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Anatomical Changes to the Retinal Region in Normal Ageing and Age-Related Macular Degeneration; Implications for Age-Related Macular Degeneration Pathophysiology

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Abstract

Purpose:

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness and research has shown that ageing is the most significant risk factor. Despite the increasing prevalence of AMD, its pathophysiology remains poorly understood. This review evaluates anatomical changes that take place in the retinal region (RR) during normal ageing (NA) and in AMD to improve current understanding of AMD pathophysiology.

Materials and Methods:

A comprehensive literature review of peer reviewed publications related to anatomical changes in the retinal region in NA and AMD was performed using the PubMed® database to identify relevant research articles.

Results:

Reductions in Bruch's membrane permeability are crucial in the general pathophysiology of AMD and macular thickening facilitates hard drusen accumulation. Extracellular deposits modulate AMD pathophysiology; breakdown of hard drusen and coalescing basal linear deposits preferentially predispose to atrophic AMD and neovascular AMD, respectively. Patterns of retinal pigment epithelial cell loss are similar in NA (principally modulated by lipofuscin) and atrophic AMD, both demonstrating foveal sparing. Choroidal neovascularisation has a predilection for the fovea and the foveal choriocapillaris also undergoes the greatest degree of degeneration during NA, compared to other regions. Choriocapillaris degeneration appears to be the primary insult in neovascular AMD pathophysiology.

Conclusions:

Enhanced lipofuscin accumulation and subsequent retinal pigment epithelial cell loss during senescence is more likely to result in atrophic AMD when combined with decreases in macular Bruch's membrane permeability and subsequent hard drusen formation. In contrast, accelerated loss of the choriocapillaris during ageing probably predisposes to neovascular AMD. However, further research is required to elucidate the exact mechanics of this process.

1 List of Abbreviations

AMD - Age-related Macular Degeneration
RR - Retinal Region
NA - Normal Ageing
GA - Geographic Atrophy
CNV - Choroidal Neovascularisation
RPE - Retinal Pigment Epithelium
BrM - Bruch's Membrane
PR - Photoreceptor
RP - Rod Photoreceptor
CP - Cone Photoreceptor
FAF - Fundus Autofluorescence
OCTA - Optical Coherence Tomography Angiography
aAMD - Atrophic Age-related Macular Degeneration
nAMD - Neovascular Age-related Macular Degeneration
BLinD - Basal Linear Deposit
OCT - Optical Coherence Tomography
VEGF - Vascular Endothelial Growth Factor

2 Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in adults over 50 years old (Pennington & DeAngelis, 2016). Age is believed to be the most significant risk factor for AMD development (Chen et al., 2008). In a cross continental study, AMD prevalence was 0.2% in people aged 55 to 64 years old, rising exponentially to 13% in those older than 85 years (Smith et al., 2001). At the same time, people are living longer than ever before. In 2001, for the first time in history, there were more persons over 60 years of age than children in the UK (Office for National Statistics (ONS), 2019). Given this association, it is unsurprising that by 2020, the number of people with AMD globally is expected to be around 200 million, increasing to nearly 300 million by 2040 (Wong et al., 2014). Despite the irrefutable epidemiological links between ageing and AMD, there is scant research into the clinical association between these processes which has contributed to limited knowledge regarding the natural history of this condition compared to many others.

AMD is a degenerative disease in people of 50 years old or above, which involves distinct anatomical changes to the retinal region (RR) visible on fundus examination (Bird et al., 1995). Such anatomical changes observed in AMD include debris accumulation, geographic atrophy of cells (GA) and choroidal neovascularisation (CNV) (Ferris et al., 2013). Virtually every measure of our visual function, including visual acuity, contrast sensitivity, field sensitivity and dark-adaptation threshold, shows a functional decline during normal ageing (NA) (Salvi, Akhtar, & Currie, 2006). Degeneration of retinal function during NA is considered a key factor in visual deterioration and anatomical changes to the RR play a major role in this process; debris accumulation, loss of retinal cellular populations and changes to the choroidal circulatory complex have all been implicated in the NA process (Delori, Goger, & Dorey, 2001; S. H. Sarks, Arnold, Killingsworth, & Sarks, 1999; Gao & Hollyfield, 1992; Ramrattan et al., 1994).

Three overarching concepts of anatomical changes therefore appear to pertain to both NA and AMD development; debris accumulation, loss of cellular populations and vascular changes to the choroidal complex. However, whilst such anatomical changes have previously been investigated, it has tended to be on an individual basis. As a result establishing the relative contributions of each to the NA and AMD processes and providing a comprehensive critical review of the range of anatomical changes documented, has not been possible. The majority of anatomical changes to the RR in NA and AMD appear to affect the outer aspects of the RR comprising the photoreceptor layer, retinal pigment epithelium (RPE), Bruch's membrane (BrM) and the underlying choroidal complex (Grossniklaus, Nickerson, Edelhauser, Bergman, & Berglin, 2012; Salvi et al., 2006; Bonilha, 2008). Understanding the structural and functional anatomy of this aspect of the RR is therefore of paramount importance in exploring the association between NA and AMD to investigate its pathophysiology. With this in mind, the review will focus on exploring the outer retinal anatomy followed by changes to the anatomy in these regions during both NA and AMD, respectively.

To date, very few papers have directly compared NA and AMD, and a much smaller minority have used anatomical changes that occur in each of the processes as a means of comparison to investigate AMD pathophysiology. Considering the obvious epidemiological link between NA and AMD, this review will be in the unique position of utilising a comparative anatomical approach with the aim of consolidating understanding of AMD's complex pathophysiology. Moreover, differentiating between anatomical changes in NA and AMD has important implications for clinical practice; establishing key anatomical differences between these processes may lead to earlier AMD diagnosis and clinical intervention. Comparative analysis between the anatomical changes of the RR in the senescent process and AMD should improve current understanding of AMD pathophysiology.

3 Methods

A comprehensive literature search of the PubMed® database was used to identify relevant research articles. The literature search was confined to the English language. Additional articles were selected from a review of the references of the original articles identified in the search. The following key words and combinations of these words were used in compiling the search: age-related macular degeneration; retinal pigment epithelium; ageing; aging; senescence; anatomy; drusen; lipofuscin, basal linear deposits; geographic atrophy; choroidal neovascularisation; atrophic; neovascular.

4 Anatomical Changes to the RR during NA

Ageing can be broadly defined as the chronological deterioration of the physiological functions that are necessary for survival and fecundity, affecting all the individuals of a species (Gilbert, 2000). Whilst the ageing process continues in both those with and without AMD, this review will only consider

individuals whom do not show clinical hallmarks, and are therefore not diagnosed with AMD, to have undergone NA. One of the most important considerations when discussing ageing is its different associations with dividing and non-dividing cellular populations (Marshall, 1987). By virtue of the high degree of differentiation exhibited by cellular populations of the RR, they are accordingly extremely limited in regenerative ability. This has profound consequences for the anatomical changes taking place during the senescent process.

5 Accumulation of Lipofuscin

Lipofuscin is a complex material that can be found in many metabolically active post 144 mitotic cells (such as RPE cells) and a key anatomical change occurring in the RR during the NA process. RPE lipofuscin is mostly composed of lipids with proteinaceous content comprising less than 2% (K.-P. et al., 2008). Evidence that a significant proportion of RPE cell lipofuscin precursors are found in the photoreceptor (PR) outer segments have been demonstrated whereby in rodent models, RPE cells unable to phagocytose PR outer segments, have a much-diminished level of intracellular lipofuscin (Katz, Drea, Eldred, Hess, & Robison, 1986). It is therefore widely acknowledged that lipofuscinogenesis results chiefly from RPE mediated incomplete phagolysosome degradation of PR outer segments throughout life. Regulated by both an innate circadian rhythm and environmental diurnal cycle, outer segment shedding of rod photoreceptors (RPs) occurs at a much faster rate than cone photoreceptors (CPs) (Boulton, Dontsov, Jarvis-Evans, Ostrovsky, & Svistunenko, 1993). Consequently, the accumulation of lipofuscin should occur at a faster rate in regions where there is a high density of RPs.

