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Genetics of reticular pseudodrusen in age-related macular degeneration

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Abstract

Reticular pseudodrusen (RPD) are subretinal deposits and when observed with age-related macular degeneration (AMD) form a distinct phenotype, often associated with late-stage disease. To date, RPD genetic risk-associations overlap six well-established AMD-risk regions. Determining RPD-specific underlying genetic causes by utilising adequate imaging methods should improve our understanding of the pathophysiology of RPD.

Keywords: Reticular pseudodrusen, age-related macular degeneration, genetic risk factors, multi-modal imaging, genome-wide association studies

Age-related macular degeneration (AMD) is a disorder of the central retina (macula), resulting in the death of retinal pigment epithelium (RPE) and photoreceptors that, in turn, often leads to vision loss. Lipid-rich deposits ('drusen') are a hallmark of AMD. These conventional drusen are seen at the macula in the early and late stages of disease, underneath the RPE. More recently, with the benefit of novel **multimodal imaging (MMI)** (see Glossary) techniques, such as the use

of **fundus autofluorescence (FAF**), **infrared reflectance (IR)** and **optical coherence tomography (OCT),** a previously considered rare deposit, above the RPE, called reticular pseudodrusen (RPD) or subretinal drusenoid deposits, have been seen more commonly. They are now the subject of increased research interest due to i) their distinct appearance and location in the outer-retina when compared to conventional drusen, ii) their newly recognized high prevalence in AMD, and iii) their increase in prevalence in later vision threatening stages of AMD [1, 2]. In reviewing current literature, it appears that RPD are present in around a quarter to a third of those with the earlier stages of AMD and in over half of those with the late stages of AMD [3]. Despite their prevalence and association with the later stages of disease very little is known about their aetiology and why some individuals with AMD develop these deposits and others do not. Increasing our understanding of underlying pathogenic pathways, including genetic risk variants that predispose to the development of RPD, is crucial as we work towards treatments to prevent vision loss in AMD which will benefit from targeting of specific subtypes.

Uncovering any genetic associations with RPD, in the context of AMD, have to date, been significantly hampered by the lack of sufficient imaging available on AMD cohorts. There has been some attempt to search for a distinct genetic risk profile underlying RPD, in AMD cohorts. However, to obtain large enough AMD data sets to analyse, many genetic studies have been performed on population-based studies, or case cohort studies, which have only had colour fundus photographs (CFP) available to determine the presence or absence of RPD. However, we now know that CFP grossly underestimate RPD, often by at least 50%, when compared to other imaging modalities studied that have MMI available [4, 5] (Box 1, Figure I).

With the advances in MMI, many new insights into the pathological features that define AMD have been possible. Whilst RPD have distinct patterns seen on infra-red and auto-fluorescent imaging, their subretinal location above their RPE, can be discerned on OCT imaging. To date, however there is no consensus on the number of distinct lesions required to define an eye as having RPD or not. For example, some studies on RPD only require one RPD to be identified in an eye with RPD, whilst others require at least a minimal number of lesions (5 or more) to be present. Many AMD cases collected, especially for the large genetic cohort studies come from late-stage AMD clinics, where the presence of RPD is often difficult to determine due to the

extensive AMD pathology. Thus, trying to determine if there is any distinct genetic association with RPD in AMD remains challenging, at the initial stage of correctly identifying the phenotype. Additionally, prior genetic studies were often not ideal due to the imbalance in cases, with early or late AMD between groups with and without RPD as well as the lack of adjustment for other risk factors such as sex and age.

Despite these limitations, studies have been undertaken to investigate genetic associations with RPD, but only focusing on select AMD-associated risk loci (from 2 to 26 SNPs) [2, 4-13]. Of 11 studies that investigated the genetic risk of developing RPD, the definitions of what determined an eye with RPD varied in the number of deposits required and the imaging modalities used to determine their presence. Only five studies used MMI to determine the presence or absence of RPD (Table 1).

