increased with drusen burden in the macula.[16,30] However, in this report, no association was found between drusen area outside the macula and within the macula, and EMD did not provide additional risk of progression to late AMD over 5 years.[30] Seddon and colleagues[15] also found that AMD severity was associated with peripheral drusen and reticular pigment with an OR of 1.9 for advanced AMD. Ersoy et al.[20] reported that individuals with EMD had an approximately fourfold risk of concomitant AMD. This suggests that EMD share a common pathogenesis with AMD and could represent another manifestation of the disease. Corbelli et al.,[31] however, reported no association between peripheral drusen characteristics and macular criteria for defining AMD, arguing that most peripheral drusen exhibited a hyperreflective content on SD-OCT, whereas macular drusen content had less than 50% hyperreflective material. This observation, together with the fact that there were eyes with only peripheral changes without AMD, led them to conclude that EMD represents a separate entity rather than an expansion of AMD.[31] We too observed that EMD without AMD features represented almost 30% of the total sample in analysis, and in this context, they tendentially presented with milder phenotype.

Regarding the genetic risk, an interesting finding was that despite EMD being strongly associated with AMD, the calculated GRS was not associated with EMD when AMD was

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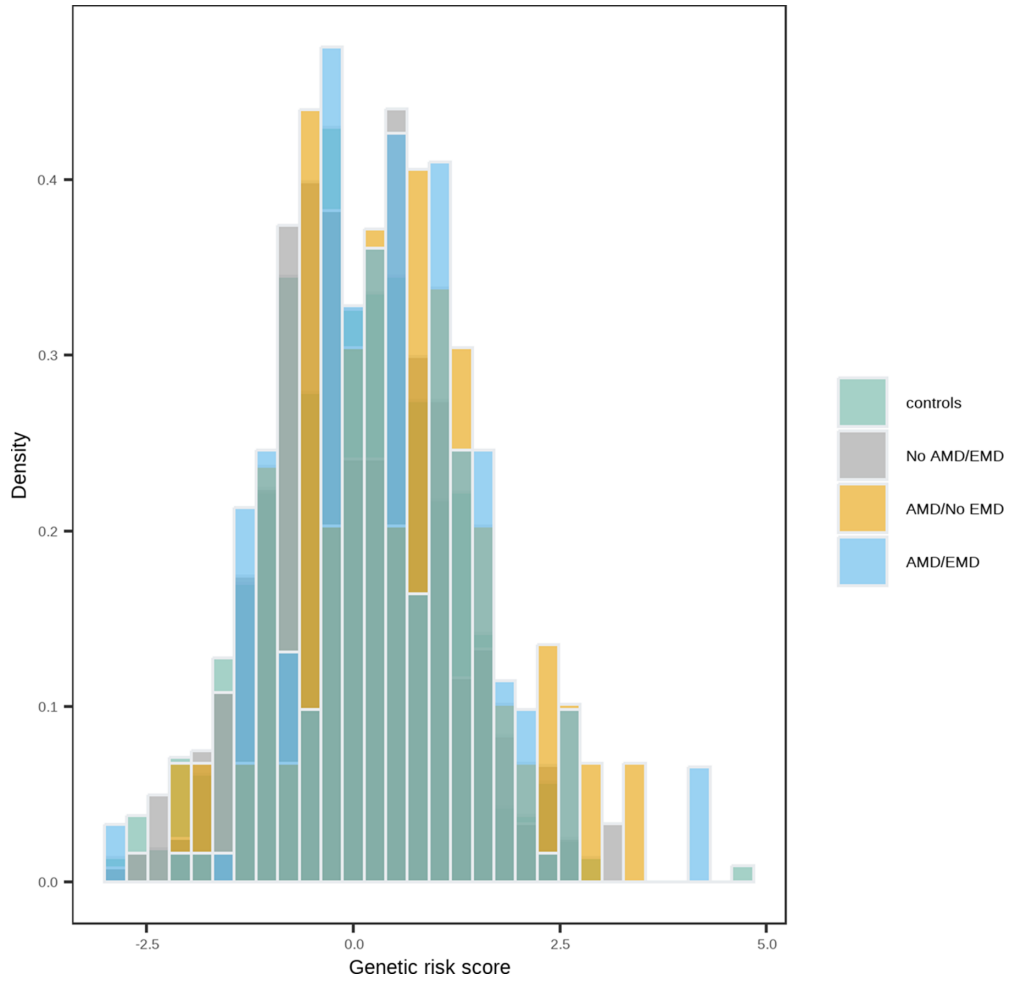

Figure 4. Distribution of the overall GRS between groups, depicting significant overlapping between categories.

