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ORIGINAL ARTICLE

Common and rare genetic risk variants in age-related macular degeneration and genetic risk score in the Coimbra eye study

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Abstract

Purpose: To determine the contribution of common and rare genetic variants in age-related macular degeneration (AMD) in a Portuguese population from the Coimbra Eye Study (CES), and the genetic risk score (GRS).

Methods: Participants underwent ophthalmologic examination and imaging. A centralized reading centre performed AMD staging. Genetic sequencing was carried out with the EYE-RISK assay. Sixty-nine single nucleotide polymorphisms (SNPs) were genotyped and tested for association with AMD. Case–control and progression-to-AMD analyses were performed using logistic regression to assess allelic odds ratio (OR) at a 95% confidence interval (CI) for each variant. GRS was calculated for cases/controls and progressors/non-progressors. Cumulative impact of rare variants was compared between cases/controls using logistic regression.

Results: In case–control analysis (237 cases/640 controls) variants associated with risk of disease were: *ARMS2* rs10490924, *ARMS2_HTRA1* rs3750846, *CFH* rs35292876, *SLC16A8* rs8135665, *TGFBR1* rs1626340. Major risk variants *ARMS2/HTRA1* rs3750846, *CFH* rs570618 and *C3* rs2230199 had unexpected lower allele frequency (AF), and the highest risk-conferring variant was a rare variant, *CFH* rs35292876 (OR, 2.668; p-value = 0.021). In progression-to-AMD analysis (137 progressors/630 non-progressors), variants associated with risk of progression were *ARMS2* rs10490924, *ARMS2_HTRA1* rs3750846, *CFH* rs35292876. GRS of cases/controls was 1.124 ± 1.187 and 0.645 ± 1.124 (p-value < 0.001), and of progressors/non-progressors was 1.190 ± 1.178 and 0.669 ± 1.141 (p-value < 0.001). Higher proportion of pathogenic rare *CFH* variants was observed in cases (OR, 9.661; p-value < 0.001).

Conclusions: Both common and rare variants were associated with AMD, but a *CFH* rare variant conferred the highest risk of disease while three major risk variants had a lower-than-expected AF in our population originary from a geographic region with lower prevalence of AMD. GRS was still significantly higher in AMD patients. Damaging *CFH* rare variants were cumulatively more common in AMD cases.

KEYWORDS
age-related macular degeneration, Coimbra eye study, common genetic variants, genetic risk score, rare genetic variants, single nucleotide polymorphism

Cláudia Farinha and Patricia Barreto both equally contributed to this study.

1 | INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the older population of industrialized countries (Colijn et al., 2017; Li et al., 2020; Wong et al., 2014). As the burden of disease is expected to increase in the next decades (Colijn et al., 2017; Li et al., 2020), further understanding on the pathophysiology of disease is of utmost importance, not only in order to develop therapeutic strategies capable of halting disease progression but also to provide the best advice to patients on their individual risk.

In the last two decades several research groups provided important information on AMD genetics with identification of several common and rare variants associated to risk of disease development and progression. In fact, the heritable component in AMD is estimated to be as high as 45%–70% (Fritsche et al., 2013, 2016; Geerlings, de Jong, et al., 2017; Jordan-Yu et al., 2021). Recently, a landmark genome-wide association study (GWAS) identified 52 variants at 34 genomic regions to be independently associated with AMD. Forty-five were common variants while seven were rare variants (minor allele frequency [MAF] <0.01). Susceptibility genes were grouped into four main pathways: (1) complement system, (2) high density lipoprotein metabolism, (3) angiogenesis and (4) extracellular matrix remodelling. Most of the identified variants were in or near a gene of the complement system: complement factor H (CFH), complement factor I (CFI), complement component 3 (C3), complement component 2 (C2), complement component 9 (C9), complement factor B (CFB) and vitronectin (VTN). Furthermore, a significant burden of rare variants was observed in the *CFH* and *CFI* genes (in addition to TIMP metallopeptidase Inhibitor 3 (*TIMP3)* and solute carrier family 16 member 8 (*SLC16A8)*) (Fritsche et al., 2016). In fact, the interest in rare variants in AMD is significantly growing since they can have strong impact due to high penetrance and may predispose to more severe disease in a given cluster of subjects or population. Several other rare and low-frequency variants (MAF 0.010–0.050) were already identified and might explain the missing heritability in AMD (Geerlings, de Jong, et al., 2017). Since population-specific rare variants tend to have a strong functional effect, case–control studies are, therefore, of most importance to be carried out in different populations (de Breuk et al., 2020; Fritsche et al., 2016; Gibson, 2012).

Strategies such as calculating the genetic risk score (GRS), the cumulative risk of developing AMD based on the genotype of variants known to be associated with disease, can also be useful. This is especially true when integrating the genetic information with other interacting environmental and demographic factors to better predict disease risk (Colijn et al., 2021; Cooke Bailey et al., 2016; de Breuk et al., 2020; Lambert et al., 2016; Wang et al., 2014). Furthermore, the GRS is important to explore in different cohorts as its calculation depends on the presence of risk variants that may be differently distributed across populations.

The Coimbra Eye Study (CES) is a 2-visit epidemiologic population-based study on the prevalence and incidence of AMD in a Portuguese population (NCT01298674, NCT02748824) (Cachulo et al., 2015, 2016; Farinha et al., 2019, 2020). The environmental and nutritional risk factors associated with AMD prevalence were previously explored and reported (Cachulo et al., 2016; Raimundo et al., 2018). Subjects who participated in the 6.5-year follow-up visit for the estimation of incidence also had blood samples collected for further genetic characterization (Farinha et al., 2020).

The purpose of this study is to determine the contribution of common and rare genetic variants in the development of AMD in a Portuguese population, to explore the burden of pathogenic rare variants, and to determine differences between the GRS of AMD patients compared to non-AMD participants.

2 | MATERIALS AND METHODS

2.1 | Study design and population

The Epidemiological Study (NCT01298674) is a single-centre population-based study whose cohort included two geographically distinct populations aged ≥55 years for the estimation of AMD prevalence: one from a coastal town (Mira), and the second from an inland town (Lousã) (Cachulo et al., 2016, 2015).

The AMD Incidence Study (NCT027048824) was conducted 6.5 years later and included only the subjects from the coastal town Mira, which had been recruited in the primary health care unit. This population was extensively characterized in this follow-up visit from a demographic and clinical perspective, including multimodal imaging (MMI). Complete information on the identification and description of the study population, as well on the patients' recruitment details, have been published elsewhere (Cachulo et al., 2016, 2015; Farinha et al., 2019).

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 favourable opinion for the conduction of the study.

2.2 | Data collection and AMD staging

Briefly, all participants from the follow-up incidence study underwent a detailed questionnaire-based interview on demographic, clinical and lifestyle related information by a trained nurse from the primary health care centre, and blood samples were collected from the participants who consented for further genetic and laboratorial analysis. Afterwards, all participants underwent bilateral ophthalmological assessment, including best-corrected visual acuity (BCVA) tested with Early Treatment Diabetic Retinopathy Study (ETDRS) charts and MMI. This multimodal approach included Colour Fundus Photography (CFP) (Topcon® fundus camera, TRC-NW8; Topcon Corp., Tokyo, Japan),

Spectral Domain Optical Coherence Tomography (SD-OCT), Fundus Autofluorescence (FAF) and Infrared (IR) imaging with Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany) (Farinha et al., 2020, 2019).

In respect to AMD grading the Rotterdam staging system was used: early AMD was defined as stages 2a, 2b and 3 (this is, 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), and late AMD as stage 4 (neovascular AMD (nAMD), and/or geographic atrophy (GA)) (Klaver et al., 2001; Vingerling et al., 1995). Staging of an individual participant was based on the eye with more severe status if both eyes were gradable, and on the gradable eye if only one eye was gradable. AMD staging was performed at a centralized reading centre (Coimbra Ophthalmology Reading Center, AIBILI, Portugal), by senior medical retina specialist graders.

2.3 | Genetic sequencing procedures and selection of cases/controls

Genomic DNA samples of the CES participants were genotyped according to standard procedures in the context of collaboration with The European Eye Epidemiology Consortium (E3). As reported elsewhere, our cohort genetic data was obtained through the recently published EYE-RISK genotype assay, which was designed to genotype 87 single nucleotide polymorphisms (SNPs), including the 52 independently associated SNPs identified by the International AMD Genomics Consortium (IAMDGC) (de Breuk et al., 2020; Fritsche et al., 2016). The assay also includes genes that have been described to carry rare variants in AMD (*C3, C9, CFH, CFI, TIMP3, SLC16A8*), candidate genes possibly carrying rare variants in AMD ((age-related maculopathy susceptibility 2 (*ARMS2*), *CD46 molecule* (*CD46*), *CFB, htrA serine peptidase 1* (*HTRA1*)), and genes involved in AMD-mimicking macular dystrophies ((ATP binding cassette subfamily a member 4 (*ABCA4), catenin alpha1 (CTNNA1), peripherin2 (PRPH2*)). Sequencing was performed by combining genomic capture using single-molecule molecular inversion probes (smMIPs) and next-generation sequencing, as described by de Breuk et al. (2020). After quality control, 69 SNPs were successfully genotyped in our cohort. To ensure a complete dataset of the 52 AMD-associated variants 10 SNPs were genotyped by KASP genotyping assays.

