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Epidemiology of Reticular Pseudodrusen in Age-Related Macular Degeneration: The Rotterdam Study

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Purpose. Reticular pseudodrusen (RPD) are considered to be a distinct feature in AMD. Population studies have studied the epidemiology of RPD using standard color fundus photographs (CFP). However, recent studies have shown that RPD are better imaged using near-infrared (NIR) imaging. We studied the epidemiology of RPD in a large population-based study using NIR and CFP.

Methods. Participants aged 65+ years from the Rotterdam Study underwent ophthalmologic examination including NIR and CFP. Both images were graded for the presence of RPD and soft indistinct drusen (SID). Associations with demographic and environmental factors, 26 genetic variants, and total genetic risk score were analyzed using logistic regression analysis.

Results. Reticular pseudodrusen were detected in 137 (4.9%) of 2774 study participants; of these, 92.7% were detected with NIR imaging and 38% on CFP. Most eyes with RPD showed presence of SID, whereas other drusen types coincided less frequently. Reticular pseudodrusen were significantly associated with age (odds ratio [OR] 1.21, 95% Confidence Interval [CI] 1.17–1.24) and female sex (OR 2.10, 95% CI 1.41–3.13). Environmental factors did not show a significant association with RPD. Major AMD risk variants were significantly associated with RPD and SID; however, *ARMS2*, *C3*, and *VEGFA* were more associated with RPD (RPD vs. SID $P < 0.05$). Total genetic risk score did not differ significantly ($P = 0.88$).

Conclusion. Detection of RPD was better with NIR imaging than on CFP in a population-based setting. Presence of RPD often coincided with presence of SID; however, they showed quantitative differences in genetic risk profile.

Keywords: reticular pseudodrusen, near-infrared imaging, epidemiology, risk factors, genetics

Reticular pseudodrusen (RPD), also known as subretinal drusenoid deposits (SDD), are depositions located in the subretinal space between the outer segments of the photoreceptors and the RPE.[1–3] Reticular pseudodrusen carry a much higher risk of developing end-stage AMD than other AMD lesions, such as soft indistinct drusen (SID).[4–7] Reticular pseudodrusen were first described in 1990 and it was already then pointed out that RPD are better visualized using blue reflectance photography than regular color fundus photographs (CFP).[8] Reticular pseudodrusen can also be detected using other imaging modalities. A comparative study between various image modalities showed that near-infrared (NIR) imaging is one of the image modalities that has the highest sensitivity for RPD detection,[9] and that sensitivity of RPD detected only on CFP can be as low as 36%.[10]

Several risk factors have been identified for RPD, which include age, smoking, higher body mass index (BMI), female predominance, and genetic risk factors, including *CFH*(Y402H) and *ARMS2*(A69S).[4,6,7,11–13] Previous studies have shown that RPD were present in 60% to 90% of patients with late AMD.[14–16] Boddu et al.[13] studied risk profiles of RPD versus large soft drusen in a small clinic-based study. These researchers could not find many significant differences between these patient groups; however, individuals with RPD were older and more often female.

Because the population-based studies on RPD based their grading solely on CFP, and the clinic-based studies were carried out only in small groups, risk estimates are likely to be imprecise.[11] Improved detection of RPD in a large population-based study may provide more accurate prevalence figures, and could enhance risk profiling for RPD.

In this study, we aimed to investigate the epidemiology of RPD using CFP and NIR images and compare this with SID in a large, unselected population. We have chosen to use the term RPD instead of SDD, because this is more commonly used in clinical-based articles; however, where RPD is written SDD can be read.

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Table 1. Prevalence per AMD Severity Grade According to the Rotterdam Classification Based on CFP Only Versus CFP and NIR Imaging

Grade	Definition	Grading CFP, % $n = 2774$	Grading CFP + NIR, % $n = 2774$
0	No signs of AMD at all **OR** hard drusen (<63 μm) only	42.70	42.30
1	Soft distinct drusen (≥63 μm) only **OR** pigmentary abnormalities only	36.10	35.00
2	SID (≥25 μm)/reticular drusen only **OR** soft distinct drusen (≥63 μm) **AND** pigmentary abnormalities	13.60	14.50
3	SID (≥125 μm)/reticular drusen **AND** pigmentary abnormalities	5.40	6.00
4	Atrophic, neovascular, or mixed AMD	2.20	2.20

Methods

Study Population

The Rotterdam Study is a prospective population-based cohort study that focuses on chronic ophthalmologic, neurologic, cardiovascular, and locomotor diseases in middle-aged and elderly subjects living in Ommoord, a suburb of Rotterdam. The aims and design of the Rotterdam Study have been described elaborately elsewhere.[17] In brief, the study started in 1989, and since then, every 2 to 4 years, follow-up examinations were performed. During these follow-up examinations, new techniques and devices were implied, such as the Heidelberg Retina Angiograph 2, a scanning laser ophthalmoscope. The Rotterdam Study was approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare, and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)." All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

