Genetic Risk for Extramacular Drusen and Age-Related Macular Degeneration in the Coimbra Eye Study

Risco Genético de Drusen Extramaculares e Degenerescência Macular Relacionada com a Idade no *Coimbra Eye Study*

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ABSTRACT

INTRODUCTION: The genetic risk for age-related macular degeneration (AMD) was previously evaluated in the AMD incidence study (Coimbra Eye Study, NCT02748824) including a genetic risk score (GRS) analysis. In AMD most genotype-phenotype associations rely on the phenotypic analysis of the macular area, with few analyzing extramacular drusen (EMD). Genetic associations between these peripheral changes and genotypes known to be associated with AMD were explored by few groups and with conflicting results.

Our purpose was to evaluate the relationship between GRS for AMD and the presence of EMD in the participants from AMD incidence study with and without AMD, and to explore whether the EMD phenotype could represent another expression of genetic susceptibility.

METHODS: Multimodal imaging with color fundus photography, optical coherence tomography, autofluorescence and near-infrared imaging was used to assess the presence and staging of AMD and EMD. To test for differences in the GRS between 4 groups: AMD only, AMD+EMD, EMD only and controls, an adjusted clustered Wilcoxon rank sum test was used. Associations of GRS with EMD and AMD were assessed using adjusted logistic regression models.

RESULTS: A total of 1846 eyes (939 subjects) were included: 570 eyes had EMD only, 252 eyes had EMD+AMD, 122 eyes had AMD only, and 902 eyes were controls.

For genetic analysis, the phenotype-genotype sample comprised 1612 eyes (829 subjects) – 346 eyes with AMD and 1266 eyes without AMD. The GRS from control eyes was inferior compared to AMD eyes with and without EMD (p=1.4e-07; p=0.0001), and the GRS from eyes with only EMD was also inferior compared with AMD eyes with and without EMD (p=2.5e-05, p=0.0019). There was a strong association between EMD and AMD (OR=3.118,95% CI 2.239-4.342, p<0.001). GRS was associated with AMD (OR=1.444,95% CI 1.248-1.670, p<0.001), but no association with EMD

was found when adjusting for the coexistence of AMD (OR=1.092,95% CI 0.964-1.235, *p*=0.16).

CONCLUSION: Our results highlight a strong relationship between AMD and EMD. However, GRS, calculated based on risk variants for AMD, was surprisingly not associated with EMD *per se* in our population. Further studies are required to understand the clinical relevance of genetic risk factors in EMD, with or without AMD.

KEYWORDS: Genetic Association Studies; Genetic Risk Score; Macular Degeneration; Retinal Drusen.

RESUMO

INTRODUÇÃO: O risco genético da degenerescência macular relacionada com a Idade (DMI) foi previamente avaliado no Estudo de Incidência da DMI (*Coimbra Eye Study*, NCT02748824), incluindo uma análise do *score* de risco genético (SRG). Na DMI, a maioria das correlações genótipo-fenótipo são baseadas na análise fenotípica da área macular, sendo raras as análises que avaliam *drusen* extramaculares (DEM). A associação genética entre alterações periféricas e genótipos associados à DMI foi explorada em poucos estudos, com resultados contraditórios.

O nosso objetivo foi avaliar a associação entre o SRG para a DMI e a presença de DEM nos participantes do Estudo de Incidência, e avaliar se o fenótipo de DEM será uma expressão alternativa de suscetibilidade genética.

MÉTODOS: Utilizou-se uma abordagem multimodal com retinografia, tomografia de coerência ótica, autofluorescência e imagens *near-infrared* para avaliar a presença e estádio da DMI e DEM. Para avaliar diferenças entre os quatro grupos: apenas DMI, DMI+DEM, apenas DEM e controlo, foi utilizado o Wilcoxon rank sum test ajustado. As associações entre o SRG e os DEM e DMI foram avaliadas com modelos de regressão logística ajustados.

RESULTADOS: Foram incluídos 1846 olhos (939 participantes): 570 olhos apenas com DEM, 252 olhos com DEM+DMI 122 olhos apenas com DMI e 902 olhos controlo.

