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Emerging roles for nuclear receptors in the pathogenesis of age-related macular degeneration

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Abstract

Age-related macular degeneration (AMD) is the leading cause of vision loss in the elderly in the Western world. Over the last 30 years, our understanding of the pathogenesis of the disease has grown exponentially thanks to the results of countless epidemiology, genetic, histo-logical, and biochemical studies. This information, in turn, has led to the identification of multiple biologic pathways potentially involved in development and progression of AMD, including but not limited to inflammation, lipid and extracellular matrix dysregulation, and angiogenesis. Nuclear receptors are a superfamily of transcription factors that have been shown to regulate many of the pathogenic pathways linked with AMD and as such they are emerging as promising targets for therapeutic intervention. In this review, we will present the fundamental phenotypic features of AMD and discuss our current understanding of the pathobiological disease mechanisms. We will introduce the nuclear receptor superfamily and discuss the current literature on their effects on AMD-related pathophysiology.

Keywords

Drusen; Geographic atrophy; Choroidal neovascularization; Transcription factors; Aryl hydrocarbon receptor; Liver X receptors; Peroxisome proliferator-activated receptors; Retinoid X receptors

Introduction

With the increase in the aging population and life expectancy, the race to uncover molecular processes that regulate key pathogenic mechanisms involved in neurodegenerative aging diseases, such as age-related macular degeneration (AMD), continues. Major challenges in identifying successful therapeutic interventions for AMD can be attributed not only to the

complexity of the organ system effected, in this case the eye, but also to the multi-factorial, polygenic nature of the disease. This is best exemplified by examining the research on AMD, which over the last three decades has resulted in (1) a clearer understanding of the pathology of disease and clinical sub-types (early, intermediate, advanced), (2) the identification of numerous genetic and environmental risk factors, and consequently (3) the generation of a sundry of probable pathogenic biological mechanisms and hypotheses. Though the discovery of successful therapies capable of preventing and/or significantly reversing vision loss remains to be realized, careful consideration of data that have been reported so far can be critical in formulating new, testable research avenues to pursue. Questions which remain unanswered that otherwise may bridge the gap between our current knowledge and potential therapies include: what are the pathological drivers and signaling pathways regulating the biology of the disease and/or promoting progression from the early and intermediate forms to the more advanced forms of AMD?

In the quest to identify such signaling pathways, nuclear receptors, a superfamily of transcription factors, are emerging as strong candidates. Indeed, nuclear receptors have been shown to regulate many of the pathogenic pathways associated with AMD such as angiogenesis, lipid metabolism, and inflammation. We and others have shown that nuclear receptors are abundantly expressed within cells vulnerable in AMD and carry out distinct roles under physiological and pathological conditions, further supporting the idea that the eye may be a secondary endocrine organ. This review focuses on the therapeutic potential of nuclear receptors in AMD and includes two parts. First, we will first summarize the clinical pathology, etiology, and pathophysiology of AMD. In the second part, we will review the human nuclear receptor superfamily and discuss recent studies providing insights into the mechanisms by which these receptors may modulate pathogenic pathways of AMD.

Overview of AMD

Age-related macular degeneration is a late-onset, progressive, chronic degeneration of the central retina and the major cause of irreversible vision loss in industrialized countries, affecting approximately 30 % of individuals over 70 years of age, or 60 million people world wide [1, 2]. Tissues and cells vulnerable in AMD include the photoreceptors, retinal pigment epithelium (RPE, the cells providing support to the photoreceptors and responsible for maintaining the outer blood–retina barrier), Bruch's membrane (a pentalaminar extracellular matrix), and the outer blood supply of the eye including the choriocapillaris and choroidal endothelial cells. Morphological and physiological changes to ocular tissues result in a collection of clinical findings such as small yellow deposits known as drusen, RPE disturbances including pigment clumping and dropout, RPE detachment, retinal and RPE cell loss or geographic atrophy (GA), choroidal neovascularization (CNV) and disciform scar. Not all of these manifestations are required for the diagnosis of AMD.

Clinically, AMD stages are defined via the Age-Related Eye Disease Study (AREDS) classification scheme [3, 4], based on results obtained from examining retinal and fundus color photographs (Table 1). However, optical coherence tomography (OCT) and autofluorescence imaging have been employed for over a decade to provide additional images of the AMD retina and have become a central tool for clinical diagnosis, disease

staging, and management of the disease [5–7]. Additionally, histopathology studies have provided critical complementary information not obtainable from various imaging modalities, carefully characterizing the transition between normal aging and early AMD, and the evolution to late AMD [8–13]. Taken together, these studies have defined AMD as a disease that progresses along a continuum starting from small drusen (drupelets), advancing to larger drusen and pigmentary changes, and eventually to development of late AMD (Fig. 1).

Clinical aspects of early dry AMD

The clinical hallmarks of early AMD are the accumulations of extracellular lipid-protein-rich deposits between the RPE and Bruch's membrane called drusen [11, 14] (Figs. 1a, 2a), which vary in size, border, thickness, and confluence [15–17] (Fig. 1b–d), and are often associated with choroidal vascular changes including choriocapillary loss [18–20] (Fig. 1d). Histopathological evaluation of donor tissue has further characterized additional forms of deposits below the RPE (sub-RPE), not directly identifiable on fundoscopic examination, including basal laminar deposits (BLamD), basal linear deposits (BLinD), and focal nodular drusen [9, 21]. BLamD consist of amorphous material of intermediate electron density with long-spacing collagen, electron-dense fibrillar or granular material and membranous debris [21] located between the RPE and its plasma membrane. They are among the most prevalent histopathologic findings in early AMD eyes [9, 12]. BLinD are diffuse, amorphous accumulations located between the inner collagenous layer of Bruch's membrane and the RPE basement membrane [8, 12], with similar content variations. Nodular drusen are discrete, dome shaped deposits within the inner collagenous layer of Bruch's membrane, often contiguous with basal linear deposits.

It is noteworthy that the incidence of individuals with drusen, as observed on retinal examination, increases as a function age, and yet, presence of drusen (which is listed under most clinical definitions of AMD) may or may not be associated with advanced changes, such as retinal and RPE dysfunction. Size of drusen plays a determining role on disease progression, such that occurrences of small, isolated drusen (drupelets) are considered normal aging changes (Fig. 1b), while intermediate size drusen ([63 microns), corresponding to continuous basal deposits on histology, is a clinical sign of early AMD (Fig. 1d) [12]. Analyses from the AREDS suggest that the development of intermediate-sized drusen signify increased risk for progression to late AMD, while small drusen do not confer increased risk of progression to large drusen and late AMD [4]. Specifically, the AREDS demonstrated that the 5-year risk of progressing to large drusen is approximately 5 % for eyes with small-sized drusen compared to 40–50 % for eyes with medium-sized drusen [4]. Importantly, the presence of drusen in the absence of other retinal abnormalities does not seem to be associated with vision loss, as patients in the early to intermediate phases of AMD have normal best-corrected visual acuity. They do however report struggling to see under dim lighting conditions, resulting in difficulty with driving and mobility, causing significant emotional distress and affecting quality of life [22, 23]. These symptoms can be attributed to decreased sensitivity of the rod system, which is responsible for vision in the dark, and to delayed dark adaptation [23, 24]. Visual impairments in patients with early and intermediate AMD are also consistent with results from pathology observed in eyes with

AMD, which demonstrate that degeneration of the rod photoreceptors precedes cones in all stages of the disease [25, 26], and clinical studies that report a loss in color contrast sensitivity of the blue cones in most patients with early AMD [27].

