Review

Aberrant Lipid Metabolism and Complement Activation in Age-Related Macular Degeneration

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Age-related macular degeneration (AMD) stands as a leading cause of severe visual impairment and blindness among the elderly globally. As a multifactorial disease, AMD's pathogenesis is influenced by genetic, environmental, and age-related factors, with lipid metabolism abnormalities and complement system dysregulation playing critical roles. This review delves into recent advancements in understanding the intricate interaction between these two crucial pathways, highlighting their contribution to the disease's progression through chronic inflammation, drusen formation, and retinal pigment epithelium dysfunction. Importantly, emerging evidence points to dysregulated lipid profiles, particularly alterations in high-density lipoprotein levels, oxidized lipid deposits, and intracellular lipofuscin accumulation, as exacerbating factors that enhance complement activation and subsequently amplify tissue damage in AMD. Furthermore, genetic studies have revealed significant associations between AMD and specific genes involved in lipid transport and complement regulation, shedding light on disease susceptibility and underlying mechanisms. The review further explores the clinical implications of these findings, advocating for a novel therapeutic approach that integrates lipid metabolism modulators with complement inhibitors. By concurrently targeting these pathways, the dual-targeted approach holds promise in significantly improving outcomes for AMD patients, heralding a new horizon in AMD management and treatment.

Keywords: AMD, complement system, CFH, lipids metabolism

Age-related macular degeneration (AMD) is a progressively degenerative disorder affecting the macula, the central region of the retina crucial for optimal visual precision.[1] As the fourth leading cause of moderate to severe vision loss in individuals over 50, AMD accounted for 1.8 million of the 33.6 million cases of blindness globally in 2020.[2] Moreover, as the world's population ages, the collective prevalence of any form of AMD has been steadily rising. It was estimated that approximately 8.69% (95% credible interval [CI], 4.26–17.40) of the global population aged 30 and above, equating to 196 million people, were affected in 2020. This prevalence is projected to escalate to 288 million by 2040, thus presenting a significant socioeconomic burden on global health.[3]

According to the classification system proposed by the Classification Committee of the Beckman Initiative for Macular Research, AMD is categorized into three stages based on the presence of pigmentary abnormalities and the size of drusen, a pathological feature located between the basal membrane of the retinal pigment epithelium (RPE) and the inner collagen layer of Bruch's membrane (BrM).[4] Early AMD is characterized by individuals with medium drusen (63 µm to 125 µm) in the absence of pigmentary abnormalities. Intermediate AMD encompasses individuals with large drusen (>125 µm) or pigmentary abnormalities associated with at least medium drusen. The onset of the disease typically becomes apparent in its advanced manifestations, exhibiting two primary forms. One is the development of confluent areas of atrophy involving the loss of photoreceptors and RPE cells, known as geographic atrophy (dry) AMD or geographic atrophy (GA). The other is characterized by choroidal neovascularization (CNV) in the macular region, referred to as neovascular (wet) AMD.[5] This neovascularization is believed to be driven by increased

expression of vascular endothelial growth factor A (VEGF-A) and subsequently binds with VEGFR2 in response to stimuli such as oxidative stress and complement activation.[6,7] Although approximately 85% to 90% of AMD patients have the non-neovascular form,[8] the neovascular subtype is responsible for the majority of severe central visual acuity loss, often accompanied by complications like edemas, hemorrhages, and fibrosis.

The management of AMD currently requires a multidisciplinary approach, incorporating lifestyle modifications, pharmacotherapy, complement inhibitors, regulation of lipid components, and surgical interventions. Studies, notably the Age-Related Eye Disease Study (AREDS), have demonstrated that antioxidant vitamin and mineral supplements (e.g., vitamins C and E, zinc, and copper) offer modest benefits in slowing the progression to advanced AMD.[9] Furthermore, intravitreal injections of anti-VEGF agents (e.g., bevacizumab, ranibizumab, aflibercept, brolucizumab, and faricimab) have significantly improved the treatment of neovascular AMD by inhibiting neovascularization and the leakage of new blood vessels, representing the primary treatment modality.[10,11] Additional therapeutic strategies under exploration include autophagy enhancers, mitochondria-targeting molecules, and programmed cell death inhibitors.[12] Recent approvals by the U.S. Food and Drug Administration (FDA) of complement inhibitors, pegcetacoplan (a complement C3 inhibitor, Syfovre; Apellis Pharmaceuticals, Waltham, MA, USA) in February 2023 and avacincaptad pegol (a complement C5 inhibitor, Izervay; Astellas Pharmaceuticals, Tokyo, Japan) in August 2023, represent significant advancements.[13] However, an effective intervention for slowing progression in the early stages or preventing the late stage of AMD, especially dry AMD, remains largely elusive.

Efforts to develop novel therapies targeting dry AMD should be expedited by gaining a more comprehensive understanding of the primary pathomechanisms underlying AMD onset. AMD is a multifactorial and complex disease influenced by various aging, genetic, and environmental factors. Among these, aging stands out as a pivotal factor disrupting retinal homeostasis[14] leading to increased resistance, rarefaction, and loss of choriocapillaris, as well as lipid and lipoprotein deposition in Bruch's membrane and reduction in photoreceptor density.[1,15] Despite the exact pathomechanisms of transition from chronological aging to AMD remains elusive, it is hypothesized that these aging-related alterations, combined with chronic inflammation, altered lipid and lipoprotein deposition, increased oxidative stress, leading to extracellular lipids, lipoproteins, and protein-containing deposits such as drusen or subretinal drusenoid drusen (SDD; deposit localizes to the subretinal space between photoreceptors and RPE) in the posterior eyes.

Moreover, from human donor tissues and large genome-wide associated studies (GWAS), the associations of AMD and genes related to inflammation and immunity, lipid metabolism and transport, cellular stress and toxicity, and extracellular matrix remodeling have also been further identified.[16] Specifically, 52 common and rare genetic variants at 34 loci have been identified to be associated with AMD, and polymorphisms in genes that regulate complement activation and lipid metabolism particularly are among the biggest genetic risk factors for AMD.[17] Moreover, several lines of collaborative GWAS have identified specific gene variants encoding complement factors, particularly complement factor H (CFH), as well as CFI, CFB, C2, C3, and C9, linked to an increased risk of AMD, with the CFH gene Y402H polymorphism making a substantial contribution to the disease susceptibility.[18] A comprehensive meta-analysis has demonstrated that the CFH Y402H variant approximately doubles the risk of developing late-stage AMD per allele in individuals of European descent,[19] underscoring the significance of the complement system in AMD pathology.

Beyond the complement cascade, there is also growing evidence to suggest that dysregulated lipid metabolism also plays a pivotal role in the progression of AMD. This is supported by genetic analyses identifying AMD-associated polymorphisms in genes governing lipid metabolism[20–22] and biochemical studies that have identified lipids and lipoproteins as predominant constituents of drusen.[23–25] Furthermore, recent epidemiological research has consistently indicated a potential link between high-density lipoprotein (HDL)—traditionally considered protective—and AMD progression.[26] This correlation has aroused great interest in elucidating the precise molecular mechanisms by which aberrant lipid metabolism may influence AMD pathogenesis. Additionally, the involvement of these pathways in conditions such as cardiovascular and central nervous system disorders, which share partially overlapping analogous etiologies with AMD,[27] raises intriguing possibilities for the crosstalk of the two systems. It is tempting to speculate that, by exploring the interplay between the complement system and lipid metabolic pathways, we could unveil novel therapeutic targets, offering promising avenues for AMD treatment and prevention.

In this context, our review article aims to provide a concise summary of the advances in our understanding of AMD's etiopathogenesis, with a particular emphasis on the role of aberrant lipids metabolism and dysregulated complement activation and their interplay in the development and progression of the disorder. Additionally, we will review the latest approved or preclinical therapeutic approaches and shed light on alternative drug candidates.

Overview of Complement System and Lipids Metabolism in AMD

Complement System

The complement system is a vital component of the innate immune response, comprising a network of proteins that act in a coordinated manner to eliminate pathogens, clear immune complexes, and modulate inflammatory responses.[28] It consists of three main activation pathways: the classical pathway, the lectin pathway, and the alternative pathway (Fig. 1). Regardless of the activation pathway, the subsequent steps involve the formation of the C5 convertase (C4bC2aC3b or C3bBbC3b), which cleaves C5 into C5a and C5b. The generation of C5b triggers the assembly of the membrane attack complex (MAC) composed of C5b, C6, C7, C8, and multiple units of C9. The MAC creates a pore in the pathogen's membrane, leading to cell lysis and destruction. In addition to their role in immune defense, complement proteins also participate in inflammation, clearance of immune complexes, and modulation of adaptive immune responses. It's worth noting that this is a simplified overview of the complement system, and there are additional regulatory proteins and factors involved in this intricate regulation and function (Fig. 1).[29,30]

In the context of AMD, research has shown that dysregulation of the complement system plays a significant role in