Para a análise genética, a amostra genótipo-fenótipo incluiu 1612 olhos (829 participantes) – 346 olhos com DMI e 1266 olhos sem DMI. O SRG do grupo controlo foi inferior relativamente ao grupo com DMI, com ou sem DEM (*p*=1,4e-07; *p*=0,0001) e o SRG do grupo com apenas DEM também foi inferior em relação ao grupo com DMI, com ou sem DEM (*p*=2,5e-05, *p*=0,0019). Encontrou-se uma forte associação entre DEM e DMI (OR=1,444,95% CI 1,248-1,670, *p*<0,001). Encontrou-se uma associação entre o SRG e a DMI (OR=1,444, 95% CI 1,248-1,670, *p*<0,001), no entanto, não foi encontrada nenhuma associação com os DEM, quando ajustada para a coexistência de DMI (OR=1,092, 95% CI 0,964-1,235, *p*=0,16).

CONCLUSÃO: Os resultados demonstram uma forte associação entre DMI e DEM. No entanto, não existe associação entre o SRG e os DEM na nossa população. Serão necessários estudos adicionais para interpretar a relevância dos fatores de risco genético nos DEM.

PALAVRAS-CHAVE: Degenerescência Macular; Drusas Retinianas; Estratificação de Risco Genético; Estudos de Associação Genética.

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in the elderly population of the Western world.[1] By the year 2040, it is estimated that as many as 18.6 million individuals worldwide will develop a blinding stage of AMD.[2,3] Therefore, further understanding of AMD underlying pathophysiology is key, to aim for new therapeutic strategies tailored to the individual risk profile.[4]

Drusen are considered the hallmark of AMD and are frequently seen association with retinal pigment epithelium (RPE) abnormalities in early-stage disease.[5,6] Drusen evaluation for AMD diagnosis is restricted to the macular area, however, neither drusen formation nor RPE changes are restricted to the macula in the ageing subject.[6,7] With the advent of ultra-widefield imaging (UWF), peripheral retinal lesions including pigmentary changes and drusen, have been described in eyes with AMD but its significance is yet to be well established, since these have also been re-

ported in subjects without AMD.[7,8] In the multicentre, randomized and interventional Age-Related Eye Disease Study 2 (AREDS2), extramacular drusen (EMD) were observed in 86.9% of eyes with AMD and were more prevalent with an increased drusen burden within the macula, suggesting that AMD could be more than just a macular disease.[5]

AMD results from a complex interaction between environmental risk factors, such as smoking, diet and oxidative stress, and genetic risk factors.[9,10] Over the past two decades, numerous common and rare genetic variants in different pathways (complement system, extracellular matrix remodelling, angiogenesis, and cholesterol metabolism pathway) have been linked to the risk of development and progression of AMD.[11] In a previous study, we reported that variants in *ARMS2*, *AMRS/HTRA1*, *CFH*, *SCL16A8* and *TGFBR1* were associated with greater risk of disease progression in a Portuguese cohort.[4] However, most genotype-phenotype correlations performed to date rely only on the phenotypic analysis of the macular area, not considering extramacular findings. Recently, polymorphisms in *ARMS2* and *CFI* were found to be highly predictive for the presence of EMD even after accounting for the presence of AMD, supporting the hypothesis of a shared genetic and pathological pathway between EMD and AMD.[6]

Genetic risk for AMD was previously evaluated in the populational-based AMD Incidence study (Coimbra Eye Study, NCT02748824).[1] The genetic risk score (GRS), which uses both common and rare variants associated with AMD to calculate the cumulative risk of developing the disease, was also identified as a valuable tool for assessing an individual's overall genetic predisposition and risk of progression.[4,11,12] However, to date, the relationship between the GRS for AMD and the presence of EMD has not been assessed to the best of our knowledge.

The present work aims to evaluate the relationship between the GRS for AMD and the presence of EMD in the participants of the AMD Incidence study,[1] to determine whether the peripheral phenotype might serve as a marker of genetic susceptibility to AMD among individuals with or without the disease.