Reticular pseudodrusen (RPD) are another class of deposits found in AMD eyes (Fig. 2b) that are characterized by an interlacing pattern in the macula, sparing the fovea, on clinical examination and color fundus photography. Unlike drusen, the detection of RPD by color fundus photography is far less reliable and requires the use of multi-modal high-resolution imaging modalities such as near-infrared reflectance/scanning laser ophthalmoscopy, fundus autofluorescence and spectral domain OCT (SDOCT) [28–31]. They present as hyper-reflective material located sub-retinally and above the RPE [32, 33], as visualized on SD-OCT [34], with a characteristic targetoid pattern on scanning laser ophthalmoscopy and autofluorescence imaging [35] (Fig. 2b). In an early clinicopathology study, Sarks et al. [36] first believed that RPD identified on red-free photography or infrared reflectance represented choroidal fibrosis in an AMD specimen lacking the neurosensory retina. In a later work, they attributed RPD to sub-retinal drusenoid deposits based on the analysis of another postmortem AMD eye with an attached retina [37]. Additional indepth examination of these structures has proposed that sub-retinal drusenoid deposits are indeed RPD [32, 34]. These deposits demonstrated high but variable prevalence in AMD eyes [31, 38–40], sharing ultrastructural and compositional similarities with drusen and soft sub-RPE deposits, including membrane-bounded particles with neutral lipid interiors, unesterified cholesterol, apolipoprotein E (apoE), complement factor H (CFH), and vitronectin [38]. Most importantly, RPD have been associated with a higher risk of progression to severe late AMD [31, 36, 41–43]. Arnold and colleagues reported that 66 % of patients with RPD had or later developed CNV in one or both eyes [36], while studies by Klein et al. [44] show that the incidence of RPD increases significantly with age, female gender, and the presence of the CFH gene variant Y402H. Although the precise nature and morphology of RPD may be a matter of debate, the AMD research community agrees on the importance of further characterizing RPD in histopathology research and longitudinal natural history studies. Current clinical studies are investigating the evolution of RPD during disease progression and their relation to development of GA and neovascular AMD.

Clinical aspects of late AMD

Most AMD-related vision loss occurs during the late stages of the disease from either the neovascular (“wet” or “exudative”) form of AMD, or development of RPE/retinal atrophy (“GA” or “late dry”; Figs. 1e–g, 2c, d). Though only approximately 12 % of AMD cases develop the wet form of the disease, neovascular AMD accounts for the majority of AMD-related severe vision loss [45–47]. Late wet AMD is characterized by CNV, the invasion of pathological new vessels from the choriocapillaris into the sub-retinal space. In this subtype of AMD, choroidal neo-vessels break through the retina, leading to plasma exudation, hemorrhage, fibrosis, and formation of a disciform scar (Figs. 1f, g, 2d). Ultimately, late neovascular AMD results in significant disruption of the normal retinal architecture and blindness. Substantial central visual loss, including legal blindness, can also occur due to GA, the atrophic form of advanced AMD, in which large areas of RPE degenerate (Fig. 2c). Late AMD significantly affects activities of daily living, causing those affected to lose their

independence in their retirement years. People with vision loss secondary to late disease often report AMD as their worst medical problem and the main cause for their diminished quality of life and well-being [24, 48] even more so than individuals affected with chronic obstructive pulmonary disease, acquired immunodeficiency syndrome, and other systemic chronic conditions [49].

Etiology of AMD

Age-related macular degeneration is a complex and heterogeneous disease, multi-factorial with genetic, systemic health, and environmental factors regulating its initiation and progression [50, 51]. Herein, we highlight some of the risk factors associated with AMD as identified by epidemiology, histology, and molecular biology studies. It is though our understanding of these risk factors in combination with pathology of the disease, that numerous studies have been able to develop in vivo animal models exhibiting some characteristic feature(s) of the human disease, reviewed extensively previously [52, 53], and investigate the impact of these factors in biochemical and molecular biological experiments in vitro.

Environmental factors linked to AMD

To date, great strides have been made in the identification of environmental factors that contribute to the development of AMD [54–56]. Epidemiology studies have shown that the incidence and progression of AMD are related to modifiable risk factors such as smoking, obesity, and dietary factors including antioxidants and dietary fat intake [51, 57]. The negative impact of smoking is due to a decrease in blood flow, levels of high-density lipoprotein (HDL) and plasma antioxidants, and an increase in platelet aggregation, fibrinogen, oxidative stress, lipid peroxidation and levels of inflammatory cytokines [57]. In addition, nicotine exposure in laser-induced CNV animal models results in increased size and severity of the neovascular lesions [58]. As a result of these findings, patients are advised to follow a lifestyle that includes a healthy diet, physical activity, weight control and smoking avoidance to reduce the risk and progression of AMD [59–64].

The molecular mechanisms underlying the effects of environmental factors in AMD are complex. As an example, environmental insults can result in the formation of radical oxidative species in the retina due to the abundance of polyunsaturated fatty acids (PUFAs) in the photoreceptor membranes [65, 66]. These oxidized lipids could lead to the formation of undegradable higher molecular polymers, and accumulation of material, such as lipofuscin, in the lysosomal compartment of RPE cells. Lipofuscin may sensitize RPE to light, intensifying cellular oxidative damage, ultimately triggering RPE dysfunction and photoreceptor cell death [65, 66]. The eye has an active antioxidant system comprised of carotenoids, in particular the macular pigments lutein and zeaxanthin, which can minimize oxidative damage [67, 68]. In fact, clinical prospective and questionnaire studies have revealed that dietary intake of lutein, zeaxanthin, and fruits rich in antioxidants decreases the incidence of neovascular AMD [63], while a low plasma level of zeaxanthin is associated with increased risk of developing early AMD [69]. The landmark AREDS clinical trial showed that oral daily supplementation with the antioxidants zinc, copper, vita-min C, vitamin E, and beta-carotene significantly reduced the risk of developing advanced AMD in

participants with intermediate AMD in at least one eye [70]. The follow-up randomized, double-masked, placebo-controlled clinical trial, AREDS2, determined whether or not adding supplements containing lutein/zeaxanthin or the omega-3 PUFAs, docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA), or both, to the AREDS formulation decreases the risk of developing advanced AMD. AREDS2 also investigated the effects of omitting beta-carotene and reducing the concentration of zinc from the original AREDS formulation. Although the primary analysis did not reveal a benefit of daily supplementation with lutein/zeaxanthin on disease progression risk, secondary exploratory analyses concluded that lutein/zeaxanthin were helpful in reducing AMD progression, while the beneficial effects of low versus high dose of zinc were equivalent [71]. The clinical recommendation that has emerged from these AREDS studies is that lutein and zeaxanthin are appropriate substitutes for beta-carotene in the original AREDS formulation.