Cases were defined as participants from the AMD Incidence Study with early or late AMD, this is stages 2, 3 and 4. Controls were participants that in the Incidence Study were staged as 0 (no signs of AMD or only hard drusen) if their age was above 60 years old, or stage 1 (only soft distinct drusen (≥63 μm) or pigmentary changes) if their age was above 70 years old. This was done to avoid including controls that could develop AMD. All cases that consented to the genetic analysis and with viable DNA samples were genotyped by the EYE-RISK consortium, as well as age and sex-matched controls.

2.4 | Genetic analysis – Association to disease/ no disease and genetic risk score

The successfully genotyped samples and 69 SNPs were tested for association under an additive model, using the presence of AMD as a binary outcome. A logistic regression analysis was performed to assess allelic odds ratio (OR) at 95% confidence interval (CI) for each variant, adjusted for age and sex, with a significance level set to 0.05.

We compared SNP allele frequencies (AFs) of control individuals and AMD patients in the CES cohort to those of the EYE-RISK and IAMDGC datasets, and we explored if the allelic ORs for all SNPs in our study showed the same direction and magnitude of effect compared with those reported in the EYE-RISK study and IAMDGC primary analysis (de Breuk et al., 2020; Fritsche et al., 2016).

The GRS was also computed in our population. Fifty-two independent variants identified by Fritsche et al. (2016) were selected and the OR from the IAMDGC GWAS fully conditioned analysis was used to compute the GRS. For each participant the GRS was generated according to the formula: $\mathrm{GRS} = \sum_{i=1}^{52} \left(G_i \beta_i\right)$, 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 i natural logarithm of the odds ratio of the minor allele variant$i$, based on the GWAS of the IAMDGC fully conditioned analysis. No data imputation was performed. The GRS was considered as missing if the genotype of one of the major risk variants (*CFH* rs570618, *CFH* rs10922109, *C2*/*CFB*/ ski2 like RNA helicase [*SKIV2L*] rs429608, *ARMS2*/*HTRA1* rs3750846 and *C3* rs2230199) was not available.

2.5 | Progression to AMD – Genetic associations and GRS

Since the CES is a longitudinal study, it was possible to also explore genetic associations with progression to AMD in the 6.5-year follow-up. For this analysis we compared progressors to non-progressors. Progressors were participants that progressed from no AMD at baseline (stages 0 or 1) to having AMD at the follow-up visit in the Incidence study (this is, stages 2,3 or 4). Non-progressors were those participants that were classified as not having AMD (stages 0 or 1) in both baseline and follow-up visits. Genetic associations were performed using the same methodology described in the previous section, as well as the calculation of the GRS for progressors versus non-progressors.

2.6 | Rare variants analysis

For the rare variant analysis, we performed logistic regression analyses to assess the cumulative effect of rare variants

with AMD for the *CFH*, *CFI* and *ARSM2* genes. All genetic variants with a MAF < 0.01 were included in the analysis.

Filtering of variants to ensure quality of the data was carried out by the EYE-RISK Consortium. Variants with less than 40 reads coverage on reference allele were changed to missing values. For homozygous reference samples, genotype was kept unchanged, even if it did not have 40 reads coverage in alternate alleles. Following the EYE-RISK quality control steps regarding rare variants, samples with more than 10% missing calls were removed from our dataset.

To predict the functional effect of the rare variants found in our population, two algorithms were explored: the PolyPhen 2 prediction score and the combined annotation-dependent depletion (CADD) score. According to the PolyPhen 2 prediction score the variants included were stratified into: benign (b), possibly damaging (P) and probably damaging (D). Variants with a described loss-of-function (LoF) effect based on functional studies were included as a separate category. According to the CADD score the functional effect of genetic variants was stratified in: score of less than 20, of 20 or more, or LoF. Loss-of-function variants were defined as nonsense, splice-site and frameshift variants and as missense variants with a described functional effect based on functional studies (de Breuk et al., 2020).

2.7 | Macular dystrophies mimicking AMD in the CES

For *ABCA4*, *CTNNA1* and *PHPR2* genes, sequenced with the EYE-RISK assay, we filtered for carriers of variants of class 3 or higher, based on the American College of Medical Genetics and Genomics classification (de Breuk et al., 2020). Retinal images of carriers were re-evaluated by a retinal specialist (C.F.) to identify patients with potential misdiagnose of AMD caused by mimicking inherited macular dystrophies.

3 | RESULTS

From the original cohort of 1.617 participants in the AMD incidence study, where 237 (14.7%) were early AMD cases and 28 (1.73%) were late AMD cases, a total of 922 samples were successfully genotyped for a total of 69 SNPs, in association with the EYE-RISK/E3. In addition, to include only controls respecting the above-mentioned age criteria, 45 samples from controls were excluded. The final cohort in analysis comprised 877 genotyped samples from 237 cases and 640 controls (Figure 1). Regarding AMD cases, 24.3% (n = 213) were early AMD (stages 2 and 3) and 2.73% (n = 24) were late AMD (stage 4). The global mean age of the cohort was 72.6 ± 6.8 years and 57.8% were female. The mean age was 71.9 ± 6.4 years in controls versus 74.7 ± 7.3 in cases, and 56.2% of controls versus 62.0% of cases were female. Characterization of the analysed genotyped population is presented in Table S1.

The AFs of the tested SNPs in AMD cases and controls are presented in Table 1 and Figure 2. Comparing the AFs from the CES cohort to the AFs of the EYE-RISK and the IAMDGC datasets, the following inverse trends in MAF distribution between cases and controls in our study were found: the MAF was higher in controls for acyl-CoA dehydrogenase family member 10/BRCA1 associated protein (*ACAD10/BRAP*) rs61941272, *C3* rs2230199, *C9* rs62358361, collagen type VIII alpha 1 chain (*COL8A1*) rs13081855 and rs140647181 and NPL4 homologue, ubiquitin recognition factor/tetraspanin 10 (*NPLOC4/TSPAN10*) rs656559; and the MAF was higher in AMD cases for ATP binding cassette subfamily A member 1 (*ABCA1*) rs1883025 and rs2740488, apolipoprotein E (exocyst complex component 3 like 2/microtubule affinity regulating kinase 4) *APOE (EXOC3L2/MARK4)* rs73036519, *CFH* rs3753394, collagen type IV alpha 3 chain (*COL4A3*) rs11884770, transforming growth factor beta 1 (*TGBR1*) rs334353, transforming growth factor beta receptor 1 (*TGFBR1*) rs1590, rs1626340 and rs334349, and vascular endothelial growth factor A (*VEGFA*) rs943080. Another interesting finding was that for *ARMS2/HTRA1* rs3750846, a major risk variant for AMD, the allele frequency in cases was much lower than expected when comparing our cohort to EYE-RISK and IAMDGC reports (AF: 0.197 CES versus 0.432 EYE-RISK/0.436 IAMDGC). The same was true for *CFH* rs570618 and *C3* rs2230199, other major risk variants, albeit to a lesser extent.

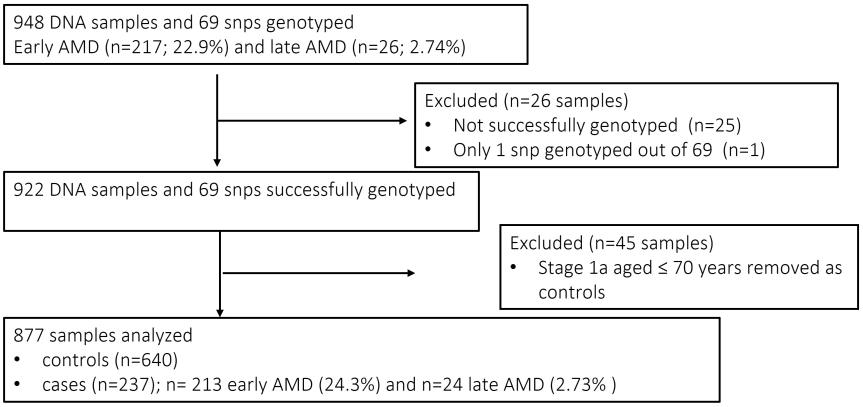

FIGURE 1 Flowchart of genotyped samples from the CES

TABLE 1 Allele frequencies (AFs) of the SNPs from AMD cases and controls in the CES and comparison to the EYE-RISK and the IAMDGC datasets