MATERIAL AND METHODS

STUDY POPULATION AND DATA COLLECTION

The Epidemiological Coimbra Eye Study (NCT01298674) was conducted from 2009 to 2013 to estimate the prevalence of AMD in the central region of Portugal covering two geographically different Portuguese populations aged ≥ 55 years: one from an inland town and other from a coastal town.[13] The AMD Incidence study (NCT02748824),[1] a single-center population-based study, was conducted 6.5 years later and included only participants from the coastal town of Mira. A detailed characterization of the population is reported elsewhere.[1] In this study, participants of the AMD incidence study were extensively characterized from a demographic, clinical, and imagological point of view, applying multimodal imaging (MMI)[14] with color fundus photography (CFP) (3 fields, at 45º), spectral domain optical coherence tomography (SD-OCT), near infra-red (NIR), and autofluorescence (FAF) imaging.

Signed informed consent was acquired from all participants. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Commission of the Faculty of Medicine of the University of Coimbra and of AIBILI – Association for Innovation and Biomedical Research on Light and Image.

OPHTHALMICE XAMINATION AND GRADING METHODS

All participants went through a complete bilateral ophthalmologic exam, including best-corrected visual acuity (BCVA) with Early Treatment Diabetic Retinopathy Study (ETDRS) charts.

CFP images (Topcon® fundus camera, TRC-NW8; Topcon Corp., Tokyo, Japan), Fields 1M, 2, and 3M (45º), from the AMD Incidence Study[1] were reassessed to evaluate the presence of extramacular drusen (EMD) and extramacular pigmentary changes. These images were enhanced using an automatic image-improving software (Colorbalance)[15] to achieve maximum detail of the features.

SD-OCT, NIR, and FAF exams (Spectralis HRA+OCT, Heidelberg Engineering, Heidelberg, Germany) were consulted for clarification and correct grading of questionable findings on CFP. SD-OCT acquisitions involved 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 IR images were acquired for field 2 at 30° (High Resolution with ≥15 frames each).[14]

Extramacular alterations were defined as those located outside the ETDRS macular grid, in the posterior pole nasal to the disc, temporal to the macula, and near/above the vascular arcades. EMD were defined as an accumulation of extracellular material between the Bruch's membrane and the retinal pigment epithelium (RPE). Other extramacular changes commonly found in AMD individuals include: extramacular pigmentary changes characterized as hyperpigmentation, clumps of hyperreflective foci overlying the RPE or drusen, or hypopigmentation, <250 μm and without visible choroidal vessels. Reticular drusen/subretinal drusenoid deposits (SDD) were defined as multiple yellowish-white lesions arranged in a reticular network pattern.

Certified ophthalmologic graders with experience in grading AMD at Coimbra Ophthalmology Reading Centre (CORC, AIBILI) performed all grading.

DISEASE DEFINITIONS AND AMD CLASSIFICATION

The Rotterdam staging system[16,17] was used to classify the AMD severity in all eyes.

The types of drusen present in the macula were also registered: soft, hard, calcified, and cuticular drusen. Late AMD was defined by the existence of neovascular AMD (nAMD), or geographic atrophy (GA), affecting the 6mm macular grid and at least 250 μm in diameter, and both forms were confirmed by consulting the corresponding OCT.

Extramacular changes were evaluated using all 3CFP acquisitions per eye, outside the ETDRS macular grid. EMD were graded as present or absent (EMD were considered present if >10 small drusen only, or >5 drusen with at least one intermediate drusen in the 3 fields combined), and according to location (nasal to the disc, temporal to the macula, near the arcades), type (hard, soft, soft-confluent, cuticular, crystalline, calcified), size (large ≥125 μm in diameter, intermediate ≥63μm; <125 μm, and small <63 μm) and distribution (more drusen in the macula, outside the macula or similar distribution in the two regions). We also analyzed the presence or absence as well as the location (nasal to the disc, temporal to the macular, near the arcades) of hypopigmentary (<250 μm) and hyperpigmentary changes and the presence or absence of SDD and their location (macula and outside the ETDRS grid). Finally, cases of atrophy, choroidal neovascularization (CNV), fibrosis, or retinal/subretinal hemorrhage outside the macula were identified.