High total dietary fat [64], saturated fat, and cholesterol intake [61] increase the risk of developing AMD. In contrast, omega-3 PUFAs, found in fish and nuts, are thought to exert a protective effect via their antioxidative, anti-inflammatory and anti-angiogenic effects [72–74]. Mechanistically, omega-3 fatty acids are thought to attenuate the effects of environmental insults to photoreceptors such as ischemia, light toxicity, inflammation, and aging, exerting anti-inflammatory effects by decreasing monocyte cell surface antigen presentation, tumor necrosis factor alpha (TNF α) and interleukin-1 beta (IL-1 β) expression, neutrophil superoxide presentation, natural killer lymphocyte activation, and lymphocyte presentation [75]. They also exert anti-angiogenic effects seen in vitro and in vivo mediated through vascular endothelial growth factor-A (VEGFA) and platelet-derived growth factor [76]. Specifically, examination of animal models has shown that the omega-3 PUFA, DHA, the main structural lipid of retinal photoreceptor outer segments [77], increases photoreceptor survival and prevents apoptosis [78], while epidemiology studies have shown that omega-3 fatty acids reduce AMD risk by 30–50 % [54, 64] and decrease the risk of progression to advanced disease [62]. Interestingly, AREDS2 did not find a benefit for omega-3 supplementation, likely due to a difference in the dosage of DHA and EPA between studies [71, 79].

The seminal observations of increased lipid and cholesterol deposition within Bruch's membrane and drusen, as seen during evaluations of human donor tissue [80, 81], motivated additional studies examining the relationship between cholesterol and AMD. The Pathologies Oculaires Liées à l'Age (POLA) [69] and Rotterdam Studies [82] both demonstrated that high HDL levels are beneficial in decreasing AMD risk. A study by Reynolds et al. [83] showed that subjects with advanced AMD had lower HDL levels, and higher low-density lipoprotein (LDL) and total cholesterol levels relative to controls. The importance of HDL/LDL ratios is further underscored by the finding that cardiovascular disease, specifically the presence of cholesterol-containing atherosclerotic lesions as identified by ultrasound, is also associated with an approximately 4.5 fold increase risk of developing late AMD [84]. Additional research is necessary to dissect the exact mechanisms through which HDL levels and genetic variants in the cholesterol pathway promote AMD genesis and progression.

Inflammation and inflammatory factors linked to AMD

Inflammation is believed to be a central player in the pathobiology of AMD, beginning as early as drusen formation [85–87]. Histologic studies have demonstrated that RPE debris becomes engulfed in the RPE basal lamina and Bruch's membrane, inciting a chronic inflammatory response that may contribute to drusen formation [85]. Inflammatory components, involved in both acute and chronic inflammation, have been found within drusen, basal deposits, and RPE of human donor eyes as well as in sub-RPE deposits and neovascular lesions of a murine model of AMD [88, 89]. These include amyloid-beta (b), complement factors, amyloid P component, and carboxyethyl pyrrole adducts generated by lipid oxidation [87, 90]. Some of these proteins also tend to co-localize within drusenoid deposits, as is the case for amyloid-b peptide and activated complement components [91, 92]. The fact that many of these inflammatory proteins accumulate in tissues affected in other aging degenerative diseases, such as Alzheimer's disease, favors the involvement of a common pathogenic mechanism. Inflammation is also associated with angiogenesis, and therefore is an important player in the neovascular form of AMD. Some inflammatory markers identified to date associated with progression to late AMD include C-reactive protein, homocysteine, IL-6, and plasma complement activation fragment Bb and C5a [93–96].

From a mechanistic perspective, examination of animal models of neovascular AMD [97–99] and some human AMD case series [100–102] indicate cellular inflammation, specifically involving retinal-resident and -recruited macrophages, may play a fundamental role in the progression of the disease. These studies demonstrate that acute, laser-induced breaks in Bruch's membrane of mice result in CNV lesions, decreased retinal function, and retinal degenerative changes including synaptic disruption and activation of Müller glial cells concomitant with retinal infiltration of macrophages early on, in the degenerative process [97, 98]. Furthermore, proliferation, hypertrophy, and recruitment of macrophages to the retina, and Bruch's membrane in proximity of sub-RPE deposits have been reported in a small number of human AMD cases [101–103]. In the latter report macrophage recruitment was found to be associated with an alteration in immunophenotype [103]. Ultimately, larger studies are necessary to definitely characterize the distribution of macrophage subpopulations, their pro- versus anti-inflammatory status at the level of the retina and RPE/choroid in human AMD eyes, and consequently their contribution to the development and progression of AMD.

Genetic factors linked to AMD

Genetics play a definitive role in AMD. Recently, results from the AMD gene consortium increased the number of loci associated with AMD to 19 [104], within which common gene variants have been found to confer a small to moderate increase in AMD susceptibility, while rare gene variants contribute to a higher risk for the disease [105, 106]. Meta-analyses studies, independently validated by the results of genome-wide association studies (GWAS) [107], have shown that chromosomes 1q25–31 and 10q26 are two key regions involved in estimating genetic risk of AMD. The most notable single nucleotide polymorphisms (SNP) linked with AMD is the variant Y402H in the *CFH* gene located on chromosome 1 [108–111]. Individuals homozygous for this risk variant have an estimated odds ratio of between

2.45 and 3.33 for all forms of AMD. Other genetic variants include several within the complement cascade such as complement factor B (BF)/complement component 2 ((C2)) [112, 113], C3 [113, 114], and complement factor I ((CFI)) [115]. Together, these support a considerable role for inflammation in the pathogenesis of AMD and suggest that uncontrolled alternative complement pathway activity contributes significantly to disease progression. There are many other genes not part of the complement cascade, linked to AMD, including the age-related maculopathy susceptibility 2/HtrA serine peptidase (*ARMS2/HTRA1*) locus on chromosome 10 [116, 117]. Although the function of this gene is not yet fully understood, it is thought to be involved in angiogenesis, extracellular matrix mineralization, and transforming growth factor, beta 1 (*TGFBI*) signaling [118, 119].

