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Genetic and environmental risk factors for reticular pseudodrusen in the EUGENDA study

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Purpose: The purpose of this study was to analyze genetic and nongenetic associations with reticular pseudodrusen (RPD) in patients with and without age-related macular degeneration (AMD).
Methods: This case-control study included 2,719 consecutive subjects from the prospective multicenter European Genetic Database (EUGENDA). Color fundus photographs and optical coherence tomography (OCT) scans were evaluated for the presence of AMD and RPD. Association of RPD with 39 known AMD polymorphisms and various nongenetic risk factors was evaluated. Stepwise backward variable selection via generalized linear models (GLMs) was performed based on models including the following: a) age, sex, and genetic factors and b) all predictors. Receiver operating characteristic (ROC) curves and the areas under the curve (AUCs) were determined.
Results: RPD were present in 262 cases (no AMD, n = 9 [0.7%; early/intermediate AMD, n = 75 [12.4%]; late AMD, n = 178 [23.8%]). ROC analysis of the genetic model including age, *APOE* rs2075650, *ARMS2* rs10490924, *CFH* rs800292, *CFH* rs12144939, *CFI* rs10033900, *COL8A1* rs13081855, *COL10A1* rs3812111, *GLI3* rs2049622, and *SKIV2L* rs4296082 revealed an AUC of 0.871. Considering all possible predictors, backward selection revealed a slightly different set of genetic factors, as well as the following nongenetic risk factors: smoking, rheumatoid arthritis, steroids, antiglaucomatous drugs, and past sunlight exposure; the results showed an AUC of 0.886.
Conclusions: RPD share a variety of genetic and nongenetic risk factors with AMD. Future AMD grading systems should integrate RPD as an important risk phenotype.

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Age-related macular degeneration (AMD) is a progressive disease of the posterior pole, and its onset is influenced by various genetic and nongenetic risk factors [1,2]. The progression of AMD represents an enormous burden because the late stage of the disease is associated with severe visual impairment [3,4]. Although the presence of reticular pseudodrusen (RPD) has been increasingly recognized as a risk factor for AMD progression [5-8], recent AMD classification and grading schemes do not include RPD as a biomarker for AMD [9].

RPD were initially described as an ill-defined yellowish interlacing network on color fundus photography [10]. However, advances in retinal imaging over the years have allowed more accurate visualization and better detection of RPD via near-infrared (NIR) reflectance images and spectral-domain optical coherence tomography (SD-OCT) compared with their sensitivity on color fundus photographs (FPs; sensitivity on FPs, 29%–88% vs. sensitivity on NIR or SD-OCT, 71%–100%) [11]. With the use of multimodal imaging, it has become apparent that in contrast to soft drusen, RPD are located in the subretinal space [12]. Nevertheless, RPD share compositional similarities with soft drusen, such as

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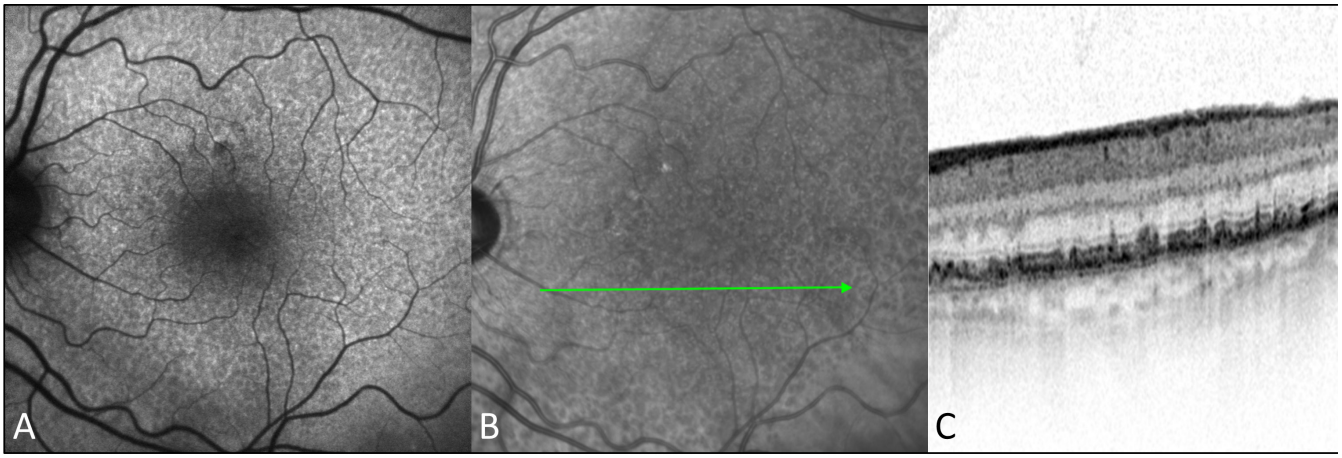