Signal intensity from fundus autofluorescence (FAF) imaging is used as an index of RPE lipofuscin content (Lois, Hatfyard, Bird, & Fitzke, 2000). In the largest study to date, Delori (2001) found that RPE lipofuscin accumulated quasi-linearly between 20 to 70 years using FAF imaging. Feeney-Burns (1984) also showed that there was an 11% increase in the volume of RPE cells occupied by lipofuscin between the ages of 40 to 80 years. FAF intensity peaked at 11° temporally and 7° nasally; both are regions whereby RP density is reaching maximal levels (Delori et al., 2001). FAF intensity was significantly lower in the fovea (Delori et al., 2001). These data are supported by other FAF and histological studies (Wing, Blanchard, & Weiter, 1978; Ach et al., 2014). Ach et al., (2014) found that the topography of FAF intensity is extremely similar to RP topography as FAF intensity was lowest in the foveal centre and peaked in the perifoveal annulus. However, outside of the macula region, FAF intensity has been noted to decrease more rapidly at increasing eccentricities than RP density (Ach et al., 2014; Delori et al., 2001). This may be partly explained by the reduction in length of RP outer segments with increasing eccentricity (Hendrickson & Drucker, 1992). Nevertheless, it is clear from these data that the accumulation of lipofuscin is extremely closely correlated with RP topographical densities and appears to result from the greater rate of outer segment shedding of RPs compared to CPs.

6 Extracellular Deposits

Whilst there is little contention regarding mechanism of formation and spatial distribution of lipofuscin, there is a degree of discord in literature regarding the implications of RPE cell lipofuscin accumulation. Yasakawa et al. (2007) proposes that lipofuscin accumulation principally acts to congest RPE cell cytoplasm which may result in progressive impedance to bimodal RPE metabolic flux. As a result, there may be sub-RPE accumulations of retinal debris such as drusen or basal laminar deposits. Basal laminar deposits are excrescences that are located between the basolateral plasma membrane of the RPE and RPE basal lamina (van der Schaft, de Bruijnz, Mooy, & de Jong, 1993a). Drusen are focal deposits, over 40% of which are comprised of lipids with a greater proteinaceous content than lipofuscin (L. Wang et al., 2010). They accumulate between the basal lamina of the RPE and the inner collagenous layer of BrM ((Spaide & Curcio, 2010). Hard (hyalinised) drusen are particularly associated with senescence and are generally $<$63m in diameter (S. H. Sarks et al., 1999).

In a histological analysis of aged eyes, Sarks et al. (1999) concludes that there are two processes for drusen formation; entrapment sites in BrM that may enlarge during nodular accumulation of debris, and also pathological drusen formation through accumulation of debris only. Hard drusen formation during NA therefore appears to chiefly result from structural changes to BrM which create entrapment sites for hard drusen accumulation during senescence. Structural changes to BrM during NA are already well documented in medical literature and therefore substantiate the theory of Sarks et al. (1999). In one study, BrM was observed to increase in thickness from 2m in the second decade to 4.7m in the tenth decade (Ramrattan et al., 1994).

Moreover, increasing elastin fibre calcification and collagen fibre cross-linking have both been observed during ageing of the BrM (Booij, Baas, Beisekeeva, Gorgels, & Bergen, 2010). Accumulation of advanced glycation end-products have also been observed to accumulate within BrM during NA (Booij et al., 2010). Advanced glycation is a feature of BrM thickening and, combined with focal lipid-based hard drusen, reduces BrM permeability during NA (Moore, Hussain, & Marshall, 1995). Moore (1995) also found that hydraulic conductivity of macular BrM halves every 9.5 years, compared to every 19 years in the periphery. Although the reduction in permeability may reduce macromolar exchange to a greater extent that smaller metabolic solutes, impaired metabolic exchange between the choriocapillaris and RPE is likely to occur in addition to impedance of fluid dynamics (Moore & Clover, 2001).

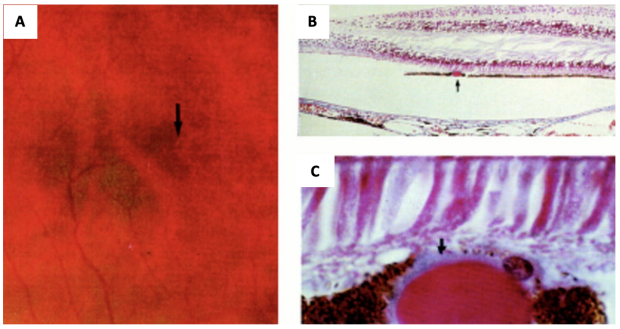

Figure 1. (From Sarks *et al.,* 1999). **Fundoscopic and Histological Imaging of Hard Drusen *in situ*.**

Figure 1A: Fundoscopic image of the smallest clinically detectable hard drusen (arrow). Figure 1B: Light microscopy of histological section (x75 magnification) through fovea showing a hard druse (arrow). Figure 1C: Light microscopy (x500 magnification) of druse in figure 1B, highlighting displacement and attenuation of the RPE layer above.

Although isolated small hard drusen are a common observation in normal, aged eyes occurring in around 93.6% of people between 43 to 86 years old (R. Klein, Klein, & Linton, 1992). Rudolf et al. (2008) reports finding a loss of RPE coverage and associated focal atrophy in regions where hard drusen were present. Whilst a limitation of this study is that the retinas used had been previously diagnosed with age-related maculopathy and therefore not truly comparable to NA, such findings have also been documented by other groups. Johnson et al. (2003) also reported finding structural abnormalities in retinal cells, including PRs, overlying regions where hard drusen were present.

Even small, subclinical drusen were found to compromise regional anatomy. In the large Beaver Dam Eye Study, those with large numbers (8) of hard drusen were found to have an increased 15-year age-adjusted incidence of soft drusen formation and pigmentary abnormalities of 16.3% and 10.6%, respectively, compared to 4.7% and 2.7% for those with <8 hard drusen (R. Klein et al., 2007). It seems that hard drusen can lead to significant changes to retinal anatomy, although this is likely to be according to the number of drusen present. Figure 1 shows drusen of the hard, hyalinised type observed in the right eye of a 71-year-old male with visual acuity 6/6. Although displacement and a degree of attenuation of the RPE cell layer is evident,there is an absence of significant RPE atrophy.

7 RPE Density

Unlike Yasakawa et al. (2007), some authors advocate that lipofuscin principally increases oxidative stress of the RPE by acting as a photoinducible generator of reactive oxygen species (Boulton et al., 1993; Davies et al., 2001; Mazzitello, Arizmendi, Family, & Grossniklaus, 2009). Cumulative exposure to ultraviolet irradiance throughout life creates an ideal oxidative environment to procure reactive oxygen species, which can induce apoptosis of the RPE cell (Boulton et al., 1993). Lipofuscin loaded RPE cells have demonstrated an increased rate of reactive oxygen species generation and reduced viability when they are exposed to visible light, supporting the notion that lipofuscin acts to induce RPE cell death by increasing oxidative stress (Wihlmark, Wrigstad, Roberg, Nilsson, & Brunk, 1997; Davies et al., 2001). Regions of the retina with a higher RP density appear to have increased lipofuscin content and therefore, should have more profound RPE cell loss also.

The concept of changing RPE cell densities to some degree during NA is fairly well supported in the literature. Gao (1992) examined foveal and equatorial (defined as 13mm/45° eccentricity from foveal centre) RRs from pan-retinal tangential sections of 35 eyes from 35 donors, spanning from the second to ninth decade of life using light microscopy. The density of foveal RPE cells did not significantly change during NA ($P>0.2$) (Gao & Hollyfield, 1992). In the equatorial RR however, the density of the RPE cell population decreased uniformly from the second to the ninth decades at 14 cells/mm2/year ($P<0.03$), supporting the above hypothesis (Gao & Hollyfield, 1992). Panda Jonas (1996) also found that there is a marked reduction in RPE cell density during NA in the mid-periphery, a region around 13-35° eccentricity with high RP density and low CP density as shown by figure 1 previously. Interestingly, a similar trend was noted for the foveal RPE cell population. The mean RPE density across the retina was found to decrease by an average of 0.3% per year (Panda-Jonas, Jonas, & Jakobczyk-Zmija, 1996).