For the current cross-sectional analysis, we included 3108 participants from the last examination round from two independent cohorts of the Rotterdam Study: 1542 participants from the Rotterdam Study I (RS-I), aged 70 years and older, and 1566 participants from the Rotterdam Study II (RS-II), aged 65 years and older. Participants were excluded from the analyses if they had ungradable CFP ($n = 17$) or ungradable NIR images ($n = 68$). Since the Heidelberg Retina Angiograph 2 was operational a few weeks after the start of the examination round, an additional 266 participants were excluded from the study due to absence of NIR images. From these two cohorts, 2774 participants with gradable CFP and gradable NIR imaging were eligible for analysis.

Grading of AMD, SID, and RPD

All eligible participants underwent 35° digital CFP of the macula (Topcon TRC-50EX; Topcon Optical Company, Tokyo, Japan, with a Sony DXC-950P digital camera, 0.44 megapixel; Sony Corporation, Tokyo, Japan) after pharmacologic mydriasis. Next, NIR images ($\lambda = 820$ nm) $30° \times 30°$ of the macula, were taken with a Heidelberg Retina Angiograph 2 (Heidelberg Engineering, Heidelberg, Germany).

Color fundus photographs were graded for presence of all AMD-related features according to the Wisconsin Age-Related Maculopathy Grading[18] and Rotterdam classification (for definition see Table 1), a modified International Classification System, using the standard grading grid for AMD, equal to the Early Treatment Diabetic Retinopathy Study grading grid (central circle 1000 μm, inner circle 3000 μm, and outer circle 6000 μm in diameter).[19,20] In short, the Rotterdam classification consists of five grades with grade 0 defined as no AMD, grade 1 as preliminary early AMD, grades 2 and 3 together as early AMD, and grade 4 as late AMD. Soft indistinct drusen were defined as yellow lesions with indistinct borders and ≥125 μm in size. These type of soft drusen are associated with a higher risk of developing advanced AMD compared with soft distinct drusen.[20] Reticular pseudodrusen were defined as indistinct yellowish lesions interlacing in networks 125 to 250 μm in width.[18,19]

Near-infrared images of the macula were graded based on the presence of RPD, detectable as groups of hyporeflectant lesions against a mildly hyperreflectant background in regular patterns.[9,10,21,22] Because SID and other drusen types are less visible on NIR imaging, these lesions were not graded on NIR, only on CFP.

On CFP, RPD and/or SID were graded inside and outside the standard grading grid. On NIR, RPD was graded if present on the image, this equals grading inside and outside the grading grid on CFP.

All images were graded by trained graders, while being masked for the grading of the other image modality, under the supervision of senior retinal specialists (PTVMdJ, JRV, CCWK). Between-grader comparisons were assessed. For drusen grading on CFP, the weighted κ values ranged from 0.60 for hard drusen to 0.82 for soft distinct drusen. For RPD grading on CFP, the κ value was 0.72. For NIR imaging, this κ value was 0.84. The eyes of each participant were graded and classified separately, and the eye with the more severe grade was used to classify the person.

Genotyping and Selection of Genetic Variables

Genomic DNA was extracted from peripheral blood leukocytes. All study participants in the RS-I were genotyped with the Illumina Infinium II HumanHap550 array or Taqman assays (Applied Biosystems, Foster City, CA, USA). Study participants from the RS-II were genotyped with the Illumina Human610-Quad array. HapMap CEU data (release #22) was used for imputation. Genetic variables associated with AMD were selected based on previous publications.[23,24]

Risk Score Three Continent AMD Consortium Prediction Model

The Three Continent AMD Consortium (3CC) developed a validated prediction model including a total risk score based on 31 variables; 26 genetic variants associated with AMD, age, sex, smoking, BMI, and AMD phenotype.[23] The total risk score was based on the sum of the β coefficients from a Cox proportional hazard analysis, which included all the selected variables. In this analysis, we used the total genetic risk score, which is a variant of the total risk score and has been calculated using only the β coefficients from the genetic variables of the 3CC prediction model.