Figure 1. Imaging of reticular pseudodrusen. Example of reticular pseudodrusen (RPD) visible via fundus autofluorescence (**A**), infrared imaging (**B**) and spectral-domain optical coherence tomography (SD-OCT; **C**).

membranous debris, complement components, lipids, vitronectin, and extracellular matrix proteins [13-19].

In recent years, several studies have evaluated the associations of RPD with known AMD risk polymorphisms. A strong association of *ARMS2* polymorphism with RPD has repeatedly been reported; however, the association of *CFH* variants with RPD is controversial [6,11,20-29]. Nevertheless, most of these studies have focused on the association of major AMD risk polymorphisms in *CFH* and *ARMS2* genes.

In this study, we aimed to conduct a comprehensive analysis of the association of various risk factors with RPD in patients with and without AMD in a cohort of 2,783 individuals. For this purpose, we evaluated the association of RPD with 39 polymorphisms known to be associated with AMD and several AMD-associated nongenetic risk factors and used NIR, SD-OCT, and FP images for the detection of RPD and staging of AMD. Furthermore, we aimed to create a multivariable prediction algorithm for the presence of RPD.

METHODS

This case-control study evaluated 2,783 consecutive cases from the European Genetic Database (EUGENDA). EUGENDA is a multicenter prospective epidemiological study enrolling patients with AMD, as well as healthy control individuals ≥ 55 years of age (Department of Ophthalmology, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany; and Department of Ophthalmology, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, the Netherlands). The database included prospectively collected questionnaires, retinal imaging data, and blood samples to evaluate genetic and nongenetic risk factors. The study was performed according to the Declaration of Helsinki and the Medical Research Involving Human Subjects Act (WMO); it was approved by the local ethics committee of each university hospital. Before enrollment in EUGENDA, written informed consent was obtained from all participants. Patients with confounding macular and retinal diseases and insufficient image quality were excluded from the analysis.

Questionnaires: Collected patient information included age, gender, body mass index (BMI), family history of AMD, marital status, highest education level, and iris color. Medical history for arterial hypertension, cardiovascular diseases (CVDs, including myocardial infarction, angina pectoris, stroke/transient ischemic attack, congestive heart failure, vascular bypass surgery, and blood clotting disorder), diabetes, rheumatoid arthritis, thyroid disease, cancer, migraine, and history of allergy were documented. Furthermore, the daily use of acetylsalicylic acid (ASA), nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, coumarin derivates, and antiglaucomatous drugs was evaluated (daily vs. nondaily use). Documented lifestyle factors included smoking (never vs at some point); regular alcohol use (regular vs. almost never); intake of fruit, vegetables, fish, and red meat (≥2 times a week vs. almost never); physical exercise (≥2 times a week vs. almost never); and current and past sunlight exposure (≥8 h a day).