Unlike Gao (1992) and Panda-Jonas (1996), Dorey et al. (1989) reported no significant decrease in equatorial RPE cell density in a similar study analysing 30 eyes aged between the first and ninth decades. A more general age-related loss of RPE cell density was still observed, however. Differences can partly be explained by the nature of the analyses. Cross-sectional analysis permits investigation of a restricted cellular population whereas tangential pan-retinal sections and whole mount preparations used by Gao (1992) and Panda-Jonas (1996), allow analysis of a greater proportion of the cellular population. Quantification of RPE cells is also likely to be made more difficult by the disruption to the regular, hexagonal pattern with adoption of a more heterogenous morphology during NA (et al. Forrester, 2002).

One histological study proposes that there is no decline in RPE cell numbers during NA and rather, changes in RPE densities in NA are probably due to retinal area fluctuations (Harman, Fleming, Hoskins, & Moore, 1997). However, biochemical studies have proved that increased apoptosis of RPE cells does occur with advancing age (Del Priore, Kuo, & Tezel, 2002; Dunaief, Dentchev, Ying, & Milam, 2002). Del Priore (2002) used TUNEL labelling to investigate RPE apoptosis and report that apoptotic RPE cells were principally confined to the macula region in aged eyes. No significant change in density was found in the central 3mm diameter region (containing the fovea) supporting the observations of Gao (1992). Whilst the highest rates of apoptosis were also found in this region, whether the rates were higher in the foveal or parafoveal regions were not detailed. The proportion of apoptotic cells increased significantly in the periphery (>12.5 mm from the foveal centre) during NA, although remained lower than the central zone. Del Priore (2002) calculated RPE

cell loss of 2.3% per decade which is similar to the reduction in density of 0.3% per year (3% per decade) calculated by Panda-Jonas (1996). Del Priore (2002) postulate that migration of peripheral RPE cells from the periphery to the macula may compensate for macula cell loss in NA (Del Priore et al., 2002). The results of Del Priore (2002) offer some support to the theory that RPE lipofuscin-induced apoptosis results in a reduction in RPE density that roughly correlates with RP and lipofuscin topography. However, a parallel correlation is difficult to ascertain as pleomorphism and dynamic shifting of the RPE mosaic are complicating factors (Del Priore et al., 2002).

8 PR Density

Closely opposed to the RPE, PRs have been reported to undergo specific age related changes by several studies. Panda-Jonas (1995) found that outside of the foveal centre, the PR density decreased significantly during NA. Both RP and CP cell losses were greatest at an eccentricity of 5-8mm (17.4-27.8°), the region in which the density of RPs is maximal (?, ?). More specifically, the decline of PR density was calculated to be 0.37% and 0.18% per annum for RPs and CPs, respectively. Panda-Jonas (1995) therefore conclude that RPs decrease in density at a faster rate than CPs during NA. Foveal CP density changes as a function of NA could not be evaluated due to technical reasons. Curcio (2001) reported that the density of foveal CPs remains static during NA. In contrast, para-foveal RP densities significantly decreased by 30% over adulthood (Curcio, 2001). Gao (1992) found that there were no significant changes in foveal CP density during NA, supporting the findings of Curcio (2001). However, whilst Curcio (2001) found that there was an undetectable reduction in RP density at 8.4mm eccentricity (29.2°), Gao (1992) recorded significant RP (and CP) density losses in the equatorial retina during NA. Although other studies have shown significant decreases in foveal CP densities during NA, the concept of preferential vulnerability of RPs compared to CPs in NA prevails through medical literature (Song, Chui, Zhong, Elsner, & Burns, 2011; Gao & Hollyfield, 1992; Curcio, 2001; Panda-Jonas, Jonas, & Jakobczyk-Zmija, 1995).

It is estimated one RPE cell provides trophic support for thirty to fifty PRs superiorly (Zinn, K. M. and Marmor, 1979). There is less literature available regarding PR apoptosis in NA compared to the equivalent RPE. Despite this, given the metabolic reliance of PRs on a well-functioning RPE, it can be deduced that losses of RPE cells should promote apoptosis of the superiorly located PRs. Indeed, PRs are known to undergo cell death by apoptosis when separated from the RPE during retinal detachment (Lo, Woo, Wong, & Wong, 2011). The theory that regions of RPE cell loss correlate with regions of PR loss is supported by two of the three main studies investigating PR changes during NA (Panda-Jonas et al., 1995; Gao & Hollyfield, 1992). The only study to investigate PR and RPE changes together, Gao (1992) explicitly conclude that PRs and RPEs show parallel losses during NA.

Such anatomical changes during NA generally concur with observed functional changes. The loss of scotopic sensitivity and dark adaptation (accorded by RPs) throughout the senescent process is greater than the equivalent loss of photopic sensitivity (accorded by CPs) (Jackson, Owsley, & Curcio, 2002). Elliott (1987) established that decreases in the contrast sensitivity of aged individuals were chiefly due to neural changes instead of optical factors such as pupillary miosis and lens photo absorption. Some aspects of visual function changes are not as readily explained by the aforementioned anatomical changes. Central visual acuity facilitated by CPs is stable until 44 years, after which there is a slow deterioration (Sjöstrand, Laatikainen, Hirvelä, Popovic, & Jonsson, 2011). A 0.3 LogMAR reduction (equivalent to Snellen reduction from 6/6 to 6/12) occurs between 44 and 88 years, equating to a yearly reduction of 1.7% (Sjöstrand et al., 2011). Curcio (2001) calculated that there must be a loss of 75% of CPs to cause a corresponding decrease in visual acuity from 6/6 to 6/12, and such profound losses have not been documented during NA. The reasons behind this remain to be determined but may involve other changes to the neurosensory retina such as ganglion cells (Gao & Hollyfield, 1992).

9 Choriocapillaris

Wang et al. (2016) reported that choriocapillaris density was not significantly associated with age (Q. Wang et al., 2016). However, the study method was not designed to analyse the relationship between choriocapillaris density and NA. Moreover, the spectral domain optical coherence tomography angiography (OCTA) device used to measure choriocapillaris density in the study is believed to have limited capability in penetrating the RPE layer, thus is likely to lead to a major underestimation of choriocapillaris thinning. Contrary to the findings reported by Wang et al. (2016),Ramrattan et al. (1994) reported a decrease in choriocapillaris density of 45% and a reduction in overall choroidal thickness of 57%, over 10 decades. A limitation of this study is that the analysis was of donor eyes and therefore, in vivo factors were not accounted for. However, these histological findings also correlate with changes observed in vivo in a cross-sectional study involving 72 healthy participants aged 20 to 80 years (mean 47.4 years) (Sacconi et al., 2019).

Sacconi et al. (2018) found a significant negative correlation between choriocapillaris perfusion density and age for foveal, perifoveal and parafoveal regions, respectively ($p<0.001$ for each region). Foveal perfusion density was observed to decrease from a mean of 77% in participants aged 20-29 to 70% in those aged 70-80 years old. Perfusion density decreased the most in the foveal region, followed by parafoveal and then perifoveal; this is similar to the findings of another recent swept source OCTA study (Nassisi et al., 2018). Sacconi et al. (2018) postulates that the reductionnin choriocapillaris perfusion density during NA results from a reduction in choriocapillaris vessel calibre instead of a reduction in number, as vessel diameter also showed a strong negative correlation with age ($p<0.001$). Ramrattan et al. (1994) substantiates this, arguing that the reduction of choriocapillaris density during senescence is secondary to a reduction in choriocapillaris vessel diameter. Reduced perfusion of the RR due to morphological

changes to the choriocapillaris may cause an increased level of oxidative stress in the outer RR and contribute to the loss of cellular populations and other senescent changes (Bhatt, Groeger, McDermott, & Cotter, 2010; Boulton et al., 1993; Davies et al., 2001; Friedman, Smith, & Kuwabara, 1963).