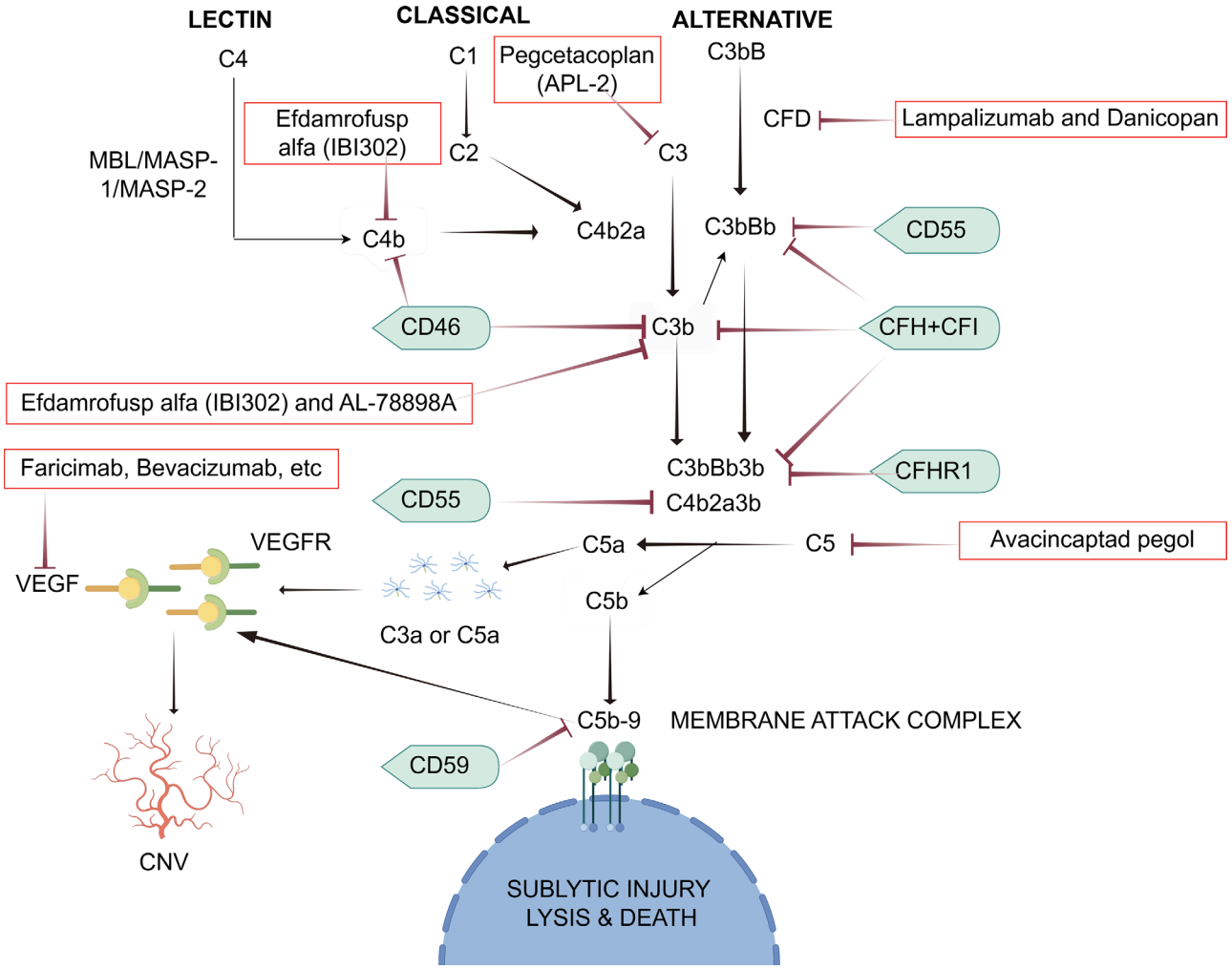

Figure 1. The main complement component involved in the pathologic process of AMD. The role of complement depends on three pathways: classical pathway, alternative pathway, and lectin pathway. These three pathways intersect at C5 and then complete the common pathway, eventually leading to the formation of MAC, leading to cell lysis and destruction. At the same time, multiple complement regulatory proteins act at different levels to modulate this system. Typically, cells mitigate excessive complement activation through fluid-phase regulators such as CFH and membrane-bound molecules such as CD46 and CD55, which control C3 and C5 convertases, alongside CD59, which obstructs the assembly of C5b-9 complexes. C3a and C5a can not only act as inflammatory factors to aggravate the inflammatory response, but also promote the production of VEGF, thereby inducing the formation of CNV and causing neovascular AMD. At present, some drugs targeting complement molecules have entered or completed clinical trials, such as Pegcetacoplan (APL-2) targeting C3, Avacincaptad pegol targeting C5, Lampalizumab and Danicopan targeting CFD, Efdamrofusp alfa (IBI302) targeting C4b and C3b, AL-78898A targeting C3b, reducing complement system-induced RPE damage and alleviating AMD symptoms. C5, complement component 5; MAC, membrane attack complex; CFH, complement factor H; C3, complement component 3; CD46, cluster of differentiation 46; CD55, cluster of differentiation 55; CD59, cluster of differentiation 59.[217]

the development and progression of the disease.[31] Remarkably, complement activation levels correlate with disease stage, with higher levels observed in intermediate and late dry AMD compared to early stages,[32] and complement inhibitors were demonstrated to be effective.[33] As for this system, a critical pathway involved is the alternative complement pathway,[34] which is responsible for the amplification of the cascade reaction.

Genetic studies indicate that variants in the alternative complement pathway significantly elevate the risk of developing AMD, with mutations in the CFH gene showing the most pronounced effects.[35,36] CFH plays a critical role in regulating the complement system, primarily by competing with complement factor B (CFB) to bind C3b, accelerating the resultant dissociation of the alternative system C3 convertase and partnering with serine protease factor I to convert C3b to its inactive form, iC3b.[37] Of note, a risk haplotype is strongly associated with a non-synonymous single nucleotide polymorphism, resulting in a tyrosine (Y) to histidine (H) substitution at position 402 of the CFH protein (the "Y402H polymorphism") and is linked to an increased risk of developing soft drusen (soft drusen and basal linear drusen [BLinD] represent two forms [lump and layer] of the same AMD-specific sub-RPE deposit) and both forms of advanced AMD (GA or neovascular AMD).[38,39] CFH consists of 20 short consensus repeats (SCRs),[40,41] with the Y402H polymorphism located in SCR 7 (Fig. 2).[40–43] This region has been shown to mediate CFH binding to polyanions such as heparin, glycosaminoglycans, and C-reactive protein (CRP), among others, but it is not directly involved in the regulation

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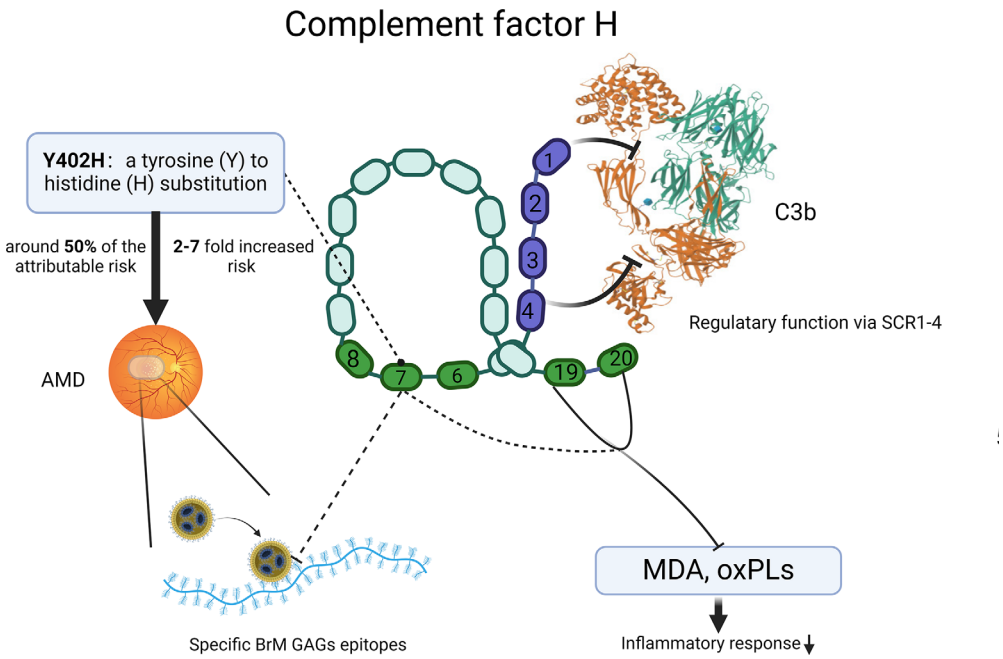

Figure 2. Schematic representation of CFH domain structure. The haplotype of CFH significantly increases the risk of AMD by 2 to 7 times and accounts for approximately 50% of the population attributable risk. CFH interacts with C3b, GAGs, MDA, oxPLs, and other ligands through its various domains to regulate the complement pathway. SCRs 1-4 are essential for CFH's role as a cofactor for Factor I, aiding in the cleavage of C3b to iC3b and facilitating the decay of the C3 convertase complex (C3bBb). SCRs 6-8 and 19-20 primarily bind GAGs in Bruch's membrane, whereas SCR 7 and 19-20 mediate CFH binding to MDA and oxPLs. The Y402H mutation affects CFH's binding to specific BrM GAGs and oxidized lipids such as MDA and oxPLs, implicating it in disease mechanisms. The C3b crystal structure is from RCSB PDB - 5FO7.

of complement.[36,44] Previous studies have demonstrated that this CFH haplotype significantly increases the risk of AMD, with odds ratios ranging from 2.45 to 5.57.[45] Possession of at least one histidine at amino acid position 402 raises the risk of AMD by 2.7-fold, potentially accounting for up to 50% of the attributable risk of AMD.[46] Notably, individuals homozygous for the risk allele have a 7.4-fold increased likelihood of developing AMD (95% confidence interval, 2.9 to 19).[44] In the Beaver Dam Eye Study, Klein et al.[47] further demonstrated that the estimated population attributable risk fraction for early and late AMD was 9.6% and 53.2%, respectively when at least 1 CFH risk allele was present. Intriguingly, a more recent study revealed that people who carry the well-establish risk AMD alleles at CFH/ARMS2 exhibit considerably variable AMD risks, ranging from close to zero in low polygenic risk score individuals to >50% in high polygenic risk score individuals.[48] These studies concomitantly revealed that this common haplotype in the CFH gene predisposes individuals to AMD.

As well-known risk alleles for AMD, CFH haplotypes have been extensively studied in both in vitro and in vivo settings to elucidate their role in disease progression. At the cellular level, studies on induced pluripotent stem cells carrying the Y402H variant have revealed early accumulation of lipid droplets and AMD-like deposits.[49–51] Furthermore, Velazquez et al.[52] demonstrated that APRE19 cells harboring the CFH Y402H variant were more susceptible to oxidative stress and exhibited significantly elevated levels of inflammatory and pro-apoptotic factors, such as cytochrome C and NF-κB, under oxidative stress conditions. They also confirmed that exogenous CFH could mitigate oxidative damage and apoptosis in these cells.[52] In animal models, transgenic mice expressing equal amounts of the full-length normal human CFH Y402 (CFH-Y/0) or the AMD-risk associated CFH H402 (CFH-H/H) variant on a Cfh (−/−) background also showed a phenotype-dependent accumulation of lipoproteins and complement activation.[53] Additionally, in commonly used models such as laser-treated C57BL/6 mice, researchers have observed increased laser-induced CNV lesion size and MAC deposition in mice treated with subretinal injections of siRNA targeting CFH or in CFH-deficient mice.[54,55] Subsequent studies on aged Cfh (+/−) and Cfh (−/−) mice further observed thickened BrM, accumulation of basal laminar deposits, RPE layer pathology, and visual function deficits compared to age-matched controls, underscoring the crucial role of CFH in the pathological features of AMD in vivo.[41]

Furthermore, polymorphisms of CFI, along with C2, C3, CFB, FD, C9, CFHR1, and CFHR3 are also involved in the pathogenesis of AMD.[31,56–58] Crowley et al.[59] found that the use of an anti-CFD agent inhibited complement activation in the eye and attenuated the inflammatory response. Cohort studies have shown that the intron 10 variant of C2 and the R32Q variant of CFB (H7) significantly reduce the risk of AMD.[60] The C3 gene, located on chromosome 19p13, has a fast-migrating variant, C3F, which is strongly associated with AMD. This variant plays a crucial role in uncontrolled alternative complement activation and the accumulation of complement-containing extracellular deposits.[61,62] A rare variant of C3 (p.Lys155Gln; rs147859257) exhibits a reduced ability to bind CFH, attenuates the catabolic cycle of C3b, and increases alternative pathway activity. This variant is more frequently observed in GA cases.[63] C3 and C5 are predominantly found in drusen, and their breakdown

products, such as C3a and C5a, serve as inflammatory mediators. These mediators not only induce the production of VEGF but also promote the formation of CNV, contributing to the development of neovascular AMD.[64,65] CFI, the major regulator of C3, has variants near its gene on chromosome 4q25 that have been linked to advanced AMD.[66] Additionally, C7 (rs2876849) and a variant of the SERPING1 gene encoding a C1 inhibitor (rs2511989) may offer protective effects against AMD.[67,68] The detailed role of the regulation of the complement system and all complement factors individually has recently been reviewed elsewhere.[58]

Based on these findings, various therapeutic approaches targeting the complement system have been explored for the treatment of AMD. These strategies aim to modulate the complement cascade, inhibit specific complement components, or restore the balance of complement regulation.[69] Clinical trials evaluating complement inhibitors and other related therapies are ongoing and hold promise for future AMD treatments.[70]

Lipids Metabolism

The retina is notable for its high lipid content, amounting to 20% of its dry weight. In general, over half of all retinal fatty acids (FAs) are unsaturated, and, of these, approximately 60% are polyunsaturated FAs (PUFAs). Outer segments (OS) of photoreceptors have the highest docosahexaenoic acid (DHA) concentration in the body, which makes the retina susceptible to oxidative stress.[71] In addition, lipids are an important source of energy and a precursor of many signaling molecules in the eyes. To maintain normal biofunctions, lipid hemostasis is tightly regulated in the retina through local enzymes and trafficking.