Imaging data and grading: Retinal images, including SD-OCT volume scans registered over NIR images (Spectralis SDOCT, Heidelberg Engineering, Heidelberg, Germany) and stereo color FP (FP, Cologne: Canon UVI fundus camera using 40° field of view; Canon, Tokyo, Japan, and Nijmegen: Topcon TRC 50IX fundus camera using 50° field of view; Topcon, Tokyo, Japan), were collected from

Table 1. Baseline characteristics of all subjects.

Variables	No RPD	RPD
Number of patients, n	2457	262
Female sex, n (%)	1436 (58.4%)	156 (59.5%)
Age (years), mean ±SD	70.77±8.35	80.74±7.97
No AMD, n (%)	1356 (99.3%)	9 (0.7%)
Early/intermediate AMD, n (%)	530 (87.6%)	75 (12.4%)
Late AMD, n (%)	571 (76.2%)	178 (23.8%)

AMD: Age-related macular degeneration, RPD: Reticular pseudodrusen CI: Confidence Interval, RPD: Reticular Pseudodrusen, OR: Odds ratio, MAF: Minor allele frequency MAF<5%: CFI rs141853578, CFB rs4151667, ABCA4 rs76157638, TIMP3.

each participant. In cases of suspected macular neovascularization (MNV), additional fluorescein angiography was performed (Spectralis HRA2, Heidelberg Engineering). AMD staging was performed for both eyes of all cases based on evaluation of FP, fluorescein angiograms (if available), and SD-OCT volume scans according to the standard protocol of the Cologne Image Reading Center (CIRCL) by certified Reading Center graders (TS, LA).

Early AMD was defined as the presence of pigmentary changes together with more than 10 small drusen (<64 µm) or the presence of <15 intermediate drusen (64-125 µm). Intermediate AMD was defined by the presence of large drusen (>125 µm) or by presence of > 15 intermediate drusen (intermediate AMD) in the early treatment diabetic retinopathy study (ETDRS) grid centered on fovea. Late forms of AMD included the presence of MNV (neovascular AMD [nAMD]) or geographic atrophy (GA). AMD staging of individuals was performed based on AMD staging of both eyes as described. The presence of RPD was evaluated on SD-OCT volume scans and NIR imaging. RPD were considered present if subretinal drusenoid deposits were visible in at least one eye, appearing as subretinal cones or flattened roundish lesions above the RPE in the OCT or as discrete hyporeflective dots with a central reflective round area and a surrounding hyporeflective annulus (Figure 1) [12].

Genetic data: Genomic DNA was extracted from peripheral blood samples using standard procedures. Single-nucleotide polymorphisms (SNPs) in or near AMD-associated risk genes that were available in the EUGENDA cohort were chosen for analysis (39 SNPs in 31 AMD risk–associated genes). Genotyping of SNPs in the *ARMS2* (rs10490924), *CFH* (rs1061170, rs800292, rs12144939), *CFI* (rs10033900, rs141853578), *C3* (rs2230199, rs1047286, rs433594), *CFB* (rs4151667, rs641153), *TIMP3* (rs9621532), *APOE* (rs2075650, rs4420638), *LIPC* (rs10468017, rs493258), *LPL* (rs12678919), *CETP* (rs3764261), *FADS1* (rs174547), *VEGFA* (rs943080), *TGFBR1* (rs334353), *SKIV2L* (rs429698), *RAD51B* (rs8017304), *ABCA4* (rs76157638), *ABCA1* (rs3758294), *COL8A1* (rs13081855), *COL10A1* (rs3812111), *SLC16A8* (rs8135665), *ADAMTS9* (rs6795735), *IER3DDR* (rs3130783), *MYRIP* (rs2679798), *HSPH1* (rs9542236), *GLI3* (rs2049622), *GLI2* (rs6721654), *TYR* (rs621313), *PON1* (rs705381), *CYP24A1* (rs1570669), *IGFR1* (rs2872060), and *TNFRSF10A* (rs1327806) genes was performed as previously described [30]. SNPs with minor allele frequency (MAF) < 0.05 were not included in this analysis.