10 Anatomical Changes to the RR during AMD

10.1 Extracellular Deposits

There are several forms of extracellular deposits that are linked to AMD pathophysiology. Soft drusen and basal linear deposits (BLinD), (collectively referred to as membranous debris) are recognised as specific anatomical changes of AMD (S. Sarks, Cherepanoff, Killingsworth, & Sarks, 2007). Other AMD associated extracellular deposits include basal laminar deposits and sub-retinal drusenoid deposits. basal laminar deposits correlate with AMD only when they thicken or incorporate vesicular structures and can occur during NA as described in previously. Figure 2 shows that basal laminar deposits are located between the RPE basement membrane (innermost layer of Bruch's membrane) and the RPE plasma membrane (Van Der Schaft, Mooy, De Bruijn, & De Jong, 1993b). Basal linear deposits (BLinDs) by contrast are found between the RPE basement membrane and the inner collagenous zone of Bruch's membrane (BrM) also shown by Figure 2 (S. H. Sarks, Van Driel, Maxwell, & Killingsworth, 1980).

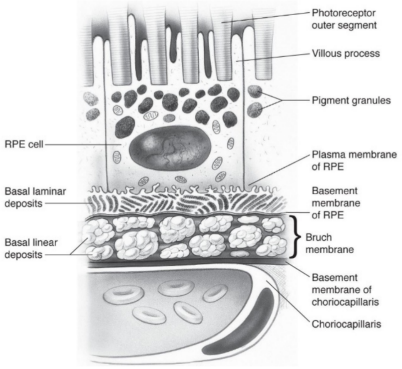

Figure 2. (From American Academy of Ophthalmology, 2019). **Anatomy of basal laminar deposits and BLinDs.**

Schematic depicting the relative anatomical locations of basal laminar deposit and BLinD in the RR.

Although BLinDs are often diffusely spread through BrM, they very commonly coalesce to create focal mounds manifesting as soft drusen (J. P. Sarks, Sarks, & Killingsworth, 1994). Soft drusen appear as yellow excrescences beneath the RPE layer but unlike hard drusen, tend to be larger and have amorphous borders (Kanski, 2003). More anatomically distinct is another subtype of extracellular deposit, called sub-retinal drusenoid deposits (also known as reticular drusen). Sub-retinal drusenoid deposits aggregate between the apical surface of the RPE and overlying PRs (Huisingh et al., 2016). The Beckman Classification is a commonly used method to define chronological AMD stages based on anatomical changes to the RR with particular emphasis on the aforementioned extracellular deposits (Ferris et al., 2013). Table 1 below depicts the Beckman Classification, illustrating the differences between extracellular deposit morphology and association with NA and AMD.

Table 1. (From Ferris *et al.*, 2013).

Diagnosis and Staging of AMD Based on Anatomical Features Associated with the RR.

Stage	Anatomical Features
Normal (aged eyes)	Small, hard drusen <63μm
Early AMD	Medium (soft) drusen 63-125μm
Intermediate AMD	Extensive medium soft drusen *Or* ≥1 large druse(n) >125μm *Or* Both
Late AMD	Signs of geographic atrophy (GA) *Or* Signs of choroidal neovascularisation (CNV) *Or* Both

During histopathological analysis of 41 human eyes, Curcio (1999) found that eyes with AMD were 24 times more likely to have BLinDs or large drusen when compared to age-matched controls (p=0.002) (Curcio & Millican, 1999). Curcio (1999) reached the conclusion that membranous debris may be significant for the progression to the late stages of the disease (Curcio & Millican, 1999). Sub-retinal drusenoid deposits have also been found to increase the risk of AMD development by 2.24 times when compared to controls and are associated with AMD progression to both GA and CNV (Huisingh et al., 2016). The mechanism of soft drusen formation is complex. Based on analysis of clinico-pathological cases, it has been mooted that there are two main mechanisms leading to the formation of soft drusen (J. P. Sarks et al., 1994). Firstly, soft drusen may form from the aggregation and fusion of multiple small, hard drusen in a process described as 'hard drusen clustering'. Subsequent breakdown of these drusen clusters results in a varied degree of morphological softening and is thought to predispose to GA. Secondly, accumulation of diffuse extracellular debris in BrM (BLinD in particular) can create soft drusen, a method which is more associated with onset of CNV (J. P. Sarks et al., 1994).According to these findings, soft drusen pathogenesis seems highly important in determining the course and manner of AMD progression.

There is an association between anatomical topography of the retina and the formation of extracellular lesions. Indeed, soft drusen tend to form in regions of the retina where the concentration of CPs is higher, such as the fovea (Curcio, 2018; J. Sarks, Arnold, Ho, Sarks, & Killingsworth, 2011). Figure 3 illustrates through various ophthalmic imaging techniques, the appearance, morphology and anatomical locations of typical soft drusen. Drusen appear concentrated and enlarged in the macula compared to the peripheral retina; it is relatively uncommon for soft drusen to develop in the retinal periphery (Ardeljan & Chan, 2013). Such morphological findings concur with current evidence regarding functional implications of the

deposits. Frennesson (1995) noted that the mean colour contrast sensitivity in patients with soft drusen was significantly lower than that measured in age-matched controls ($p<0.0002$ for each of the three CP types) (Frennesson, Nilsson, & Nilsson, 1995).

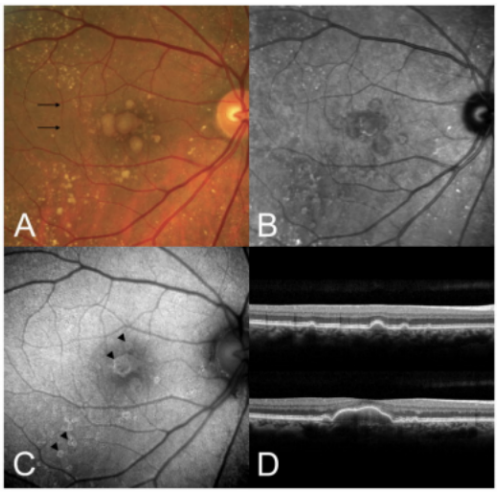

Figure 3. (From Spaide, 2010). **Anatomy of Soft Drusen.** 458 Figure 3A: *En face* colour fundus photography showing extensive large soft drusen 459 in macula and much less, in peripheral retina. Arrows indicate scan lines for 460 transverse section optical coherence tomography (OCT) in figure 3D. Figure 3B: 461 scanning laser ophthalmoscopic image with darkened areas at soft drusen sites. 462 Figure 3C: Scanning laser ophthalmoscope image illustrating hyperfluorescence at 463 soft drusen boundaries indicated by the arrowheads. Figure 3D: Transverse section 464 OCT at sites identified in 6A. There is clear accumulation of membranous debris 465 under the RPE, with main accumulations indicated by arrows.

Prevailing hypotheses have tended to place emphasis on drusen components being derived from either RPE cells or choroidal vasculature (Hageman et al., 2001; Penfold, Madigan, Gillies, & Provis, 2001). Crabb et al. (2002) proposes a more holistic mechanism of drusen formation. They suggest that RPE waste products and extravasation of choroidal vasculature components, together, provide abundant material for drusen formation. Over time, immunological involvement may lead to more diffuse expansion and accumulations. The findings of Johnson et al. (2000) suggest immune pathogenesis of RPE cells may indeed contribute to drusen formation. Immunoreactivity analysis revealed that immunoglobulin and complement components were concentrated in soft drusen and drusen-associated RPE cells. However, some of these measures of immunological involvement were also present in hard drusen and associated RPE cells. Although immunoreactivity of drusen has been described by other research groups (Mullins, Russell, Anderson, & Hageman, 2000), others have failed to detect any form of immunoglobulin component of drusen (Van Der Schaft et al., 1993b), thus the exact role of the immunological system in drusen pathogenesis remains unclear.

10.2 Geographic Atrophy

GA is the underlying process that occurs during aAMD. GA involves progressive and irreversible atrophy to the RPE layer, overlying PRs and underlying choriocapillaris epithelial cells (J. P. Sarks, Sarks, & Killingsworth, 1988; ?, ?; F.G., E.C., S., & M., 2014). aAMD is the most common form of late AMD accounting for around 90% of such cases (Morris, Imrie, Armbrecht, & Dhillon, 2007).