Assessment of Nongenetic Variables

Information on the history of diabetes mellitus, education level, and cigarette smoking were derived from computerized

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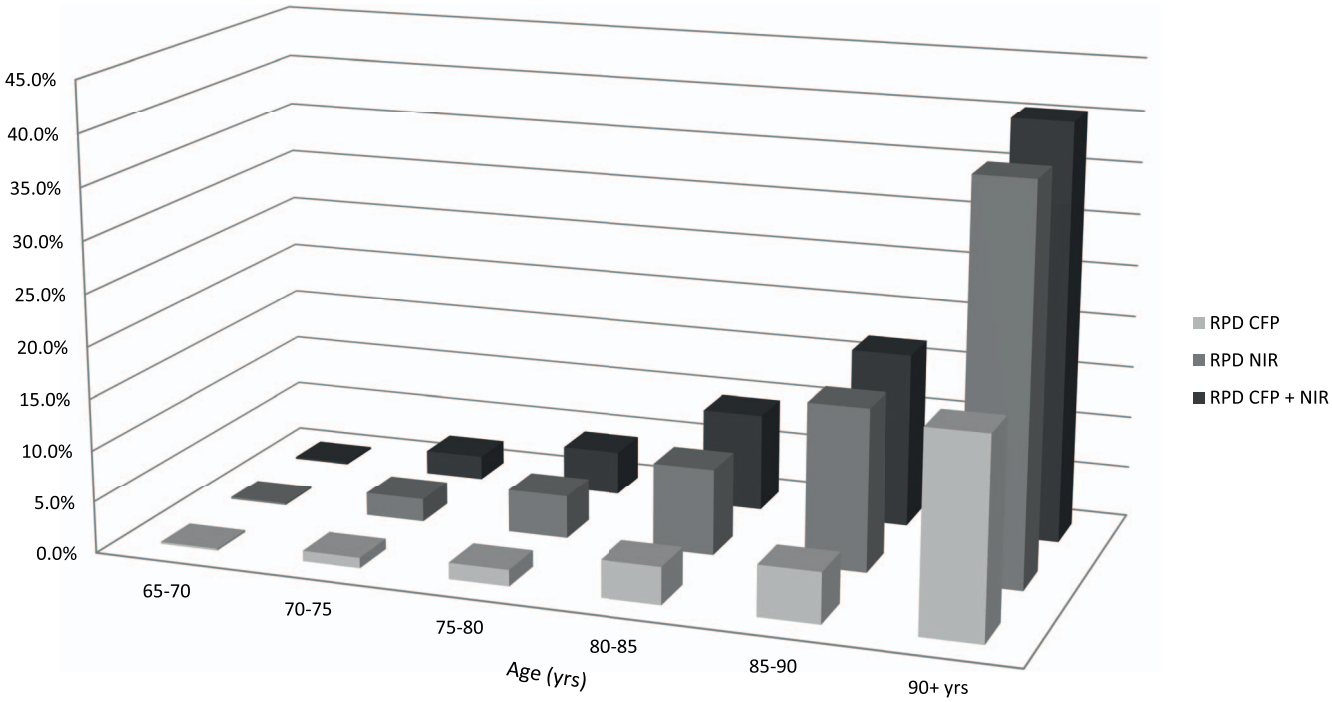

Figure 1. Reticular pseudodrusen detection with CFP and NIR. Frequency of RPD was plotted per age category for the different imaging types: CFP, NIR, and both imaging types combined. *x*-axis, age category in years; *y*-axis, percentages; yrs, years.

questionnaires administered during home interviews. Smoking was categorized in never, past, and current smokers. Blood pressure, systolic and diastolic, was calculated as the average of two consecutive measurements, using a random-zero mercury sphygmomanometer. Hypertension was defined as having a systolic blood pressure $\geq$140 mm Hg or a diastolic blood pressure $\geq$90 mm Hg or using antihypertensive medication at baseline. Cholesterol, high-density lipoprotein (HDL), and triglycerides were measured at baseline by the Central Clinical Chemical Laboratory of the Erasmus University Medical Center. A subgroup of measurements was carried out in the laboratory of the Department of Epidemiology and Biostatistics, Erasmus University Medical School. BMI was calculated as weight in kilograms divided by height squared in meters. Waist circumference and hip circumference were measured in centimeters.

Statistical Analysis

Prevalence of RPD as a function of age was calculated per image modality. The prevalence of early AMD based on CFP diagnosis of AMD lesions was compared with the prevalence of early AMD based on both NIR and CFP grading.

We investigated the association with demographic, genetic, and environmental variables using various outcomes: SID versus no drusen, RPD versus no drusen, and RPD versus SID. Outcomes were binary and the association was assessed with logistic regression analysis. Total genetic risk scores were calculated for each individual using only the genetic variables from the 3CC prediction model. Risk scores were grouped and stratified for RPD, SID, and no RPD/SID; strata were compared using χ^2 statistic test. Analyses investigating concurrence of drusen types were eye-based, all other analyses were person-based. All statistical analyses were performed using SPSS version 21 (SPSS IBM, New York, NY, USA).