Table 3. Associations of EMD, AMD, and GRS

Characteristic	Odds Ratio	95% CI	*P* Value*
Association of EMD with AMD			
EMD	**3.333**	2.388–4.653	**<0.001**
Age	**1.068**	1.041–1.095	**<0.001**
Sex, male	1.043	0.741–1.467	>0.927
Association of GRS and EMD with AMD			
GRS	**1.416**	1.218–1.646	**<0.001**
EMD	**3.330**	2.376–4.666	**<0.001**
Age	1.068	1.041–1.095	**<0.001**
Sex, male	1.030	0.731–1.453	>0.927
Association of GRS and AMD with EMD			
GRS	1.006	0.881–1.148	0.929
AMD	**3.300**	2.356–4.623	**<0.001**
Age	**1.043**	1.020–1.066	**0.0004**
Sex, male	**0.299**	0.220–0.407	**<0.001**
Association of GRS by pathway and AMD with EMD (adjusted by age and sex)			
GRS *ARMS2*	1.1889	0.937–1.509	0.232
GRS complement	0.9578	0.822–1.117	0.789
GRS lipids	1.6444	0.939–2.879	0.136
GRS ECM	1.1489	0.652–2.024	0.789

P in Bold is for statistical significance.
* *P* values were obtained using FDR for adjustment for multiple comparisons.

accounted for in the model. We calculated the GRS based on risk alleles for AMD identified in the large GWAS by the IAMDGC, and as expected, the GRS was associated with AMD.[5] Because of the association between EMD and AMD, adjustment for AMD presence was necessary when evaluating the association of EMD with the GRS. Because of this adjustment, the association between the GRS and EMD was not significant. This was somehow surprising and indicates that genetic factors for AMD and EMD are not fully identical and possible common genetic risk factors are lost if assessed only by a global risk score such as the GRS. To further support our results that EMD are not associated with a GRS as calculated for AMD, we found that there was no difference between the GRS of control eyes and EMD eyes, neither between AMD eyes with and without EMD. The GRS was only different between non-AMD eyes (control and EMD) and AMD eyes (AMD and AMD + EMD). Considering this, EMD per se may be only a nonpathological feature related to aging and do not represent increased genetic risk for AMD. On the other hand, individuals with AMD who present with more extensive drusen deposition outside the macular area probably have a more aggressive phenotype, perhaps genetically determined by other variants not fully weighted by the GRS. In fact, the morphologic characteristics of EMD were more severe in our cohort when associated with AMD, and a study comparing AMD phenotype in probands from single-

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Table 4. Significant Associations of Common Variants With EMD, Adjusted by Presence of AMD, Sex, and Age

Genotype	EMD, *n* (%)	Odds Ratio	95% CI	*P* Value*
ARMS2 rs10490924				
GG	501/1215 (41)	1.00 (reference)	—	—
GT/TT	233/477 (49)	1.415	1.050–1.906	**0.042**
C3 rs2230199				
GG	522/1139 (46)	1.00 (reference)	—	—
GC/CC	197/533 (37)	0.697	0.519–0.936	**0.042**
CETP rs5817082				
CC	393/860 (46)	1.00 (reference)	—	—
CAC/CACA	325/807 (40)	0.740	0.562–0.974	**0.042**
SLC16A8 rs8135665				
CC	393/960 (41)	1.00 (reference)	—	—
CT/TT	185/397 (53)	1.355	0.972–1.888	0.073

P in Bold is for statistical significance.
* *P* values were obtained using FDR for adjustment for multiple comparisons.

ton and multiplex families found that the latter had more peripheral drusen, and the same was found in carriers of pathogenic but rare *CFH* variants.[13,32] Common *CFH* variants play a substantial part when calculating the GRS, and possibly other genetic variants more associated with EMD are not being covered and/or weighted with this approach.