Extracellular deposits, soft drusen in particular, are strongly associated with development of AMD into the GA phase (M. L. Klein et al., 2008). The mechanism by which GA arises is an area of contention in current literature, split between those advocating apoptotic or necrotic pathways, respectively (Dunaief et al., 2002; Hanus, Anderson, & Wang, 2015). Using TUNEL immunochemistry to identify apoptotic cells, Dunaief et al. (2002) discovered that in eyes with late AMD, there was a significant increase in TUNEL-positive RPEs and PRs. Dunaief et al. (2002) concludes that GA is a sequela of cellular apoptosis. Limitations of this study however, included that AMD eyes analysed were affected by both GA and CNV thus the result is likely to be biased and a true correlation between apoptosis and GA difficult to establish.

In contrast, Hanus (2015) proposes that the process of GA is chiefly necroptotic. Figure 4 illustrates a possible simplified mechanism of GA pathogenesis as described by Hanus (2015). Hanus (2015) proposes that the formation of soft drusen leads to a progressive bidirectional impedance to membranous flux between the choriocapillaris and the RPE. Deprivation of trophic support may accentuate oxidative stresses experienced by the RPE cell, shown in figure 4A. As drusen accumulate, there is macroglial recruitment and involvement of the complement cascade with the RPE cells overlying drusen becoming oedematous and beginning to undergo necrotic and necroptotic cell death resulting in widespread GA, correlating to drusen distribution (figure 4C). Subsequently deprived of trophic support, the overlying PRs atrophy too.

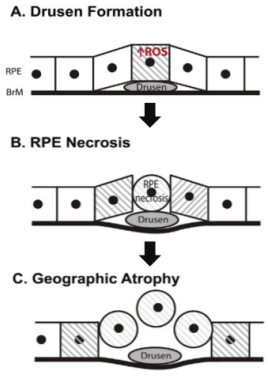

Figure 4. (Adapted from Hanus, 2015). **Possible Simplified Mechanism of GA Pathogenesis.**

Drusen formation amplifies oxidative stresses (figure 4A), causing involvement of inflammatory pathways and necroptosis/necrosis of the RPE (figure 4B), finally leading to regions of GA (figure 4C).

Drusen formation amplifies oxidative stresses (figure 4A), causing involvement of inflammatory pathways and necroptosis/necrosis of the RPE (figure 4B), finally leading to regions of GA (figure 4C). It seems that soft drusen formation plays a pivotal role in GA development; excessive diffusion distance from the choriocapillaris to the RPE and impedance to membranous flux due to the hydrophobic nature of the soft drusen are two theories that may cause primary insult to the RPE (Curcio, Zanzottera, Ach, Balaratnasingam, & Freund, 2017; Rudolf et al., 2008). Soft drusen in situ can be observed in figure 5C, in association with a typical GA pattern illustrated in figure 5A. Whilst apoptosis may be a supplementary mechanism for RPE cell death in aAMD, the inflammatory changes associated with GA highlight necroptosis and necrosis as potentially more significant means for cell death (Anderson, Mullins, Hageman, & Johnson, 2002). However, their relative contributions remain an area of intense research.

In areas of large drusen formation and focal atrophy of the RPE, there appears to be a corresponding RPE hypopigmentation, presumably due to RPE cell death and loss of the intracellular pigments such as melanin (Bressler, Silva, Bressler, Fine, & Green, 1994). Figure 5A shows a perifoveal RPE hypopigmentation indicating regions affected by GA. Focal hyperpigmentation is correlated with areas of hypertrophy of the RPE which possibly develops as a response to the initial RPE atrophy, although no overt hyper pigmentary response can be observed in figure 5A (Bressler et al., 1994). Although these findings are in keeping with current knowledge of GA processes, it must be noted that the aforementioned conclusions were reached from clinicopathological analysis of only 3 eyes from 2 patients (Bressler et al., 1994).

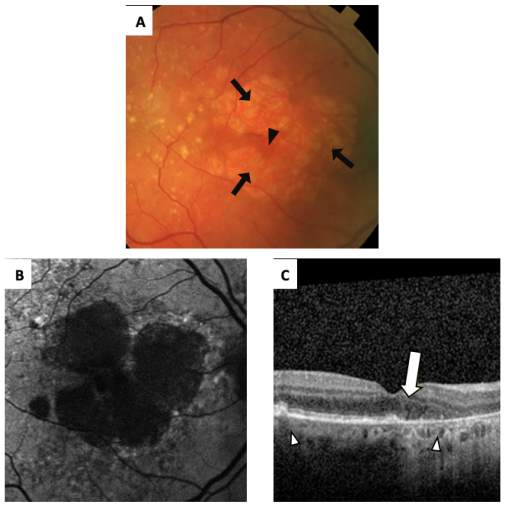

Figure 5. (Adapted from Danis, 2015). **Retinal Images of the Right Eye in a Patient with aAMD.**

Figure 5A: *En face* colour fundus photography with RPE hypopigmentation indicating regions of extensive GA (arrows) with foveal sparing (arrowhead). Figure 5B: FAF image showing clear demarcation between area of GA (dark) and lighter, functional RPE. Figure 5C: Transverse section OCT. Arrow shows demarcation between normal RPE (inferior and immediate left) and RPE affected by GA (inferior and immediate right). Soft drusen are also present under normal RPE (arrowheads).

Although GA is characterised by both RPE and choriocapillaris epithelial cell atrophy, RPE atrophy probably precedes choriocapillaris breakdown. An experimental study by Korte (1984) provided evidence that isolated destruction of the RPE subsequently leads to choriocapillaris atrophy. The choriocapillaris remained normal in areas where the RPE was preserved. Although the study was animal based, histological studies carried out on human tissue reach the same conclusion. McLeod et al.(2009) found that in regions of GA, there was well-defined sub-macular RPE atrophy and degeneration of the underlying choriocapillaris. External to the GA, RPE showed signs of pigmentary changes and drusen accumulations whilst choriocapillaris morphology appeared relatively normal. Mean capillary diameters also showed a reduction from 13.9m ($\pm$1.4m) in non-atrophic regions, to 10.34 ($\pm$0.7m) in border regions to 7.9 ($\pm$1.2m) in GA regions ($p<0.01$). There was no significant statistical difference between the diameters in non-atrophic regions of GA eyes and normal controls ($p=0.44$). These data substantiate the conclusion made by Bhutto (2012) that during GA, RPE atrophy is the primary insult and choriocapillaris atrophy is secondary to this.

Visual loss due to GA tends to be slow and progressive, taking around 5-10 years to result in legal blindness (Arnold & Heriot, 2007). GA progression rates show a large variation in the literature ranging from 0.53 mm2/year to 2.6mm2/year (median 1.78mm2 573 /year) (Batiolu, Ouz, Demirel, & Özmert, 2014; Sunness et al., 2007). A dual combination of both colour fundus photography and FAF have been reported as the optimal method to analyse GA progression patterns (Khanifar et al., 2012). Domalpally et al. (2016) analysed GA progression, reporting a GA enlargement rate of 1.44mm2 /year averaged across the two methods (colour fundus photography= 1.45mm2/year, FAF= 1.43mm2/year).

This figure approximates closely to the median 1.78mm2/year quoted previously, thus appears a reliable general estimate. There appears to be a degree of contention in the literature regarding the expansion of GA areas as a function of time. Some believe that GA lesions, with the exception of a few very small or very large lesions, show a relatively linear progression over time (Feuer et al., 2013). However, others suggest that the enlargement rates differ exponentially according to the size of the GA lesion. Sunness et al. (2007), found that GA lesions of size $<1.3mm^2$ and $8.3mm^2$, progressed at rates of 0.8 and $3.0mm^2$/year, respectively.