Gene	SNP	Major/minor allele	MAF controls CES	MAF cases CES	MAF controls EYE-RISK	MAF cases EYE-RISK	MAF controls IAMDGC	MAF cases IAMDGC
ABCA1	**rs1883025**	**C/T**	**0.264**	**0.288**	**0.266**	**0.239**	**0.261**	**0.243**
ABCA1	**rs2740488**	**A/C**	**0.292**	**0.297**	**0.285**	**0.244**	**0.275**	**0.255**
ACAD10/BRAP	**rs61941272**	**C/A**	**0.009**	**0.006**	**0.010**	**0.015**	**0.018**	**0.024**
ADAMTS9	rs6795735	C/T	0.521	0.530	0.458	0.450	0.433	0.465
ADAMTS9-AS2	rs62247658	T/C	0.525	0.537	0.472	0.457	0.433	0.466
APOE	rs429358	T/C	0.106	0.072	0.114	0.108	0.135	0.099
APOE (EXOC3L2/ MARK4)	**rs73036519**	**G/C**	**0.216**	**0.257**	**0.286**	**0.281**	**0.302**	**0.284**
ARHGAP21	rs12357257	G/A	0.317	0.297	0.270	0.230	0.223	0.243
ARMS2	rs10490924	G/T	0.142	0.201	0.181	0.437	0.208	0.436
ARMS2/HTRA1	rs3750846	T/C	0.140	0.197	0.181	0.432	0.208	0.436
B3GALTL	rs9542236	T/C	0.461	0.483	0.466	0.474	0.437	0.452
B3GALTL	rs9564692	C/T	0.329	0.319	0.302	0.260	0.299	0.277
C2	rs4151667	T/A	0.017	0.004	0.029	0.028	0.046	0.025
C2/CFB/SKIV2L	rs2746394	G/A	0.012	0.008	0.008	0.009	0.012	0.016
C2/CFB/SKIV2L	rs429608	G/A	0.142	0.078	0.134	0.087	0.148	0.090
C2/CFB/SKIV2L (PBX2)	rs204993	A/G	0.182	0.191	0.201	0.259	0.260	0.284
C3	rs147859257	T/G	*0.000*	*0.000*	0.001	0.014	0.004	0.012
C3	**rs2230199**	**G/C**	**0.183**	**0.168**	**0.182**	**0.249**	**0.208**	**0.266**
C3 (NRTN/FUT6)	rs17855739	C/T	0.001	0.000	0.001	0.001	0.049	0.038
C9	rs34882957	G/A	0.013	0.011	0.009	0.017	0.009	0.016
C9	**rs62358361**	**G/T**	***0.013***	***0.011***	**0.009**	**0.017**	**0.009**	**0.016**
CFB	rs641153	G/A	0.125	0.085	0.102	0.050	0.090	0.048
CETP	rs17231506	C/T	0.292	0.301	0.313	0.335	0.315	0.348
CETP	rs3764261	C/A	0.303	0.311	0.320	0.341	0.317	0.350
CETP	rs5817082	C/CA	0.290	0.236	0.260	0.221	0.264	0.232
CFB	rs4151672	C/T	0.015	0.004	0.029	0.029	0.045	0.025
CFH	rs10922109	C/A	0.443	0.361	0.461	0.243	0.426	0.223
CFH	rs121913059	C/T	0.000	0.000	0.000	0.001	0.000	0.003
CFH	rs1410996	G/A	0.443	0.360	0.460	0.242	0.426	0.223
CFH	rs148553336	T/C	*0.002*	*0.002*	0.005	0.003	0.009	0.003
CFH	rs191281603	C/G	*0.005*	*0.002*	0.008	0.007	0.006	0.007
CFH	rs35292876	C/T	*0.010*	*0.023*	0.011	0.018	0.009	0.021
CFH	**rs3753394**	**C/T**	**0.304**	**0.308**	**0.281**	**0.262**	**0.291**	**0.266**
CFH	rs570618	G/T	0.310	0.340	0.347	0.578	0.364	0.580
CFHR5	rs10922153	G/T	0.550	0.506	0.531	0.360	0.499	0.342
CFI	rs10033900	C/T	0.304	0.341	0.421	0.510	0.477	0.511
CFI	rs141853578	C/T	*0.000*	*0.000*	0.001	0.004	0.001	0.003
CNN2	rs10422209	C/G	0.228	0.165	0.165	0.136	0.123	0.142
COL10A1	rs3812111	T/A	0.451	0.415	0.426	0.355	0.387	0.372
COL4A3	**rs11884770**	**C/T**	**0.355**	**0.374**	**0.330**	**0.267**	**0.278**	**0.258**
COL8A1	**rs13081855**	**G/T**	**0.083**	**0.080**	**0.088**	**0.113**	**0.092**	**0.104**
COL8A1	**rs140647181**	**T/C**	**0.028**	**0.024**	**0.020**	**0.022**	**0.016**	**0.023**
COL8A1	rs55975637	G/A	0.114	0.118	0.114	0.141	0.117	0.132
CSK_MIR4513	rs2168518	A/G	0.339	0.327	0.335	0.328	0.345	0.328
CTRB2/CTRB1	rs55993634	C/G	0.127	0.105	0.105	0.069	0.089	0.075
HTRA1	rs11200638	G/A	0.131	0.164	0.177	0.424	0.207	0.431

(Continues)

TABLE 1 (Continued)

Gene	SNP	Major/minor allele	MAF controls CES	MAF cases CES	MAF controls EYE-RISK	MAF cases EYE-RISK	MAF controls IAMDGC	MAF cases IAMDGC
LIPC	rs2043085	C/T	0.402	0.382	0.390	0.370	0.384	0.354
LIPC	rs2070895	G/A	0.209	0.209	0.203	0.195	0.217	0.195
LIPC	rs493258	C/T	0.526	0.500	0.494	0.444	0.465	0.442
LPL	rs12678919	A/G	0.102	0.078	0.118	0.100	0.099	0.100
MIR	rs4351242	C/T	0.094	0.086	0.075	0.038	0.067	0.063
MIR6130/RORB	rs10781182	G/T	0.327	0.335	0.300	0.314	0.306	0.328
MMP9	rs142450006	TTTTC/T	0.100	0.118	0.098	0.080	0.141	0.124
NPLOC4/TSPAN10	**rs6565597**	**C/T**	**0.318**	**0.287**	**0.339**	**0.380**	**0.381**	**0.400**
PILRB/PILRA	rs7803454	C/T	0.200	0.205	0.199	0.210	0.190	0.209
PRLR/SPEF2	rs74767144	C/G	0.010	0.006	0.021	0.021	0.022	0.017
RAD51B	rs2842339	A/G	0.140	0.144	0.110	0.092	0.094	0.107
RAD51B	rs8017304	A/G	0.527	0.489	0.425	0.348	0.372	0.349
RDBP_CFB	rs760070	T/C	0.124	0.086	0.106	0.050	0.091	0.049
SLC16A8	rs8135665	C/T	0.150	0.203	0.181	0.228	0.195	0.217
SYN3/TIMP3	rs5754227	T/C	0.116	0.096	0.133	0.109	0.137	0.109
TGFBR1	**rs334353**	**T/G**	**0.231**	**0.249**	**0.257**	**0.227**	**0.248**	**0.231**
TGFBR1	**rs1590**	**T/G**	**0.236**	**0.256**	**0.268**	**0.236**	**0.260**	**0.242**
TGFBR1	**rs1626340**	**G/A**	**0.181**	**0.219**	**0.211**	**0.182**	**0.209**	**0.189**
TGFBR1	rs334348	A/G	0.238	0.257	0.265	0.235	0.260	0.242
TGFBR1	**rs334349**	**G/A**	**0.224**	**0.248**	**0.259**	**0.236**	**0.261**	**0.242**
TMEM97/VTN	rs11080055	C/A	0.481	0.478	0.498	0.485	0.486	0.463
VEGFA	**rs943080**	**T/C**	**0.465**	**0.467**	**0.484**	**0.460**	**0.497**	**0.465**
ZBTB41	rs12724106	A/G	0.088	0.105	0.088	0.151	0.105	0.168

Note: To compare allele frequencies in cases and controls in CES with EYE-RISK and IAMDGC datasets, major and minor alleles were selected to match the ones from Fritsche et al., 2016 (table S11).