GWAS studies have also uncovered an association between genes in the HDL pathway and AMD including lipase C (*LIPC*), cholesterol ester transfer protein (*CETP*), and ATP-binding cassette subfamily A member 1 (*ABCA1*) [120]. Additionally, extracellular matrix molecules including TIMP3 [120], collagen type X, alpha 1/fyn-related Src family tyrosine kinase (*COL10A1/FRK*), and *COL8A1* [104, 120], and angiogenesis-related genes such as *VEGF-A* [121] and TGFβ receptor 1 (*TGFBR1*) [104] have been identified. Other loci discovered via GWAS include *TNFRSF10A*, *APOE*, *IER3/DDR1*, *SLC16A8*, *RAD51B*, *ADAMTS9/MIR548A2*, and *B3GALT1* [104, 122]. The precise roles of many of these genes in the pathogenesis of AMD remain to be elucidated.

While rare genetic variants affect only a minority of the population, they have been shown to confer a strong effect on AMD risk and are likely to play a pivotal role in disease genesis. Rare variants that confer higher risk and earlier disease onset include the *CFH* haplotype with a missense mutation, R1210C, which has been shown to lead to *CFH* loss of function [123] and 59 rare *CFI* variants and mis-sense mutations in *C3* and *C9*, which have been reported to cause excessive activation of the alternate complement pathway. Together, these findings emphasize the importance of genetic polymorphisms in *CFH*, *C3*, *CFI* and *C9* in AMD pathogenesis.

Some AMD-related genes are associated with disease progression in addition to increased risk of AMD development. For example, *CFH* and *ARMS2/HTRA1* have been linked to progression from early or intermediate stages to late stages of AMD [56, 124]. By contrast, *LIPC* is associated with decreased risk of progression from large drusen to CNV [125]. An *ABCA1* variant has been shown to be associated with decreased progression from intermediate to large drusen, while *CFH*, *C3*, *CFB*, and *ARMS2/HTRA1* appear to be associated with increased progression from intermediate drusen to large drusen and from large drusen to both late neovascular (CNV) and dry (GA) AMD [125]. Furthermore, both peripheral retinal drusen and reticular pigment changes, which are thought to confer a higher risk of developing advanced AMD, have been associated with variations in *CFH* [126].

Although future identification of additional risk factors for AMD, with progressive improvement in genetic tools, is inevitable (and welcomed), the risk factors already identified to be associated with AMD and reviewed herein provide a meaningful glimpse into AMD biology. Together with our understanding of the disease phenotype, they have given rise to the identification of many potential pathogenic pathways modulated in the

disease. These pathways are worth investigating at a mechanistic level with the important goal of identifying therapeutic avenues to pursue.

Potential AMD pathogenic mechanisms and other considerations in the pursuit to identify disease-related signaling pathways and therapeutic targets

Though no consensus exists on the pathophysiological and biochemical mechanisms responsible for development of AMD, converging data collected from the clinical, epidemiologic, genetic, histological, and biochemical studies described above suggest the involvement of the following potential pathogenic paradigms and mechanisms each currently under extensive investigation: (1) degenerative changes due to aging, (2) apoptosis, (3) lipid and cholesterol dysregulation, (4) accumulation of lipofuscin, a potential cytotoxic byproduct of faulty or incomplete lysosomal degradation of photoreceptors by RPE cells [127, 128], (5) deposition and infiltration of glycoproteins, proteins and in particular lipids into inner Bruch's membrane creating a diffusion barrier, which impedes flow of nutrients and oxygen from the choroid to the RPE and waste products from the RPE to the choroid [81, 129], (6) inflammation, (7) cellular mechanical stress, (8) angio-genesis, (9) choroidal hypoperfusion, (10) fibrosis and extracellular matrix dysregulation, (11) oxidative stress, (12) autophagy, (13) mitochondrial dysfunction, (14) hypoxia, and (15) complement activation. These paradigms provide important guidelines in the quest to identify potential regulators. One group of transcription factors, nuclear receptors, are probable candidates as they have previously been shown to regulate many aspects of these purported AMD-related pathways in a myriad of biological and disease processes. The rest of this review will focus on the biology of a number of these receptors studied in the eye as they relate to AMD. We will also highlight the rationale behind the efforts to explore targeting these receptors for therapies in AMD.

Overview of the nuclear receptor superfamily

Nuclear receptors (NRs) are a superfamily of evolution-arily related DNA-binding/ligand-inducible transcription factors [130–132]. They operate as sensors, effectors and modulators of signaling pathways, translating endocrine and metabolic cues such as fat-soluble hormones, vitamins, and dietary lipids into gene expression programs that regulate a broad spectrum of physiological processes including embryonic development, metabolic homeostasis, apoptosis, cell growth, differentiation, and proliferation, to name a few [130–132]. As one of the largest families of transcription factors, they are comprised of a total of 48 members (Table 2) that were identified through sequencing of the human genome [133]. The superfamily can be divided into three classes based on their ligand- and DNA-binding properties (Fig. 3; Table 2). The first and most extensively characterized subfamily includes the classical steroid hormone receptors, which are responsive to glucocorticoids, mineralocorticoids, estrogens, progestins, and androgens. These receptors usually bind to DNA nuclear receptor response elements (NRRE), located within the promoter region of their target genes, as homodimers in a ligand-dependent fashion. The second class are the orphan receptors, termed “orphan” because though they have the structurally conserved

features of the nuclear receptor superfamily, their endogenous regulatory ligands have not been identified or they may even function in a ligand-independent manner. These receptors have several modes of DNA binding, including binding as monomers, homo-dimers, or heterodimers with the 'promiscuous' retinoid X receptors (RXRs) [134]. The third subfamily consists of "adopted" orphan receptors. These receptors were originally categorized as "orphan" receptors; however, upon identification of their naturally occurring ligands and physiological roles they have been reclassified into this subfamily. These include the retinoic acid receptors (RARs), RXRs, thyroid hormone receptors, the vitamin D receptor, peroxisome proliferator-activated receptors (PPARs) and the liver X receptors (LXRs). These receptors typically bind their specific NRREs as heterodimers with RXRs in the presence or absence of a ligand [135, 136].

Structurally, nearly all members of the NR family are modular proteins and have a common architecture composed of three domains: the carboxy (C)-terminal ligand-binding domain (LBD), the DNA-binding domain (DBD), and the amino (N)-terminus (Fig. 3) [136]. The LBD, where ligands acting as pure agonists, antagonists, mixed or partial agonists or antagonists bind, is localized at the C-terminus. Ligand-induced allosteric changes in the NR structure following its binding to the LBD include a conformational change, creating, exposing, or removing interaction surfaces. This in turn modifies the interaction profile of the receptors' co-regulatory proteins and the recruitment of transcriptional co-activators and/or corepressors generating positive or negative regulation of target gene expression. The DBD is composed of two zinc fingers in the middle of the protein. This allows NRs to bind to a sequence-specific NRRE in the promoter region of target genes. The N-terminal domain shows variation in amino acid sequence and length, and does not have an organized structure. Additional regions of NRs include a hinge domain that connects the DBD to the LBD, and two activation function domains (AF-1 and AF-2), necessary for co-activator recruitment. The location, amino acid sequence, and contribution of the AFs to regulation of NR activity vary with each receptor. Two exceptions to the overall structural organization of NRs are seen in the NRs DAX-1 and SHP, both of which lack a DBD and by virtue of not binding to DNA, exert dominant-negative transcriptional activities [137].