Statistical analysis: Associations of RPD with genetic and nongenetic risk factors were analyzed by univariate and multivariable generalized linear models (GLMs). Variables with > 15% missing cases were not included in the GLMs. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated. The probability of RPD was estimated based on the selected models, and receiver operating characteristic (ROC) curves with the corresponding areas under the curve (AUCs) were obtained. Bootstrapping was used to derive 95% CIs for the ROC curves. Statistical analysis was performed using R software version 4.1 (packages: peperr, rms, fbroc).

RESULTS

Patient data: Out of 2,783 individuals available in the EUGENDA cohort at the time of analysis for this study, 64 cases were excluded because of insufficient image quality and confounding macular or retinal diseases. RPD were considered present in 262 cases (9.6%). The mean age of individuals with RPD was higher compared with cases without RPD (80.74 ± 7.97 versus. 70.77 ± 8.35 years, $p = 4.47 \times 10^{-5}$, OR 1.13, 95% CI 1.12–1.15). No association was observed between gender and the presence of RPD. AMD was detected in at least one eye in 1,354 individuals (49.8%).

The presence of RPD showed a strong association with AMD (adjusted for age, $p = 1.11 \times 10^{-18}$, OR 21.03, 95% CI

Table 2. Variables selected based on model a) including age, sex and genes.

Variables	Subset	Estimate	OR	Lower 95% CI	Upper 95% CI	P- Value
Age		0.15	1.16	1.14	1.18	6.65E-47
APOE rs2075650	GA versus AA	−0.42	0.66	0.43	1.01	0.056
	GG versus AA	−15.34	0.00	0.00	n/A	0.977
ARMS2 rs10490924	TG versus GG	0.64	1.90	1.31	2.77	0.001
	TT versus GG	1.62	5.05	3.20	7.95	2.89E-12
CFH rs800292	GA versus GG	−0.86	0.42	0.29	0.62	9.12E-06
	AA versus GG	−0.82	0.44	0.17	1.14	0.090
CFH rs12144939	TG versus GG	−0.93	0.40	0.25	0.62	3.9E-05
	TT versus GG	−1.48	0.23	0.07	0.76	0.016
CFI rs10033900	TC versus CC	0.33	1.39	0.92	2.10	0.122
	TT versus CC	0.48	1.61	1.00	2.59	0.050
COL8A1 rs13081855	GT versus GG	0.61	1.83	1.24	2.71	0.002
	TT versus GG	−0.30	0.74	0.16	3.54	0.708
COL10A1 rs3812111	AT versus AA	−0.09	0.91	0.57	1.46	0.707
	TT versus AA	−0.43	0.65	0.40	1.07	0.087
GLI3 rs2049622	GA versus GG	−0.39	0.68	0.46	1.00	0.048
	AA versus GG	−0.37	0.69	0.44	1.09	0.116
SKIV2L rs4296082	GA versus GG	−0.54	0.58	0.37	0.91	0.019
	AA versus GG	−1.88	0.15	0.02	1.34	0.090

CI: confidence interval, RPD: reticular pseudodrusen, OR: odds ratio

10.69–41.36). The prevalence of RPD was 0.7% (9/1365) in no AMD, 7.7% (25/323) in early AMD, 17.7% (50/282) in intermediate AMD, 26.7% (27/101) in pure GA, 22.68% (139/610) in pure nAMD and in 31.6% (12/38) in mixed type with GA in one eye and MNV in the fellow eye (Table 1). The distribution of RPD in late AMD subgroups was not statistically different (p = 0.81).