Abbreviations: *ABCA1*, ATP binding cassette subfamily A member 1; *ACAD10/BRAP*, acyl-CoA dehydrogenase family member 10/BRCA1 associated protein; *ADAMTS9*, ADAM metallopeptidase with thrombospondin type 1 motif 9; *ADAMTS9-AS2*, ADAMTS9 antisense RNA 2; AFs, allele frequencies; AMD, age-related macular degeneration; *APOE (EXOC3L2/MARK4)*, apolipoprotein E (exocyst complex component 3 like 2/microtubule affinity regulating kinase 4); *APOE*, apolipoprotein E; *ARHGAP21*, rho GTPase activating protein 21; *ARMS2/HTRA1*, age-related maculopathy susceptibility 2/htrA serine peptidase 1; *B3GALTL*, beta 3-glucosyltransferase; *C2/CFB/SKIV2L*, complement component 2/complement factor B/ski2 like RNA helicase; *C3*, complement component 3; *C9*, complement component 9; CES, Coimbra Eye Study; *CETP*, cholesteryl ester transfer protein; *CFB*, complement factor B; *CFH*, complement factor H; *CFHR5*, complement factor h related 5; *CFI*, complement factor I; *CNN2*, calponin 2; *COL10A1*, collagen type X alpha 1 chain; *COL4A3*, collagen type IV alpha 3 chain; *COL8A1*, collagen type VIII alpha 1 chain; *CSK_MIR4513*, c-terminal src kinase/microRNA 4513; *CTRB2/CTRB1*, chymotrypsinogen B2/chymotrypsinogen B; *HTRA1*, htrA serine peptidase 1; IAMDGC, International AMD Genomics Consortium; *LIPC*, lipase c, hepatic type; *LPL*, lipoprotein lipase; MAF, minor allele frequency; *MIR6130/RORB*, microRNA 6130/RAR related orphan receptor b; *MMP9*, matrix metallopeptidase 9; *NPLOC4/TSPAN10*, NPL4 homologue, ubiquitin recognition factor/tetraspanin 10; *NRTN/FUT6*, neurturin /fucosyltransferase 6; *PBX2*, PBX homeobox 2; *PILRB/PILRA*, paired immunoglobulin like type 2 receptor beta/ paired immunoglobulin like type 2 receptor alpha; *PRLR/SPEF2*, prolactin receptor/sperm flagellar 2; *RAD51B*, RAD51 paralog b; *SLC16A8*, solute carrier family 16 member 8; SNPs, single nucleotide polymorphisms; *SYN3/TIMP3*, synapsin III/TIMP metallopeptidase inhibitor 3; *TGFBR1*, transforming growth factor beta receptor 1; *TMEM97/VTN*, transmembrane protein 97/vitronectin; *VEGFA*, vascular endothelial growth factor A; *ZBTB41*, zinc finger and BTB domain containing 41.

Single nucleotide polymorphisms with an inverse trend in AF between cases and controls in the CES in comparison to the EYE-RISK and IAMDGC datasets are presented in bold. Rare variants in our cohort are presented in grey.

3.1 | Associations with AMD risk

To test for variants associated with AMD cases, the total of 877 samples and 69 SNPs were tested for association under an additive model, using the presence of AMD as a binary outcome, in a univariate logistic regression analysis adjusted for age and sex.

Five risk variants were associated to increased risk of AMD: *ARMS2* rs10490924 (OR 1.474; CI 95% 1.121–1.933, *p*-value = 0.005), *ARMS2/HTRA1* rs3750846 (OR 1.462; CI 95% 1.106–1.924, *p*-value = 0.007), *CFH* rs35292876 (OR 2.668; CI 95% 1.136–6.171, *p*-value = 0.021), *SLC16A8* rs8135665 (OR 1.436; CI 95% 1.052–1.951, *p*-value = 0.021) and *TGFBR1* rs1626340 (OR 1.321; CI 95% 1.014–1.713, *p*-value = 0.037). Moreover, we identified seven variants with protective effect: *CFH* rs10922109, *CFH* rs1410996, *C2/CFB/SKIV2L* rs429608, *CETP* rs5817082, calponin 2 (*CNN2*) rs10422209, *CFB* rs641153 and *RDBP/CFB* rs760070. Significant associations are depicted in Table 2, as well as comparisons to the EYE-RISK and the IAMDGC datasets (de Breuk et al., 2020; Fritsche et al., 2016). For purpose of completeness all risk associations tested are depicted in Table S2.

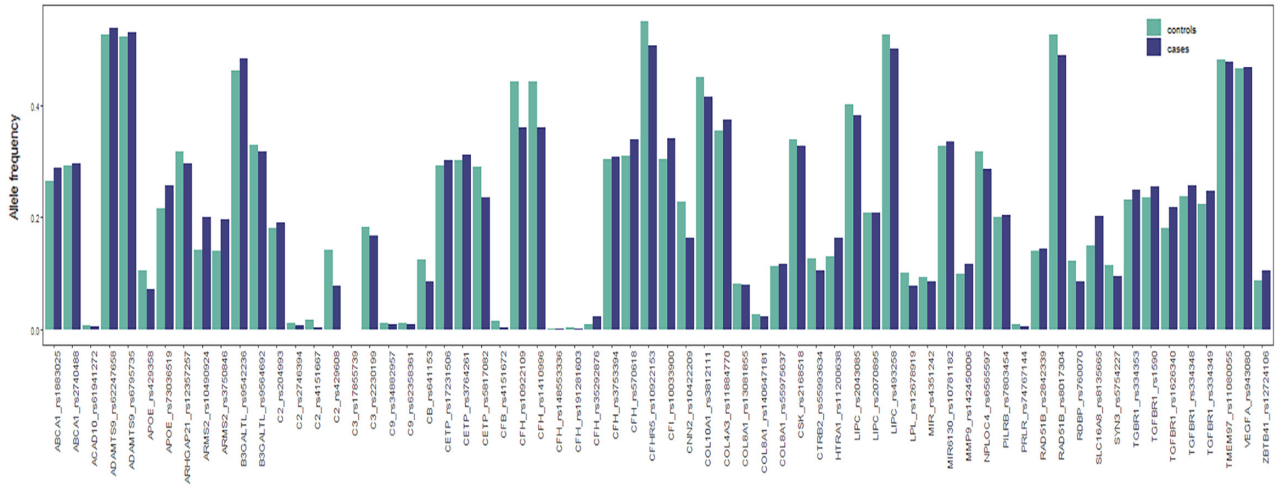

FIGURE 2 Minor allele frequencies in the CES incidence cohort. Comparison between cases and controls

3.2 | Genetic risk score

To assess individual genetic risk, the GRS was calculated. The SNPs from the IAMDGC fully conditioned analysis used in the GRS calculation in the CES study are presented in Table S3. However, if the genotype of one of the major risk variants (*CFH* rs570618, *CFH* rs10922109, *C2*/*CFB*/*SKIV2L* rs429608, *ARMS2* rs3750846 and *C3* rs2230199) was not available, the GRS was considered as missing. For this reason, the analysed cohort to compute the GRS comprised 829 subjects: 607 controls and 222 cases.

Significant differences between the GRS from controls and AMD cases were found in our population: 0.645 ± 1.124 versus 1.124 ± 1.187, respectively ($p < 0.001$). The GRS varied from −2.905 to 5.526, and there was a clear shift towards a higher GRS in AMD cases comparing to controls. It was, however, not possible to completely distinguish between cases and controls based on the GRS alone, as there is substantial overlap (Figure 3). We further explored on the GRS from early ($n = 213$) and late ($n = 24$) AMD cases, but no significant differences were found between them.

3.3 | Progression to AMD – Genetic associations and GRS

We obtained 137 samples from progressors and 630 samples from non-progressors. Variants associated to risk of progression were: *ARMS2* rs10490924, *ARMS2*/*HTRA1* rs3750846, *CFH* rs35292876; and variants protective for progression were again *C2*/*CFB*/*SKIV2L* rs429608, *CFH* rs10922109, *CFH* rs1410996, *CNN2* rs10422209 but also complement factor h related 5 (*CFHR5)* rs10922153, synapsin III (*SYN3)*/*TIMP3* rs5754227 and collagen type X alpha 1 chain (*COL10A1)* rs3812111 (Table 3).

Non-progressors and progressors also had a significantly different GRS: 0.669 ± 1.141 and 1.190 ± 1.178, respectively ($p < 0.001$). Again, and despite the substantial overlap, there was a shift towards a higher GRS in those who progressed to AMD (Figure 4).

3.4 | Rare and low-frequency variants analysis

A total of 859 samples and 1031 rare variants were successfully genotyped in our cohort. After filtering, 973 SNPs and 804 samples from 591 controls and 213 AMD cases were analysed. We investigated the presence of rare variants and their association with disease for the *CFH*, *CFI* and *ARMS2* genes.

For the *CFH* gene, a total of 90 rare variants were included (Table S4). The cumulative analysis revealed that AMD patients had more rare variants with a CADD score ≥ 20 or LoF variants compared to controls (OR, 9.661; p-value < 0.001) (Tables 4 and 5).

As for the *CFI* gene the rare variants found are reported in Table S5. Controls had more benign variants according to PolyPhen-2 score and higher frequency of a CADD score < 20; however, the cumulative difference did not reach statistical significance when comparing controls with cases (Table S6).

For the *ARSM2* gene the only two rare variants assessed were 10:124214262:G:C (Gly7Arg) and 10:124214475:C:G (Pro78Ala), and none was found in our population.

3.5 | Macular dystrophies mimicking AMD

No AMD cases in the CES had two class 3 or higher variants previously reported as pathogenic in the *ABCA4* gene. Two controls were homozygotes for class 3 variant Asn1868Iln (rs1801466) in *ABCA4* gene, but the fundus imaging did not show features compatible with macular dystrophy after revising the exams. Furthermore, the cumulative analysis of variants for the *ABCA4* gene did not reveal more rare variants in AMD cases compared to controls (Table S7). No pathogenic variants were found for genes *CTNNA1* and *PRPH2*.