NR signaling is remarkably complex as evident by their diverse mechanisms of action and transcription modulation. NRs follow both canonical and non-canonical activation rules. The canonical pathway involves diffusion of ligands across the cell membrane and binding to a NR located within the cytoplasm, often stabilized by chaperon proteins. Upon ligand binding and dissociation from the chaperon proteins, the ligand/NR complex translocates to the nucleus where they bind to the NRRE of their target genes. Conversely, NRs that follow the non-canonical pathway may be constitutively active in the nucleus regardless of the presence of the ligand. NR transcriptional modulation also occurs through several distinct mechanisms including both activation and repression. These activities can be ligand dependent or independent and can be genomic or non-genomic, mediating gene repression, the release of gene repression, gene activation, or gene transrepression.

Given the multi-faceted roles of NRs in the development and function of both endocrine and non-endocrine tissues, multiple neurodegenerative and systemic diseases have been found to be associated with mutations and altered or impaired activity of these receptors. Diverse

disease functions modulated by NRs include but are not limited to inflammation and immunity, lipid metabolism, extracellular matrix regulation, and angiogenesis. Conspicuously, and of great interest to the ophthalmology field, these functions are also impaired in the different clinical sub-types of AMD [138–141]. While the roles of some of these receptors have been well characterized in other diseases and have been shown to ameliorate pathogenesis in animal models of disease, their actions in relation to cells and tissues impacted in AMD have either not been investigated or only recently have become an area of interest.

It is appropriate to quickly restate that some of the challenges we face in definitely determining the role of various signaling pathways, in this case involving NRs, in the biology of AMD lies in the complexity of the tissue affected. Therefore, in our desire to identify targetable pathways, it is important to merge the information derived from various pathology, epidemiology, and genetic studies with our understanding of the basic biology of the cellular compartments and tissue complexes vulnerable in AMD. These tissues and cells include the RPE cells, photoreceptors, cells present in the choroid, such as the endothelial cells, and immune cells, including macrophages and microglia (Fig. 4). Among the cells listed, the RPE cells, a monolayer of pigmented cuboidal epithelial cells, have been studied most extensively, since the overall health of RPE is particularly important in all clinical sub-types of AMD. Akin to epithelial cells found in other organs, RPE cells are polarized, characterized by an asymmetrical distribution of plasma membrane proteins and by a preferential release of secreted proteins into apical and basal compartments [142]. They are tightly sandwiched between the overlying neurosensory retina on their apical side, facing the light-sensitive photoreceptor outer segments, and the underlying choroidal blood supply on their basal side, facing Bruch's membrane. The network of apical junctional complexes that surrounds and links adjacent RPE cells forms the ocular blood-retinal barrier. The functions of RPE cells are complex and absolutely essential for visual function, as they provide support to the photo-receptors and retina in multiple ways, serving as a conduit allowing delivery of nutrients from the outer blood supply and transporting ions, water, and metabolic end products from the sub-retinal space back to the blood. The RPE cells play a necessary role in daily phagocytosis and digestion of shed photoreceptor outer segments, recycling and then returning retinaldehyde to the photoreceptors to rebuild light-sensitive outer segments, required for phototransduction and the visual cycle [143]. They also secrete a variety of growth factors and immunosuppressive factors that help maintain the structural integrity of the chorio-capillary endothelium and photoreceptors, and help establish the immune privilege of the eye [144]. Given the multi-functional characteristics of these cells, it is easy to see that a failure of any of these functions could lead to degeneration of the overlying retina, loss of visual function and blindness, as seen in AMD. Because of the vital role of RPE cells in the eye and in AMD, we will place additional emphasis on the role of NRs in RPE cells throughout the remainder of this review.

Nuclear receptors in RPE physiology

Several groups have shown that select nuclear receptors play various roles in RPE physiology. However, there are significant inconsistencies in the concluding results. For example, activation of the glucocorticoid receptor (GR) has mitogenic properties in RPE

cells causing cellular proliferation in cases of proliferative vitreoretinopathy following retinal reattachment surgery [145]. Conversely, Ayalasomayajula et al. [146] recently showed that the corticosteroid fluocinolone can inhibit VEGF expression in the human RPE cell line, ARPE19, in a GR-dependent manner. In vivo murine models of estrogen receptor beta (ER β) knockout have been associated with altered murine matrix metalloprotease-2 activity, increased collagen production, and sub-RPE deposit formation as seen in human dry AMD [147, 148], yet ER β mRNA is reportedly undetectable in in vitro RPE cell cultures and freshly isolated human RPE [149]. The PPAR gamma (γ) has consistently been observed in ARPE19 cells and often in primary RPE cell lines harvested from human donors [150] and can become activated following photoreceptor phagocytosis [151], yet it is unclear if this occurs in the eye, as disparate results have been reported regarding its expression in freshly isolated human RPE cells and human sections [149, 152]. Interestingly, the PPAR γ agonist pioglitazone has been shown to inhibit the epithelial to mesenchymal transition and, therefore, fibrotic change in cultured monkey RPE cells, a hallmark of proliferative vitreoretinopathy [153]. Nonetheless, differential effects on oxidative stress and apoptosis have been seen in ARPE19 cells depending on the agonist used, with pioglitazone- and rosiglitazone-potentiating cell death and troglitazone functioning as a potent cytoprotective agent [154].

These reported discrepancies can be explained in part because of variability in expression of NRs between mice and humans and in part due to the different RPE cell model systems available and studied, which include a spontaneously arising human RPE cell line (ARPE19), primary cell lines derived from adult donors, and RPE cells isolated from freshly obtained adult donor tissue. These cell models are the most commonly used in research labs, and each has inherent advantages and disadvantages. For example, ARPE19 cells are easy to culture, form a hexagonal cobblestone layer, and exhibit morphological polarization under certain culture conditions, yet lack pigmentation and lose some RPE cell-specific markers [155]. Early passage human primary RPE cells, which are cultured from human donor eyes, are often limited in availability, generally used only until passage 10, and therefore have a finite lifespan. However, they retain some normal physiological functions, including polarization, phagocytosis and the ability to transport retinoids [156]. RPE cells freshly isolated from human donor eyes for biochemistry and molecular biology are ideal, as they have not been subjected to culturing conditions and potential de-differentiation. However, accessibility to donor tissue obtained within a short postmortem time is limited and, due to the small amount of obtainable tissue per eye, there are constraints on the number of studies that can be conducted. Furthermore, there is variability in the biology of the samples obtained, which is reflective of normal heterogeneity within the donor population. These are important caveats that must be taken into consideration when interpreting the results of RPE-focused studies as we try and gain insight into human in vivo biology.