When exploring for individual genetic associations with EMD, we found that only the *ARMS2* rs10490924 was associated with EMD risk, while *C3* rs2230199 and *CETP* rs5817082 decreased the risk. In this respect, Ersoy et al.[20] reported in a case-control study that after adjusting for AMD, there was no association between *CFH* and *ARMS2* risk genotypes with EMD, and Shuler et al.[33] reported that in AMD cases, the presence of extramacular drusen (around the vascular arcades, as in our study) and peripheral drusen (anterior to the equator) was not significantly associated with the *CFH* Y402H variant. In contrast, other studies described that peripheral drusen were associated with AMD and with the *CFH* Y402H and *CFH* rs1410996 genotypes, while the *C3* rs2230199 was not. However, they did not perform any adjustment for the presence of AMD in the analysis.[15,34] Plus, in the case-control study by Altay et al.,[21] only the *ARMS2* rs10490924 and *CFI* rs10033900 (among 31 tested SNPs) were associated with EMD, even after adjustment for AMD, and the *CFH* rs1061170 risk allele again showed no statistically significant association. This heterogeneity in the results of the abovementioned studies points to variability in the genetics of EMD in different populations but could also reflect different study designs, including different imaging protocols and lesion classification definitions.

Age was associated with both AMD and EMD in our study. Altay et al.[21] also found that age was one of the most predictive risk factors for EMD, even after adjusting for AMD, supporting the idea of EMD as a feature of the aging retina. They also reported female sex to be associated with EMD, as in our study.[21]

Our study has some limitations that should be considered. We did not perform UWF imaging, which could, most likely, have shown a higher rate of extramacular and peripheral features. Despite this, we were able to achieve a significant rate of detection of extramacular lesions, and we found differences between the assessed groups. Plus, this is a cross-sectional analysis, and there is no information regarding those individuals without macular alterations but with EMD that progressed to develop AMD over time. This is fundamental to define the role of EMD as risk factor for AMD. Therefore, longitudinal observational studies should be performed to explore further the true risk conferred or not by these extramacular changes. We also recognize that other genetic risk variants for AMD itself are being uncovered, and thus the shared genetic risk with EMD is not fully disclosed as well.[35] Still, we were able to report on the association between specific genetic risk variants for AMD and EMD in our population, and we are the first study to investigate the link between the GRS for AMD and the presence of EMD. Finally, replication of our genetic findings in an independent cohort would be ideal, and there is a lack of racial/ethnic diversity, but these limitations were not possible to overcome due to the design of this populational study and genetic sequencing data available. We are currently conducting a similar study in the population of the second cohort of the epidemiologic CES (inland town), which will be used in the future to strengthen our results.

In conclusion, there is a strong association between EMD and AMD. However, the GRS calculated based on risk variants for AMD was surprisingly not associated with EMD per se, which might be due to the substantial part that *CFH* genotypes play when measuring the GRS in AMD, as defined by the IAMDGC, and the lack of representativeness of other genes and risk variants that could be more relevant in EMD development. Based on this, we can infer that EMD alone do not seem to represent a significant increase in the global genetic risk for AMD, but there is a common genetic basis revealed when assessing individual risk variants. More studies exploring specific major but also rare genetic variants will be important to understand the influence of genetic factors in the pathophysiology of EMD, with and without AMD findings in the macula, in different populations.

Acknowledgments

The authors thank the EYE-RISK consortium for its support through the genotyping of the CES participants.

The epidemiologic Coimbra Eye Study (NCT01298674) was financially supported by Novartis.

Disclosure: **C. Farinha**, Novartis (C), Bayer (C), Abbvie (C); **P. Barreto**, None; **R. Coimbra**, None; **M.B. Machado**, None; **I. Figueiredo**, None; **M.L. Cachulo**, Bayer (C), Novartis (C); **J. Cunha-Vaz**, Precision Ocular Ltd (C), Roche (C), Carl Zeiss Meditec (C), AlimeraSciences (C), Allergan (C), Bayer (C), Gene Signal (C), Novartis (C), Pfizer (C), Sanofi-Aventis (C), Vifor Pharma (C); **R. Silva**, Bayer (C), Alcon (C), Thea (C), Novartis (C), Alimera Sciences (C), Allergan (C)

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