Summarizing these findings, it was found that several **single nucleotide polymorphisms (SNPs)** within *CFH*, *ARMS2*/*HTRA1*, *C3*, *VEGFA*, *C2*/*CFB*, *LIPC* genes have been reported to be associated with RPD in the setting of AMD (Table 1) [2, 5-11]. Comparing selected AMD-risk loci between eyes with both RPD and conventional drusen and eyes with only conventional drusen, Buitendijk *et al.* showed that loci within the *C3* gene (rs22130199), the *ARMS2* (rs10490924) and *VEGFA* (rs943080) genes present different association strength between the two groups of patients, suggesting different genetic loci underlying the risk for RPD and conventional drusen [9]. Amongst specifically identified risk loci for RPD in AMD cohorts, the rs10490924 in the *ARMS2* gene has been the one most consistently associated with greater RPD prevalence [14]. These findings, thus far, have not considered the genetic contribution of the remaining AMD risk loci nor other non-AMD unrelated variants, that explore other potential pathways such as hypoxia, inflammation and lipoprotein metabolism [15]. RPD are found in other retinal diseases and indeed without any other disease being present, supporting the view that there will be independent and novel pathological pathways driving the formation of RPD, potentially unrelated to pathways driving the development of conventional drusen [16]. Investigating the impact of genetic mutations that cause rare Mendelian conditions that have been reported to develop RPD, such as Retinitis punctatus albescens, Pseudoxanthoma elasticum and late-onset retinal degeneration, may also provide clues of causative genetic variants and pathogenic

mechanisms that contribute to RPD, although these cohorts are much rarer to find than RPD co-presence with AMD.

Identifying existing cohorts, with the required imaging available, to robustly determine if RPD is present or not, is required to establish a consortium with an adequate sample size, that should enable gaining new insights from **genome-wide association studies (GWAS)**, as the latter will possess greater power than before for the discovery of new findings. It will then be possible to apply advanced genetic techniques such as **multi-trait analysis of GWAS (MTAG)** and **Mendelian Randomization (MR)** to help explore GWAS signals. These genetic methods can be applied to summary statistics from future RPD-specific GWAS, together with existing AMD results to improve discovery power for RPD-specific risk within highly correlated traits. Genetically estimated comorbid traits can be partly negated using conditional GWAS to extract SNPs that are independently associated with AMD plus RPD or only in AMD without RPD. MTAG has already been applied to several GWAS for the early stages of AMD (with RPD status undefined) and has led to distinguishing those loci associated with early disease from the advanced stages of AMD [16]. Given AMD and RPD are strongly correlated, MR method should be able to test whether RPD is directly associated with the same genes/pathways that lead to AMD, or its progression, or rather reveal a correlation with other systemic intermediate phenotypes. For instance, the MR method recently discovered a causal role for systemic serine depletion in macular telangiectasia, a different macular disease to AMD [17].

Novel approaches to help determine the clinical phenotype of RPD in larger cohorts, perhaps with less suitable imaging for RPD detection, would also supplement the genetic studies. The advances in retinal imaging lend themselves to the use of artificial intelligence and the retinal research field is well advanced in developing algorithms to help detect RPD. These novel approaches, together with more advanced genetic bioinformatic approaches, will be invaluable to help detect RPD-specific genetic associations. Understanding the genetic associations will then allow pathways and mechanisms to be elucidated, facilitating targeted development of strategies for intervention. Treatment strategies might well be different in those with RPD compared to those without, as was recently found in an interventional study in AMD that reported different treatment effect depending on the presence or absence of RPD [18].