Results

Reticular pseudodrusen were detected in 137 (4.9%) of 2774 study participants, of whom 52 (38.0%) were identified on CFP and 127 (92.7%) on NIR imaging (numbers do not add up to 100% due to overlap). Reticular pseudodrusen were mostly present from age 70 years onward (Fig. 1) and were bilaterally present in 69.3%. Only one person had been diagnosed with RPD at the age of 64.0 years, and RPD were visible on CFP as well as on NIR imaging. Frequency of RPD increased per age category; however, the steep rise in those aged 90+ years was most pronounced when RPD were diagnosed on NIR imaging.

In the Rotterdam classification system, RPD are part of the criteria for staging. To investigate how much the improved detection of RPD by NIR imaging influenced AMD prevalence, we classified each person by using the two gradings: one solely based on CFP and the other based on CFP and NIR imaging. Inclusion of the NIR imaging increased the prevalence of early AMD (stages 2 and 3) from 19.0% to 20.5% (Table 1).

We then investigated the number and type of other drusen present in eyes with RPD ($n = 232$) (Fig. 2, Supplementary Fig. S1). We performed this analysis for drusen within and outside the grid. Most eyes with RPD had SID, both within and outside the grid. These eyes also presented with other types of drusen, but they were less frequent compared with SID. Of eyes with RPD, only 14 eyes (6.0% of eyes with RPD) did not have any type of soft drusen, and 9 eyes (3.9% of eyes with RPD) had no other type of drusen at all. Of these, the contralateral eye of two persons had drusen. Only five persons with RPD had no drusen at all, not even in the contralateral eye.

Table 2 shows the frequency of characteristics between persons with SID, RPD, and those without SID or RPD. Table 3 shows the associations of these characteristics with SID only, with RPD, and with RPD versus SID. The odds ratio (OR) represents the risk for a certain parameter on a drusen type (RPD or SID) in reference to the other drusen type or to

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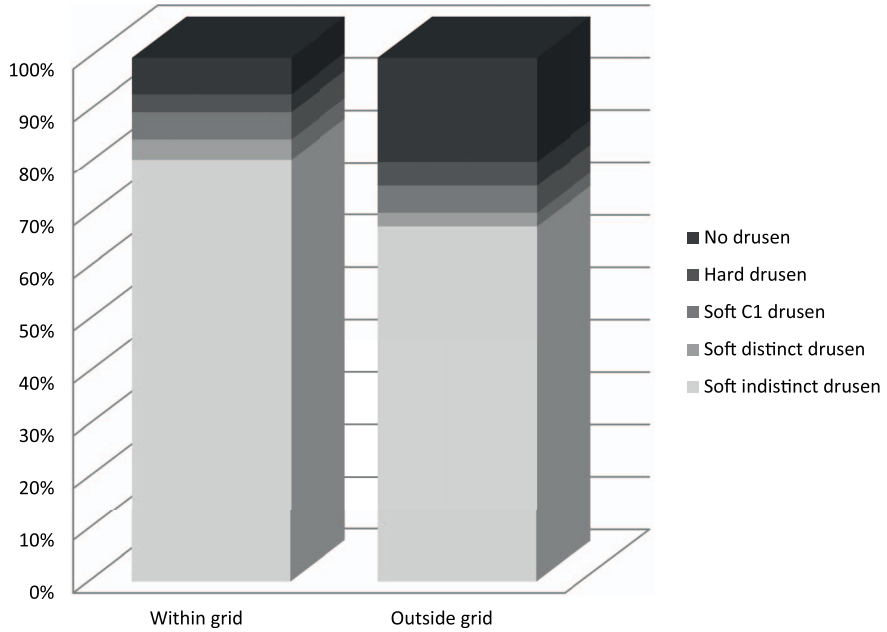

Figure 2. Frequency of other drusen types in eyes with RPD. Other drusen types coinciding with RPD. Grading based on most severe drusen type, besides RPD, on CFP, according to the Wisconsin Age-Related Maculopathy Grading. Frequencies of other drusen types were stratified for drusen presence within and outside the grading grid.

absence of RPD/SID. Both SID and RPD were associated with age (OR 1.09, 95% confidence interval [CI] 1.06–1.11 and OR 1.21, 95% CI 1.17–1.24), respectively. For SID there was no association with sex (OR 1.02, 95% CI 0.77–1.34), whereas occurrence of RPD was strongly associated with female sex (OR 2.10, 95% CI 1.41–3.13). High-density lipoprotein cholesterol (OR 1.83, 95% CI 1.30–2.59) and triglycerides (OR 0.66, 95% CI 0.51–0.86) in serum were both significantly associated with SID, but not with RPD. No other demographic or environmental risk factors were significantly associated with SID or RPD. Table 4 shows the association of RPD and SID with genetic factors. Genetic variants in the *CFH* gene (rs1061170, rs12144939, rs800292), the *ARMS2* gene (rs10490924), and the *C2/CFB* gene (rs641153) were significantly associated with both drusen types. A variant in the *IER3/DDR1* gene (OR 2.54, 95% CI 1.32–4.86, for rs3130783, GG vs. AA) was associated only with SID, whereas a variant in the *C3* gene (OR 1.61, 95% CI 1.08–2.40, for rs22130199, CG vs. CC) was associated only with RPD. Two variants were significantly more associated with RPD than SID. These included the variant in the *ARMS2* gene (OR 2.48, 95% CI 1.001–6.17, for rs10490924, TT vs. GG) and a variant in the *VEGFA* gene (OR 2.00, 95% CI 1.05–3.8, for rs943080, CT vs. CC). Genetic risk score frequencies did not differ significantly between SID and RPD ($P = 0.88$, χ^2 test) (Supplementary Table S1).