Role of age and genetic risk factors: The univariate associations of each genetic risk factor with RPD and their MAF are presented in Appendix 1. A genetic risk model was created after inclusion of all genes, sex, and age (model a). The following variables were included in the genetic model after backward variable selection: *APOE* rs2075650, *ARMS2* rs10490924, *CFH* rs800292, *CFH* rs12144939, *CFI* rs10033900, *COL8A1* rs13081855, *COL10A1* rs3812111, *GLI3* rs2049622, and *SKIV2L* rs4296082 (Table 2). This genetic model showed a high AUC of 0.871. The ROC curve of the genetic model with its bootstrapping curve is presented in Figure 2.

Additional value of nongenetic risk factors: Univariate associations of nongenetic risk factors with RPD are presented in Table 3. A new model with all available predictors (model b) was created by backward selection to estimate the possible effects of nongenetic risk factors. Besides age, this model revealed the following genetic variants: *APOE* rs2075650, *ARMS2* rs10490924, *CFH* rs800292, *CFH* rs12144939, *CFI* rs10033900, *COL8A1* rs13081855, *CYP24A1* rs1570669, *LIPC* rs10468017 *SKIV2L* rs4296082, and *TYR* rs621313. Moreover, it revealed the following nongenetic risk factors: smoking, rheumatoid arthritis, corticosteroids, antiglaucomatous drugs, and past sunlight exposure (Table 4). However, the AUC of this model was only marginally better than that of the genetic model (AUC 0.883).

DISCUSSION

This comprehensive association study between known genetic and nongenetic AMD risk factors with RPD revealed common genetic risk pathways between RPD and AMD and highlighted the strong association of RPD with age, AMD, and *ARMS2* polymorphism. Our results support the notion that RPD, as an important risk phenotype, should be integrated into the future AMD classification systems used for patient prognosis.