Box 1: Review strategy and methods to detect RPD

We reviewed studies investigating RPD genetic risk factors described in individuals with AMD, published in English until September 2021. The PubMed database was searched for research articles using the terms “reticular pseudodrusen”, “subretinal drusenoid deposits”, “reticular macular disease”, “reticular drusen”, “drusen” and “genetics” keywords. In total, 11 studies investigated the association of AMD-related candidate genetic loci with RPD (Listed in Table 1 and two genetic studies with no findings, references 12-13). Of 11 studies that investigated the genetic risk of developing RPD, the definitions of what determined an eye with RPD varied in the number of deposits required and the imaging modalities used to determine their presence. Early studies and cohorts collected to study AMD genetics which are now being analysed for RPD associations, are based solely upon colour fundus photographs (CFP) that do not optimally detect eyes with RPD when imaged with newer modalities, such as OCT (Figure I). Therefore, the reported associations must be cautiously interpreted.

Figure I. Top panel: example of an eye with only conventional drusen as seen on a colour fundus photograph (left) and optical coherence tomography (OCT) (right), **bottom panel:** example of an eye with both conventional drusen and reticular pseudodrusen (RPD). The OCT scan reveals the apparent deposits of RPD in the subretinal space (white arrow) below the retinal pigment epithelium (RPE). The RPD deposit starts in the subretinal space but as they grow, they push further into the photoreceptor later and in stage-3 they break through the Ellipsoid zone. Conventional drusen form and stay beneath the RPE. Note that using only colour fundus images does not allow RPD to be easily distinguished from conventional drusen.

Table 1. List of studies that report reticular pseudodrusen (RPD)-specific risk loci (only significant results are shown in this table).

Study	**Sample size (AMD grade*)**	**Imaging method to define RPD**	**Identified RPD-associated genes (loci)**	***P*-value; Odd ratio (OR)/ Hazard Ratio (HR)#**	**RPD definition/detection**
Smith *et al.* (2011) [6]	67 AMD+RPD subjects (early- or late-stage AMD) compared to 64 AMD-RPD	Infrared reflectance (IR), Fundus autofluorescence (FAF)	*CFH* (rs1061170)	0.003; 0.46	The diagnosis of RPD was accomplished by multimodal imaging (MMI) but no OCT imaging.
			ARMS2 (rs10490924)	0.045; 1.73	
Ueda-Arakawa *et al.* (2013) [5]	216 subjects (late AMD)	Colour fundus photography (CFP), IR, FAF, Spectral-domain optical coherence tomography (SD-OCT)	*ARMS2* (rs10490924)	0.007	RPD were diagnosed where the reticular pattern was observed in ≥2 imaging modalities.
Joachim *et al.* (2014) [7]	2,230 subjects (follow-up of RPD+ patients to late-stage AMD)	CFP	*CFH* (rs1061170)	0.0008 ; 1.8	RPD were defined as individual lesions usually more than 125 µm in diameter using all available retinal photographs from baseline and follow-up visits. The questionable RPD images were verified by two senior experienced grading researchers.
			ARMS2 (rs10490924)	<0.0001 ; 3.0	
Yoneyama *et al.* (2014) [8]	408 subjects (wet AMD)	CFP, FAF, IR, SD-OCT	*ARMS2* (rs10490924)	0.008; 3.23	The diagnosis of RPD was made by independent evaluators based upon the peculiar reticular pattern around the macula, whose visibility was enhanced by IR, FAF, or red-free images on CFP, and upon the SD-OCT. When the evaluators did not agree on the diagnosis of RPD, the final decision was made by a third evaluator.
Finger *et al.* (2016) [2]	5,208 subjects (early, intermediate, and late AMD)	CFP	*ARMS2* (rs10490924)	< 0.05	RPD were classified based on the certainty of the grading into 3 categories (100% certainty, <100%–≥80% certainty, and <80% certainty based on a pattern of distribution), and any discrepancies between the graders were resolved through regrading for an agreement in the definition.
			HTRA1 (rs11200638, rs3793917)	< 0.05	
			CFH (rs393955, rs1061170, rs2274700)	< 0.05	