The aforementioned issues, along with the lack of a comprehensive expression study of all human nuclear receptors in RPE cells, in which standardized methodologies have been utilized to collect data, led to the development of a nuclear receptor atlas of human RPE cells [149]. Creation of this atlas [nuclear receptor signaling atlas (NURSA) website (<http://www.nursa.org>)] involved the systematic profiling of all 48 human nuclear receptors, two variants of the PPAR β /d and c receptors, as well as the aryl hydrocarbon receptor (AhR),

owing to its similar mechanisms of action to NRs, in the three model systems of human RPE cells discussed above. The goals of developing this RPE cell-specific NR atlas were threefold: first, to create a comprehensive baseline of NR expression to elucidate NR-driven RPE biochemical and physiological pathways under normal conditions; second, to identify potential NR pathways that may contribute to pathogenic changes seen in retinal diseases; and third, to determine the validity of using the more experimentally accessible in vitro cell culture models to pursue focused studies on NR-related signaling pathways [149]. Using the relative standard curve method of real-time quantitative RT-PCR, receptors were classified based on expression level into the following categories: absent or undetectable, low, medium, or high expression. While most NRs were expressed in all three RPE cell models, the relative expression levels varied, with many NRs expressed at high levels in freshly isolated RPE cells compared to the in vitro models. In contrast, a few NRs were absent in the cell culture systems but present in freshly isolated human RPE cells (Table 2). Overall, the results of the NR atlas supported the validity of using cell culture models to initiate investigation of the biology of NRs, but only after careful consideration of differences in mRNA expression levels between the cell culture line to be used and RPE cells in vivo. Finally, it was suggested that confirmation of protein expression levels of the NR of interest would provide additional justification for future studies using in vitro RPE cell models. Importantly, this study identified a list of nuclear receptors that may be important in AMD pathogenesis, by dividing the receptors into functional groups with relevance to AMD, based on a literature search using gene ontology terms (Table 2; Fig. 5).

Nuclear receptors as regulators of AMD biology

The majority of the work investigating the potential contributions of NRs and associated signaling pathways to AMD development and progression comes from genetic studies and animal models of wet AMD utilizing the laser-induced CNV model in mice and rats.

Genetic studies demonstrating NR-associated risk for AMD

Though some epidemiology studies have failed to show an association between female gender and AMD risk [157], others have reported that diminished exposure to endogenous estrogens, as is the case in early menopause, is associated with AMD, and that postmenopausal hormone replacement therapy may be protective against the disease [158]. Mechanistically, this may also be attributable to impaired cellular estrogen receptor function. In the Rotterdam prospective population-based study, an ER α polymorphism was shown to be associated with wet AMD progression [159, 160]. Follow-up in vitro studies confirmed that 17-beta estradiol can protect ARPE19 cells from oxidative stress [161]. It should be noted that the authors reported that this protection occurs through ER β , whose expression in ARPE19 cells has been confirmed by several studies, but as of yet not confirmed in aged human donor RPE cells [149]. The retinoic acid receptor-related orphan receptor (*ROR α*), which is associated with maturation of cone photoreceptors in the retina, is another gene shown to be associated with late AMD pathogenesis. Linkage analysis and expression studies have found that decreased expression of *ROR α* and common variants and haplotypes of this receptor, in concert with an *ARMS2* polymorphism are associated with an increased risk for AMD [162]. The functional consequence and biological implications of

RORA in cells vulnerable in AMD remains to be determined. Finally, it is important to point out that impairment of NRs in disease may not necessarily be detectable in genetic studies of affected and unaffected cases. This is, in part, due to the fact that receptor expression is not a reflection of activity of the receptor and it is impaired activity, due to changes in DNA binding, ligand binding and/or recruitment of co-regulators that may result in a substantial effect on the responsiveness of the NR to ligands. Since deficiency of NR function may not be detectable in genetic association studies, evaluating NR activity using in vitro activity assays may be required. This is best illustrated by reviewing data from a haplotype analysis of AMD versus control cases that concluded the aryl hydrocarbon receptor (*AhR*), a receptor critical in the biological response to endogenous and xenobiotic toxins, may be associated with the development of AMD [163]. Though a significant difference in expression was not seen, this minor genetic association along with the overlap between molecular events regulated by *AhR*, such as toxin clearance, extracellular matrix proteolysis, cellular degradation, and events important in AMD, led to the hypothesis that the *AhR*-signaling pathway may be important in AMD pathogenesis and would need to be investigated in detail using both in vitro and in vivo methods [164]. In fact, in vitro, evaluation of the activity of *AhR* in primary cultures of young and old human RPE cells found a significant decrease as a function of age, suggesting that the RPE cells ability to clear toxin and “garbage” becomes less efficient with age. While in vivo examination of the eye phenotype of 11- to 12-month-old mice harboring the null allele at the *AhR* locus, revealed the presence of sub-RPE deposits, focal RPE atrophy, thinning of Bruch's membrane, focal choroidal atrophy, and visual function deficits, characteristic features also reported in human AMD patients. Collectively, these findings indicate a role for the *AhR*-signaling pathway in the evolution of sub-RPE deposits, a mechanism that becomes less active with age and may therefore, contribute to disease pathogenesis.

Animal studies demonstrating NR-regulating biologic roles in AMD

A potential association has surfaced between the PPAR/ RXR-signaling pathway and AMD. This is due to PPARs and specifically the PPAR γ 's known participation in various mechanisms and biologic pathways related to lipid regulation, immune modulation, and oxidant/antioxidant pathways, processes also associated with the pathogenesis of AMD. Furthermore, RXR along with some of the PPAR isoforms has been found to be expressed in human macrophages (α , β/δ , γ), RPE cells (α , β/δ), and endothelial cells (α , β/δ , γ ; Fig. 4). Though reports directly linking PPARc dysfunction with human AMD pathology are limited, follow-up functional studies using ARPE19 and/or primary cells have speculated that it plays a role in AMD by targeting oxidative stress and inflammation [154, 165– 169] (though as discussed earlier, caution is necessary in interpreting these cell culture models, until the NR of interest has been shown to be definitely expressed in the aged human eye cells). Still, it is probable that in vivo targeting of PPAR γ may have a therapeutic benefit given the role of macrophages in AMD and data from the atherosclerosis field demonstrating PPARs as negative regulators of macrophage activation. Specifically, PPAR γ as well as PPAR β/δ agonists have been shown to inhibit inflammatory responsive genes in macrophages including *TNFA*, *IL1B*, inducible nitric oxide synthase, *MCP-1* and numerous chemokine genes [170–173]. Also, macrophages from PPAR γ knockout mice show impaired engulfment and clearance of apoptotic cells, a pathological proinflammatory

phenotype, along with altered regulation of essential genes for phagocytosis, including complement genes [174]. Indeed, AMD-specific in vivo studies have evaluated the effect of intravenous treatment with a PPAR γ agonist on laser-induced CNV in mice and found that this treatment inhibits the growth of new vessels through Bruch's membrane as well as fibrosis [175–177]. Future studies are needed to evaluate the PPAR/RXR-signaling pathway and its effect on other pathogenic processes relevant in AMD, including determining if activation of these receptors could suppress microglial-mediated inflammatory responses and promote the degradation of amyloid- β peptides through upregulation of genes responsible for reverse cholesterol transport, as has been reported in Alzheimer's disease [178, 179].