GENOTYPING AND GENETIC RISK SCORE ASSESSMENT

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.[1,11] The EYERISK genotype assay is designed to genotype 87 single nucleotide polymorphisms (SNPs) including 52 independently associated SNPs identified in the GWAS by the International AMD Genomics Consortium (IAMDGC).[11,18]

For GRS calculation, these fifty-two independent variants identified by Fritsche *et al*[18] were selected. For each participant the GRS was generated according to the formula: $GRS{=}i{=}152Gi\beta i$, where Gi represents the genotype of variant i coded as 0, 1 or 2 based on the number of minor alleles and βi represents the effect size of variant, based on the GWAS of the IAMDGC fully conditioned analysis. No data imputation was performed. Only subjects with all the major risk variants genotyped (*CFH* rs570618, *CFH* rs10922109, *C2/CFB/SKIV2L* rs429608, *ARMS2/HTRA1* rs3750846 and *C3* rs2230199) were considered for the GRS computation. In the absence of at least one major risk variant, GRS was considered null.

STATISTICAL ANALYSIS

Descriptive statistics was used to describe all variables assessed. Categorical variables were summarized using frequencies and percentages and numerical variables with mean and standard deviation.

To test for differences in the GRS between 4 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 false discovery rate (FDR).

Associations of the GRS with EMD and AMD were assessed using logistic regression models adjusting for age and sex. Generalized estimating equations (GEE) were used to consider inter-eye correlations. For this purpose, odds ratio (ORs) at 95% CI were computed for each analysis. An exchangeable working correlation structure was used.

All statistical analysis were performed using R Core Team (2022) and Stata (16.1, StataCorp LLC, College Station, TX) and *p*-values less than 0.05 were considered statistically significant.

RESULTS

Of the 1617 eligible subjects who participated in the AMD Incidence study,[1] 948 subjects (1896 eyes) were genotyped. A total of 15 eyes were excluded from the analysis because of absent images or poor imaging quality, or other retinal conditions hampering grading. The 1881 eyes remaining (945 subjects) were graded for extramacular features. Subjects with doubtful EMD presence were excluded for accurate grading, with a final group of 1846 eyes (939 subjects) to be analysed (Fig. 1). The mean age of the 939 subjects was 72.33 ± 6.8 and 764 (70.6%) were men. No significant differences were found in age between the four groups.

Of the 1.846 eyes (939 subjects) included: 902 (48.9%) were control eyes, 570 eyes (30.9%) had EMD without AMD, 122 eyes (6.6%) had AMD without EMD, and 252 eyes (13.7%) AMD with EMD. General characteristics, namely, type, size, location, and distribution of extramacular drusen are given in Table 1. The characteristics of all other study variables are described in the Appendix.

Of the 939 subjects included, 110 were excluded since their genetic samples did not pass quality checks or had major variants missing. As a result, our final sample available for phenotypic-genotypic associations comprised 1612 eyes from 829 subjects - 346 with AMD diagnosis and 1266 without (Table 2).

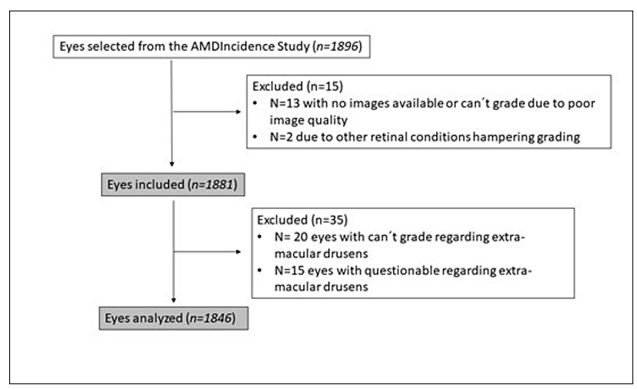

Figure 1. Flow-chart of the participants.

Table 1. Extramacular drusen characteristics in eyes with AMD and without AMD.

EMD characteristics	All EMD eyes (N= 822)	EMD without AMD (N = 570)	EMD with AMD (N = 252)
Drusen type			
Hard (< 63 µm)	702.0 (85.4%)	506.0 (88.8%)	196.0 (77.8%)
Soft distinct (≥ 63 µm)	573.0 (69.7%)	370.0 (64.9%)	203.0 (80.6%)
Soft indistinct (≥ 63 µm)	410.0 (49.9%)	228.0 (40.0%)	182.0 (72.2%)
Soft confluent	167.0 (20.3%)	86.0 (15.1%)	81.0 (32.1%)
Cuticular	4.0 (0.5%)	2.0 (0.4%)	2.0 (0.8%)
Crystallinne/Calcified	2.0 (0.2%)	0.0 (0.0%)	2.0 (0.8%)
Drusen size			
(< 63 µm)	712.0 (86.6%)	511.0 (89.6%)	201.0 (79.8%)
≥ 63 µm but <125 µm	694.0 (84.4%)	460.0 (80.7%)	234.0 (92.9%)
≥ 125 µm	290.0 (35.3%)	137.0 (24.0%)	153.0 (60.7%)
Drusen location			
Nasal to the disc	623.0 (75.8%)	412.0 (72.3%)	211.0 (83.7%)
Temporal to the macula	530.0 (64.5%)	333.0 (58.4%)	197.0 (78.2%)
Near/above superotemporal vascular arcade	612.0 (74.5%)	408.0 (71.6%)	204.0 (81.0%)
Near/above inferotemporal vascular arcade	382.0 (46.5%)	238.0 (41.8%)	144.0 (57.1%)