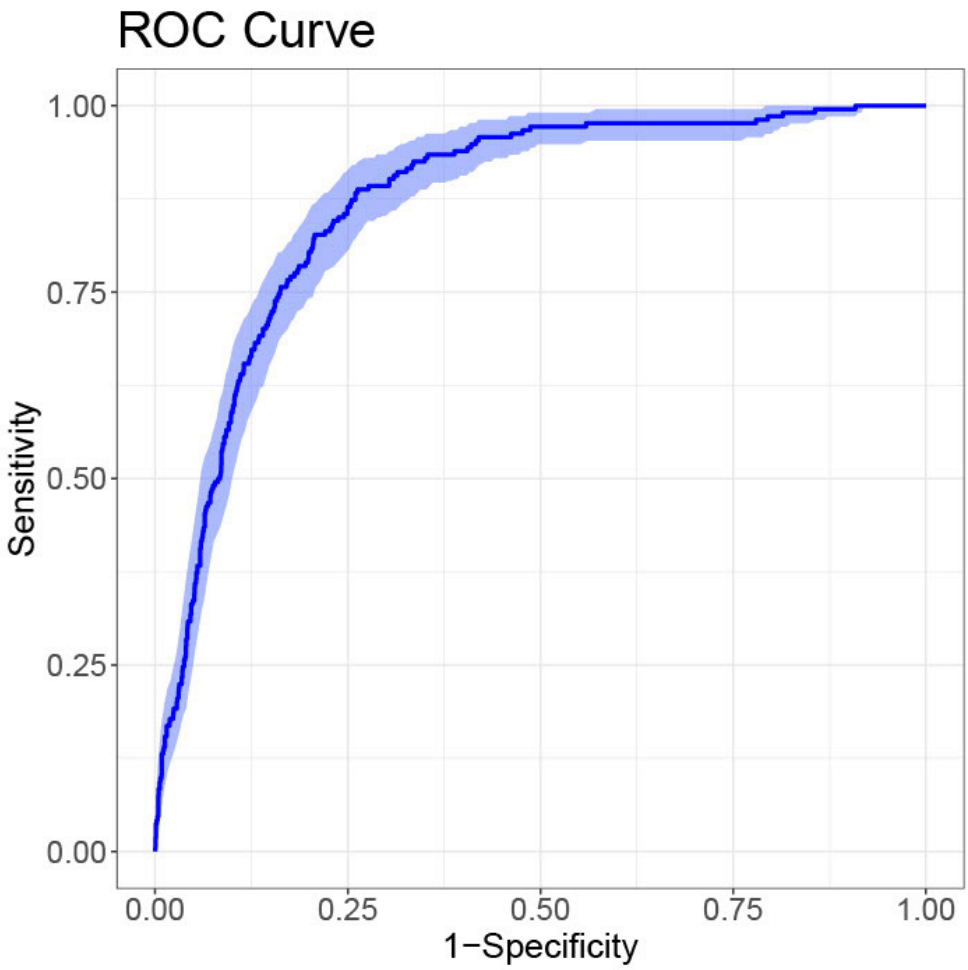

Figure 2. Receiver operating characteristic (ROC) curve with 95% confidence interval (CI) for variables selected based on genetic model).

In this study, a strong relationship between RPD and AMD was detected, as reported previously [6,22-25,28,31]. RPD in the absence of AMD were detected in only nine cases (<1%). AMD onset is strongly linked to age and genetic susceptibility involving multiple genetic variants related to the complement system, extracellular matrix, and lipid metabolism [1]. In concordance with previous reports, we detected a strong association between RPD and the *ARMS2* rs10490924 variant, one of the major AMD susceptibility polymorphisms [6,20-28]. The *ARMS2* gene encodes for the ARMS2 protein, which is an extracellular matrix protein surrounding choriocapillaris adjacent to Bruch's membrane (BrM), presumably contributing to BrM homeostasis [32,33]. Although RPD are frequently observed in AMD and their presence is associated with AMD progression [5-7], RPD also appear in other diseases related to BrM pathologies, such as Sorsby dystrophy and pseudoxanthoma elasticum [34,35]. These findings support the hypothesis that alterations in the BrM/RPE complex may be related to RPD formation.

Although genetic variants encoding for complement system components and regulators count as important risk factors for AMD and its progression [1,36-39], the associations of RPD with *CFH* variants are controversial [6,20-28,31]. Some studies have attributed the presence of *CFH* variants to an increased risk of RPD (CFH rs1061170 [22,28], *CFH* rs393955 [22], *CFH* rs2274700 [22]), whereas others have indicated that *CFH* rs1061170 (Y402H)—the major AMD risk polymorphism—is even associated with a lower incidence of RPD [20,24]. In contrast, some studies have found neither a positive nor negative association of *CFH* polymorphisms with RPD [21,23,26,31]. In this cohort, *CFH* rs800292 and *CFH* rs12144939 were associated with a decreased rate of RPD. Our results indicate the influence of *CFH* variants and the *ARMS2* variant on the presence of RPD.

In this study, further known AMD risk polymorphisms in *APOE*, *COL8A1*, *COL10A*, *GLI3*, and *SKIV2L* genes were observed to have significant associations with the presence of RPD. Of these polymorphisms, an association with variants in

Table 3. Univariate association of non-genetic risk factors with presence of RPD.