Table 2. Frequency of Clinical Parameters Stratified for Drusen Type

Variables	No RPD/SID $n = 2416$	SID $n = 221$	RPD $n = 137$
Age, y (SD)	75.0 (5.6)	77.8 (6.2)	82.1 (6.2)
Sex, % females	54.6	54.8	71.5
Education, y, %			
<12	26.8	23.3	29.1
≥12	73.2	76.7	70.9
Smoking, %			
Never	31.2	32.1	37.0
Past	57.0	57.0	48.9
Current	11.9	10.9	14.1
Diabetes mellitus, %	13.0	12.4	13.0
Hypertension, %	86.0	86.8	89.7
Systolic blood pressure, mm Hg (SD)	152.3 (21.4)	154.0 (21.4)	154.0 (23.7)
Diastolic blood pressure, mm Hg (SD)	85.2 (11.0)	85.9 (11.1)	85.3 (13.3)
Cholesterol, mM (SD)	5.4 (1.1)	5.4 (0.9)	5.3 (1.1)
HDL cholesterol, mM (SD)	1.5 (0.4)	1.6 (0.4)	1.5 (0.4)
Triglycerides, mM (SD)	1.4 (0.7)	1.3 (0.6)	1.4 (0.8)
BMI, kg/m^2	27.5 (4.1)	27.1 (4.1)	27.2 (3.9)
Waist circumference, cm (SD)	93.7 (12.0)	92.0 (11.7)	91.5 (11.0)
Hip circumference, cm (SD)	102.9 (8.2)	102.6 (9.3)	102.6 (8.7)

Discussion

In this cross-sectional analysis of a population-based study, we showed that NIR imaging is superior to traditional CFP in the detection of RPD. We also found that RPD generally coincide with soft drusen, and were seldom seen as the only AMD feature. However, the AMD risk profile of RPD showed quantitative differences with that of SID. Those with RPD were more likely to be women, have older age, and carry risk variants in the *C3*, *ARMS2*, and *VEGFA* genes.

Our study confirms that NIR imaging is preferred over the use of CFP for detection of RPD.[11,13,21,25] Of all persons diagnosed with RPD, 62.7% were not visible on CFP, only on NIR. By contrast, 7% of those with RPD were diagnosed on CFP and failed to be detected on NIR. This implies that multimodal imaging for complete AMD grading is better than using single imaging devices. Suzuki et al.[26] described three subtypes of RPD: two were better visualized by NIR imaging, and one, the ribbon-like subtype, was more easily spotted on CFP. Indeed, we acknowledge that diagnosis of RPD on CFP is mostly based

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Table 3. Comparison of Clinical Parameters Among Drusen Types

Variables	SID vs. No RPD/SID OR (95% CI) Age and Sex Adjusted	RPD vs. No RPD/SID OR (95% CI) Age and Sex Adjusted	RPD vs. SID OR (95% CI) Age and Sex Adjusted
Age*	1.09 (1.06–1.11)	1.21 (1.17–1.24)	1.12 (1.07–1.16)
Sex†			
Male	1	1	1
Female	1.02 (0.77–1.34)	2.10 (1.41–3.13)	2.16 (1.34–3.49)
Education, y			
<12	1	1	1
≥12	1.31 (0.93–1.83)	1.24 (0.82–1.89)	0.90 (0.53–1.52)
Smoking			
Never	1	1	1
Past	0.98 (0.70–1.35)	1.06 (0.69–1.62)	0.97 (0.57–1.65)
Current	0.98 (0.60–1.62)	1.77 (0.97–3.23)	1.54 (0.71–3.32)
Diabetes mellitus			
No	1	1	1
Yes	0.96 (0.59–1.55)	1.05 (0.57–1.94)	1.41 (0.64–3.09)
Hypertension			
No	1	1	1
Yes	0.88 (0.58–1.33)	0.89 (0.49–1.61)	0.91 (0.44–1.89)
Cholesterol, mM	1.06 (0.93–1.21)	0.89 (0.75–1.06)	0.78 (0.61–1.00)
HDL cholesterol, mM	1.83 (1.30–2.59)	1.28 (0.80–2.05)	0.68 (0.39–1.20)
Triglycerides, mM	0.66 (0.51–0.86)	0.95 (0.70–1.28)	1.39 (0.97–1.98)
BMI, kg/m^2			
≤25	1	1	1
>25	0.83 (0.61–1.11)	1.13 (0.75–1.70)	1.50 (0.90–2.48)
Waist circumference, cm			
≤ 90	1	1	1
> 90	0.82 (0.61–1.11)	1.21 (0.82–1.80)	1.51 (0.93–2.47)
Hip circumference, cm			
≤ 100	1	1	1
> 100	0.87 (0.65–1.15)	1.18 (0.81–1.72)	1.29 (0.81–2.06)