AMD – age-related macular degeneration, EMD – extramacular drusen.

Table 2. GRS comparison between the 4 groups. N (%) of EMD and AMD eyes.

AMD Staging	No EMD	EMD	Total
No AMD (stages 0,1)	806 (63.7 %)	460 (36.3%)	1266
AMD (stages 2, 3, 4)	113 (32.7 %)	233 (67.3 %)	346
Total	**919**	**693**	**1612**

AMD – age-related macular degeneration, EMD – extramacular drusen.

GRS COMPARISON BETWEEN THE 4 GROUPS

The GRS was compared between 4 groups: 1) AMD without EMD, 2) AMD with EMD, 3) EMD without AMD, and 4) healthy controls. Overall, a p-value=1.75e-5 was obtained using a clustered Wilcoxon rank sum test. The pairwise comparisons between the categories were calculated, adjusting for multiple comparisons using false discovery rate (FDR). The GRS from control eyes was inferior compared to AMD eyes with and without EMD (p=1.4e-07; p=0.0001), and the GRS from eyes with only EMD was also inferior compared with AMD eyes with and without EMD (p=2.5e-05, p=0.0019). However, the GRS distribution was not discriminative between groups, since there was substantial overlapping in the 4 groups (Figs. 2 and 3). Furthermore, the GRS in eyes with only EMD was not significantly different from GRS from controls, and in AMD eyes there was no difference when comparing eyes with EMD and eyes without (p=0.25615 and p=0.98491, respectively).

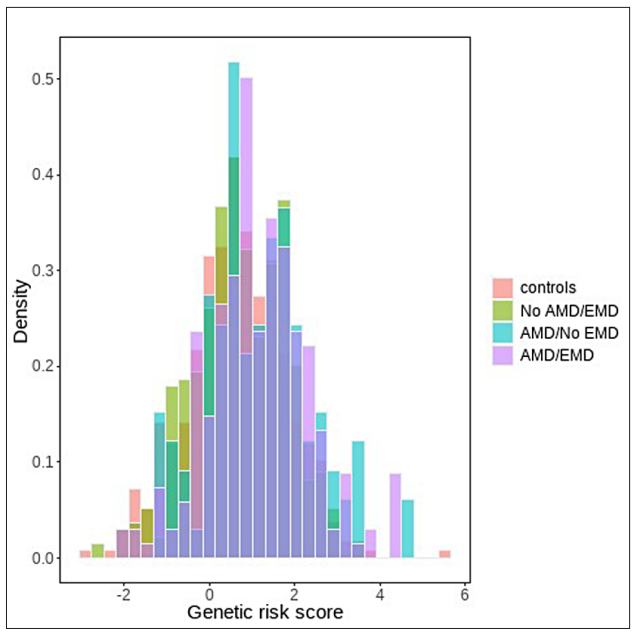

Figure 2. Bar graph showing the distribution of the overall GRS between groups, depicting significant overlapping between categories.

ASSOCIATION OF EMD WITH AMD

A strong association was found between EMD and AMD as displayed in Table 3. Eyes with EMD showed an approximately threefold increased risk for AMD compared to eyes without EMD (p<0.001, odds ratio [OR] 3.137, 95% confidence interval [CI]: 2.266-4.344). Age was also significantly associated with AMD (p<0.001, OR 1.067, 95% CI 1.041-1.094), whereas sex was not associated with AMD.