The theory of GA lesions expanding at different rates is further supported by the observations of Lindner et al. (2015) who noticed that progression of atrophic areas is 2.8 times faster toward the retinal periphery than towards the fovea. In a study by Schmitz-Valckenberg et al. (2016), foveal GA progressed at 1.28mm2/year and extrafoveal at 2.05mm2/year ($P=0.001$). Indeed, another study showed that foveal sparing occurred until there was an area of GA greater in size than the optic disc (J. P. Sarks et al., 1988). Although visual acuity may be preserved for a period, perifoveal atrophy in itself is understood to affect general visual performance and scotopic vision in particular (Brown, Goldstein, Chan, Massof, & Ramulu, 2014). It has been hypothesised that

such trends indicate that RPs exhibit a higher vulnerability to GA induced cell death than CPs. Bhatt et al. (2010) and colleagues demonstrated that both RPs and CPs produce reactive oxygen species in response to stress of serum deprivation. Findings from other studies have suggested that RPs seem to be die preferentially to CPs in response to an oxidative stress stimulus (Komeima, Rogers, Lu, & Campochiaro, 2006). These data imply that RPs are more sensitive to increases in reactive oxygen species and therefore undergo cell death before CPs, resulting in further increases in oxidative stresses which eventually leads to CP death.

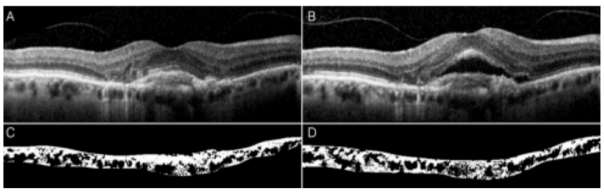

Figure 6. (From Invernizzi *et al.*, 2018). **CNV Imaged by OCT.** Figures 6A and 6B are transverse section OCT scans of the foveal region of a single patient. Figure 6A is a baseline image and figure 6B is taken after a 12-month period from which there is a progression from inactive CNV (6A) to active CNV (6B). Figures 6C and 6D are transverse section reconstructions of the choroidal complex represented in 6A and 6B, respectively. The arrow indicates a region of sub-retinal exudate accumulation resulting from classic CNV.

10.3 Choroidal Neovascularisation

The process of CNV defines nAMD, the second form of late stage AMD wherein there is a pathological growth of new and largely incompetent choroidal vasculature,extending from the choroidal complex into the sub-retinal or sub-RPE spaces (Kanski, 2003). Subsequent extravasation and haemorrhage create neovascular membranes, consisting of proteinaceous fluid, lipid and blood in the aforementioned anatomical spaces. Although less common than aAMD, comprising around 10% of late AMD cases, nAMD results in around 75-90% of AMD associated blindness(Morris et al., 2007; R. Klein, Klein, Jensen, & Meuer, 1997).

CNV lesions can be classified as classic or occult based on their fluorescein angiography appearance; occult lesions are poorly defined and haemorrhage less intensely whilst classic lesions are well defined and haemorrhage intensively (Kanski, 2003; Domalpally, A. and Danis, 2008). Occult neovascular membranes accumulate under the RPE (can also be termed type 1 lesions) whilst classic neovascular membranes lie above the RPE in the sub-retinal space (can also be termed type 2 lesions) (Lim, Mitchell, Seddon, Holz, & Wong, 2012). Occult lesions are the more common type; classic lesions tend to comprise around 20% of all neovascular membranes (Cohen et al., 2007; Olsen, Feng, Kasper, Rath, & Steuer, 2004).

There is much contention in the literature regarding anatomical changes to the choroidal complex during nAMD, but it seems this may be due to the different stages at which studies took place. Invernizzi et al. (2018) found that during the active disease phase of both CNV types, there was a significant increase in both sub-foveal and mean choroidal thicknesses compared to the control group ($p<0.0001$). Sub foveal choroidal thickness increased the most during active nAMD from 164m to 175m . Figure 6 is a collection of OCT scans depicting the findings of Invernizzi et al. (2018). There is an increase in the thickness of the choroidal layer in figure 6D (active CNV) compared to figure 6C (inactive CNV) over a follow-up period of 12 months. The arrow in figure 6B shows a large sub-retinal fluid accumulation, resulting from classic CNV extravasation.

Since age and choroidal thickness are negatively correlated, the findings of Invernizzi et al. (2018) demonstrate involvement of anatomical changes in active nAMD that are not present in NA (Margolis & Spaide, 2009). Other authors have also investigated anatomical changes to the choroidal complex during nAMD (Manjunath, Goren, Fujimoto, & Duker, 2011; Govetto et al., 2017). Manjunath et al. (2011) carried out a retrospective review and discovered that the mean sub-foveal choroidal thickness in nAMD patients was 194.6m compared to a mean sub-foveal choroidal thickness of 213.4m in aAMD patients. Sub-foveal and temporal choroidal thicknesses were also found to be significantly lower in nAMD patients than in aAMD patients ($p=0.037$) by Govetto et al. (2017). In this study, participants were diagnosed with nAMD in one eye and aAMD in the other, providing a like-for-like comparison between the two disease types and reducing the inter-individual variability in choroidal measurements.

Both Manjunath et al. (2011) and Govetto et al. (2017) studied patients who were already diagnosed with nAMD based on clinical observations such as CNV, intraretinal or subretinal fluid. On the other hand, Invernizzi et al. (2018) enrolled participants as cases only if they had no signs of neovascular activity at the baseline appointment, and subsequently developed neovascular activity at the follow-up appointment 12 months later. It can be proposed based on these data that whilst there may be a transient increase in choroidal thickness during early active CNV in nAMD, the choroidal complex subsequently undergoes significant atrophy to become much thinner. Data from these studies also suggests that CNV and subsequent atrophy of the choroidal complex affects the foveal region of the macula to a greater degree than in GA (Invernizzi et al., 2018; Manjunath et al., 2011; Govetto et al., 2017).

A preferential vulnerability of the fovea to CNV compared to the rest of the macula may be explained in part by the particularly high density of foveal PRs which are likely to mean the metabolic demands of this region are higher than the rest of the macula (et al. Purves, 2001). Provis et al. (2005) argues that adaptations of the sub-foveal choriocapillaris such as wider lumens and a thinner elastic lamina, may in the long term make the region more vulnerable to senescent changes such as membranous debris accumulation. Indeed, membranous debris accumulation has a predilection

for the fovea and is especially linked to the manifestation of CNV (Curcio, 2018; J. Sarks et al., 2011; J. P. Sarks et al., 1994). The choriocapillaris also supplies little in excess of the metabolic demand under normal conditions and combined with its lobular morphology, is likely to both inadequately perfuse and insufficiently remove waste from the foveal region in particular (Provis, Penfold, Cornish, Sandercoe, & Madigan, 2005; Alten, Clemens, Heiduschka, & Eter, 2013).

Degeneration of the choriocapillaris may be the instigating mechanism behind CNV development. McLeod et al. (2009), examined the relationship between choriocapillaris and RPE changes in AMD by analysing post-mortem choroids. They found that there was a 50% reduction in viable choriocapillaris immediately adjacent to areas of CNV (i.e. in advance of CNV involvement). Interestingly, areas of significant choriocapillaris degeneration around sites of active CNV were associated with viable RPE. A limitation of this study is that findings were based on observations of 3 post-mortem specimens only. However, other studies substantiate the findings of McLeod et al. (2009). Moult et al. (2014) performed swept source OCTA in vivo imaging of 63 eyes of 32 healthy controls and 19 eyes of 15 nAMD patients. They discovered that in all 16 eyes with identifiable CNV on SS-OCT, severe choriocapillaris alteration (atrophy and/or flow impairment) was present under regions of CNV. Moreover, in 14 of the 16 eyes, they discovered that the CNV lesions were surrounded by a region of severe choriocapillaris alteration. These observations are strong evidence to suggest that the initial insult to the RR in nAMD is the loss of choroidal vasculature (Bhutto & Lutty, 2012).