Strong evidence suggests that aberrant lipid metabolism is involved in dysfunction of RPE. Particularly, more than 40% of drusen, the hallmark histopathologic feature of AMD, is comprised of lipids dominated by esterified cholesterol, unesterified cholesterol, and phosphatidylcholine.[23] Almost a decade ago, Curcio et al.[72,73] proposed the "oil spill" model to characterize the process by which lipids accumulate within the retina during physiologic aging and in the transition to AMD, culminating in the formation of "lipid wall" and subsequent decreased productivity of BrM.

Moreover, a recent meta-analysis of several GWAS confirmed that many AMD-associated loci were located near or within genes mainly involved in such as high-density lipoprotein particle remodeling, cholesterol transporter activity, and more.[74] In recent years, numerous studies have further identified associations between AMD and genetic variants near or within specific genes, notably the hepatic lipase gene (LIPC), cholesterol ester transfer protein (CETP), ATP binding cassette subfamily A member 1 (ABCA1), and apolipoprotein E gene (APOE), all of which are integral to the HDL metabolic pathway. These findings illuminate potential genetic correlations with AMD, offering insights into its molecular underpinnings.[75,76]

Furthermore, in the largest metabolome association analysis in AMD to date, 60 plasma metabolites were identified to be associated significantly with AMD, including increased levels of large and extra-large HDL subclasses and decreased levels of very low-density lipoprotein, low-density lipoprotein (LDL) and high total serum cholesterol.[77] Intriguingly, previous studies have yielded conflicting results regarding the use of systemic HDL or LDL measurements as risk indicators for the development of AMD, with no consistent consensus emerging.[78] However, recent evidence suggests that elevated serum HDL levels may increase the risk of AMD, challenging the conventional view of low-density lipoprotein-cholesterol (LDL-C) as "bad" cholesterol and high-density lipoprotein-cholesterol (HDL-C) as "good" cholesterol, a paradigm established in cardiovascular disease research.[79,80] Furthermore, a recent review by Eckardstein and colleagues[81] revisited the functions of HDL, proposing that extremely high HDL-C levels are not only associated with a heightened risk of AMD but also with increased risks of infectious diseases and all-cause mortality. These findings suggest a need to reevaluate the role of HDL in lipid metabolism, particularly in conditions such as AMD, diverging from traditional perspectives.[81]

In addition, lipofuscin, characterized as a complex mixture of cross-linked proteins and lipids, is of keen interest for its popular existence during physiological aging. Intriguingly, particularly in the retina, lipofuscin primarily comprises lipid-bisretinoids with minimal protein content, with which amino content of only 2%, distinguishing it from lipofuscins in other tissues.[82] These autofluorescent vitamin A derivatives, known as bisretinoids, are synthesized through random non-enzymatic condensation reactions between retinaldehyde and phosphatidylethanolamine within the photoreceptor OS discs.[83] After phagocytosis of these outer segments, bisretinoids accumulate in the RPE lysosomes, facilitated by pH-dependent protonation.[84] However, despite its increasing recognition, the exact mechanisms underlying retinal lipofuscin's cytotoxicity remain poorly understood, and current intervention strategies are limited in efficacy.

Interaction Between Lipid Metabolism and Complement in AMD

Given the intricate association between lipid components and complement, recent research has focused on the crosstalk between lipids and the complement system. Lipid alterations, including dysregulated cholesterol homeostasis mediated by lipoproteins,[85] accumulation of oxidized lipids,[26] and intracellular deposits of lipofuscin,[86] are implicated in complement system activation. Pioneering multi-omics analyses, as demonstrated by Crabb et al., have identified the presence of various complement components (C3, C5, C6, C7, C8, C9) within drusen,[31] a hallmark of AMD characterized by its rich lipid content.[36] Notably, complement-regulatory molecules such as CFH, vitronectin, and clusterin are also abundant in these lipid-rich deposits.[24]

Meanwhile, numerous GWAS have elucidated that the unaccounted heritability of AMD, not explained by most studied genes in the CFH pathway or the ARMS2/HTRA1 locus, may be partially attributed to the HDL cholesterol metabolism pathway.[87] Additional studies suggest that increasing levels of HDL cholesterol, particularly via inhibition of CETP, may lead to an increased risk of AMD.[88] Notably, a study involving a Chinese case-control group of neovascular AMD and polypoidal choroidal vasculopathy (PCV) patients identified a statistically significant interaction between rs3764261 in the CETP gene and rs800292 in the CFH gene for both neovascular AMD and PCV.[89] This suggests that CETP may act as a modifier gene for CFH in the development of these conditions, indicating interactions between genes in the complement system and the lipid metabolism pathway. Furthermore, the intricate relation-

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ship extends to circulating lipoprotein particles, several of which are functionally and structurally linked to the complement system. Chylomicron particles, the largest and least dense, play a crucial role in lipid transport from the intestine to the body. Intriguingly, these particles also transport transthyretin, which has been shown to stimulate Complement C3 (C3) and acylation-stimulating protein (C3desArg) synthesis in a dose-dependent manner.[90] Proteomic studies of HDL have identified several complement components, including C3, C4B, Factor B, C5, and to a lesser extent, C1 subcomponents and C2.[91] This finding aligns with epidemiological evidence from the EYE-RISK and European Eye Epidemiology Consortia, linking systemic complement activation, indicated by the C3d to C3 ratio, with AMD-associated metabolites, such as various lipoprotein subfractions and lipid-related genes like CETP and LIPC.[92] Notably, elevated levels of large and very large HDL are also associated with increased complement activation in AMD patients compared to controls[26,93] and dysfunction in lipoprotein transport can lead to increased hydraulic resistance and aberrant formation of various oxidized lipids, which will be discussed in detail below.

Additionally, the RPE is crucial for physiological visual function, transporting nutrients into and metabolites out of the retina, and recycling vitamin A to maintain the visual cycle.[94] Under high metabolic stress, RPE may lose its function of clearance, resulting in the accumulation of undegraded photoreceptor outer segment (POS) components containing autofluorescent bisretinoids adducts in RPE lysosomes, forming lipofuscin over time.[95] Within the first decades of life, these intracellular debris start to accumulate in RPE and represent almost 20% of the cytoplasmic volume of RPE cells by age 80 years.[96] The mutation in ABCA4 is known to disrupt the normal visual cycle by affecting the transport of N-retinylidene-PE across the lipid bilayer to the cytoplasmic side, where it is hydrolyzed to release all-trans-retinal. When ABCA4 is nonfunctional, as in Stargardt disease, N-retinylidene-PE can irreversibly react with a second molecule of all-trans-retinal to form bisretinoids such as A2E.[84,97] Several studies have shown that lysosomal lipofuscin is significantly associated with complement activation through byproducts of photooxidation[98] or pathological accumulation of A2E and ceramides.[97] In summary, further comprehensive research into lipid metabolism, specifically the roles of lipoprotein profiles, oxidized lipids, and lysosomal lipofuscin, and their interactions with the complement system, is warranted.

Aberrant Lipoprotein Trafficking and Metabolism

Lipoproteins are a group of particles with diverse biochemical compositions, as well as distinct physicochemical and biological properties, that mediate the transportation of insoluble lipids, such as cholesterol and triglycerides.[99] Multiple studies have suggested a strong association between lipoprotein trafficking metabolism and AMD.[26,81,85,92,100] As a high metabolic tissue, the retina has numerous lipids demands and must meet those needs either by synthesizing its supply of cholesterol (de novo synthesis)[101] or by importing cholesterol from extraretinal sources, such as lipoprotein-based transport.[102] Physiologically, lipoproteins mechanically ensure the efflux of retinal cholesterol in its native form. Those lipoproteins can cross the basement membrane of the RPE via ApoE/A-I, ABCA1/G1, and SR-BI, II receptors.[103] As highlighted by Leeuwen et al.,[103] retinal lipid homeostasis is meticulously maintained through various mechanisms that facilitate lipid trafficking between neurons and glial cells, as well as lipid elimination via the bloodstream. RPE cells manage the removal of photoreceptor metabolites, including oxidized lipids and vitamin A derivatives, and produce large quantities of lipoproteins containing ApoB100 and esterified cholesterol, termed "Bruch's membrane lipoprotein particles" (Fig. 3A).[24,25,72] RPE expresses ApoB and microsomal triglyceride transfer protein, secreting these cholesterol-rich lipoparticles into Bruch's membrane, where they are retained and cleared through the choriocapillaris. Additionally, lipoproteins in the neural retinal environment can cross the inner blood-retinal barrier via SR-BI expressed by retinal capillary endothelial cells.[104]

In the context of AMD, however, these lipoproteins could be captured in the Bruch's membrane and form the "lipids wall" (Fig. 3A) as we have discussed in the Lipids Metabolism Section. Moreover, captured lipoproteins in the Bruch's membrane are modified over time and form inflammatory/toxic species with harmful properties such as linoleate hydroperoxide, leading to the formation of lipoprotein-derived debris that are significant components of drusen. Curcio et al.[24] described this process as a "response to retention" because it alters the lipid wall and initiates the formation of AMD lesions, such as BLinD and soft drusen. Collaborative studies further support this hypothesis, suggesting a unified pathogenic mechanism for SDD formation that involves cholesterol lipoprotein particles. This mechanism is attributed to abnormal lipid trafficking through lipoproteins and subsequent accumulation of lipoprotein-derived oxidized and pro-inflammatory lipids in the sub-RPE or subretinal space, which occurs with aging.[25,72] However, the exact molecular mechanisms how these lipoparticles retain in the posterior eye remain to evolve.

Competitive Binding Between Lipoproteins and Complement. Interestingly, recent studies suggested that the variant of CFH Y402H polymorphism may independently contribute to the retention of lipoprotein particles in eyecup.[41] It is hypothesized that the normal form of CFH can compete with RPE-derived lipoproteins at the three-dimensional glycosaminoglycans (GAGs) motif of the BrM and that this interaction is lost after CFH mutation (Y402H), leading to dysregulated complement activation by a lower level of BrM-associated CFH[40,105] and excessive accumulation of lipoproteins in the BrM (Fig. 2).[36] Consequently, increased BrM lipoprotein accumulation would lead to earlier development of drusen and resultant AMD pathology.