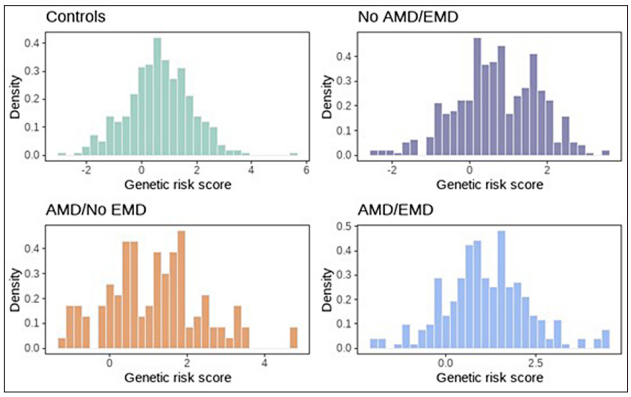

Figure 3. Bar graphs showing the distributions of the GRS for controls, eyes with EMD without AMD, eyes with AMD without EMD, and eyes with AMD and EMD.

ASSOCIATION OF THE GRS WITH AMD

Logistic regression analysis with GEE showed a strong association of the GRS to AMD (p<0.001, OR 1.444, 95% CI 1.248-1.670). The association between AMD and EMD remained significant (p<0.001, OR 3.118, 95% CI 2.239-4.342), and with age (p<0.001, OR 1.067, 95% CI 1.041-1.095). Sex was not associated with AMD.

ASSOCIATION OF GRS WITH EMD

Due to the depicted association between EMD and AMD, the correlation between EMD and the GRS must be adjusted for AMD presence. Only AMD, age and sex were found to be associated with EMD (Table 3). The GRS was not associated with EMD.

DISCUSSION

This is the first population-based cohort study to evaluate the relationship between the GRS calculated for AMD and the presence of EMD, and whether the EMD phenotype could potentially represent an additional expression of genetic susceptibility for AMD development. We found that eyes with EMD have higher risk for AMD versus eyes without EMD. However, no association was found between EMD and the calculated GRS based on risk alleles for AMD, suggesting that genetic factors for AMD and EMD are not exactly the same and genetic risk variants other than those evaluated in the GRS are probably linked to the EMD phenotype.

Our results show that EMD are more prevalent in eyes with AMD than in eyes without AMD (present in 67.3% and 36.3% of cases, respectively). In fact, eyes with EMD showed an approximately threefold increased risk for AMD compared to eyes without EMD (p<0.001, OR 3.137, 95% CI: 2.266-4.344). Eyes with AMD also appeared to have more severe characteristics of EMD compared with eyes without AMD, such as: higher percentage of extramacular large soft drusen (60.7% in eyes with AMD *vs* 24.0% in eyes without AMD) and wider and more uniform distribution in all four quadrants around the posterior pole.

Our findings agree with those from AREDS2 clinical trial, which demonstrated that EMD and peripheral changes were present in 87% of eyes with intermediate AMD, with its prevalence increasing with drusen burden within the macula.[5] In their study, however, no relationship was found between drusen area outside the macula and within the macula, and EMD was not found to provide additional risk of progression to late AMD over 5 years, neither to geographic atrophy nor neovascular AMD.[5] Similarly, Seddon and colleagues[8] conducted an analysis of clinical and photographic data from 2013 individuals and found an approximately 2-fold increased risk of peripheral drusen in eyes with AMD, highlighting an association between macular and peripheral AMD phenotypes. Ersoy *et al* also found that individuals with EMD had an approximately four-fold risk of AMD phenotype when compared with

Table 3. Associations of EMD, AMD and GRS.

Characteristics	Odds Ratio	95% CI	*p*-value
Associations of EMD with AMD			
EMD	3.137	2.266 - 4.344	**< 0.001**
Age	1.067	1.041 - 1.094	**< 0.001**
Sex, male	1.027	0.734 - 1.436	0.878
Association of GRS and EMD with AMD			
GRS	1.444	1.248 - 1.670	**<0.001**
EMD	3.118	2.239 - 4.342	**<0.001**
Age	1.067	1.041 - 1.095	**< 0.001**
Sex, male	1.011	0.721 - 1.419	0.950
Association of GRS and EMD with AMD			
GRS	1.092	0.990 - 1.270	0.16
AMD	1.694	1.326 - 2.165	**<0.001**
Age	1.046	1.024 - 1.069	**< 0.001**
Sex, male	0.326	0.243 - 0.438	**<0.001**