4 | DISCUSSION

Several variants were found to be associated with the presence of AMD and its progression in our epidemiological

TABLE 2 Variants significantly associated with risk of AMD in the CES, and comparison to EYE-RISK and IAMDGC reports

Gene	SNP	REF	ALT	Major/minor allele	MAF controls CES	MAF cases CES	OR CES (95% CI)	*p*-Value CES	OR EYE-RISK[a]	*p*-Value EYE-RISK[a]	OR IAMDGC primary analysis[a]	*p*-Value IAMDGC primary analysis[a]
C2/CFB/SKIV2L	rs429608	G	A	G/A	0.142	0.078	0.507 [0.338–0.741]	0.001	0.62	1.00^{-6}	0.57	1.2^{-103}
CFH	rs1410996	G	A	G/A	0.443	0.360	0.713 [0.571–0.888]	0.003	0.37	1.64^{-47}	0.38	0
CFH	rs10922109	C	A	C/A	0.443	0.361	0.717 [0.574–0.893]	0.003	0.37	3.93^{-47}	0.38	9.6^{-618}
ARMS2	**rs10490924**	G	T	G/T	0.142	0.201	**1.474 [1.121–1.933]**	0.005	3.29	9.04^{-55}	2.81	0
ARMS2/HTRA1	**rs3750846**	T	C	T/C	0.140	0.197	**1.462 [1.106–1.924]**	0.007	3.18	5.26^{-52}	2.81	6.5^{-735}
CNN2	rs10422209	C	G	C/G	0.228	0.165	0.655 [0.464–0.913]	0.014	0.80	0.02	1.15	2.7^{-8}
CFB	rs641153	G	A	G/A	0.125	0.085	0.629 [0.424–0.915]	0.018	0.44	9.00^{-12}	0.51	1.1^{-89}
CETP	rs5817082	C	CA	C/CA	0.290	0.236	0.731 [0.562–0.945]	0.018	0.81	0.003	0.84	3.6^{-19}
SLC16A8	**rs8135665**	C	T	C/T	0.150	0.203	**1.436 [1.052–1.951]**	0.021	1.33	0.001	1.14	5.5^{-11}
CFH	**rs35292876**	C	T	C/T	0.010	0.023	**2.668 [1.136–6.171]**	0.021	1.62	0.09	2.42	8.2^{-37}
RDBP_CFB	rs760070	T	C	T/C	0.124	0.086	0.648 [0.438–0.939]	0.025	0.44	3.56^{-12}	0.51	9.5^{-91}
TGFBR1	**rs1626340**	G	A	G/A	0.181	0.219	**1.321 [1.014–1.713]**	0.037	0.83	0.02	0.88	3.8^{-10}

Abbreviations: ALT, alternative allele; AMD, age-related macular degeneration; *ARMS2*, age-related maculopathy susceptibility 2; *ARMS2/HTRA1*, age-related maculopathy susceptibility 2/htrA serine peptidase 1; *C2/CFB/SKIV2L*, complement component 2/complement factor B/ski2 like RNA helicase; CES -Coimbra Eye Study; *CETP*, cholesteryl ester transfer protein; *CFB*, complement factor B; *CFH*, complement factor H; CI, confidence interval; *CNN2*, calponin 2; IAMDGC, International AMD Genomics Consortium; MAF, minor allele frequency; OR, odds ratio; REF, reference allele; *SLC16A8*, solute carrier family 16 member 8; SNP, single nucleotide polymorphisms; *TGFBR1*, transforming growth factor beta receptor 1.

Variants in bold are associated with an increased risk of having AMD; the remaining variants are associated with a protective effect towards AMD.

[a]Data from de Breuk et al., 2020.

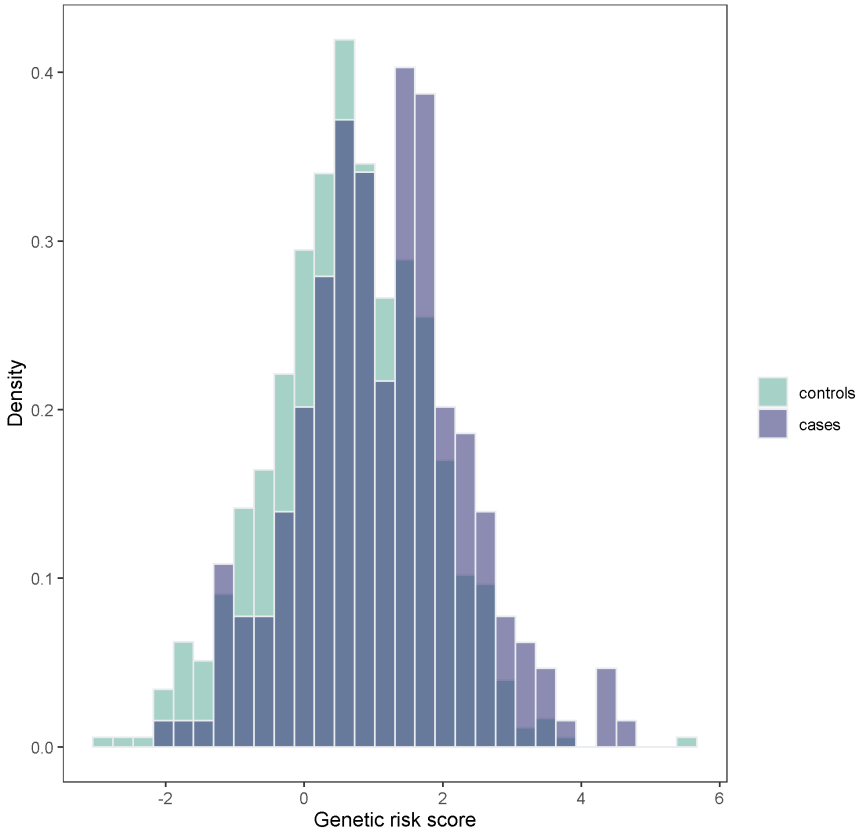

FIGURE 3 GRS of cases and controls

longitudinal study, while others had a protective role. These genes act in different pathophysiologic pathways sustaining the multifactorial aetiology of AMD. Their effects in our population agree with major reports, including large GWAS studies, although some risk variants considered major were found in lower frequency than expected. Despite this, the GRS was still significantly different between AMD and non-AMD cases and between progressors and non-progressors, supporting its role when assessing individual risk. Furthermore, we also found that rare and low-frequency variants in the *CFH* gene with damaging effects were more common in our AMD patients.

Genome-wide association studies have identified several genetic risk variants that are strongly associated with AMD: 52 variants at 34 genomic regions, of which 45 were common variants while 7 were rare variants (Fritsche et al., 2016). In our study 12 variants sequenced by the genotype assay developed by the EYE-RISK consortium were found to be associated with AMD. Eleven are common variants while one in the *CFH* gene (rs35292876) is a rare variant that increases the risk of AMD.

The analysis of the MAF of all sequenced SNPs in AMD cases versus controls revealed that some variants had an inverse trend in our cohort compared to what was found in the larger databases of the EYE-RISK and IAMDGC. These differences can be due to the relatively low number of our sample or most probably due to real specificities of our study population, which originates from a small populational area in central Portugal. These discrepancies were found in different pathways, such as the complement system (*CFH*, *C3* and *C9*), extracellular matrix (*COL4A3*, *COL8A1*, matrix metallopeptidase 9 [*MMP9*]), cholesterol metabolism (*ABCA1*, *ACAD10*/*BRAP*, *APOE*) and the *TGFBR1* gene (de Breuk et al., 2020; Fritsche et al., 2016).

Another interesting finding when analysing the MAF distribution was that regarding the major risk variants for AMD, we observed that for both *ARMS2*/*HTRA1* rs3750846 and *CFH* rs570618, the allele frequency in our cases was much lower compared to the AFs of cases from the EYE-RISK and IAMDGC datasets (de Breuk et al., 2020, Fritsche et al., 2016). In addition, not only the same was true for *C3* rs2230199, another major risk variant, but even an inverse distribution between cases and controls was found in our cohort for this variant. This lower-than-expected AF in major risk variants in AMD cases translates into lower odds ratios with implications in AMD risk in our cohort. We previously reported in our epidemiologic study that this coastal population had significantly lower prevalence of both early and late AMD compared to the inland cohort. Furthermore, in the subsequent incidence study of the coastal population we found that incidence of late AMD was lower than expected compared to other European cohorts (Cachulo et al., 2016; Farinha et al., 2019). These differences could be due to different habits and lifestyle profiles, as well as for different genetic patterns such as we now describe in this report. Furthermore, we also previously reported that a higher adherence to the Mediterranean diet was significantly protective for AMD, and that the coastal population had a significantly higher adherence to it (Nunes et al., 2018). The interplay between these lifestyle and genetic background differences could be the cause of our previous epidemiologic findings for this population

TABLE 3 Variants significantly associated with risk of progression to AMD in the 6.5-year follow-up from the CES