Anatomically, Bruch's membrane is a pentalaminar extracellular matrix located between the RPE and the choroid, consisting of sublaminae made up of collagen and elastin-based fibrous connective tissues, as well as a variety of proteoglycans (PGs), predominantly those rich in heparan sulfate (58%)[106] produced by the RPE.[107] In the BrM, PGs are heavily glycosylated glycoproteins covalently linked to all classes of GAGs chains (e.g., heparan sulfate, chondroitin sulfate, dermatan sulfate, and keratin sulfate).[107] Previous studies have documented interactions between heparan sulfate proteoglycans (HSPGs) and lipoproteins,[108] and the CFH molecule was also demonstrated to have two acetyl HS binding domains (SCRs 6-8 and SCRs 19-20), with heparan sulfate identified as the primary binding partner for CFH in human Bruch's membrane.[109–111] Thus the normal form of CFH can compete with ApoE and ApoB-containing lipopro-

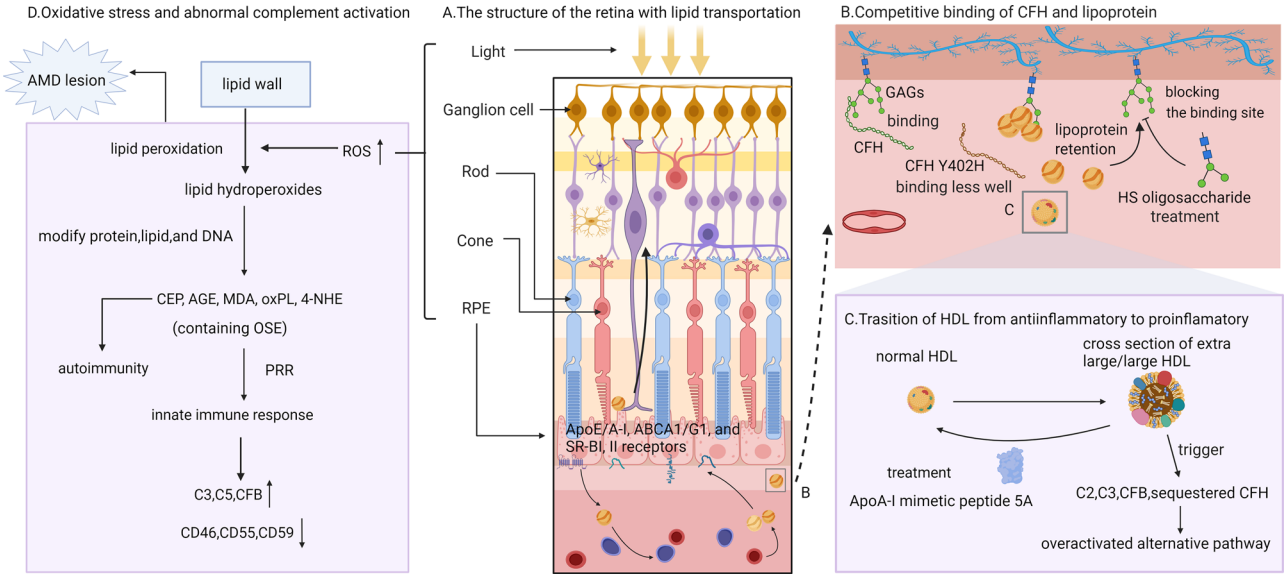

Figure 3. The process of transport and oxidation of lipids metabolism in the posterior eyes. (**A**) The structure of the retina with lipid transportation. Retinal lipid homeostasis is meticulously maintained through various mechanisms that facilitate lipid trafficking between neurons and glial cells (Muller cell, astrocyte, and microglia), as well as lipid elimination via the bloodstream. RPE cells regulate the elimination of metabolites produced by photoreceptors and release lipoproteins-containing particles as byproducts of lipid metabolism. These lipoproteins can cross the basement membrane of the RPE via ApoE/A-I, ABCA1/G1, and SR-BI, II receptors. (**B**) The process of competitive binding of CFH and lipoprotein in the retina. Normally, CFH can compete with ApoE and ApoB-containing lipoproteins for binding to GAGs in BrM, preventing the excessive accumulation of lipoproteins in BrM. However, the variant of CFH Y402H binds less well to heparan sulfate and dermatan sulfate glycosaminoglycans within Bruch's membrane, contributing to the retention of lipoprotein particles in eyecup. Short heparan sulfate oligosaccharide can specifically block the binding sites in lipoproteins while showing little inhibitory effect on CFH, thus effectively removing lipoprotein-containing deposits in BrM. (**C**) The transition of HDL from anti-inflammatory to proinflammatory. The normal HDL exerts significant influence on the intricate mechanisms of RCT, as well as exhibiting indispensable functions in antioxidation and anti-inflammatory responses. However, once it assumes as cross section of extra-large/large HDL such a form, it triggers the upregulation of C2, C3, CFB, and sequestered CFH, ultimately leading to the activated alternative pathway. As for the ApoA-1 mimetic peptide 5A, it can increase RCT, turning the cross-section of extra-large/large HDL into the normal form. (**D**) The oxidative stress and abnormal complement activation in the fundus. In the context of AMD, lipoproteins could be captured in the Bruch's membrane and form the "lipids wall." Then the light, the metabolic activity of RPE, cone photoreceptors, and retinal ganglion cells could give rise to elevated ROS. These excessive ROS from various sources can initiate the formation of highly reactive oxidized lipids, a process known as lipid peroxidation. The lipid peroxidation process results in the creation of lipid hydroperoxides. Subsequently, these products can modify intracellular and extracellular macromolecules including proteins, lipids, and DNA. These modifications, termed OSEs, are present in various products, such as CEP, AGEs, MDA, oxPLs, 4-HNE. These compounds would elicit autoimmunity. Furthermore, they can engage with corresponding PRRs, activating the innate immune response, culminating in overexpress C3, CFB, and C5, while downregulating negative regulators of the complement system such as CD46, CD55, and CD59. Eventually, all of this process acts as a "response to retention," altering the lipid wall and initiating the formation of AMD lesions, such as BLinD and drusen.

teins for binding to GAGs in both porcine and human BrM,[41] whereas the CFH Y402H binds less well to heparan sulfate and dermatan sulfate GAG within Bruch's membrane[112] (Figs. 2 and 3B). Intriguingly, a recent study proposed by Keenan et al.[113] was that specific sulfation patterns within the BrM extracellular matrix form "zip codes" and the Y402H polymorphism may alter the "zip code" recognition of CFH.

In addition, age-related changes in BrM include increased cross-linking of collagen fibers and accelerated turnover of GAGs, resulting in a shift towards larger PGs with a reduced proportion of heparan sulfate (25%) in newly synthesized PGs.[106] Moreover, Keenan et al.[113] also identified a significant age-related decrease in heparan sulfate levels in BrM from postmortem human ocular tissue, leading to fewer CFH binding sites, particularly the binding efficiency of the CFH Y402H variant. This reduction in heparan sulfate facilitates the accumulation of lipoparticles in BrM and the formation of extracellular deposits like drusen, predisposing the macular to AMD. In contrast, an increased proportion of heparan sulfate was observed in the retinas of mice following photoreceptor degeneration,[114] where lipoprotein accumulation was linked to altered heparan sulfate binding properties. Intriguingly, a recent study analyzing aged transgenic cfh$^{-/-}$ mice on a high-fat cholesterol-rich diet revealed genotype-dependent changes in plasma and eyecup lipoproteins and basal laminar deposit accumulation, despite equivalent complement activation in both aged CFH-H/H and CFH-Y/0 mice.[53] This correlation with an AMD-like phenotype in older CFH-H/H mice further suggests that competition for HSPG binding sites[41,53] could be an independent mechanism. These findings collectively suggest that targeting lipoproteins could be a viable therapeutic strategy for treating AMD.

Pathological Lipoprotein Transformation in Composition and Function. Additionally, an expanding body of evidence suggests that the dynamic composition of lipoprotein derivatives may significantly influence complement activation.[81,85,115] This effect is probably mediated through the regulation of specific complement components (e.g., C1q, C5, C6, C8, and C9) and regulators (e.g., CFH), which have been identified within extracellular deposits associated with AMD.[116] These extracellu-

lar deposits, including BLinD, drusen, and SDD, encompass significant amounts of apolipoproteins (E, B, A-I, C-I, and C-II)-rich lipoproteins, alongside complement components that play a significant role in the progression of AMD, as previously discussed.[25,31,58,117] The lipoprotein-like particles observed by Curcio et al.[24] are round vesicles, 60 to 80 nm in diameter, with a core-shell structure, forming the "lipid wall" between the inner collagenous layer and the elastic layer of BrM. Further lipid profile analysis in cultured RPE-J cells, an immortalized rat RPE cell line, revealed the presence of ApoB-containing lipoprotein-like particles similar to those observed in the BrM of AMD patients. In contrast to circulating HDL and LDL, which range from 8 to 13 nm and 15 to 25 nm, respectively, these findings suggest an alteration in lipoprotein profiles and production by RPE cells.[118] Landowski and colleagues[53] also demonstrated changes in lipoprotein composition in aged CFH-H/H mice compared to age-matched controls, with increases in apolipoproteins B48 and A1 in the RPE/choroid tissue. Collectively, these studies suggest that both circulating and local lipoprotein profiles are altered in AMD. However, the exact mechanisms by which these components interact and evolve during AMD progression remain poorly understood. Therefore a comprehensive understanding of these dynamic changes is essential for advancing AMD treatment.

Specifically, in a human RPE cell-cultured model, activation of the complement cascade appears to be mediated via the classical pathway by the binding of C1q to certain ligands in APOE-rich sub-RPE deposits, triggering direct activation of complement by C1q, deposition of terminal complement complexes and inflammatory sequelae.[119] In contrast, when cultures are exposed to serum depleted of C1q, C5b-9 immunoreactivity associated with sub-RPE deposits is dramatically reduced. It is not surprising to hypothesize that there might be some components arrayed on lipoparticles and interact with the complement system. In addition, in pathological conditions such as AMD, lipoproteins could be converted into dysfunctional particles that instigate oxidative stress and chronic inflammation (Fig. 3).[81,120] Moreover, a detailed proteomic analysis of HDL subclasses has revealed the presence of several complement components, such as C1q, C2, C3, C4B, C5, vitronectin, clusterin, and CFH in extra-large or large HDL, suggesting a potential link between lipoprotein dynamics and the immune response, particularly complement activation.[91,115]

Previous studies have demonstrated that components of HDL, such as APOA1/2 and apolipoprotein J (also known as clusterin), can downregulate complement activation.[121,122] Moreover, CFH was found to bind ApoE on HDL via SCR5-7 and this binding is dependent on the proportion of ApoE on the HDL particles.[123] It was expected that CFH may be sequestered by large HDL particles and contribute to their anti-inflammatory properties, inhibiting complement activation.[115] However, because recent studies found that the systematic complement activity was associated with elevated large and extra-large HDL,[26,92] a possible explanation is that increased uptake of CFH by large HDL leads to a reduced level of unbound circulating CFH in AMD patients[26] (Fig. 3). Additionally, complement factor H-related (CFHR) proteins 1, 4, and 5, which compete with CFH at several binding sites and are less efficient, have been associated with a small subfraction of HDL (approximately 2% of APOA1-containing HDL, with high-density) that has been subsequently named the Factor H–related protein-associated lipoprotein particle.[124,125] Under pathological conditions such as AMD, HDL is prone to modification by harmful products such as oxidized phospholipids (oxPLs), which may impair its function in reverse cholesterol transport (RCT) and reduce its antioxidative and anti-inflammatory properties.[81] It has been reported that the composition of HDL exhibits elevated levels of C3, C4, and CFB, and reduced levels of clusterin in conditions like coronary artery disease or rheumatoid arthritis.[126,127] Interestingly, the alteration of HDL composition in these conditions could be reversed by the use of statins and niacin.[128] Eckardstein and colleagues[81] recently reviewed the adverse functions of HDL in chronic diseases or during infections, suggesting that the composition and biological activity of HDL might play a significant role in the pathogenesis of diseases like AMD. Taken together, these findings indicate that further exploration of the precise functions of lipoprotein fractions and the presence of complement components and regulators is still warranted.

Oxidized Lipids and Dysregulated Complement Activation

The retina stands out as one of the most metabolically active organs in the human body, rendering it highly susceptible to oxidative stress emerging as a by-product of physiological processes (Fig. 3A). Remarkably, macular harbors the highest density of cone photoreceptors, numbering around 150,000 to 180,000 cones/mm^2,[129] which imposes substantial metabolic and photochemical stress on the retina. Moreover, the metabolic activity of RPE, such as routine phagocytosis of photoreceptor OS, also gives rise to elevated reactive oxygen species (ROS) in posterior eyes.[130] Meanwhile, retinal ganglion cells are characterized by considerable metabolic demand to produce a large amount of ATP to compensate for their limitation of being unmyelinated,[131] thus giving rise to an elevated amount of ROS similar to RPE cells. Furthermore, exogenous oxidative stressors such as continuous light exposure, a well-known catalyst for photooxidation reactions, and cigarette smoke, are extensively documented as triggers of oxidative stress.[132] These elements collectively make the macular region highly vulnerable to oxidative damage (Fig. 3A).