AMD - age-related macular degeneration, CI - confidence interval, EMD - extramacular drusen, GRS - genetic risk score.

a control group.[19] In a different study, however, Corbelli *et al*[20] 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 exhibiting solely peripheral changes without AMD, led them to conclude that EMD represents a separate disease entity rather than an expansion of AMD.[20]

Current AMD staging systems do not include extramacular phenotypic variations.[21] However, the recent introduction of UWF imaging modalities has further motivated numerous researchers to explore the influence of extramacular lesions on both the pathogenesis and symptoms of AMD.[22] Phenotypic characteristics observed during routine examinations that indicate a high-risk genotype could provide valuable information to clinicians, especially if these characteristics were detectable early in the disease course.[21] It has been proposed that the combination of UWF imaging and genetic testing could serve as basis for individual risk estimation, with peripheral abnormalities serving as potential biomarkers to grade disease severity or even having prognostic implications in predicting the risk of AMD progression.[23,24]

Based on the EYE-RISK Consortium methodology used in our cohort,[3,18] we calculated the GRS based on risk alleles for AMD identified in large GWAS by the IAMDGC[18] and, as expected, was strongly associated with AMD, proving the robust genetic susceptibility of AMD. Because of this strong association between EMD and AMD phenotype, adjustment for AMD was necessary when evaluating the association of EMD with GRS.

Our results revealed no association between the calculated GRS for AMD and the presence of EMD in our population. 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. Thus, the GRS calculated for AMD is not considered appropriate to solely assess the risk for EMD. In fact, EMD may even constitute non-pathological variants related to aging. Individuals with GRS for AMD develop clinical manifestations of the disease and those with AMD and EMD have a more aggressive phenotype, due to possible additive or synergistic interactions between risk factors and macular and extramacular drusenoid deposits.

To further validate our results that EMD are not associated with GRS for AMD, we performed a subgroup analysis in subjects without AMD. In this analysis, the GRS from control eyes was inferior compared to eyes with AMD and the GRS from eyes with only EMD was also inferior compared to eyes with AMD. Furthermore, the GRS in eyes with only EMD was not significantly different from GRS from controls, and in AMD eyes there was no difference when comparing eyes with EMD and eyes without.

Our results align with those by Ersoy and colleagues,[19] who demonstrated in a case-control study a strong association between AMD with *CFH* and *ARMS2* genotypes, however, after adjusting for AMD, they also could not find any association between *CFH* and *ARMS2* genotypes with EMD. Similarly, Shuler *et al* had already previously reported that drusen around the vascular arcades were not significantly associated with the CFH genotype.[21] In contrast, two studies by Seddon and Munch described that peripheral drusen were both associated with AMD and with CFH Y402H genotype, however, they did not perform any adjustment for the presence of AMD.[8,25]

CFH variants play a substantial part when calculating GRS and possibly other genetic variants are more associated with EMD that are being covered by GRS. A twin cohort study by Belmouhand *et al*[26] found that the increased heritability for ≥20 hard extramacular drusen and ≥20 small hard macular drusen were mostly explained by additive genetic effects of various genes influencing or modifying the phenotype (A [additive genetic effects] =0.86% [95% CI, 0.73%-0.99%] and A=0.79% [CI 0.53%-1.00%], respectively). One study by Mantel and colleagues also reported that the complement factor B (*CFB*) R32Q polymorphism was associated with a more frequent finding of peripheral drusen, but also not adjusting for the presence of AMD.[27] Altay *et al*[6] described a significant correlation between EMD and *ARMS2* and *CFI* polymorphisms were predictive for the presence of EMD, even after adjustment for the presence of AMD.

In our study, male gender was a protective factor for EMD, which is in agreement with Altay´s work that mentioned that the presence of EMD was associated with female gender.[6] Since there is not much information available on this topic, further studies are needed to elucidate the involvement of sex as a risk factor for EMD. The role of gender as a risk factor for AMD is still controversial. In the review by Heesterbeek *et al*,[28] the female gender was associated with a higher progression rate to early AMD (OR 2.2) and late AMD (HR 1.6-2.6), which might be attributable to the greater life expectancy of females and hormonal differences between sexes, such as in estrogen levels.[29] However, in a previous study by Fraser-Bell,[30] it was the male sex that was found associated to AMD. One proposed explanation was the fact that this was a population-based study that included only Latinos living in California, so geographical and ethnic differences could also have influenced the results.