The LXR/RXR heterodimer is another NR complex that, although genetic studies have not shown a direct association with AMD, may likely play an important role in the pathogenesis of the disease. LXRs regulate reverse cholesterol transport, normalizing cholesterol efflux pathways to prevent excessive lipid accumulation and, as such, serve to maintain total body cholesterol and fatty acid homeostasis. These mechanisms are also altered in AMD [180, 181]. Moreover, GWAS studies have shown an association between AMD and SNPs in genes involved in cholesterol metabolism, which include the LXR target genes *ABCA1*, *CETP*, and *LIPC* [104]. Additionally, both the α and β isoforms are expressed to varying levels within the peripheral neural retina, macrophages, RPE cells and endothelial cells [149, 182–184]. Again, interest in determining the potential role of LXR in AMD arose from animal studies of atherosclerosis and Alzheimer's disease. This work demonstrated that LXR alone, RXR alone, or in some cases LXR and RXR in combination can ameliorate disease-related pathology including regression of athero-sclerotic plaques [185], positive clearance of amyloid- β and apoE-mediated proteolysis in the brain as well as repression of pro-inflammatory mediators by activated microglial cells [186–189]. Furthermore, deletion of either of the LXR isoforms individually, or both, in an animal model of Alzheimer's disease results in worsening of symptoms [190]. AMD-specific in vitro studies have shown that A2E (*N*-retinylidene-*N*-retinylethanolamine), a metabolic fluorophore of lipofuscin, which accumulates with age in RPE cells, can stimulate intracellular accumulation of free and esterified cholesterol preventing cholesterol efflux, and that treatment of RPE cells with LXR agonists can effectively restore cholesterol homeostasis within the cells [191]. In vivo studies to date provide further convincing support for the role of LXR in AMD. Mice deficient in *CYP27a1*, a novel LXR target gene, develop pathological retinal and choroidal neovascularization, though this pathology is complicated by the background of the mice used in the study, which carries a spontaneous mutation in the crumbs homolog 1 gene causing retinal degeneration [192]. Most notably, Apte et al. [182] have shown treatment of old mice with a synthetic LXR agonist in eye drop formulation that can restore their tissue-specific cholesterol efflux capacities and reduce the severity of laser-induced CNV.

Though not an exhaustive list, multiple other NRs not investigated directly in AMD (Table 2; Figs. 4, 5) are worth mentioning, including RAR, which has been shown to upregulate vascular endothelial growth factor expression in RPE cells [193, 194] and as such may be important in the biology of neovascularization [195–197]. GRs are ubiquitous within cells and have been used for the treatment of a broad spectrum of inflammatory diseases, acting

on the innate and adaptive immune systems [198]. The orphan nuclear receptors NOR-1, NURR1 and Nur77 are key transcriptional regulators in metabolism and vascular disease [137]. Last, but not least, the COUP-TFs have been shown to be involved in angiogenesis reparative processes including wound healing and in the regulation of lipid and glucose metabolism [199].

Concluding remarks

Nuclear receptor biology in the posterior segment of the eye is a relatively new area of study and one that shows great promise. There is a significant overlap between pathogenic pathways in AMD and other diseases currently under treatment in the clinic with validated NR-targeted drugs. Given that, to date, there are no known NR-targeted therapies for AMD, it is imperative to search for the potential relevance of NR drugs in AMD therapy. Certainly, the widespread expression of NRs in cells vulnerable in AMD provide support for the premise that the eye shares fundamental signaling pathways with other endocrine organs and exhibits endocrine-like functions that ultimately may play a central role in the pathobiology of disease. Future studies centered on determining the biology of these receptors in the eye hold significant promise in the identification of potential NR-driven AMD therapies.

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Abbreviations

A2E	<i>N</i> -retinyle- din- <i>N</i> -retinylethanolamin
ABCA1	ATP binding cassette subfamily A1
AF	Activation function
AhR	Aryl hydrocarbon receptor
AMD	Age-related macular degeneration
ApoE	Apolipoprotein E
AREDS	Age-related eye disease study
BLamD	Basal laminar deposit BLinD Basal linear deposit
CFH	Complement factor H
CNV	Choroidal neovascularization
CYP450	Cytochrome P 450
DBD	DNA-binding domain
DHA	Docosahexaenoic acid

EPA	Eicosapentaenoic acid
ER	Estrogen receptor
GA	Geographic atrophy
GR	Glucocorticoid receptor
GWAS	Genome-wide association study
HDL	High-density lipoprotein
IL	Interleukin
LBD	Ligand-binding domain
LDL	Low-density lipoprotein
LXR	Liver X receptor
NR	Nuclear receptor
NRRE	Nuclear receptor response element
PPAR	Peroxisome proliferator-activated receptor
RAR	Retinoic acid receptors
ROR	Retinoic acid receptor-related orphan receptor
RPD	Reticular pseudodrusen
RPE	Retinal pigment epithelium
RXR	Retinoid X receptor
SD-OCT	Spectral domain-optical coherence tomography
SNP	Single nucleotide polymorphism
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor

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Fig. 1.

Diagram illustrating the stages of AMD: **a** Normal. **b** Few small drusen. **c** Early AMD with thin continuous sub-RPE deposits. **d** Intermediate AMD with thick sub-RPE deposits overlying degenerating choriocapillaries. **e** Geographic atrophy. **f** Choroidal neovascularization. **g** Disciform scar. *NFL* nerve fiber layer, *GCL* ganglion cell layer, *IPL* inner plexiform layer, *INL* inner nuclear layer, *OPL* outer plexiform layer, *ONL* outer nuclear layer, *PR* photo receptors, *RPE* retinal pigment epithelium, *BM* Bruch's membrane, *chor* choroid

Fig. 2. Multi-modal retinal imaging illustrating cases of AMD with corresponding histopathology. **a** Dry AMD with drusen color fundus photograph, spectral domain-optical coherence tomography (SDOCT), and histopathology. **b** Reticular pseudodrusen color fundus photograph, fundus autofluorescence, SD-OCT, and histopathology. **c** Geographic atrophy color fundus photograph, fundus autofluorescence, SD-OCT and histopathology. **d** Classic CNV lesion in neovascular AMD color fundus photograph, fluorescein angiography, SD-OCT and histopathology. Histopathology of reticular pseudodrusen is adapted with permission from ref [38]

Fig. 3. Primary structure of a nuclear receptor along with a listing of members of three classes of nuclear receptors: steroid hormone receptors, orphan nuclear receptors and adopted orphan nuclear receptors. *DBD* DNA-binding domain, *LBD* ligand-binding domain, *NRRE* nuclear receptor response element, *NR* nuclear receptor, *AF* activation function, *Hsp* heat shock protein

Fig. 4.