Tested Non-genetic risk factor	OR	95% CI	p-value
Age	1.13	1.12–1.15	<0.001
Gender	0.96	0.74–1.24	0.732
Smoking (never/ever)	1.09	0.84–1.42	0.517
Body Mass Index (BMI)	0.99	0.95–1.02	0.423
Hypertension (no/yes)	0.99	0.76–1.29	0.936
Diabetes (no/yes)	1.40	0.91–2.09	p=0.109
CVD (no/yes)	1.88	1.43–2.45	<0.001
Rheumatoid Arthritis (no/yes)	1.87	1.22–2.79	0.003
Thyroid Disease (no/yes)	1.01	0.70–1.41	0.973
Cancer (no/yes)	1.20	0.84–1.67	0.305
Migraine (no/yes)	0.90	0.55–1.41	0.664
History of Allergy (no/yes)	0.56	0.39–0.79	0.001
ASA intake (no/yes)	2.14	1.56–2.90	<0.001
NSAID intake (no/yes)	1.21	0.56–2.33	0.596
Corticosteroid intake (no/yes)	2.58	1.60–4.02	<0.001
Coumarine intake (no/yes)	1.63	0.94–2.66	0.065
Antiglaucomatous drops (no/yes)	1.34	0.71–2.35	0.330
Alcohol use (no/ regular)	0.68	0.51–0.93	0.014
Fruits Intake (almost never versus regular)	0.89	0.48–1.85	0.736
Vegetables Intake (almost never versus regular)	1.22	0.24–22.22	0.851
Fish Intake (almost never versus regular)	0.86	0.64–1.18	0.351
Red Meat Intake (almost never versus regular)	1.29	0.93–1.83	0.141
Physical exercise (never versus ≥3 times/week)	0.65	0.46–0.91	0.015
Past Sunlight exposure (<4h versus ≥8h/day)	2.30	1.60–3.25	<0.001

AMD: Age-related macular degeneration, ASA: Acetylsalicylic acid, CVD: cardiovascular disease, CI: Confidence Interval, NSAID: non-steroidal anti-inflammatory drugs, OR: Odds ratio

the *APOE* gene was evaluated previously by Puche et al., but no association was found [27]. Apolipoprotein E immunoactivity has been previously described in patients with RPD and soft drusen [16]. In this cohort, *APOE* rs2075650 was associated with a low risk of RPD. Further associations of RPD were observed with *COL8A1* and *COL10A*, both encoding for the chains of collagen types VIII and X; collagen type VIII was previously shown to be an important part of BrM and choroidal stroma [40,41]. Thus, associations of RPD with *COL8A* and *COL10A* might further support the involvement of BrM alterations in RPD pathogenesis. Altogether, the role of these variants remains to be evaluated carefully in larger cohorts.

In line with previous studies, we also observed a strong association between RPD and increasing age [4,23,26,28,31]. Moreover, the RPD rate was higher among patients with a history of smoking, rheumatoid arthritis, steroids, and past sunlight exposure. Smoking was previously reported as a risk factor for RPD [4,28]. In line with Wu et al. [23], the distribution of RPD was similar between women and men in this cohort, although several previous studies have reported otherwise [4,6,20,26,28,31]. Nevertheless, the ROC analysis demonstrated that the addition of nongenetic risk factors to a model consisting of age and genetic factors merely influences the discrimination ability between RPD and no RPD.

To date, RPD are increasingly accepted as an important risk factor for AMD progression [5-7], but the RPD pathogenesis is still not fully understood. Alteration of choroid-BrM-RPE is suggested as a contributor to RPD formation [18,34,42-46]. Impaired RPE might secrete proteins in an inverse fashion to the apical “subretinal” space instead of the sub-RPE, causing the accumulation of RPD [19]. The strong link of RPD with genetic variants affecting BrM and extracellular matrix remodeling supports the hypothesis that

Table 4. Variables selected based on model b) including all predictors.