Gene	SNP	REF	ALT	Major/minor allele	MAF progressors CES	MAF non-progressors CES	OR CES (95% CI)	P-value CES	OR EYE-RISK[a]	P-value EYE-RISK[a]	OR IAMDGC primary analysis[a]	P-value IAMDGC primary analysis[a]
C2/CFB/SKIV2L	rs429608	G	A	G/A	0.066	0.136	0.428 (0.243–0.707)	0.002	0.62	1.00–6	0.57	1.2–103
CFH	rs1410996	G	A	G/A	0.335	0.437	0.655 (0.493–0.862)	0.003	0.37	1.64–47	0.38	0
CFH	rs10922109	C	A	C/A	0.344	0.437	0.682 (0.514–0.897)	0.007	0.37	3.93–47	0.38	9.6–618
CFHR5	rs10922153	T	G	G/T	0.471	0.556	0.704 (0.539–0.916)	0.009	0.5	3.77–27	0.52	0
ARMS2/HTRA1	**rs3750846**	T	C	T/C	0.203	0.141	**1.519 (1.078–2.120)**	0.015	3.18	5.26–52	2.81	6.5–735
CFH	**rs35292876**	C	T	C/T	0.029	0.012	**3.060 (1.183–7.412)**	0.015	1.62	0.09	2.42	8.2–37
ARMS2	**rs10490924**	G	T	G/T	0.204	0.144	**1.508 (1.073–2.098)**	0.016	3.29	9.04–55	2.81	0
SYN3/TIMP3	rs5754227	T	C	T/C	0.070	0.115	0.560 (0.326–0.912)	0.026	0.8	0.02	0.77	1.1–24
CNN2	rs10422209	C	G	C/G	0.154	0.223	0.631 (0.403–0.958)	0.036	0.8	0.02	1.15	2.7–8
COL10A1	rs3812111	T	A	T/A	0.408	0.463	0.761 (0.581–0.992)	0.045	0.75	4.00–6	0.94	1.2–4

Note: Variants in bold are associated with an increased risk of progression to AMD; the remaining variants are associated with a protective effect towards progression.

Abbreviations: ALT, alternative allele; AMD, age-related macular degeneration; *ARMS2*, age-related maculopathy susceptibility 2; *ARMS2/HTRA1*, age-related maculopathy susceptibility 2 /htrA serine peptidase 1; *C2/CFB/SKIV2L*, complement component 2/complement factor B/ski2 like RNA helicase; CES -Coimbra Eye Study; *CFH*, complement factor H; *CFHR5*, complement factor h related 5; CI, confidence interval; CNN2, calponin 2; *COL10A1*, collagen type X alpha 1 chain; IAMDGC, International AMD Genomics Consortium; MAF, minor allele frequency; OR, odds ratio; REF, reference allele; SNP, single nucleotide polymorphisms; *SYN3/TIMP3*, synapsin III/TIMP metallopeptidase inhibitor 3.

[a]Data from de Breuk et al., 2020.

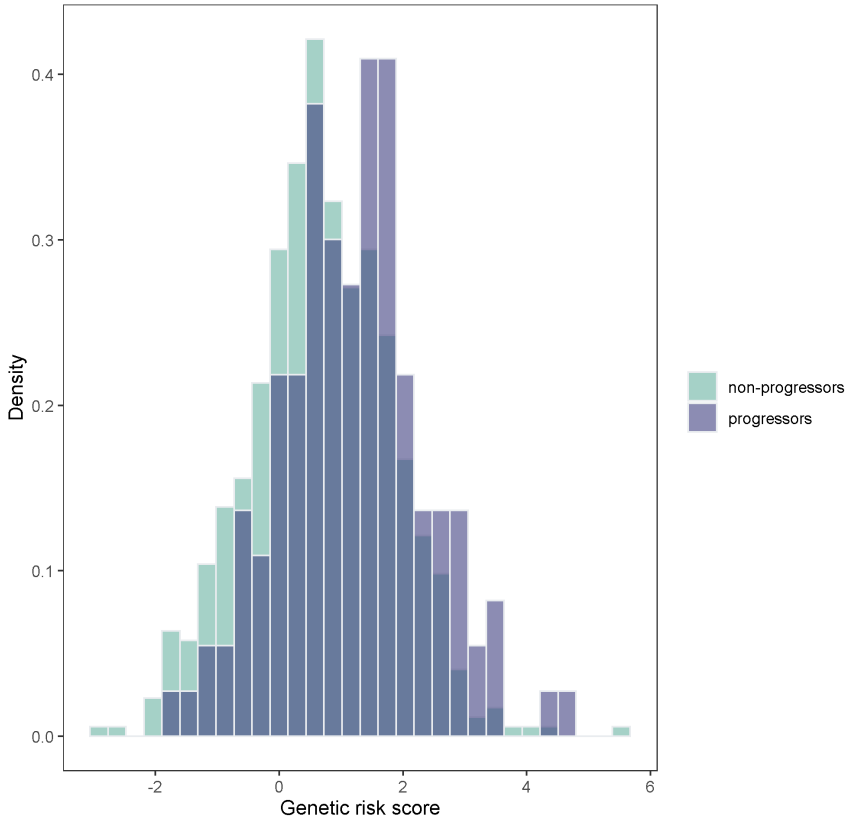

FIGURE 4 GRS of progressors and non-progressors

and are in accordance to the findings on genetic and lifestyle interaction by Colijn et al. (2021).

The variants significantly associated to AMD in our population were fewer than expected but as discussed above, specific genetic differences in our population cannot be excluded, as for instance there were sequenced variants in the complement pathway totally absent in our cohort. Furthermore, genes associated to having the disease were just in part the same as those associated with conversion to AMD in the longitudinal analysis and located only in *CFH* and *AMRS2*/*HTRA1* genes. This discrepancy might be related to the more prominent role of these genes in disease progression. The variant *ARMS2* rs10490924, is known to be associated with incidence of early AMD and progression to both neovascular AMD and geographic atrophy (Seddon et al., 2015; Heesterbeek et al., 2020).

The development of AMD is influenced not only by common variants but also by rare genetic variants, and the impact of such rare variants can be quite significant. The *CFH* rs121913059 (Arg1210Cys) for instance is associated to a 47 times higher risk of developing AMD but was not found in our cohort (Geerlings, de Jong, et al., 2017). The *CFH* rare variant rs35292876 was identified in our population as a *low-frequency* variant and in addition conferred the highest risk of AMD (OR, 2.67), even when compared to major common risk variants. Moreover, it was associated to the highest risk of progression to AMD in follow-up analysis (OR, 3.06). Interestingly, for this variant the EYE-RISK did not find association to AMD risk while the IAMDGC reported an OR of 2.42 (de Breuk et al., 2020; Fritsche et al., 2016). Geographic variations might explain the discrepancies, and this specific variant was found to be more common in western Europe compared to other globe regions, justifying its superior prevalence and effect in our population (Geerlings et al., 2018). This variant was not found to be associated to FH or FHR concentrations in serum, but other rare variants have, and their burden analysis is important to pursue in different cohorts. The *CFH* rs757785149 (Arg53Cys), has been previously identified in AMD families with high disease burden and was identified in our study only in cases. It is reported to possibly affect the local conformation of Factor H, slightly reducing the binding affinity to C3b (Geerlings, de Jong, et al., 2017; Lorés-Motta et al., 2021). Our cumulative analysis of rare variants in the *CFH* gene revealed that they had impact in disease development in our cohort, as damaging rare variants were more frequent in AMD patients compared to non-AMD controls. More functional studies are necessary to determine their pathophysiological effect.

Rare variants in the *CFI* gene have also been associated with a four-fold increased risk of AMD, younger age at AMD onset and with late AMD (de Breuk et al., 2020; de Jong et al., 2020; Seddon et al., 2013). In our population controls had more benign variants, while none of the reported high risk rare variants. The *CFI* variant Pro553Ser was observed more in controls and is reported to be benign in respect to Factor I levels measured in the plasma of carriers (de Jong et al., 2020; Geerlings, de Jong, et al., 2017; Geerlings, Kremlitzka, et al., 2017). Our lack of significance when cumulatively comparing between cases and controls is probably related to the small number of carriers. No *ARMS2* rare variants were found, as observed in the EYE-RISK report, which is