Schematic view of a cross-section of the photoreceptor/RPE/ choroid complex with a druse. Selected nuclear receptors, functionally associated with each cell type and potential cellular processes regulated by these receptors are listed. *PR-OS* photoreceptor outer segments, *RPE* retinal pigment epithelium, *BM* Bruch's membrane, *mφ* macrophages, *EC* choroidal endothelial cells

Fig. 5.

List of potential nuclear receptors that may regulate AMD pathogenic pathways through their involvement in angiogenesis, oxidative stress, inflammation and the immune response, fibrosis and ECM (extracellular matrix) dysregulation, neurodegeneration and apoptosis, and lipid metabolism and dysregulation

Table 1

AREDS AMD classification scheme

	AMD level	Criteria
Category 1	No AMD	None or a few small drusen (<63 microns diameter)
Category 2	Early AMD	Any or all of the following: <ul style="list-style-type: none"> • Multiple small drusen (63–124 microns in diameter) • RPE abnormalities
Category 3	Intermediate AMD	Any or all of the following: <ul style="list-style-type: none"> • Extensive intermediate drusen and at least one large druse (>125 microns) • Geographic atrophy not involving the foveal center
Category 4	Advanced AMD	Is characterized by one or more of the following in one eye (in the absence of other causes): <ul style="list-style-type: none"> • Geographic atrophy of the RPE and choriocapillaris involving the centre of the fovea • Neovascular maculopathy such as: <ul style="list-style-type: none"> – Choroidal neovascularization (CNV) – Serous and/or hemorrhagic detachment of the sensory retina or RPE – Retinal hard exudates (a secondary phenomenon resulting from chronic leakage from any sources) – Sub-retinal and sub-RPE fibrovascular proliferation – Disciform scar

Table 2
Nuclear receptor superfamily, ligands, marketed drugs, disease function and presence in human RPE cells

Nuclear receptor	Abbreviation	Nomenclature	Ligand	Function/associated diseases	Human RPE ^a
Androgen receptor	AR	NR3C4	Androgens, testosterone	Prostate cancer, X-linked androgen insensitivity	No
Constitutive androstane receptor	CAR	NR1I3	Xenobiotics, steroids	Xenobiotic metabolism	No
COUP transcription factors	COUP-TF α , β , γ	NR2F1, 2, 6	Orphan	Prostate cancer, osteoblast differentiation	Yes, yes, yes
DAX	DAX	NR0B1	Orphan	X-linked adrenal hypoplasia congenita	No
Estrogen receptors	ER α, β	NR3A1, 2	Estrogens, 17 β -estradiol	Breast cancer, osteoporosis, atherosclerosis	Yes, no
Estrogen-related receptors	ERR α , β , γ	NR3B1, 2, 3	Orphan	Embryonic development	Yes, no, yes
Farnesoid X receptor	FXR	NR1H4	Bile acids	Dyslipidemia, liver disease	No
Germ cell nuclear factor	GCNF	NR6A1	Orphan	Fertility, contraception	Yes
Glucocorticoid receptor	GR	NR3C1	Glucocorticoids, Cortisol	Immunological and metabolic disorders, glaucoma	Yes
Hepatocyte nuclear factors 4	HNF4 α , γ	NR2A1, 2	Fatty acids	Diabetes, hemophilia, lipid metabolism	Yes, yes
Liver receptor homolog 1	LRH-1	NR5A2	Orphan	Crohn's disease, ulcerative colitis	No
Liver X receptors	LXR α, β	NR1H3, 2	Oxysterols	Cholesterol homeostatis, atherosclerosis, Alzheimer's disease	Yes, yes
Mineralocorticoid receptor	MR	NR3C2	Mineralcorticoids, aldosterone	Hypertension	Yes
Nerve growth factor induced-B	NOR1, NURR1, Nur77	NR4A1, 3, 2	Orphan	Neurological and immunological disorders	Yes, yes, yes
Photoreceptor-specific NR	PNR	NR2E3	Orphan	Enhanced S-cone syndrome autosomal recessive RD	No
Peroxisome proliferator-activated receptor	PPAR α, β, γ	NR1C1, 2, 3	Fatty acids, DHA, leukotriene, prostanoids	Diabetes, coronary heart disease, obesity, cancer	Yes, yes, no
Progesterone receptor	PR	NR3C3	Progesterones	Breast cancer, infertility, pregnancy maintenance	Yes
Pregnane X receptor	PXR	NR1I2	Xenobiotics, steroids, bile acids	Xenobiotic metabolism	No
Retinoic acid receptor	RAR α, β, γ	NR1B1, 2, 3	Retinoic acids	Inflammatory skin disorders, leukemia	Yes, yes, yes
Rev-Erbs	Rev Erb α , β	NR1D1, 2	Heme	Circadian rhythm, metabolism	Yes, yes
Retinoid-related orphan receptors	ROR α , β , γ	NR1F1, 2, 3	Cholesterol, all trans retinoic acid, orphan	Atherosclerosis, immunological disorders, osteoporosis, wet AMD	Yes, yes, yes
Retinoid X receptors	RXR α, β, γ	NR2B1, 2, 3	Retinoic acids	Alzheimer's disease, coronary heart disease	Yes, yes, yes
Steroidogenic factor-1	SF-1	NR5A1	Orphan	Sexual development adrenocortical insufficiency, insulin resistance	No
Small heterodimeric partner	SHP	NR0B2	Orphan	Mild early onset type II diabetes and obesity	No

Nuclear receptor	Abbreviation	Nomenclature	Ligand	Function/associated diseases	Human RPE ^a
Tailless homolog	TLX	NR2E1	Orphan	Retinal dystrophy	Yes
Thyroid receptors	TR α, β	NR1A1, 2	Thyroid hormone, triiodothyronine	Hypothyroidism, obesity	Yes, yes
Testicular receptors	TR 2, 4	NR2C1, 2	Orphan	Cerebellar development, motor coordination	Yes, yes
Vitamin D receptor	VDR	NR1H1	Vitamin D	Osteoporosis, psoriasis, kidney and cardiovascular diseases, AMD	No

NRs within the subfamily with marketed drugs are indicated in bold italics

AMD age-related macular degeneration, RD retinal degeneration

^a Presence or absence of NRs within aged human RPE cells