Age was both associated with AMD and EMD in our study. Advanced age stands as the main risk factor for AMD, since it is associated with both functional and structural changes of the retina and amplifies the cumulative effect of other risk factors over time.[10,28] Our previous population-based incidence study[1] had already demonstrated that both early and late AMD were more prevalent with increasing age, and age was associated also with progression of AMD. Altay *et al*[6] also found that age was one of the most predictive risk factors for EMD, even after adjusting for AMD, though the is only sparse evidence currently available on this topic.

One of the most notable observations from our analysis was that, despite previous reports mentioning a correlation between specific genes and EMD,[6,8,19] this seems to be the first study to investigate the connection between GRS for

Table A.1. Other macular features assessed.	
Extramacular pigmentary changes present	
No	1,815.0 (98.3%)
Yes	28.0 (1.5%)
CG	3.0 (0.2%)
Hypopigmentary changes present	
Nasal to the disc	3.0 (75%)
Temporal to the macula	3.0 (75%)
Near/above superotemporal vascular arcade	4.0 (100%)
Near/above inferotemporal vascular arcade	4.0 (100%)
No	24.0 (85.7%)
Hyperpigmentary changes present	
Nasal to the disc	11.0(45.8%)
Temporal to the macula	15.0 (62.5%)
Near/above superotemporal vascular arcade	2.0 (8.3%)
Near/above inferotemporal vascular arcade	2.0 (8.3%)
SDD present	
No	1,749.0 (94.7%)
Yes	87.0 (4.7%)
QT	8.0 (0.4%)
CG	2.0 (0.1%)
SDD location	
Nasal to the disc	2.0 (2.3%)
Temporal to the macula	17.0 (19.5%)
Near/above superotemporal vascular arcade	66.0 (75.9%)
Near/above inferotemporal vascular arcade	19.0 (21.8%)
Atrophy of the RPE present outside the macula	
CG	2.0 (0.1%)
No	1,840.0 (99.7%)
Yes	4.0 (0.2%)
Choroidal neovascularization present outside the macula	
CG	2.0 (0.1%)
No	1,843.0 (99.8%)
QT	1.0 (0.1%)
Subretinal fibrosis present outside the macula	
CG	2.0 (0.1%)
No	1,843.0 (99.8%)
QT	1.0 (0.1%)
Retinal/subretinal hemorrhage present outside the macula	
CG	2.0 (0.1%)
No	1,843.0 (99.8%)
Yes	1.0 (0.1%)

CG - cannot grade, QT - questionable, SDD - subretinal drusenoid deposits, RPE - retinal pigment epithelium.

AMD and the presence of extramacular manifestations. Another notable point of our study was that AMD classification was robust, as we used a multimodal approach to interpret images from the Incidence study.[1] As clarified in previous studies, in comparison with those acquired with CFP only, multimodal imaging allows a greater accuracy in the detection of AMD lesions, such as subretinal drusenoid deposits in early AMD, and initial cases of nAMD or GA.[14]

Our study has some limitations which should be considered when interpreting our results. First, we cannot elaborate on risks provided by specific known rare genetic variants nor by individual major risk variants for AMD in EMD development. Thus, in the future, a follow-up study should address this question. Second, control individuals above 55 years old were included in this analysis. This could bias our results, as we cannot exclude these individuals from developing EMD and/or AMD lesions in the future, and, therefore, there could be a genetic predisposition for the disease not clinically manifested at the time of our study. Third, we did not perform UWF imaging which could, most likely, have shown a higher rate of EMD. Despite this, we were able to achieve a significant rate of detection of extramacular lesions. Finally, this is a retrospective analysis and there was no information regarding individuals without macular alterations and the development of AMD over time. Therefore, longitudinal observational studies should be performed to explore extramacular findings in AMD.

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 suggest that EMD alone do not seem to represent another expression of the genetic risk for AMD, at least when assessing it with the conventional GRS. Therefore, future studies in this population, exploring specific major and rare genetic variants, will be important to understand the influence of genetic risk factors in the pathophysiology EMD, with or without macular AMD findings.

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