TABLE 4 *CFH* rare variants identified in the CES cohort

Gene	Position GRCh37 (hg19)	REF	ALT	Function	SNP	Nucleotide change	Protein change	SIFT[d]	Polyphen2_HDIV[d]	CADD[d]	Variants (N)	MAC cases	MAC controls	MAF cases	MAF controls
CFH	196 642 206	C	T	Non-synonymous_SNV	rs757785149	C157T	R53C[a,c]	D	D	29.8	1	1	0	0.002	0.000
CFH	196 646 659	G	T	Non-synonymous_SNV	rs777300338	G481T	A161S[b]	T	P	2.863	1	1	0	0.002	0.000
CFH	196 648 794	A	G	Non-synonymous_SNV	rs774239374	A661G	I221V[a]	T	B	0.001	1	0	1	0.000	0.001
CFH	196 658 607	G	A	Non-synonymous_SNV	rs371192606	G1022A	R341H[b]	NA	NA	7.702	1	1	0	0.002	0.000
CFH	196 658 733	T	C	Non-synonymous_SNV	rs762389370	T1148C	V383A	T	P	0.006	1	0	1	0.000	0.001
CFH	196 684 751	T	A	Non-synonymous_SNV	rs147403664	T1548A	N516K[b]	T	D	16.62	1	1	0	0.002	0.000
CFH	196 684 825	A	G	Non-synonymous_SNV	1:196684825:A:G	A1622G	E541G	T	D	21.3	1	1	0	0.002	0.000
CFH	196 695 985	C	A	Non-synonymous_SNV	rs763441589	C2151A	F717L[b]	D	B	12.93	1	1	0	0.002	0.000
CFH	196 706 659	C	A	Non-synonymous_SNV	rs114743644	C2651A	S884[b]	T	P	10.94	1	0	1	0.000	0.001
CFH	196 711 052	G	C	Non-synonymous_SNV	rs201816520	G3004C	G1002R[b]	T	D	7.236	1	1	0	0.002	0.000
CFH	196 716 415	T	A	Non-synonymous_SNV	1:196716415:T:A	T3668A	L1223Q	D	D	24.1	1	0	1	0.000	0.001
CFH	196 694 418	A	G	Non-synonymous_SNV	1:196694418:A:G	A1864G	I622V	T	B	0.194	2	1	1	0.002	0.001
CFH	196 648 906	C	T	Non-synonymous_SNV	rs768526062	C773T	P258L[b]	NA	NA	21.1	17	13	4	0.033	0.004
CFH	196 706 677	G	T	Non-synonymous_SNV	rs515299	G2669T	S890I[b]	T	B	0.463	25	9	16	0.021	0.014
CFH	196 712 596	A	T	Non-synonymous_SNV	rs35274867	A3148T	N1050Y[a,c]	T	B	3.424	32	3	29	0.007	0.025

Abbreviations: *CFH*, complement factor H; CES, Coimbra Eye Study; GRCh37(hg19), genome reference consortium human reference 37; REF, reference allele; ALT, alternative allele; SIFT, Sorting intolerant from tolerant; CADD, combined annotation-dependent depletion; MAC, Minor allele counts; D, probably damaging; P, Possible damaging; B, Benign; AMD, Age-related macular degeneration.

[a]Variants reported as significantly associated with AMD in one or more AMD case–control cohorts.

[b]Variants found in one or more studies.

[c]Variants with a functional effect on the protein or change systemic levels (Geerlings, de Jong, et al., 2017).

[d]Data from de Breuk et al., 2020.

TABLE 5 *CFH* rare variants score in AMD cases versus controls

***CFH* rare variant carriers by PolyPhen 2 score**	**Controls, *N* (%) (*n* = 591)**	**Cases, *N* (%) (*n* = 213)**	**OR (95% CI)**	***p*-Value**
Non-carrier	545	195	1 reference	
Carrier-B	44	13	0.826 (0.419–1.524)	0.558
Carrier-P	1	1	2.795 (0.110–70.904)	0.468
Carrier-D	0	2	NA	0.981
Carrier-LoF	0	1	NA	0.986
CFH rare variant carriers by CADD score				
Non-carrier	541	182		
Carrier-CADD < 20	45	17	1.123 (0.612–1.975)	0.695
Carrier-CADD ≥ 20 or LoF	4	13	**9.661** (3.371–34.636)	**<0.001**

Abbreviations: AMD, Age-related macular degeneration, N, number of subjects; B, Benign; CADD, combined annotation-dependent depletion; *CFH*, complement factor H; CI, confidence interval; D, probably damaging; LoF, loss-of-function; OR, odds ratio; P, Possible damaging.

interesting since it is a gene known to play a fundamental role in AMD progression. Despite altering the protein structure or splicing, the sequenced variants have a low CADD score (<10) and were absent from our population. Other rare variants known to be associated to increased risk of AMD such as *C3* rs147859257 or *C9* rs62358361 were not found in our cohort or were not associated to AMD, and this is probably related to the different distribution of rare alleles across populations.

Addressing the cumulative risk of damaging rare variants may be more useful when analysing differences between cohorts than focusing only on a few variants with low effect in larger study populations. This approach might even have a role in the near future in the identification of those who would benefit more of targeted therapies. Rare variants in the *CFH* and *CFI* genes were already found to cause higher levels of complement activation, thus the carriers might respond more to complement-inhibiting therapies. Phase I/II clinical trials for subretinal gene therapy in AMD are currently underway, and others targeting the complement inhibition are already in Phase III (Cabral De Guimaraes et al., 2021; de Jong et al., 2021; Jaffe et al., 2020; Liao et al., 2020).

Regarding the Genetic Risk Score, it was found to be significantly different between AMD cases and controls, and between progressors and non-progressors. This confirms that the conjoined heritable component in a given individual is important for developing the disease and should be taken into account, if personalized medicine is to be pursued in the future. However, since there was a substantial overlap, it was not possible to completely distinguish between AMD patients and controls based on the GRS alone. This is not unexpected and is in line with what was found in previous publications, since the complex aetiology of AMD depends not only of the genetic background, but is greatly impacted and modified by environmental factors (Colijn et al., 2021; de Breuk et al., 2020). Thus, a score that comprehensively assesses genetic and lifestyle risk factors, such as smoking, body mass index, nutrition and even concomitant medication, together with phenotypic characteristics of the disease, might be more informative of the risk of disease than the GRS alone (Heesterbeek et al., 2019; Seddon et al., 2015). This is more relevant as one must remember that the GRS is calculated on the basis of pre-defined risk variants, and as we found, not even major risk variants are evenly distributed across populations, compromising the generalization of such tool if used alone in risk calculation. Awareness of this is especially important if the GRS is to be implemented in settings such as clinical trials and clinical practice to assess the individual risk of a patient.

Genetic studies on AMD are based on the principle that diagnosis is correct. However, it is sometimes challenging to differentiate from mimicking inherited macular dystrophies, especially in late atrophic stages. Moreover, it is crucial to correctly identify AMD patients before their inclusion in clinical trials (de Breuk et al., 2020; Kersten et al., 2018). We evaluated the occurrence of rare genetic variants associated with AMD-mimicking dystrophies and no pathogenic variants were found in our patients, thus excluding this possible bias from our analysis, and further strengthening the genetic characterization of our cohort.

This study has some limitations that should be addressed. Despite being originally an epidemiological population-based study, for the purpose of genetic analysis it is a relatively small cohort, and the population is from a single location. As some genetic variants are geographically and regionally heterogeneous there is the risk of bias, and the analysis cannot be fully extended to the entire Portuguese population. However, this is the first and only genetic study in AMD in a Portuguese cohort, and we provide extensive characterization regarding common and rare variants. We also found differences in AFs that might explain previous findings in both prevalence and incidence in the CES, further contributing to the disease genetic knowledge in Europe and differences towards other regions. Another limitation was that in progression analysis a comprehensive understanding of the genetic risk of progression to late AMD and of fast progressors, which would be of most interest to explore, was not possible due to small sample size available for these analyses. However, we still derived important information on those who progressed to develop AMD during follow-up. Finally, as part of the EYE-RISK project our results are based in a comprehensive genotype assay recently validated in European populations.

In summary, several variants were identified in association to AMD in our cohort, and the *CFH* rare variant

rs35292876 conferred the highest risk of disease, while three major AMD risk variants in *ARMS2*/*HTRA1*, *CFH* and *C3* had a lower-than-expected AF. Damaging rare variants in the *CFH* gene were significantly more frequent in AMD patients when cumulatively analysed. The GRS was significantly higher in AMD cases, but it was insufficient to discriminate from controls and non-progressors, reinforcing the need to include lifestyle and other risk factors when personalizing risk. Our study adds new information regarding the common and rare variants associated to AMD in a European population, which can be used for comparison with other populational cohorts and further expanding the knowledge of AMD pathophysiology.

DISCLOSURE

Claudia Farinha: Novartis – Consultant, Bayer – Consultant; Patricia Barreto: No Commercial Relationship; Rita Coimbra: No Commercial Relationship; Maria Luz Cachulo: Novartis – Consultant, Bayer – Consultant; Joana Melo: No Commercial Relationship; Yara Lechanteur: No Commercial Relationship; Carel Hoyng: Bayer, Novartis, Horus Pharma Abbvie, Horama. Co-founder of Astherna; Jose Cunha-Vaz: Consultant for Precision Ocular Ltd, Roche, Carl Zeiss Meditec, Alimera Sciences, Allergan, Bayer, Gene Signal, Novartis, Pfizer, Sanofi-Aventis, Vifor Pharma; Rufino Silva: Consultant for Bayer, Thea, Novartis, Alimera Sciences, Allergan.