Variables	Subset	OR	Lower 95% CI	Upper 95% CI	P- value
Age		1.16	1.14	1.19	3.53E-36
Gender	Male versus female	0.71	0.47	1.08	0.109
APOE rs2075650	GA versus AA	0.76	0.47	1.24	0.273
	GG versus AA	0.00	0.00	N/A	0.977
ARMS2 rs10490924	TG versus GG	2.21	1.44	3.38	0.0002
	TT versus GG	4.93	2.90	8.39	3.92E-09
CFH rs800292	GA versus GG	0.36	0.23	0.57	7.60E-06
	AA versus GG	0.68	0.26	1.78	0.43
CFH rs12144939	TG versus GG	0.24	0.13	0.42	6.28E-07
	TT versus GG	0.15	0.04	0.62	0.008
CFI rs10033900	TC versus CC	1.60	1.00	2.56	0.051
	TT versus CC	1.48	0.85	2.57	0.162
COL8A1 rs13081855	GT versus GG	1.55	0.98	2.47	0.064
	TT versus GG	0.00	0.00	N/A	0.984
CYP24A1 rs15706692	GA versus AA	0.73	0.49	1.10	0.131
	GG versus AA	1.32	0.75	2.34	0.340
LIPC rs10468017	CT versus CC	1.25	0.85	1.84	0.266
	TT versus CC	0.52	0.22	1.26	0.149
SKIV2L rs4296082	GA versus GG	0.52	0.30	0.90	0.018
	AA versus GG	0.14	0.02	1.32	0.086
TYR rs6213132	TC versus TT	0.81	0.52	1.26	0.354
	CC versus TT	0.58	0.34	0.98	0.041
Smoking	Never versus ever	1.43	0.95	2.15	0.083
Rheumatoid Arthritis	Yes versus no	2.35	1.30	4.27	0.005
Corticosteroids	Daily versus Non-daily	1.75	0.90	3.42	0.098
Antiglaucomatous Drugs	Daily versus Non-daily	2.10	0.98	4.49	0.055
Past Sunlight Exposure	≥8 h a day versus <8 h a day	1.62	0.97	2.72	0.068

CI: confidence interval, RPD: reticular pseudodrusen, OR: odds ratio

RPD formation is rather dependent on the choroid-BrM-RPE complex. This is further supported by proteomic findings in the aqueous humor of RPD patients showing upregulated extracellular matrix proteins similar to soft drusen [19]. Despite several similarities between RPD and AMD [13-19], lipid and immune cell composition of RPD and AMD seem to be slightly different [18,19]. Nevertheless, patients with no drusen and RPD have been shown to have a significant risk for development of both neovascularization and geographic atrophy over the years [47]. In addition, the results of this comprehensive study highlight that RPD and AMD share genetic pathways, even if the impact of each polymorphism might be different for soft drusen and RPD.

The strengths of this study include its large sample size and its prospective nature using multimodal imaging. In this study, we determined the presence of the RPD via OCT and NIR images, which is a great advantage for detecting RPD. Furthermore, the images were graded by two independent certified graders. The RPD detection rate in this EUGENDA cohort was 9.6%, which was slightly higher than population-based studies that have used only FP for RPD detection [4,28]. Nevertheless, the prevalence rates of RPD in early, intermediate, and nAMD in this cohort were comparable to those of a recent AREDS2 report (EUGENDA vs. AREDS2: early AMD, 7.7% vs. 6.0%; intermediate AMD, 18% vs. 26%; nAMD, 23% vs. 19%), whereas the RPD rate in GA was less than in the EUGENDA cohort (27% vs. 36%) [6]. An important limitation of this study is its case-control design;

the distribution of RPD might differ from population-based trials. With correction for the multivariable approach, we attempted to minimize confounding factors. Furthermore, the information obtained from the questionnaire is subjective and was not validated. An additional limitation is that only known genetic and nongenetic AMD risk factors were included in this study, and therefore, additional risk factors for RPD could not be detected. Larger studies may reveal further important factors associated with RPD.

In conclusion, our results suggest that RPD share common genetic pathways with AMD and are strongly linked to AMD, age, and *ARMS2* and *CFH* variants. Moreover, RPD and AMD share common nongenetic risk factors, such as smoking, but their influence seems to be modest. In light of these findings, integration of RPD in future AMD grading systems would help us understand the role of RPD in AMD.

APPENDIX 1. UNIVARIATE ASSOCIATION OF SNPS WITH PRESENCE OF RPD.

To access the data, click or select the words "Appendix 1." CI: Confidence Interval, RPD: Reticular Pseudodrusen, OR: Odds ratio, MAF: Minor allele frequency, MAF< 5% : CFI rs141853578, *CFB* rs4151667, *ABCA4* rs76157638, *TIMP3* rs9621532.

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