* Adjusted for sex.
† Adjusted for age.

on recognition of the pattern of this feature. Aside from NIR and CFP, other image modalities also can reveal RPD, such as fundus autofluorescence, optical coherence tomography (OCT), confocal blue reflectance, and indocyanine green angiography. Whether incorporation of all these methods further improves detection of RPD is questionable. A recent study assessed the accuracy of RPD detection using all these modalities, and found that NIR and OCT have the highest sensitivity, both 94.6%.[9]

Using NIR imaging increased our estimate of the overall prevalence of RPD to 4.9%. All previously reported population estimates of RPD were based on CFP only, and were therefore lower; the Beaver Dam Eye Study reported an overall prevalence of 0.7% in a population aged 45+ years,[6] the Blue Mountain Eye Study reported an overall prevalence of 1.95% in a population aged 50+ years,[12] and the Melbourne Collaborative Cohort Study recently reported an overall prevalence of 0.41% in a population 48 to 86 years of age.[4] In our study, RPD were bilateral in 69% of cases, whereas other population-based cohorts reported a slightly lower frequency of bilaterality, 51% to 63%, respectively.[4,6,12] The higher rates in our study could be due to the improved detection of RPD using NIR.

We identified several risk factors for RPD and SID. For RPD, demographic risk factors were older age and female sex, factors that have been reported by several studies.[6,7,11–13,27] We did not find any statistically significant environmental risk factors, although the relationship with current smoking was suggestive. Other studies reported risks of RPD for hypertension, lower income, lower education, higher BMI, angina pectoris, HDL cholesterol, and triglycerides, but we and many others could not confirm these findings.[6,7,11–13,21,27] Associations that showed a preference for SID were increased HDL cholesterol, and decreased triglycerides. These trends were observed previously for late AMD, and have not been explained thus far.[20] With respect to AMD risk variants, those in the *CFH*, *C2/FB*, and *ARMS2* genes were significantly associated with both RPD and SID in this study; *C3,* however, was associated only with RPD. The lack of association with other risk variants may be due to our limited statistical power to find minor associations. Remarkably, *ARMS2* appeared to have a strong predilection for RPD. Homozygous carriers of the *ARMS2* risk variant were twice as likely to have RPD than SID. We did not find this differential distribution for the *CFH* or *C2/FB* risk variants. Previous reports found stronger associations with *ARMS2* than *CFH*.[27,28] Another gene showing a tendency for RPD was *VEGFA*. Although this gene was not significantly associated with either phenotype, it showed a significant risk difference between RPD and SID.

Eyes featuring RPD without any type of drusen were rare in our study. We evaluated the risk profiles of these participants

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Table 4. Genetic Risks Among Drusen Types