					Note that this study used only CFP to detect RPD.
Buitendijk *et al.* (2016) [9]	2,774 total participants (a mix of AMD severity grades: 0-4 according to the Rotterdam Classification) Note: 358 individuals included in AMD with/without RPD analysis.	CFP and near-IR (NIR)	*C3* (rs22130199)	< 0.05 ; 1.61	This study investigated genetic risk factors among drusen types including RPD. 92.7% of eyes with RPD were detected with NIR imaging and 38% on CFP.
			ARMS2 (rs10490924)	< 0.05; 2.48	
			VEGFA (rs943080)	< 0.05; 2.00	
			C2/CFB (rs641153)	< 0.05; [$]	
			CFH (rs1061170, rs12144939, rs800292)	< 0.05; [$]	
Wu *et al.* (2016) [10]	300 subjects (intermediate AMD)	OCT and NIR	*ARMS2* (rs10490924)	0.002	In this study, an experienced grader performed the grading of the images from all the participants for all used imaging modalities. To minimize bias, the images of all participants for each imaging modality were graded while being masked to other imaging modalities during different sessions.
Domalpally *et al.* (2019) [11]	1,307 subjects (intermediate AMD)	FAF	*ARMS2* (rs10490924)	< 0.0001 (Bonferroni-adjusted significance)	Note that only FAF was used in this study to detect RPD.
			C3 (rs2231099)	0.04 (at a nominal level)	
			CFH (rs1061170)	0.048 (at a nominal level)	
Dutheil C. et al. (2020) [4]	472 eyes (excluding eyes with advanced AMD or ungradable)	CFP, SD-OCT, FAF, NIR	*ARMS2* (rs10490924)	< .001; 3.57	RPD were considered as present if detected by at least 2 of the 4 used imaging methods.
			CFH (rs1061170)	0.04; 2.12	
			LIPC (rs10468017)	0.003; 2.57	

[*]Wisconsin grading protocol is used in the studies if mentioned any.

[#]Odd ratios and/or hazard ratios are not represented when more than two groups of genotypes (heterozygote and homozygote of risk alleles compared to homozygote of non-risk alleles) are compared. See the odd ratios for genotypes in the related references. The odd ratios (OR) are not shown in this table when the values are not provided by some studies.

[$]The OR represents the risk for RPD in reference to the other drusen type (in Ref. no. 9) or to absence of RPD, when the comparison is between two genotype groups.

Glossary

Fundus autofluorescence: A non-invasive retinal imaging modality used in clinical practice to record fluorescence that may occur naturally in ocular structures or as a result of a retinal disease process.

Genome-wide association studies: An approach that involves rapidly scanning of germline variants in human diseases/traits, in which the association of different variants such as single nucleotide polymorphisms (SNPs) with a given disease/trait is investigated.

Infrared reflectance: A non-invasive imaging method that uses the retina's ability to absorb, reflect or scatter infrared light to produce high quality images. This method highlights sub-retinal features which may otherwise be obscured by standard retinal photography.

Mendelian Randomization: A method in epidemiology that uses genetic variants to infer causal relationship of an exposure (risk factor) and a disease outcome.

Multimodal imaging: Multimodal imaging refers to a new retinal imaging method that is a combination of more than one imaging techniques with a high sensitivity in detecting various abnormalities in clinical studies. This method has started to be recognised as optimal methodologies to detect reticular pseudodrusen.

Multi-trait analysis of GWAS: A method for joint analysis of summary statistics from genome-wide association studies of different traits. MTAG has been utilised to discover biological mechanisms that are involved in correlated traits.

Optical coherence tomography: A non- invasive imaging technique that produces cross-sectional images of retina with high resolution. This method has been used as a gold standard to detect reticular pseudodrusen.

Single nucleotide polymorphisms: Germline variations within the genome that present substitution of a single nucleotide among people. The frequency of these variants might vary in different populations.

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