Mechanically, these excessive ROS from various sources can initiate the formation of highly reactive oxidized lipids through enzymatic or nonenzymatic degradative reactions,[133] a process known as lipid peroxidation, such as the peroxidation of polyunsaturated fatty acids (e.g., DHA). The lipid peroxidation process results in the creation of lipid hydroperoxides, which, in the presence of transition metals like iron, yield short-chain, unesterified aldehydes and a second class of aldehydes that remain esterified to the parent lipid.[134] Subsequently, oxidized lipids and their reactive lipid degradation products can modify intracellular and extracellular macromolecules including proteins, lipids, and DNA by reacting with free amino groups of protein side chains or lipids that are localized in their vicinity, leading to altered structures that can be sensed by the innate immune response. These modifications, termed "oxidation-specific epitopes" (OSEs),[135] are present on various products[133] such as carboxyethylpyrrole (CEP), advanced glycation end-products (AGEs), malondialdehyde (MDA), oxidized phospholipids (oxPLs). These compounds act as danger-associated molecular patterns, engaging with corresponding pattern recognition receptors (PRRs), scavenger receptors (SRs), and toll-like receptors, thus activating

the innate immune response—particularly the complement system—in the context of AMD[130,136] (Fig. 3B). Although the presence of OSEs typically fulfills a housekeeping role to maintain homeostasis, their accumulation can also act as a proinflammatory signal under certain conditions including AMD.[136,137]

Oxidized Lipids Participate in the Overactivation of Complement. Several studies have shown that oxidative stress can adversely affect complement functionality in various ways.[138] Wang et al.[139] demonstrated that cigarette smoke can elicit overexpress C3, CFB, and C5 while downregulating negative regulators of the complement system such as CD46, CD55, and CD59. Similarly, oxidized products, particularly lipids, can cause a decrease in CFH,[140] CD59, and CD46,[141–143] leading to an increase in MAC, which in turn leads to an increase in VEGF release, suggesting that oxidative stress-mediated reduction in complement regulators makes RPE cells more sensitive to complement-mediated injury.

As previously discussed, lipoproteins like apoB100, once captured in Bruch's membrane, can undergo oxidative and nonoxidative reactions that trigger the degradation of apolipoprotein components, leading to the formation of oxidized products such as oxLDLs.[24]In a model of ARPE-19 cells treated with oxLDLs, notably, cellular CD46 and CD59 proteins, known as complement membrane regulators were decreased by 2.9- and 9-fold ($P < 0.01$), respectively,[145] whereas CFB, C3b, and MAC were observed elevated. These decreased levels of CD46 and CD59 were in part explained by their release in exosomal and apoptotic membranous particles.[146] In addition, studies show that oxLDLs could increase the expression of IRE1α, an endoplasmic reticulum-localized ribonuclease and kinase, which cleaves CD59 mRNA and reduces CD59 load, promoting complement-mediated injury.[145,147] Interestingly, Ebrahimi and colleagues also demonstrated that oxLDLs contributed to elevated complement factor B mRNA and Bb protein levels, thereby increasing the formation of C5b-9 complexes. Although oxLDLs also raise CD55 mRNA levels, they do not impact its protein expression. In contrast, LDL does not influence the mRNA or protein levels of CD46, CD55, or CD59.[145]

Additionally, oxLDLs have been shown to bind both C1q and mannan-binding lectin, thus enhancing the downstream clearance mediated by monocytes and monocyte-derived macrophages.[148] However, it was also revealed that modified LDL activates only the classical complement pathway, with no activation of the lectin pathway detected. This activation is mediated by the interaction between C1q and OSEs on oxLDLs, underscored by the presence of C1q on MDA-LDL and malondialdehyde acetaldehyde-LDL, which is an advanced MDA-lysine adduct[148] (MDA is produced by lipid peroxidation of lipofuscin and can form covalent bonds with adjacent proteins[149]). Yin and colleagues[150] further demonstrated that all ApoE isoforms could attenuate classical pathway activity through a higher affinity for C1q. Additionally, C3a has been shown to interact with oxLDLs via MDA epitopes, and the amount of C3a bound to malondialdehyde acetaldehyde-LDL correlates with a higher level of complement activation compared to normal samples.[151]

Beyond oxLDLs, CEP also may participate in dysregulated complement and has been identified as increased in drusen and blood samples in AMD compared to the normal control and is instrumental in predicting AMD susceptibility.[152] In the macular, the most abundant PUFA at the tip of the photoreceptor is DHA, which is prone to be oxidized under oxidative stress.[130] When the oxidized fragment of DHA covalently interacts with the ε-lysyl amino group in the tissue protein, giving rise to the substantial formation of CEP in the POS.[153] Meanwhile, CEP has also been identified as an oxidized component of the pigment lipofuscin, alongside molecules like A2E.[98] Typically, CEP is found in the POS and RPE, whereas mice immunized with CEP have shown a marked accumulation of basal laminar deposit (a diffusely distributed extracellular material between the RPE basal lamina and its plasma membrane) throughout the retina and a significant thickening of BrM.[154] And the presence of C3d, a degradation product of C3b,[155] in the immunized mice's Bruch's membrane, suggests an immune response to CEP.[154,156]

In the posterior eyes, other oxidized lipids such as AGEs are formed through non-enzymatic reactions between sugars, lipids, and proteins, and their accumulation increases with age. AGEs are prevalent in basement membranes and drusen, where they can bind to various pattern recognition receptors, including RAGE (Receptor for AGEs). RAGE specifically exhibits a high affinity for C3a and C3adesArg, functioning within a complex where CpGAs act as a linker in the formation of this ternary complex.[157] Another significant compound, 4-hydroxy-2-nonenal (4-HNE), is a primary active aldehyde that arises from the interaction of oxygen-derived free radicals with double bonds in ω-6-unsaturated fatty acids.[158] The levels of 4-HNE in the retina and plasma of AMD patients are significantly elevated, and full-length CFH has been shown to mitigate 4-HNE-induced oxidative stress,[96] although it does not directly interact with 4-HNE epitopes.[42]

Interaction of Oxidized Lipids With CFH in AMD. Moreover, the Y402H variant of CFH was demonstrated to disturb lipid metabolism in RPE cells due to the disability of CFH to bind OSEs, such as oxLDLs, oxPLs[43] and MDA[42] (Fig. 2). CFH is documented to be a soluble PRR, which is capable of recognizing OSEs on oxidized byproducts such as oxLDLs.[159] Shaw et al. found that CFH mitigates oxidative stress by binding to oxPLs, thus preventing oxPLs from initiating inflammatory cascades through RPE and macrophages, thereby reducing abnormal angiogenesis.[43] However, the variant of CFH Y402H was found to disturb the binding of CFH to oxPLs and compromise the removal of these danger-associated molecular patterns, losing control of local inflammation in the eyes.

Furthermore, CFH was also identified as a major MDA-binging protein that can block both the uptake of MDA-modified proteins by macrophages and MDA-induced proinflammatory effects in vivo in mice.[42] CFH in the alternative complement pathway was found to be a novel PRR that specifically recognizes MDA[130] and the binding of CFH to MDA is specific and mediated by two of its domains—SCR7 and SCR19- 20 (Fig. 2).[42] CFH protects against MDA-induced IL-8 secretion and converts C3b to iC3b on MDA-carrying surfaces. However, this protective effect is not associated with the overall CFH levels in plasma, which suggests potential local effects of CFH in the eyes. Interestingly, CFH binding to MDA is reduced by more than 65% in patients homozygous for the Y402H variant compared to controls, and this reduction is more pronounced in homozygous than in heterozygous patients, demonstrating a "dose-response" effect.[42] Subsequent studies using a mouse model expressing a chimeric CFH transgene with the human SCRs 6–8 sequences of the H402 or Y402 CFH variants, also further demonstrated that the H402 variant in CFH

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SCR6–8 leads to higher levels of MDA-adducts compared to normal age-matched C57BL/6 controls, as well as increased microglial/macrophage uptake of MDA.[160] Additionally, CFH also binds to apoptotic fragments containing MDA epitopes, resulting in the production of iC3b inactivated fragments, and iC3b opsonin can promote the clearance of apoptotic cells without inflammation, but in the CFH Y402H variant, the production of C3b inactivated fragments is also impaired.[42] Moreover, similar to its interaction with oxPLs, CFH can inhibit the uptake of MDA-modified proteins by macrophages, mitigate their proinflammatory effects, and reduce IL-8 production.

Intriguingly, as an extensively studied molecule in AMD, MDA has been shown to bind to C1q, C3a, and complement factor H-related proteins 1, 3, and 5 (CFHR-1, CFHR-3, CFHR-5).[133,148,151] Additionally, a GWAS study identified rs1061170 in CFH and deletions in CFHR3 and CFHR1 as key genetic variants that decreased the binding of CFH or its splice variant, factor H-like protein 1, to MDA epitopes in healthy individuals and AMD patients.[161] It was further revealed that CFHR1 and CFHR3 compete with CFH for MDA-epitope binding,[162] with CFHR1 displaying the highest affinity for these epitopes.[161] Notably, CFHR1 binds to MDA-rich areas via SCR1-2,[163] obstructing CFH from inactivating C3b and consequently enhancing the alternative pathway activation. Moreover, individuals carrying deletions of CFHR1 and CFHR3 have been reported to be at a lower risk of developing AMD.[111] These interactions underscore the multifaceted role of MDA in complement pathways, significantly impacting the progression of AMD. Additionally, both CFH and CFHR3 have been reported to be recruited to CEP.[133,162] In experiments with ARPE-19 cells, CFH binds to CEP-decorated surfaces, a process that is diminished by the presence of CFHR3.[162] Furthermore, in polarized senescent ARPE-19 cells, CFHR-3 was shown to be internalized, subsequently leading to increased expression of C3 and CFB.[162]

Oxidized Lipids, Autoantibodies, and Complement Activation. Several lines of studies also suggested that these oxidized molecules could also prompt the generation of corresponding autoantibodies like IgG, due to the novel antigen recognized as a foreign substance and interacting with autoreactive T and B cells (Fig. 3).[12,164,165] Moreover, OSEs are recognized as significant antigens for natural IgM in both mice and humans,[166] participating in the elimination of damaged structures and pro-inflammatory effects of oxidized lipids. Over 30% of natural IgM antibodies, which usually feature unmutated variable regions from germline gene sequences, specifically neutralize OSEs, including oxPLs, CEP, 4-HNE, and MDA—with MDA being the predominant antigen.[136] These antibodies are also powerful activators of the complement system, effectively facilitating waste removal through the complement cascade[167,168] and recruiting several complement components such as C1q,[169] ficolin, and mannose binding lectin.[170] Joseph and colleagues[170] further demonstrated that, in oxidatively stressed RPE cells, the lectin complement pathway can also be activated by natural IgM attached to OSEs on phospholipids. This body of research indicates that both the classical and lectin pathways can be activated through OSE-recognizing autoantibodies.

Additionally, on the surface of apoptotic cells, regulators of complement activity such as C4-binding protein (C4BP) and CFH can attach to OSEs either directly or indirectly via CRP.[171] It has also been reported that CFH can prevent excessive complement activation triggered by naturally occurring antibodies that recognize MDA epitopes of various structures.[172] Moreover, OSEs-targeted passive and active immunization strategies that increase the concentration of corresponding IgM autoantibodies in the body have raised intriguing possibilities to protect against diseases such as atherosclerosis and hepatic inflammation with ways such as passive immunity and vaccination,[173,174] which also provide a potential avenue for treatment of AMD.