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REFERENCES

Cabral De Guimaraes, T.A., Daich Varela, M., Georgiou, M. & Michaelides, M. (2021) Treatments for dry age-related macular degeneration: therapeutic avenues, clinical trials and future directions. *The British Journal of Ophthalmology*, 106, 297–304. https://doi.org/10.1136/bjophthalmol-2020-318452

Cachulo, M.L., Laíns, I., Lobo, C. et al. (2016) Age-related macular degeneration in Portugal: prevalence and risk factors in a coastal and an inland town. The Coimbra eye study – report 2. *Acta Ophthalmologica*, 94, e442–e453.

Cachulo, M.D.L., Lobo, C., Figueira, J. et al. (2015) Prevalence of age-related macular degeneration in Portugal: The Coimbra eye study - report 1. *Ophthalmologica*, 233, 119–127.

Colijn, J.M., Buitendijk, G.H.S., Prokofyeva, E., Alves, D., Cachulo, M.L., Khawaja, A.P. et al. (2017) Prevalence of age-related macular degeneration in Europe: the past and the future. *Ophthalmology*, 124, 1753–1763.

Colijn, J.M., Meester-Smoor, M., Verzijden, T., de Breuk, A., Silva, R., Merle, B.M.J. et al. (2021) Genetic risk, lifestyle, and age-related macular degeneration in Europe: the EYE-RISK consortium. *Ophthalmology*, 128(7), 1039–1049.

Cooke Bailey, J., Hoffman, J., Sardell, R., Scott, W., Pericak-Vance, M. & Haines, J. (2016) The application of genetic risk scores in age-related macular degeneration: a review. *Journal of Clinical Medicine*, 5, 31.

de Breuk, A., Acar, I.E., Kersten, E., Schijvenaars, M.M.V.A.P., Colijn, J.M., Haer-Wigman, L. et al. (2020) Development of a genotype assay for age-related macular degeneration: the EYE-RISK consortium. *Ophthalmology*, 128(11), 1604–1617.

de Jong, S., Gagliardi, G., Garanto, A., de Breuk, A., Lechanteur, Y.T.E., Katti, S. et al. (2021) Implications of genetic variation in the complement system in age-related macular degeneration. *Progress in Retinal and Eye Research*, 84, 100952.

de Jong, S., Volokhina, E.B., de Breuk, A., Nilsson, S.C., de Jong, E.K., van der Kar, N.C.A.J. et al. (2020) Effect of rare coding variants in the CFI gene on factor I expression levels. *Human Molecular Genetics*, 29, 2313–2324.

Farinha, C.V.L., Cachulo, M.L., Alves, D., Pires, I., Marques, J.P., Barreto, P. et al. (2019) Incidence of age-related macular degeneration in the central region of Portugal: the Coimbra eye study-report 5. *Ophthalmic Research*, 61, 226–235.

Farinha, C., Cachulo, M.L., Coimbra, R., Alves, D., Nunes, S., Pires, I. et al. (2020) Age-related macular degeneration staging by color fundus photography vs. multimodal imaging—epidemiological implications (the Coimbra eye study—report 6). *Journal of Clinical Medicine*, 9, 1329.

Fritsche, L.G., Chen, W., Schu, M. et al. (2013) Seven new loci associated with age-related macular degeneration. *Nature Genetics*, 45, 433–439.

Fritsche, L.G., Igl, W., Bailey, J.N.C., Grassmann, F., Sengupta, S., Bragg-Gresham, J.L. et al. (2016) A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nature Genetics*, 48, 134–143.

Geerlings, M.J., de Jong, E.K. & den Hollander, A.I. (2017) The complement system in age-related macular degeneration: a review of rare genetic variants and implications for personalized treatment. *Molecular Immunology*, 84, 65–76.

Geerlings, M.J., Kersten, E., Groenewoud, J.M.M., Fritsche, L.G., Hoyng, C.B., de Jong, E.K. et al. (2018) Geographic distribution of rare variants associated with age-related macular degeneration. *Molecular Vision*, 24, 75–82.

Geerlings, M.J., Kremlitzka, M., Bakker, B., Nilsson, S.C., Saksens, N.T., Lechanteur, Y.T. et al. (2017) The functional effect of rare variants in complement genes on C3b degradation in patients with age-related macular degeneration. *JAMA Ophthalmology*, 135, 39–46.

Gibson, G. (2012) Rare and common variants: twenty arguments. *Nature Reviews. Genetics*, 13, 135–145.

Heesterbeek, T.J., De, J.E.K., Acar, I.E. et al. (2019) Genetic risk score has added value over initial clinical grading stage in predicting disease progression in age-related macular degeneration. *Scientific Reports*, 9(1), 6611.

Heesterbeek, T.J., Lorés-Motta, L., Hoyng, C.B., Lechanteur, Y. & den Hollander, A.I. (2020) Risk factors for progression of age-related macular degeneration. *Ophthalmic & Physiological Optics*, 40(2), 140–170.

Jaffe, G.J., Westby, K., Csaky, K.G., Monés, J., Pearlman, J.A., Patel, S.S. et al. (2020) C5 inhibitor Avacincaptad pegol for geographic atrophy due to age-related macular degeneration a randomized pivotal phase 2/3 trial. *Ophthalmology*, 128, 576–586.

Jordan-Yu, J.M., Teo, K., Fan, Q. et al. (2021) T and genetic variations between Asian and Caucasian polypoidal choroidal vasculopathy. *The British Journal of Ophthalmology*, 105(12), 1716–1723. https://doi.org/10.1136/bjophthalmol-2020-317537

Kersten, E., Geerlings, M.J., Pauper, M., Corominas, J., Bakker, B., Altay, L. et al. (2018) Genetic screening for macular dystrophies in patients clinically diagnosed with dry age-related macular degeneration. *Clinical Genetics*, 94, 569–574.

Klaver, C.C., Assink, J.J., van Leeuwen, R., Wolfs, R.C., Vingerling, J.R., Stijnen, T. et al. (2001) Incidence and progression rates of age-related maculopathy: the Rotterdam study. *Investigative Ophthalmology & Visual Science*, 42, 2237–2241.

Lambert, N.G., ElShelmani, H., Singh, M.K., Mansergh, F.C., Wride, M.A., Padilla, M. et al. (2016) Risk factors and

biomarkers of age-related macular degeneration. *Progress in Retinal and Eye Research*, 54, 64–102. https://doi.org/10.1016/j.preteyeres.2016.04.003

Li, J.Q., Welchowski, T., Schmid, M., Mauschitz, M.M., Holz, F.G. & Finger, R.P. (2020) Prevalence and incidence of age-related macular degeneration in Europe: a systematic review and meta-analysis. *The British Journal of Ophthalmology*, 104(8), 1077–1084. https://doi.org/10.1136/bjophthalmol-2019-314422

Liao, D.S., Grossi, F.V., El, M.D. et al. (2020) Complement C3 inhibitor Pegcetacoplan for geographic atrophy secondary to age-related macular degeneration a randomized phase 2 trial. *Ophthalmology*, 127, 186–195.

Lorés-Motta, L., van Beek, A.E., Willems, E., Zandstra, J., van Mierlo, G., Einhaus, A. et al. (2021) Common haplotypes at the CFH locus and low-frequency variants in CFHR2 and CFHR5 associate with systemic FHR concentrations and age-related macular degeneration. *American Journal of Human Genetics*, 108, 1367–1384.

Nunes, S., Alves, D., Barreto, P. et al. (2018) Adherence to a mediterranean diet and its association with age-related macular degeneration. The Coimbra eye study–report 4. *Nutrition*, 51–52, 6–12.

Raimundo, M., Mira, F., Cachulo, M. et al. (2018) Adherence to a Mediterranean diet, lifestyle and age-related macular degeneration: the Coimbra eye study – report 3. *Acta Ophthalmologica*, 96(8), e926–e932.

Seddon, J.M., Silver, R.E., Kwong, M. & Rosner, B. (2015) Risk prediction for progression of macular degeneration: 10 common and rare genetic variants, demographic, environmental, and macular covariates. *Investigative Ophthalmology & Visual Science*, 56(4), 2192–2202.

Seddon, J.M., Yu, Y., Miller, E.C., Reynolds, R., Tan, P.L., Gowrisankar, S. et al. (2013) Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. *Nature Genetics*, 45(11), 1366–1370.

Vingerling, J.R., Dielemans, I., Hofman, A., Grobbee, D.E., Hijmering, M., Kramer, C.F.L. et al. (1995) The prevalence of age-related maculopathy in the Rotterdam study. *Ophthalmology*, 102, 205–210.

Wang, J.J., Buitendijk, G.H.S., Rochtchina, E., Lee, K.E., Klein, B.E.K., van Duijn, C.M. et al. (2014) Genetic susceptibility, dietary antioxidants, and long-term incidence of age-related macular degeneration in two populations. *Ophthalmology*, 121, 667–675.

Wong, W.L., Su, X., Li, X., Cheung, C.M.G., Klein, R., Cheng, C.Y. et al. (2014) Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *The Lancet Global Health*, 2, e106–e116.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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