Genes	SID vs. No RPD/SID OR (95% CI) Age and Sex Adjusted	RPD vs. No RPD/SID OR (95% CI) Age and Sex Adjusted	RPD vs. SID OR (95% CI) Age and Sex Adjusted
CFH (Y402H) rs1061170			
TT	1	1	1
CT	1.65 (1.17–2.34)	1.45 (0.95–2.23)	0.91 (0.52–1.58)
CC	3.84 (2.50–5.90)	2.78 (1.57–4.93)	0.83 (0.42–1.66)
CFH rs12144939			
GG	1	1	1
GT/TT	0.34 (0.23–0.51)	0.28 (0.17–0.47)	0.89 (0.47–1.71)
CFH rs800292			
GG	1	1	1
GA	0.54 (0.39–0.76)	0.55 (0.36–0.84)	0.88 (0.51–1.50)
AA	0.38 (0.17–0.83)	0.30 (0.11–0.87)	0.96 (0.24–3.74)
ARMS2 (A69S) rs10490924			
GG	1	1	1
GT	1.81 (1.33–2.47)	2.41 (1.60–3.62)	1.24 (0.75–2.05)
TT	3.25 (1.70–6.20)	7.85 (3.75–16.45)	2.48 (1.001–6.17)
C2/CFB (L9H) rs4151667			
TT	1	1	1
TA/AA	0.65 (0.35–1.19)	0.43 (0.17–1.10)	0.78 (0.25–2.44)
C2/CFB (R32Q) rs641153			
GG	1	1	1
GA/AA	0.56 (0.34–0.90)	0.38 (0.19–0.75)	0.66 (0.29–1.54)
C3 (R102G) rs2230199			
CC	1	1	1
CG	1.19 (0.86–1.64)	1.61 (1.08–2.40)	1.32 (0.80–2.19)
GG	1.28 (0.64–2.56)	1.81 (0.80–4.13)	1.18 (0.41–3.40)
C3 rs433594			
GG	1	1	1
GA	0.80 (0.58–1.12)	0.84 (0.55–1.28)	1.16 (0.68–1.96)
AA	0.83 (0.52–1.32)	0.94 (0.51–1.71)	1.01 (0.47–2.16)
CFI rs10033900			
CC	1	1	1
CT	1.27 (0.87–1.85)	0.82 (0.52–1.30)	0.68 (0.38–1.24)
TT	1.33 (0.86–2.06)	0.90 (0.52–1.54)	0.62 (0.31–1.24)
LPL rs256			
CC	1	1	1
CT/TT	0.88 (0.62–1.25)	0.75 (0.48–1.19)	0.83 (0.46–1.47)
LIPC rs12912415			
AA	1	1	1
AG/GG	0.90 (0.64–1.26)	0.75 (0.48–1.17)	0.98 (0.56–1.71)
MYRIP rs2679798			
AA	1	1	1
AG	1.04 (0.73–1.49)	1.42 (0.88–2.27)	1.34 (0.75–2.42)
GG	1.23 (0.81–1.87)	1.36 (0.77–2.39)	1.22 (0.61–2.47)
SKIV2L rs429608			
GG	1	1	1
GA/AA	0.57 (0.38–0.84)	0.55 (0.33–0.91)	1.01 (0.53–1.92)
ABAC1 rs1883025			
CC	1	1	1
CT	0.78 (0.56–1.09)	1.00 (0.67–1.50)	1.03 (0.61–1.74)
TT	1.09 (0.61–1.94)	0.53 (0.19–1.53)	0.38 (0.11–1.28)
CETP rs3764261			
CC	1	1	1
CA	1.27 (0.92–1.76)	1.15 (0.76–1.73)	1.06 (0.63–1.79)
AA	1.40 (0.84–2.32)	1.02 (0.50–2.07)	0.68 (0.29–1.63)

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Table 4. Continued

Genes	SID vs. No RPD/SID OR (95% CI) Age and Sex Adjusted	RPD vs. No RPD/SID OR (95% CI) Age and Sex Adjusted	RPD vs. SID OR (95% CI) Age and Sex Adjusted
TIMP3 rs5749482			
GG	1	1	1
CG/CC	0.87 (0.60–1.27)	0.62 (0.37–1.04)	1.17 (0.40–3.40)
VEGFA rs943080			
CC	1	1	1
TC	0.81 (0.56–1.17)	1.34 (0.79–2.24)	2.00 (1.05–3.81)
TT	1.09 (0.72–1.65)	1.51 (0.84–2.73)	1.50 (0.73–3.08)
COL8A1 rs13081855			
GG	1	1	1
GT/TT	1.15 (0.78–1.68)	0.90 (0.53–1.52)	0.85 (0.45–1.59)
TNFRSF10A rs13278062			
TT	1	1	1
GT	0.92 (0.65–1.30)	0.73 (0.46–1.14)	0.89 (0.51–1.57)
GG	0.72 (0.46–1.12)	0.77 (0.45–1.31)	1.11 (0.57–2.18)
FRK/COL10A1 rs3812111			
TT	1	1	1
AT	0.93 (0.68–1.29)	0.92 (0.61–1.39)	0.96 (0.57–1.60)
AA	0.86 (0.52–1.44)	0.53 (0.24–1.15)	0.71 (0.28–1.79)
SLC16A8 rs8135665			
CC	1	1	1
CT	1.17 (0.85–1.61)	0.83 (0.54–1.28)	0.73 (0.43–1.24)
TT	1.31 (0.61–2.82)	0.74 (0.21–2.63)	0.74 (0.17–3.18)
ADAMTS9 rs6795735			
CC	1	1	1
TC	0.96 (0.68–1.36)	1.03 (0.66–1.61)	1.10 (0.63–1.92)
TT	1.34 (0.88–2.06)	1.35 (0.77–2.37)	0.90 (0.45–1.80)
TGFBR1 rs334353			
TT	1	1	1
GT	0.92 (0.66–1.27)	1.09 (0.72–1.64)	1.29 (0.76–2.18)
GG	1.03 (0.55–1.94)	0.62 (0.24–1.63)	0.61 (0.20–1.87)
RAD51B rs8017304			
AA	1	1	1
AG	0.82 (0.59–1.13)	0.85 (0.56–1.29)	1.26 (0.74–2.14)
GG	0.63 (0.38–1.03)	0.85 (0.46–1.56)	1.46 (0.67–3.19)
IER3/DDR1 rs3130783			
AA	1	1	1
AG	0.85 (0.61–1.19)	0.83 (0.54–1.28)	0.95 (0.55–1.64)
GG	2.54 (1.32–4.86)	1.48 (0.49–4.51)	0.34 (0.10–1.20)
B3GALTL rs9542236			
TT	1	1	1
CT	0.77 (0.55–1.09)	1.11 (0.70–1.74)	1.57 (0.89–2.76)
CC	0.92 (0.60–1.39)	1.02 (0.58–1.83)	1.49 (0.73–3.04)