Intracellular Accumulation of Lipofuscin in AMD

Lipofuscin Directly Prompts Complement Activation. In the RPE cells, lipofuscin accumulation is a hallmark of aging, observed within the lysosomal compartments across both normal and pathologic retinal conditions (Fig. 4A).[83] Generally, the progressive build-up of ocular lipofuscin, notably its vitamin A metabolites called bisretinoids such as N-retinylidene-N-retinylethanolamine (A2E),[175] has been related to detrimental effects observed in in vitro studies. Specifically, this age-related augmentation of A2E within RPE cells underscores a critical pathway to cellular dysfunction, suggesting a pivotal role in the pathogenesis of macular degeneration diseases such as recessive Stargardt macular degeneration (STGD1) and AMD.[176] Several lines of investigation have elucidated that A2E and similar bisretinoids pigments are instrumental in triggering disease mechanisms, namely membrane disruption,[177] lysosomal dysfunction,[178,179] and diminished antioxidant activity.[179]

Emerging evidence showed that lipofuscin and its constituents are intricately involved in both the upstream and downstream modulation of the alternative complement pathway. This intrinsic synergy fosters abnormal complement activation within the aging retina, serving as a pivotal mechanism in the pathogenesis of AMD and other retinal diseases. Notably, Zhou et al. discovered that the photooxidation products of lipid-bisretinoids such as A2E and all-trans-retinal dimer, another bisretinoids compound, could prompt the activation of the complement system through the alternative pathway, precipitating immune dysregulation, which was evidenced by the increase in iC3b production following the exposure of A2E-enriched ARPE-19 and human fetal RPE cells to 430 nm light and their subsequent treatment with human serum.[98,180] A prevailing hypothesis suggests that the phototoxic degradation of lipid-bisretinoids within the RPE cells triggers oxidative stress and apoptosis.[181] However, retinal damage by ambient light was only evident in albino mice,[84] and there is no evidence that antioxidants, light blockage, or anti-apoptotic drugs provide any benefit against degeneration in pigmented retinas overloaded with lipofuscin in which photodegradation of lipid-bisretinoids is considerably diminished.[182] This suggests that the role of lipofuscin in the pathogenesis of AMD might be more complex than previously anticipated, highlighting the need for further research to unravel its intricate involvement, particularly with the complement system.

Further investigations in Abca4$^{-/-}$ mice, a model of STGD1 sharing convergent etiologies with AMD,[183] and human RPE cells demonstrated that intracellular A2E may amplify complement activation through the down-regulation of CRPs such as CFH, thus causing photoreceptor degeneration (Fig. 4A).[184] Intriguingly, complement activation in bisretinoids-laden RPE cells also is strongly dependent on the CFH haplotype, which exhibited higher complement activation in the AMD-predisposing CFH haplotype (HH402/VV62).[185] In addition, except for RPE, Ma et

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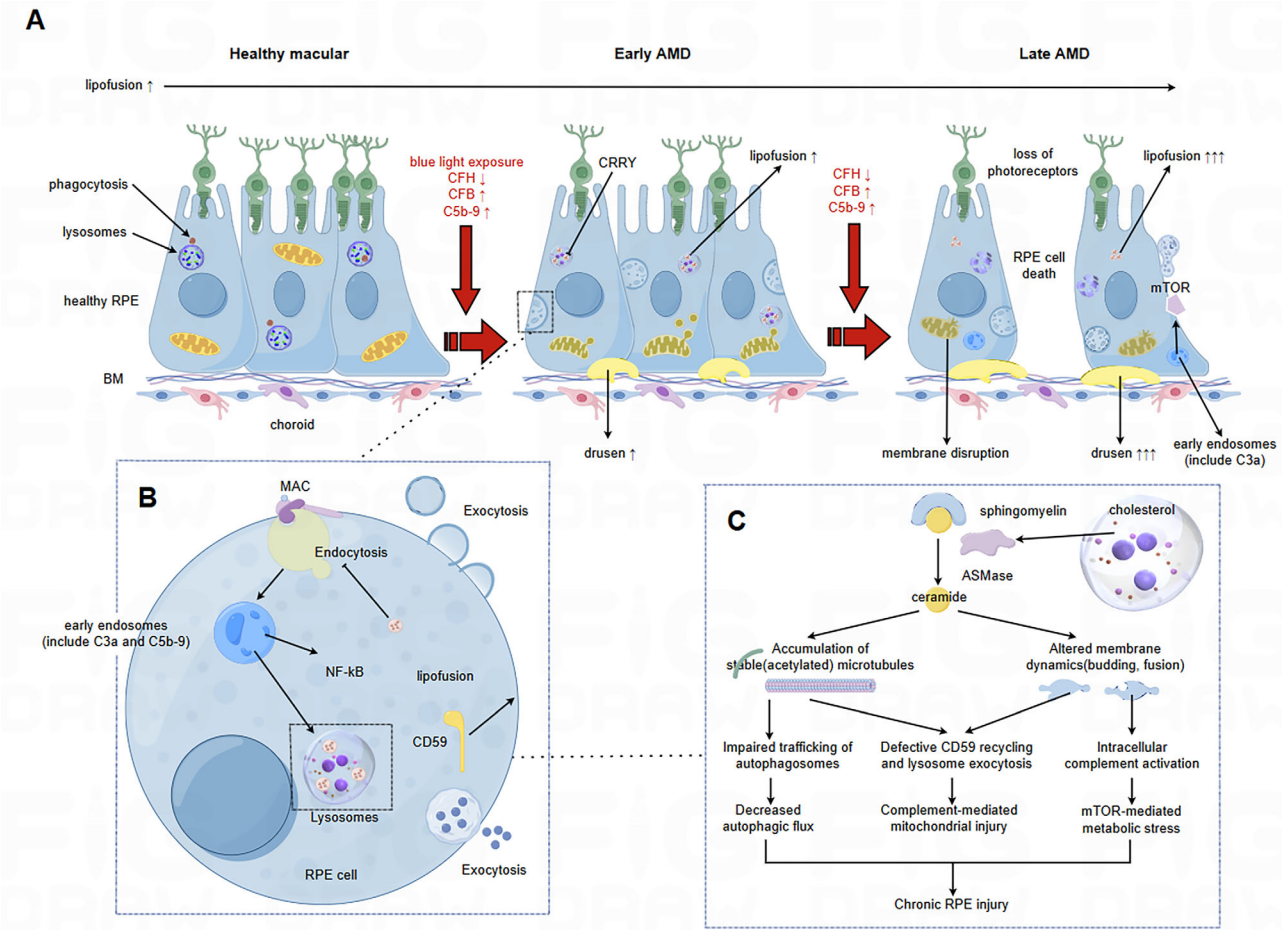

Figure 4. Lipofuscin accumulation and its byproducts in RPE cells serve as a pivotal mechanism in the pathogenesis of AMD. (**A**) Lipofuscin accumulation is a hallmark of aging and plays a pivotal role in AMD. It amplifies complement activation by downregulating CFH and increasing levels of complement effectors like CFB, especially under light exposure. This leads to a substantial generation of photooxidation products of lipofuscin and chronic inflammation in the retina, causing lysosomal dysfunction, reduced antioxidant activity, drusen formation, RPE cell apoptosis, and photoreceptor loss. (**B**) RPE cells use various mechanisms to mitigate the harmful effects of complement activation, such as endocytosis, ectocytosis, and exocytosis. Endocytosis helps clear C5b-9 complexes from the cell surface, directing them to lysosomes for degradation. Moreover, MAC-positive endosomes can modulate complement-mediated inflammation via noncanonical NF-κB activation. Additionally, accelerated recycling of membrane-bound complement regulator CD59 and lysosomal exocytosis following complement attacks have been observed in polarized RPE cells and ABCA4$^{-/-}$ mouse models. However, lipofuscin accumulation can impair autophagy by affecting lysosomal enzymes and inhibiting MAC degradation. (**C**) Excessive lipofuscin bisretinoids accumulation can lead to cholesterol buildup and subsequent generation of ceramides in RPE cells, disrupting protective mechanisms in RPE. This sequestration of cholesterol in endosomes or lysosomes increases ceramide production through the activation of ASMase. Elevated ceramide levels result in tubulin acetylation and altered membrane dynamics, impairing autophagosome trafficking and reducing autophagic flux. Additionally, these changes promote inward budding and early endosome formation, internalizing complement protein C3 and generating intracellular C3a fragments. Increased C3a activates the mTOR, leading to metabolic stress in AMD. The combined effects of defective CD59 recycling, impaired lysosomal exocytosis, and chronic RPE cell injury predispose cells to AMD.

al.[186] found that age-dependent lipofuscin accumulation in subretinal microglia, which was also showed to express complement components and CRPs, may favor complement activation exerted by increased levels of CFB and reduced levels of CFH.[187]

In line with these observations, numerous studies have concentrated on developing interventions targeting these mechanisms. One notable example includes complement modulation, as evidenced by a study in which the administration of complement receptor 1-like protein y, a crucial CRP in mice analogous to human CD46, significantly reduced both lipofuscin accumulation and complement activation in adeno-associated virus (AAV)–complement receptor 1-like protein y–injected mice.[188] These results collectively imply that the photooxidation derivatives of bisretinoid lipofuscin pigments in RPE or microglial cells may act as initiators of complement system activation. This activation could aggravate susceptibility to macular diseases and propel the chronic inflammatory responses that lead to drusen formation. Because it is a common process of lipofuscin accumulation in the posterior eyes in aging people, unveiling the correlation between these intracellular complexes and AMD may provide constructive clues in improving public health.

Aberrant Protective Mechanisms Caused by the Accumulation of Lipofuscin. Moreover, the identification of reactive complement proteins, notably C5b-9 (MAC), on RPE cell surfaces elucidates a potentially detrimental

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cycle. This cycle is characterized by impaired endocytic and exocytic pathways, alongside lysosomal dysfunction exerted by pathologic accumulation of lipofuscin, which in turn accelerates the accumulation of lipofuscin[189,190] (Fig. 4B).

Notably, in the human retina, C5b-9 accumulation within the RPE cell/Bruch's membrane interface can be observed as early as five years of age,[191] with its levels intensifying during the aging process. Furthermore, due to the anatomical configuration of the blood-retinal barrier, the basal aspect of RPE cells adjacent to the chondral capillaries is particularly susceptible to complement deposition.[192] Although sustained, low-level activation of the complement system may play a crucial role in preserving the immune privilege within the ocular environment,[193] evidence increasingly indicated that dysregulated complement activity is intricately linked with the pathogenesis of AMD.[12,36] Yang and colleagues[194] previously demonstrated that RPE is sufficiently protected against MAC deposits on cell surfaces due to the high levels of membrane-bound complement inhibitors (e.g., CD59, CD46, CD55). Recent studies also revealed that RPE cells could employ additional molecular strategies such as endocytosis, ectocytosis, or exocytosis, to mitigate against the potentially harmful effects of complement attacks similar to other cells.[195–197]

For example, research by Georgiannakis et al.[189] demonstrated that RPE cells could use the endocytic pathway to clear C5b-9 accumulations from their surface, routing the internalized complexes to lysosomes for degradation (Fig. 4B). Intriguingly, recent studies also found that MAC (+) endosomes could modulate complement-mediated-inflammation via activating noncanonical NF-κb.[198] Moreover, Tan et al.[190] demonstrated, using polarized RPE and ABCA4$^{-/-}$ mouse models, two protective responses to complement attack: accelerated recycling of the membrane-bound complement regulator CD59 to the RPE surface and immediate lysosomal exocytosis. The biogenesis of vesicles and transport of CRPs in these processes occur similarly to those described in extravesicles (Fig. 4B).[199] During these rapid responses, CD59 is transported to the RPE surface via the endosomal recycling route, inhibiting the recruitment of C9 and preventing the assembly of functional MAC pores. This response is impaired in Abca4$^{-/-}$ mouse RPE and polarized primary porcine RPE monolayers treated with the vitamin A dimer A2E.[190] Live-cell imaging using total internal reflection fluorescence microscopy and complementary biochemical assays revealed that complement attack induces the rapid mobilization of lysosomes near the plasma membrane, which then undergo exocytosis to patch nascent MAC pores.[200] Lysosomal hydrolases and lipids released during exocytosis are proposed to remodel the cell surface and limit membrane damage caused by pore-forming toxins.