Proliferation of new choroidal architecture during the CNV phase of nAMD is believed to be principally stimulated by RPE-mediated release of the pro-angiogenesis factor VEGF (Spilsbury, Garrett, Shen, Constable, & Rakoczy, 2000). Immunological involvement is inextricably linked with the production of VEGF (Thurman et al., 2009). The chronological order of the CNV process remains to be determined; some authors propose that diffuse membranous debris deposition induces hypoxia whilst others suggest pre-existing hypoxia may be the cause of debris deposition and subsequent CNV (Schlingemann, 2004; Feigl, Brown, Lovie-Kitchin, & Swann, 2007). It is likely both theories contribute to CNV development, but the latter may be more significant given that choriocapillaris degeneration appears to be the primary insult in CNV (J. P. Sarks et al., 1994; Bhutto & Lutty, 2012).

The differences between occult and classic neovascular membranes appear to have important implications on the resulting anatomical changes observed in the RR of nAMD eyes (Kanski, 2003; Lim et al., 2012). Schmidt-Erfurth (2007) explored the anatomical changes using fluorescein angiography and indocyanine green angiography in 158 patients. In fluorescein angiography of classic CNV, there was an irregular, steep elevation at the lesion site which was encircled by a prominent ring akin to a dark halo, as depicted in figure 7A below. The central prominence appears to result from the ventral displacement of the neural retina due to sub-retinal neovascular membrane, illustrated in figure 7B. Schmit-Erfurth (2007) deduced that the circumferential halo is a proliferating and actively leaking CNV zone, thus suggesting extravasation and haemorrhage occurs medially to this zone in classic CNV.

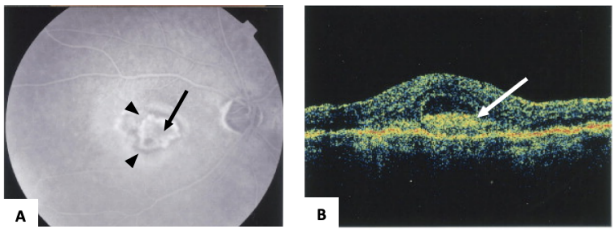

Figure 7. (From Hughes *et al.*, 2005). **Classic CNV in 91-year-old Female.**

Figure 7A: Fluorescein angiography image of well-defined classic CNV lesion (arrow) encircled by circular (halo) zone of choroidal proliferation (arrowheads). Figure 7B: Transverse section OCT image of discreet subretinal neovascular membrane with associated fluid (arrow) resulting from classic CNV.

Fluorescein angiography analysis of occult CNV demonstrated more numerous areas of confluence with a partially convex and flatter appearance (see figure 8B) (Schmidt-Erfurth, Kriechbaum, & Oldag, 2007). The flatter morphology of the occult lesion is suggested in literature to be resulting from neovascular membrane compression by a largely intact RPE layer (Grossniklaus & Green, 2004). Indocyanine green angiography observation revealed a relatively well-perfused central lesion; better perfusion of the RPE and neural retina than in classic CNV may explain the slower visual deterioration observed in occult CNV. Whilst histological analyses generally agree with fluorescein angiography and indocyanine green angiography findings, Lafaut et al., (2000), concludes that in classic CNV, there may also be smaller sub-RPE lesions. This data supports more wider theoretical opinion that nAMD may involve a greater degree of classic CNV than previously thought (J. D. Gass, Yannuzzi, Kramer, & Green, 1994; J. D. M. Gass, 1997). Indeed, figure 8B demonstrates an accumulation of subretinal fluid, in addition to the expected sub RPE fluid, in a patient with occult CNV.

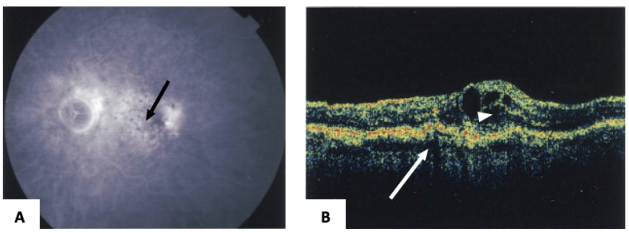

Figure 8. (From Hughes *et al.*, 2005). **Occult CNV in 82-year-old Female.**

Figure 8A: Fluorescein angiography image of poorly defined CNV consistent with the occult type (arrow). Figure 8B: Transverse section OCT image showing significant sub-RPE fluid (arrow) and resulting RPE disruption. Subretinal fluid is also present (arrowhead).

Unlike GA, CNV expansion rates are sparsely represented in medical literature, presumably due to a more rapid progression. Stevens et al. (1997) analysed the natural history of eyes in which occult or both occult and classic CNV was diagnosed over a period of 12 months. They found that 32% of the

lesions doubled or more in size and 38% increased by 4 or more MPS disc areas. 1 MPS disc area is equivalent to a retinal area of 2.54mm2 (Sunness et al., 2007). Functional deficits resulting from the anatomical changes during CNV are stark and more rapid than in GA. A systematic meta-analysis by Wong et al. (2008) showed patients with untreated nAMD can expect functional declines in visual acuity of 1 to 3 LogMAR lines over 3 months, and 3 to 4 lines over the course of a year. A loss of 3 lines represents a halving of the retina's resolving power. Severe vision loss of more than 6 lines was present in over 40% of untreated patients at 3 years. The more aggressive, classic CNV type is often associated with earlier and more substantial visual loss due to direct damage to the neural retina, whereas occult CNV is associated with a slower visual deterioration due to a greater preservation of the RPE layer (Bressler, Finklestein, Sunness, Maguire, & Yarian, 1990).

11 Discussion

This review explores and analyses the anatomical changes that take place in the RR during NA and in AMD, with explicit reference to how such changes relate to AMD pathophysiology. Based on anatomical changes, the senescent process appears to be strongly associated with, and has profound implications, on AMD pathophysiology.

Changes to BrM appear critical in the pathogenesis of all aspects of AMD. Such is the apparent role of BrM in NA and AMD modulation, Moore (2001) moots the idea of a BrM permeability threshold, below which NA changes occur and above which, AMD is more likely. Although likely to be a gross oversimplification, thickening of BrM such as via proteinaceous modification and accumulation of advanced glycation end products compounded by cross-linking as oxidative defence mechanisms deteriorate through senescence, is theorised to take place (Booij et al., 2010; Crabb et al., 2002). Such changes predispose to extracellular debris accumulations which appear to initiate early AMD. Extracellular deposits are anatomical changes common to the processes of both NA and AMD. In NA, drusen are characterised by small size (<63m), distinct borders and a hardened texture (S. H. Sarks et al., 1999). In contrast, amorphous (soft) drusen are a defining anatomical feature of AMD eyes contributing to clinical staging of the disease (Ferris et al., 2013).

Formation of hard drusen appears to be more strongly linked with BrM changes during NA than lipofuscin accumulation, creating a relatively diffuse distribution of hard drusen across the retina (S. H. Sarks et al., 1999). One way in which soft drusen may manifest is via coalescing BLinDs; this process is also linked to BrM thickening during senescence (J. P. Sarks et al., 1994; S. H. Sarks et al., 1999). Whilst appearing relatively innocuous in NA, hard drusen degradation has also been linked to AMD pathophysiology. Breakdown of hard drusen has been postulated to be the other main mechanism of soft drusen formation (J. P. Sarks et al., 1994). Whether the origin of soft drusen is from hard drusen or diffuse BLinD may therefore be important for the pathological course of AMD (Yehoshua et al., 2011; J. P. Sarks et al., 1994). A hard drusen origin appears to preferentially lead to GA in aAMD, whereas a BLinD origin appears to preferentially predispose to CNV in nAMD (J. P. Sarks et al., 1994). However, confounding factors complicate the process of deducing explicit conclusions as basal laminar deposits accumulation has also been linked to onset of GA; basal laminar deposits form across the RR like hard drusen but are not considered a key part of the NA process (S. Sarks et al., 2007; van der Schaft et al., 1993a). Soft drusen are almost universally confined to the macula, with membranous debris having a particular affiliation with the fovea (Curcio, 2018; J. Sarks et al., 2011; Ardeljan & Chan, 2013). The exact reason that hard drusen breakdown in AMD but remain relatively well preserved in NA remains to be discovered. However, this may be due to the lack of immunological involvement in NA compared to AMD (Crabb et al., 2002).