($n = 5$), and these were not remarkably different from the entire group of RPD (Tables 2, 3). Noteworthy, none of the RPD in these participants were observed on CFP, only on NIR imaging. Reticular pseudodrusen without any type of drusen may represent other phenotypes than AMD; in literature, RPD have been reported to accompany Sorsby fundus dystrophy, pseudoxanthoma elasticum, acquired vitelliform lesions, and systemic disorders, such as vitamin A deficiency, cardiovascular disease, and complement-mediated IgA nephropathy.[28–33] In our study, these five patients showed no other retinal pathology aside from RPD. A focus on unravelling the etiology of isolated RPD may help shed light on overarching molecular mechanisms in retinal disease.

Current insights into RPD pathogenesis are still highly limited, but a vascular etiology has been suggested.[27,28] The genetic predilections in our study may point toward this hypothesis. *ARMS2* was found to be expressed in the ellipsoid layer of the photoreceptors and in the intercapillary pillars of the choroid.[34,35] The first is close to the location of RPD,[34] and the latter may help explain the vascular hypothesis.[35] Another hint in this direction is the higher susceptibility of *VEGFA* and *ARMS2* for neovascular AMD,[24] although RPD do not preferentially accompany this AMD subtype.[4,6,7,36] Further-

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more, the genes *CFH*, *C3*, and *VEGFA* have been associated with cardiovascular and coronary artery disease.[27,28] Morphologic changes in eyes with RPD suggest that these lesions follow the pattern of the watershed zones of the choroid, and correspond with local thinning of this layer.[37] Spaide[38] found that choroidal thinning is even more pronounced when RPD regress and photoreceptors shorten. A recent study of retinal imaging with adaptive optics showed that photoreceptor changes precede RPD regression.[39] Several histopathologic studies have studied the molecular content of RPD and SID and found significant overlap: both contain unesterified cholesterol, complement factor H, apolipoprotein E, and vitronectin.[16,40] There are also remarkable differences: RPD lack immunoreactivity for photoreceptors, Müller cells, and RPE marker proteins, and have only low concentrations of esterified cholesterol and other neutral lipids.[16,40] Why the deposit in RPD is located above rather than below the RPE cell is unclear. Several hypotheses have been made. Rudolf et al.[41] suggested that loss of RPE cell polarity may lead to deposition of unphagocytized photoreceptor outer segments above rather than below the RPE cell. Curcio et al.[16] suggest that perturbation of cholesterol homeostasis and the lipid transfer between the RPE cell and the photoreceptor cell in the context of an outer retinal lipid-recycling program, could explain the formation of these deposits. Another hypothesis may be that shortening of the RPE villi[42] jeopardize close contact with photoreceptor outer segments, and may hamper uptake of shed discs from the photoreceptor by the RPE cell. In AMD, the formation of RPD appears to follow that of soft drusen, because the concurrence of these lesions carries a higher risk of progression to late AMD.[4,6,20] How the associated genes, the choroidal anatomic changes, and formation of drusenoid material above the RPE are related remains an intriguing question for future studies.

To our knowledge, this is the first population-based study studying RPD using another imaging device aside from CFP. Other strengths include the large sample size of unselected persons, the study of RPD within the context of other early AMD features, and the comparison of risk profiles of RPD versus SID. Other reports did not specify the frequency of concurrent AMD lesions in eyes with RPD. Furthermore, we analyzed a much more comprehensive set of demographic, environmental, and genetic risk factors for RPD than previous population-based and clinical studies, which led to new insights into the genetic background of RPD. A limitation of our study is the lack of follow-up data with NIR imaging, hampering the study of RPD incidence and progression. This needs to be addressed in prospective studies.

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