However, current evidence also points out that lysosome capacity would decrease with the accumulation of lipofuscin in aging. Moreover, studies have shown that blue light exposure in lipofuscin-laden primary human RPE cells and ARPE-19 cells triggers lysosomal membrane permeabilization, resulting in the cytosolic release of lysosomal enzymes.[201] In addition, lipofuscin accumulation could also impair autophagy, a process overlapping with the endocytic pathway,[202] by impairing enzymes in lysosomes and impeding the degradation of MAC. And in an induced pluripotent stem cell-derived RPE AMD model, dysfunctional lysosomal degradation is directly linked to the complement system overactivation.[203] Furthermore, compelling data have demonstrated that accelerated lipofuscin bisretinoids accumulation or oxLDLs may lead to cholesterol accumulation in RPE,[43,204,205] potentially interfering with the protective mechanisms mentioned above. Mechanically, progressive accumulation of lipofuscin bisretinoids would sequester cholesterol in endosome or lysosome, facilitating increased cellular ceramide production through the activation of acid sphingomyelinase (ASMase),[205] the enzyme that hydrolyzes sphingomyelin to ceramide. Ceramides are bioactive lipids whose levels are tightly regulated in the retina. They have emerged as common mediators of inflammation and RPE cell death in various retinal diseases, including AMD.[206] Elevated ceramide levels contribute to tubulin acetylation,[207,208] disrupt organelle trafficking such as autophagic flux, hinder autophagosome transport,[190,205] impair the recycling of the complement regulatory protein CD59, and relocalize lysosomes to the perinuclear region instead of near the plasma membrane in RPE cells (Fig. 4C).[190]

Moreover, Kaur et al.[209] demonstrated that pathological accumulation of lipofuscin bisretinoids also promotes inward budding and homotypic early endosomes in RPE cells. Consequently, these enlarged endosomes internalize the complement protein C3 into the RPE, resulting in the intracellular generation of C3a fragments. Additionally, increased bioactive C3a in turn activates the mechanistic target of rapamycin (mTOR), a regulator of critical metabolic adaption in AMD (Fig. 4).[97,210] Sustained mTOR signaling can impair RPE homeostasis by modulating multiple stress pathways implicated in aging and disease (Fig. 4C).[211,212] In summary, these studies suggested that an elevated lipofuscin level may interfere with protective responses in RPE by hindering the processing and degradation of C5-9 or activating the complement system via cellular signaling, potentially predisposing RPE cells to AMD.

Future Treatment of AMD Targeting Complement System and Lipid Metabolism

Recent Therapies Targeted Complement System

The age-standardized prevalence of blindness caused by AMD declined by almost 30% from 1990 to 2020.[2] This reduction is likely attributed to healthier lifestyle choices and the widespread adoption of anti-VEGF therapy for the treatment of exudative AMD.[8] Over the past two decades, significant advancements have been made in the treatment of CNV through the implementation of biologics or monoclonal antibodies that inhibit VEGF or its receptors.[213] Additionally, the development of gene therapies for wet AMD, including genome editing techniques, offers the potential for substantial improvements in the quality of life for patients.[214] However, since the majority (85%–90%)[8] of AMD cases are the currently untreatable nonexudative form, which can progress to atrophy of the foveal center (geographic atrophy), the development of specific treatments and preventive measures for non-exudative AMD remains a critical area of interest.

Currently, the only intervention available for the treatment of dry AMD is Age-Related Eye Disease Supplement (AREDS), an oral supplement containing vitamin C, vitamin E, lutein/zeaxanthin, and zinc recommended in two large randomized controlled trials (RCTs; AREDS1 and AREDS2).[215,216] AREDS enhances protection against oxidative stresses in the eye and was shown in the AREDS trials to reduce the risk of advanced AMD by about 25% over 5 years in participants with intermediate AMD, although there

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Table 1. Drugs in Clinical Trial Targeting the Complement Pathway

Therapeutic Category or Name	Mechanism	Mode of Administration	Clinical Trial ID, NCT No.*	Study Phase (Status)	Participants	Reference
AREDS	Reduce oxidative stress	Oral delivery	NCT01915238	Completed	52	251
Pegcetacoplan (APL-2)	C3 inhibitor	Intravitreal	NCT03525613	Completed	637	217
			NCT04770545	Phase 3	1200	
			NCT01603043	Phase 2	10	
Avacincaptad pegol	C5 inhibitor	Intravitreal	NCT04435366	Phase 3	448	218
NGM621	C3 inhibitor	Intravitreal	NCT04014777	Phase 1	15	252
ANX007	C1q inhibitor	Intravitreal	NCT04656561	Phase 2	270	253
Iptacopan	CFB inhibitor	Oral delivery	NCT05230537	Phase 2	146	254
Lampalizumab	Inhibit complement factor D	Intravitreal	NCT02745119	Phase 3	994	255
Danicopan	Inhibit complement factor D	Oral delivery	NCT05019521	Phase 2	365	256
Efdamrofusp alfa (IBI302)	Neutralize VEGF and C3b/C4b	Intravitreal	NCT05972473	Phase 3	600	236
AL-78898A	Selectively binds C3b and C3c	Intravitreal	NCT01157065	Phase 2	99	224

* NCT no. was unavailable for some therapeutics because formal clinical trials have yet not been conducted; NCT no. is provided for the most recent clinical trials.

was no effect in participants with early or advanced AMD and 22% of patients still experienced a 15-letter decline in visual acuity after supplementing of antioxidant.[215]

Since overactivation of the complement system is a key driver of the disease, complement inhibition is therefore a promising therapeutic strategy for this incurable and largely untreatable condition. Numerous studies have focused on finding effective complement inhibitors, mainly centering C1q, C3, C5, FB, FI, or regulators such as CD59 and CFH33 (Table 1). However, until recently, there were no proven therapies for geographic atrophy. Surprisingly, in 2023, the FDA approved pegcetacoplan (a C3 inhibitor, APL-2) in February and avacincaptad pegol (a C5 inhibitor, Zimura [ARC1905]) in August for slowing the progression of lesions of GA. The results of the phase 3, multicenter, double-masked, sham-controlled, randomized clinical trials of these two different complement inhibitors offered promising prospects.[217,218]

In detail, in the OAKS (NCT03525613) and DERBY (NCT03525600) phase 3 trials, 1258 participants (637 and 621, respectively) were randomly assigned in a 2:2:1:1 ratio, corresponding to those treated with 15 mg intravitreal pegcetacoplan injections monthly or bimonthly and sham injections monthly or bimonthly. At month 24, both OAKS and DERBY showed significant reductions in GA progression with pegcetacoplan monthly and bimonthly compared with sham controls. However, new-onset exudative nAMD occurred in 11%, 8%, and 2% in OAKS and in 13%, 6%, and 4% in DERBY for pegcetacoplan monthly, pegcetacoplan every other month, and sham, respectively, at 24 months.[217] Other adverse events like endophthalmitis was observed lower than the previous phase 2 FILLY study (NCT02503332).[219] As for avacincaptad pegol, in the GATHER2 phase 3 trial (NCT04435366), 448 patients were randomized 1:1 to receive 2 mg avacincaptad pegol injections monthly or sham injections for 12 months.[218] Avacincaptad pegol reduced geographic atrophy growth, with a rate of 0.336 mm/y compared with 0.392 mm/y for the sham group, with a 14% difference between the two subgroups in total atrophic areas (absolute difference, 0.056 mm/y [95%CI, 0.016-0.096 mm/y]; $P = 0.006$). At month 12, the occurrence of nAMD was observed in 5% of patients receiving avacincaptad pegol 2 mg and in 3% of patients in the sham group.[218] However, neither pegcetacoplan nor avacincaptad pegol demonstrated statistically significant differences in prespecified visual function endpoints compared with corresponding sham groups in their respective studies.[217,218] The high rates of ineligibility and the blurred stratification of risk for patients with GA in studies of both drugs make it challenging to determine the generalizability of these treatments for patients with any form of GA.[13] Although it is exciting to have novel and effective drugs for a condition that has no previous effective therapy, the risk-benefit profile of both drugs remains uncertain and there is no general recommendation for their use yet.[13]

In addition, several other studies are currently in clinical trials. For example, Iptacopan (LNP023) (NCT05230537, Phase 2) binds to CFB to inhibit the activation of the alternative complement pathway.[33] EG-301 (NCT05170048, Phase 2) targets RPE cells, enhancing the transport function of RPE autophagosomes, inhibiting RPE complement activation, and protecting mitochondrial function. GT005 (NCT05481827, Phase 2) is a one-time gene therapy based on AAV, designed to increase the expression of CFI, thereby exerting a negative regulatory effect on the complement system.[220] Lampalizumab and Danicopan (NCT05019521, Phase 2) inhibit complement factor D, reducing the production of C3 convertase in the alternative pathway. However, a Phase 3 clinical trial of Lampalizumab (NCT02745119), which included 994 participants, was terminated early by the sponsor because of lack of efficacy.[221] GEM103 (NCT04684394) is a recombinant form of human CFH protein intended for treating genetically defined AMD, but its Phase 2 clinical trial was terminated during the recruitment phase by the sponsors.[222,223]

Advancements in Inventions for Aberrant Lipids Metabolism

During these years, growing evidence from studies further supports the important role of dysregulated lipid metabolism in AMD progression. Several lines of studies have provided preclinical evidence supporting ther-