Static foveal RPE densities during NA have been reported, whereas RPE cell loss occurs elsewhere in the macula, thus creating a foveal sparing phenomenon (Gao & Hollyfield, 1992; Del Priore et al., 2002). Evidence suggests that foveal sparing is also demonstrated in GA of aAMD whilst there is cell loss in the parafoveal and perifoveal regions (Lindner et al., 2015; Schmidt-Erfurth et al., 2007; J. P. Sarks et al., 1988). The trends appear to be explained by increases in oxidative stress, although the mechanisms by which this occurs depends on the process. In NA, increased turnover of outer segments by RPs compared to CPs is thought to create a lipofuscin concentration profile that correlates well with RP density (Wing et al., 1978; Delori et al., 2001; Boulton et al., 1993; Ach et al., 2014). The main implication of lipofuscin appears to be photoinducible generation of reactive oxygen species and subsequent apoptosis of the RPE-PR complex, although some link it to hard drusen formation too (Davies et al., 2001; Mazzitello et al., 2009; Yasukawa et al., 2007). Literature tends to agree that RPE density shows greatest decline in regions of the retina where the density of RPs is high (Gao & Hollyfield, 1992; Del Priore et al., 2002). A close, but not parallel, correlation of RPE-PR loss with RP topography is understood to occur in NA owing to the lipofuscin pathway discussed previously.

RPE atrophy is understood to be the defining insult to the RR in aAMD (Bhutto & Lutty, 2012). In GA pathophysiology, parafoveal and perifoveal loss of RPE similar to NA is observed, likely due to preferential macular BrM thickening and reduced permeability (Moore et al., 1995). Formation of hard drusen resulting from this and subsequent immunological involvement could lead to hard drusen breakdown which is known to induce GA (S. H. Sarks et al., 1999; Crabb et al., 2002; J. P. Sarks et al., 1994). The effect of such a decrease in BrM permeability (and subsequent hard drusen formation) is likely to be less marked in the fovea due to the significantly lower concentration of lipofuscin accumulated during NA (Delori et al., 2001). Additionally, it is understood that CPs have a higher resistance to increases in reactive oxygen species than RPs (Komeima et al., 2006). It is therefore proposed that enhanced lipofuscin accumulation and subsequent RPE cell loss during senescence, is more likely to result in aAMD when combined with decreases in macular BrM permeability and

subsequent hard drusen formation.

In NA, vascular and perfusion densities decrease; the reduction is most marked in the fovea and is probably due to reduced capillary diameters (Ramrattan et al., 1994; Sacconi et al., 2019). Similarly, CNV has been proven to have a predilection for the foveal region during nAMD (Invernizzi et al., 2018; Manjunath et al., 2011; Govetto et al., 2017). Although BLinD formation is linked to BrM thickening, it is probably more extensively modulated by choriocapillaris changes of the fovea (S. H. Sarks et al., 1999; Provis et al., 2005). Reduced perfusion during NA is likely to increase oxidative stress of the RR and decreasing RPE waste removal may aid production of membranous debris (Bhatt et al., 2010; Friedman et al., 1963; Alten et al., 2013; Curcio, 2018; J. Sarks et al., 2011). Choriocapillaris degeneration, unlike in aAMD, appears to be the primary insult to the RR in nAMD (Bhutto & Lutty, 2012; Mcleod et al., 2009; Moult et al., 2014). The effects of such an insult would have greater impact on the foveal region given the changes that take place in NA. It is therefore proposed that accelerated loss of the choriocapillaris during ageing is likely to preferentially predispose to BLinD accumulation and aggregation, leading to nAMD as a sequela. However, given the lack of knowledge regarding the manner in which this primary insult may occur, further research into the role of the choroidal complex in AMD pathophysiology is required.

Mechanisms leading to RPE cell death also appear to be noticeably different between NA and AMD; proportionally greater inflammatory and immunological involvement is likely to comprise AMD pathophysiology compared to the NA process. Increased oxidative stress from lipofuscin accumulation in particular, is theorised to result in RPE-PR apoptosis (Wihlmark et al., 1997; Bhattacharya, Chaum, Johnson, & Johnson, 2012; Del Priore et al., 2002). In AMD however, apoptosis appears to be supplementary to a predominantly necroptotic pathway (Hanus et al., 2015). The findings of Hanus (2015) agree with studies that have found extensive immunological involvement in soft drusen formation (Johnson, Ozaki, Staples, Erickson, & Anderson, 2000). It must be considered that Johnson et al. (2000) also found some degree of immunological involvement in hard drusen formation, thus suggesting that cellular population changes in NA are not entirely apoptotic.

There is a noticeable disparity between the age of research investigating NA of the RR, compared to AMD. With few exceptions, literature on NA changes predates current AMD morphometric research by decades, indicating a general acceptance within the scientific community that sufficient analysis of this area has been undertaken. By comparison, there is an unprecedented intensity of AMD research at present and this is unsurprising given the prevalence and clinical impact of the disease. However, this study has uncovered noticeable contention in NA findings. Given the level of technological advancement over the past 20 years and the inextricably close relationship between NA and AMD, research into NA changes to the human RR has never been more important.

A limitation of this study is the explicit focus on late stages of AMD. Whilst investigation into the development of AMD from the early stages was undertaken, it was necessary to preferentially analyse late AMD given the overt anatomical changes that occur during these stages. It is acknowledged that changes observed in early AMD may be more closely aligned with NA changes. A further limitation is the absence of discussion regarding other structures of the neurosensory retina. Further research into anatomical changes in regions of the retina not investigated in this review may aid understanding of issues, such as the decline in visual acuity in NA with relative absence of foveal RPE-PR anatomical changes, highlighted by this paper.

12 Conclusion

The senescent process is strongly associated with AMD and has profound implications for its pathophysiology. In addition to predisposing to the pathological state, NA appears to play a key role in determining the pathophysiological course of the disease.

Reductions in BrM permeability through proteinaceous modification, collagen cross linking and advanced glycation end-products in senescence, appears to be critical in the general pathophysiology of AMD. Production of hard drusen and BLinDs are associated with the structural changes to BrM during ageing. These extracellular deposits appear to significantly influence AMD pathophysiology; breakdown of hard drusen is more associated with aAMD whilst coalescing of BLinDs seems to be more linked with nAMD development. Oxidative stress is a common theme in NA and AMD and extensively contributes to AMD pathophysiology. This review has also highlighted various areas of ambiguity in AMD pathophysiology, and the importance of ongoing research into AMD pathophysiology to simultaneously consider the retinal ageing process.

It is proposed that enhanced lipofuscin accumulation and subsequent RPE cell loss during senescence, is more likely to result in aAMD when combined with decreases in macular BrM permeability and subsequent hard drusen formation. RPE atrophy is understood to be the defining insult to the RR in aAMD. Patterns of RPE cell loss are similar in NA (principally modulated by lipofuscin) and aAMD, both demonstrating foveal sparing. Macular BrM thickening facilitates hard drusen accumulation, which can breakdown, leading to aAMD. The effect of such a decrease in BrM permeability (and subsequent hard drusen formation) is likely to be less marked in the fovea due to the significantly lower concentration of lipofuscin accumulated during NA. It is also posited that accelerated loss of the choriocapillaris during ageing is likely to preferentially predispose to BLinD accumulation and aggregation, leading to nAMD as a sequela.

Choriocapillaris degeneration appears to be the primary insult to the RR in nAMD. CNV has a predilection for the fovea during nAMD, and the fovea also appears to be disproportionately affected in NA. Although BLinD formation is linked to BrM thickening, it is probably more extensively modulated by choriocapillaris changes of the fovea. The effects of this

primary choriocapillaris insult would therefore be greater in the fovea given the changes that take place in NA, thus nAMD is likely to ensue. However, there is a lack of understanding regarding the exact mechanism by which the primary choriocapillaris insult occurs. Indeed, further research is required to elucidate the exact role of the choroidal complex in AMD pathogenesis.

Author statements

Conflicts of interest statement

No conflicts of interest have been declared by any authors.

Authorship statement

All authors fulfill ICMJE authorship criteria, which can be accessed at `http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html`. All authors have read and approved the final version, and accept responsibility for information published.

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Authors declare that no ethical approval was required for this article.

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