Table 2. Drugs in Preclinical Trial Targeting Lipid Metabolism

Name	Mechanism	Model	Mode of Administration	Use Level	Reference
Short heparan sulfate oligosaccharide	Remove the ApoB-containing HDL on aged human BrM explants	Human BrM tissue	Media	16 µg of the HS NS2S 12-mer	87
5A	ApoA-1 mimetic peptide	Mouse Pig RPE cells	Intraperitoneal Media.	30 mg 0, 8, 40, or 200 µg/mL	87
L-4F	ApoA-I mimetic peptide	Macaca fascicularis of Mauritian origin	Intravitreal	6 times 25–175 µg	226
GW3965	LXR agonist	C57BL/6J mice Wild-type C57BL/6J mice	Diet Vitreous injection	120 mg/kg 10 µM	230
TO901317	LXR agonist	ARPE-19 and RF/6A cells Mice	Media Intravitreal	10 µM 10 m/kg	232
Desipramine	Prevents the formation of enlarged early endosomes, intracellular C3 proteolysis, and mTOR activation	Primary Porcine RPE, human RPE Cell	Media	10 µM	205
6-Ureido/thioureido-2,4,5-trimethylpyridin-3-ol derivatives	NOX2/4 inhibitor	ARPE-19 cells	Media	10 µM	257
Polyphenol quercetin	Reduce 4-HNE-induced damage	ARPE-19 cells	Media	50 µM	158
Anti-CEP antibodies	Inhibit CEP	Sprague–Dawley rats C57BL/6J mice	Inserted into corneal micropockets Subretinal injections	4 µg 1 µg	234

apies specifically targeting lipid metabolism for AMD (Figs. 3B, 3C, Table 2). For example, pharmaceutical interventions aimed at lipoprotein binding in BrM as we have discussed may be effective in the treatment of AMD. Based on this theory, and as the major site of interaction between CFH and HDL occurs at heparan sulfation within BrM,[36,87] HDL composition or soluble heparan sulfate in the posterior eye may limit excessive extracellular deposits of lipoproteins such as HDL-like particles in BrM and rescue subsequent RPE cell damage and death. In support of this, Kelly et al.[87] came up with two potential therapeutic avenues targeting HDL interactions with BrM, namely short heparan sulfate (HS) oligosaccharide and an ApoA-I mimetic peptide 5A. Short heparan sulfate oligosaccharide can specifically block the binding sites in lipoproteins while showing little inhibitory effect on CFH, thus effectively removing lipoprotein-containing deposits in BrM. As for ApoA-I mimetic peptide 5A, it is similar to ApoA-1, a major protein constituent of HDL associated with AMD risk,[92,225] and deemed to be capable of transforming the pathologic pro-inflammatory HDL back to its norm form. Specifically, they demonstrate that short HS oligosaccharides can remove the ApoB-containing HDL on aged human BrM explants and treatment of the ApoA-1 mimetic peptide 5A increases RCT and ameliorates some of the changes to the HDL proteome caused by a high-fat and cholesterol diet in old CFH-H/H mice. The results were further incorporated by another study suggesting that monocular intravitreal injections of ApoA-I mimetic peptide L-4F could effectively remove neutral lipid, esterified cholesterol, and MAC in BrM and improve corresponding ultrastructure in eyes compared to placebo-treated controls (Figs. 3B, 3C).[226] Taken together, pharmaceutical interventions toward binding between HDL-like lipoproteins and BrM may emerge as a tempting avenue.

In addition, restoration of normal lipids process in RPE could also be a means to therapeutically target lipid metabolism in AMD. For example, multiple transcription factors, including sterol regulatory element-binding proteins, liver X receptors (LXRs), retinoid X receptors (RXRs), and peroxisome proliferator-activated receptors (PPARs) participate in intracellular lipid homeostasis. Among these, LXRs, an essential regulator of cholesterol homeostasis and inflammatory responses,[227] are responsible for cholesterol efflux and protect against pathologic angiogenesis by inhibiting M2 polarization via the LXR–ABCA1/G1 or APOE pathway.[228,229] In a study aimed at developing LXR-targeted therapies for the treatment of AMD, GW3965, an LXR agonist, was able to reduce AMD-relevant pathogenic phenotypes, including posterior pole ocular inflammation and lipid deposition in mice expressing apoB100, a model for aging and early AMD-associated phenotypes.[230] It is incorporated by a study in which C57BL/6 mice treated with GW3965 exhibited decreased RPE cell apoptosis caused by ROS-generated 7-ketone-cholesterol (7KCh).[231] Moreover, Tan et al.[97] also demonstrated that LXR agonists might alleviate the pathologic accumulation of ceramides caused by cholesterol-mediated activation of ASMase in Abca4$^{-/-}$ mice. Moreover, in a very recent study using siRNA transfection in cells and Vldlr (−/−) mice, the application of LXR agonist TO901317 inhibited OxLDLs-induced CNV in mice, as well as inflammation and angiogenesis in vitro.[232] All of these studies provide a new insight into regulating abnormal lipids process to its physiological states. However, of note, there are also many limitations such as various adverse events and rational design for new agonists in these pathways.[233]

Intriguingly, as mentioned in 3.3.2, Kaur et al.[209] reported enlarged early endosomes in the RPE of Abca4$^{-/-}$ mice that

allow more extracellular C3 intake and activation of the C3-proteolytic cleavage product, C3a. Inhibiting ASM and down-regulating the concentration of ceramide with desipramine can decrease the size of early endosomes and prevent C3a activation in Abca4$^{-/-}$ RPE. Promoting cholesterol efflux with a LXR agonist, TO901316, can mimic the effects of desipramine on early endosome size in the Abca4$^{-/-}$ RPE.[209] In addition, desipramine has also been documented to play a vital role in AMD. Specifically, in ABCA4$^{-/-}$ mice, intraperitoneal or oral administration of desipramine decreases RPE ceramide levels to that in age-matched wild types. This prevents the formation of enlarged early endosomes, intracellular C3 proteolysis, and mTOR activation.[209] Desipramine treatment in RPE with bisretinoids corrects impaired autophagosome trafficking and autophagic flux[205] and restores CD59 recycling to the plasma membrane, which prevents MAC assembly after complement attack, and protects RPE mitochondria from complement-induced fragmentation.[190]

Other potential therapeutic targets include the preservation of lipid oxidative pathways. For example, Studies have shown that 4-HNE-induced RPE cell death can be prevented by inhibiting the activity of NADPH oxidase 4 (NOX4). The NOX2/4 inhibitor VAS2870 and synthetic 6-ureido/thioureido-2,4,5-trimethylpyridin-3-ol derivatives are available, of which compounds 17-28 inhibit 4-HNE-induced superoxide generation and APRE-19 cell death.[133] Polyphenol quercetin could reduce 4-HNE-induced damage by enhancing ARPE-19 cell viability and reducing inflammation. Considering the role of CEP in the pathological process of AMD, it has been shown that the use of anti-CEP antibodies can neutralize its mediated increase in VEGF and neoangiogenesis with a stronger effect than the use of anti-VEGF antibodies.[234]

Exploring Multitarget Therapies in the Future

Recent advancements have resorted to bi-specific fusion monoclonal antibodies that simultaneously target multiple molecules. A prime example is faricimab, a sort of bispecific antibody targeting both VEGF-A and the angiopoietin/Tie (Ang/Tie) pathway.[11] Recent phase III clinical trials and real-world studies have demonstrated that faricimab offers a promising treatment option for neovascular AMD due to its functional and anatomical improvements in nAMD, diabetic macular edema, and other ocular conditions, such as PCV.[235] Faricimab received FDA approval and, more recently, was subsequently approved by the European Medicines Agency (EMA) in 2022 for treating wet-AMD and diabetic macular edema.[235] Furthermore, Yang et al.[70] have developed efdamrofusp alfa, a type of monoclonal antibodies that neutralizes both VEGF isoforms and the complement components C3b/C4b. Their phase II study showed that intravitreal efdamrofusp alfa was well tolerated by nAMD patients, with similar vision acuity and anatomical improvements reported.[236] These findings highlight the potential of multitarget therapies (Table 2).

Similarly, as the interplay we have discussed above, there is significant crosstalk and potential for vicious cycles in AMD etiopathology. For instance, considering therapies that target binding sites in HSPGs (or OSEs) alongside C3 (or C5) raises intriguing possibilities. Such a dual-targeting approach might effectively reduce the formation of deposits like drusen and prevent subsequent RPE cell death and atrophy in an advanced stage. Given the current gap in effectively halting the progression of early and particularly advanced dry AMD, and considering the significant limitations of single-target therapies like anti-VEGF, it is tempting to speculate that the strategic combination of lipid metabolism modulators and complement inhibitors would emerge as a promising therapeutic avenue, offering a novel approach in clinical practice (Tables 1 and 2).

Future Perspectives and Conclusions

AMD is a multifaceted disorder and remains a leading cause of blindness globally. Despite intensive research and development, effective strategies for early intervention and advanced treatment are still elusive. Current anti-VEGF therapies, while prevalent, pose significant challenges including economic burdens and the need for frequent intravitreal injections, which can lead to poor patient compliance.[237] On average, seven to eight treatments per year are required to control CNV progression effectively, yet these treatments do not cure neovascular AMD but rather slow its progression, merely delaying vision loss.[238,239] Notably, evidence from multiple studies suggested that anti-VEGF treatments might even contribute to serious adverse events such as the onset of GA,[240,241] endophthalmitis, retinal detachment, and traumatic lens injury.[242] Furthermore, many patients also suffer from anti-VEGF resistance and even develop more severe visual impairment after the treatment.[243] While promising advancements, such as the new generation of gene therapies, aim to enhance long-term efficacy with a future "one and done" in-office way, significant challenges persist in predicting the long-acting effects after a single injection of recombinant DNA and the result of ongoing phase 3 trials are warranted.[214] The use of present AAV vectors is limited by various factors like limited tropism, inefficient delivery across the retinal inner limiting membrane, small packaging capacity, neutralizing antibody/immune response, and others.[244]

Recent advancements in complement inhibitors such as pegcetacoplan and avacincaptad pegol have shown promising prospects for the treatment of GA.[13,33,217,218] However, these inhibitors have also exhibited significant adverse events, notably a dose-dependent increase in the rate of progression to nAMD in certain subgroups treated with these drugs.[217,218,245] It remains to be clarified whether these complement inhibitor-induced adverse events can be totally reversed by common anti-VEGF therapies. Moreover, waiting for the evaluation of the long-term treatment effects and incidences of adverse events, manufacturers continue to monitor and alert on the development of new serious adverse events,[246] including intraocular inflammation and occlusive vasculitis, as discussed at the 2023 American Society of Retinal Surgeons meeting. Additionally, the optimal initial dose and regimen for complement inhibitor treatment remain undetermined, and identifying quantifiable biomarkers for AMD progression is crucial, given the prevalence of early and intermediate AMD.[247]

AMD is a genetically complex disease, making it unlikely that a single common genetic variant will significantly influence its progression or response to treatment.[16] This complexity partly explains the unsatisfactory results in several clinical trials of complement inhibitors, such as the inhibition of factor D with lampalizumab.[33] Variations in the complement system do not fully account for AMD heritability, and their roles may change at different stages of the disease, necessitating further elucidation.[248] Furthermore,

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to reduce potential adverse events, total inhibition of the complement system may not be recommended.[33]

Regarding lipid modification, the results of treatments with common lipid-lowering agents, particularly statins, are still conflicting. A small multicenter open-label prospective clinical study involving 26 patients found that intensive treatment with 80 mg of atorvastatin daily resulted in the regression of many large, soft drusenoid deposits.[249] However, a 2022 meta-analysis of 21 studies with a total of 1,460,989 participants found no significant difference in the incidence or progression of AMD based on statin use.[250] Because the retina like the brain is separated from the bloodstream by a tight barrier, interference with lipid metabolism in the periphery may have no impact on retina function.[81] Therefore further research on the use of any form of statins in AMD patients is also warranted.

Our focus on lipid metabolism and the complement system—two critical pathways in AMD progression—ensures a deeper understanding of their interaction and its implications for disease etiology and treatment strategies. These pathways are deeply involved in key processes such as drusen formation, oxidative byproduct accumulation, and lipofuscin buildup, all of which are strongly linked to the pathogenesis of AMD. The presence of complement components in drusen, possibly due to impaired RPE clearance or choriocapillaris susceptibility to immune complex deposition, underscores the potential of these pathways to amplify complement activation, contributing to RPE and photoreceptor atrophy and subsequently progressive vision loss.

As numerous studies have demonstrated, the onset of AMD may be accompanied by various molecules in different systems.[16,145,214] Multitarget therapies, such as bispecific fusion antibodies, may therefore be favored. For example, simultaneous targeting of both pathways (e.g., bispecific antibodies targeting simultaneously OSEs on oxidized lipids and C3/C5 crucial for complement activation) may significantly halt AMD progression and protect against vision loss. In summary, investigating these two crucial systems provides new insights into potential multifaceted therapeutic strategies, promising more effective treatments for AMD and possibly transforming the management of this complex disease.

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