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An overview of retinal light damage models for preclinical studies on age-related macular degeneration: identifying molecular hallmarks and therapeutic targets

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Abstract: Age-related macular degeneration (AMD) is a complex, multifactorial disease leading to progressive and irreversible retinal degeneration, whose pathogenesis has not been fully elucidated yet. Due to the complexity and to the multiple features of the disease, many efforts have been made to develop animal models which faithfully reproduce the overall AMD hallmarks or that are able to mimic the different AMD stages. In this context, light damage (LD) rodent models of AMD represent a suitable and reliable approach to mimic the different AMD forms (dry, wet and geographic atrophy) while maintaining the time-dependent progression of the disease. In this review, we comprehensively reported how the LD paradigms reproduce the main features of human AMD. We discuss the capability of these models to broaden the knowledge in AMD research, with a focus on the mechanisms and the molecular hallmarks underlying the pathogenesis of the disease. We also critically revise the remaining challenges and future directions for the use of LD models.

Keywords: age-related macular degeneration; retinal light damage; animal model; preclinical and clinical studies

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1 Introduction

The visual system is involved in multiple disorders caused by the dysfunction of different components of the eye. The anterior part of the eye may develop cataracts (an opacification of the crystalline lens) or lesions of the cornea, with a consequent damage to the trigeminal nerve. Nevertheless, these damages could be repaired through surgery, corneal transplant or topical application of specific drugs (Kumar et al. 2022). Conversely, neurodegenerative disorders affect the retina, which is part of the sensory nervous system located at the back of the eye, with no regenerative capability. Retinitis pigmentosa (Newton and Megaw 2020), Stargardt's disease (Tsang and Sharma 2018), glaucoma (He et al. 2018), diabetic retinopathy (Lin et al. 2020) and age-related macular degeneration (AMD) (Guymer and Campbell 2023) are the main degenerative diseases affecting the retina, with different aetiology and incidence, but with the common fate of causing vision loss. In this review, we will focus on AMD and the involvement of light as a risk factor triggering retinal neurodegeneration. We will discuss the use of light damage (LD) rodent models in preclinical studies to deeply explore AMD pathogenesis, therapeutics and molecular underpinnings, starting from the fundamental role of light for retinal function and then how it becomes harmful in certain conditions. We will review the main literature on the existing LD models and their ability to reproduce the features of human AMD both in early and late stages. We will also explore the advantages and limitations of the LD model for preclinical studies of AMD.

1.1 Anatomical and histological considerations

The macula is a specialized region of the human retina responsible for the visual acuity. This area is approximately 6 mm in diameter with a depression in the centre, called foveola. The neuroretina is composed of multiple transparent layers including, from the outside to the inside:

photoreceptor layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer and nerve fiber layer. Photoreceptors (rods and cones) are the specialized cells able to convert light into an electrical response, placed in the back portion of the eye. In the macula, there is a lateral displacement of the inner retinal layers; hence the light could directly reach the photoreceptors avoiding light distortion. The fovea is enriched in cone photoreceptors, conversely to the peripheral and much of the macular region, which are mainly composed of rods. The high density of cones is responsible for high central visual acuity (Provis et al. 2005). Retinal pigment epithelium (RPE) is placed in proximity of photoreceptors, ensuring their homeostasis through its multiple functions (Lakkaraju et al. 2020). The RPE lays on a specialized extracellular matrix called Bruch's membrane (BrM) (Booij et al. 2010), which divides it from the choriocapillaris (CC) of the choroid. Oxygen supply in macular region occurs primarily through choroidal circulation, which has a 7-fold increased flow in the macula than peripheral retina, while there is a decreased density of the retinal vasculature in this region (Provis et al. 2005). This adaptation makes the macula more susceptible to stressful stimuli and aging (Handa 2012). RPE displays numerous protective molecules and mechanisms against stress stimuli. For instance, with the diet we absorb lutein, which is converted in mesozeaxanthin through the cytoplasmic enzyme RPE65 (Shyam et al. 2017). RPE displays several carotenoids, which are part of the retinal antioxidant machinery (Kiser and Palczewski 2016). Another protective factor expressed in the RPE and in the iris is melanin, a pigment able to limit photo-oxidative damage by absorbing the scattered light (Arunkumar et al. 2018). The macula is missing in rodents, highlighting a critical point for their use as a model for the study of AMD. However, in rats, the retina displays a region, located in the superior area, which is more susceptible to light. This area was defined "hot spot" by the researchers who first characterized it (Rapp 1990). The hotspot does not have structural and functional specializations of the human macula; nevertheless, during degenerative processes, retinal damage is confined to this area, reproducing a similar scenario to that of the macula in human AMD. This is not true for mice, in which degenerative damage is extended throughout the retinal length.

1.2 Age-related macular degeneration

Age-related macular degeneration is a multifactorial, neurodegenerative disorder characterized by a progressive impairment of the macula (Chichagova et al. 2018; Mitchell et al. 2018). This disorder is considered the major cause of blindness in Europe and developed countries, and its prevalence is approximately one person every 10, over 55 years (Zapata et al. 2021). It has been evaluated around 196 million cases for the 2020, with an estimated increase to 288 million by 2040 (Li et al. 2020; Wong et al. 2014). AMD consists of several alterations in neuronal retinal cells, RPE, BrM, choroid and retinal vessels. A lot of progress has been made in recent years, but to date, the exact pathogenesis of AMD is still under investigation, mainly to fully understand all the events occurring during the onset of the disease and its progression. According to clinical manifestations, AMD is classified in two main distinct forms (Figure 1): a dry form (atrophic or non-exudative) and a wet form (exudative or neovascular), and both can cause an irreversible visual impairment resulting in blindness in the most severe stages (Ambati and Fowler 2012). The most prevalent form is the dry AMD which accounts for 85–90 % of all cases (Evans and Syed 2013) and is characterized by a gradual vision loss. Although is less common (10 % of all cases), the exudative form of AMD is the most severe with a rapid progression, and it is characterized by abnormal choroidal neovascularization (CNV) in the macular region. The starting point of AMD is the dysfunction of RPE followed by photoreceptors death (Ferris et al. 2013; Wong et al. 2008). A Clinical Classification was proposed by Ferris et al. (2013) and it is based on the size and the degree of drusen deposits and pigmentary abnormalities, which are two main pathological hallmarks of AMD. According to this classification, AMD can be divided into early, intermediate (a critical clinical stage due to the increased risk for the progression of the pathology), and advanced stage. Starting from the intermediate stages, the pathology could be distinguished into *dry* or *wet* form (Ferris et al. 2013; Flaxel et al. 2020). The end stage of dry AMD is characterized by a typical lesion called geographic atrophy (GA) (Garcia-Garcia et al. 2022). On the other hand, in the wet or exudative form there is a dysregulated CNV. CNV could be classified into (i) type 1, which is typically localized under the RPE with its elevation, (ii) type 2 located in the subretinal space with the separation of the RPE from the neuroretina and (iii) type 3 characterized by oedema, haemorrhage and the aberrant connection between retinal and choroidal circulation (Spaide et al. 2020). The two forms of late AMD are not mutually exclusive; indeed, a secondary GA may appear following the neovascularization (Garcia-Garcia et al. 2022). A mixture of both types of AMD late-stages could be commonly found in the same eye (Stahl 2020).

The incidence of AMD mainly correlates with the age (Blasiak et al. 2022; Jonasson et al. 2014), and lifestyle; indeed, smoking, light, higher body mass index with hyper lipidic diet, comorbidity with cardiovascular disease, previous

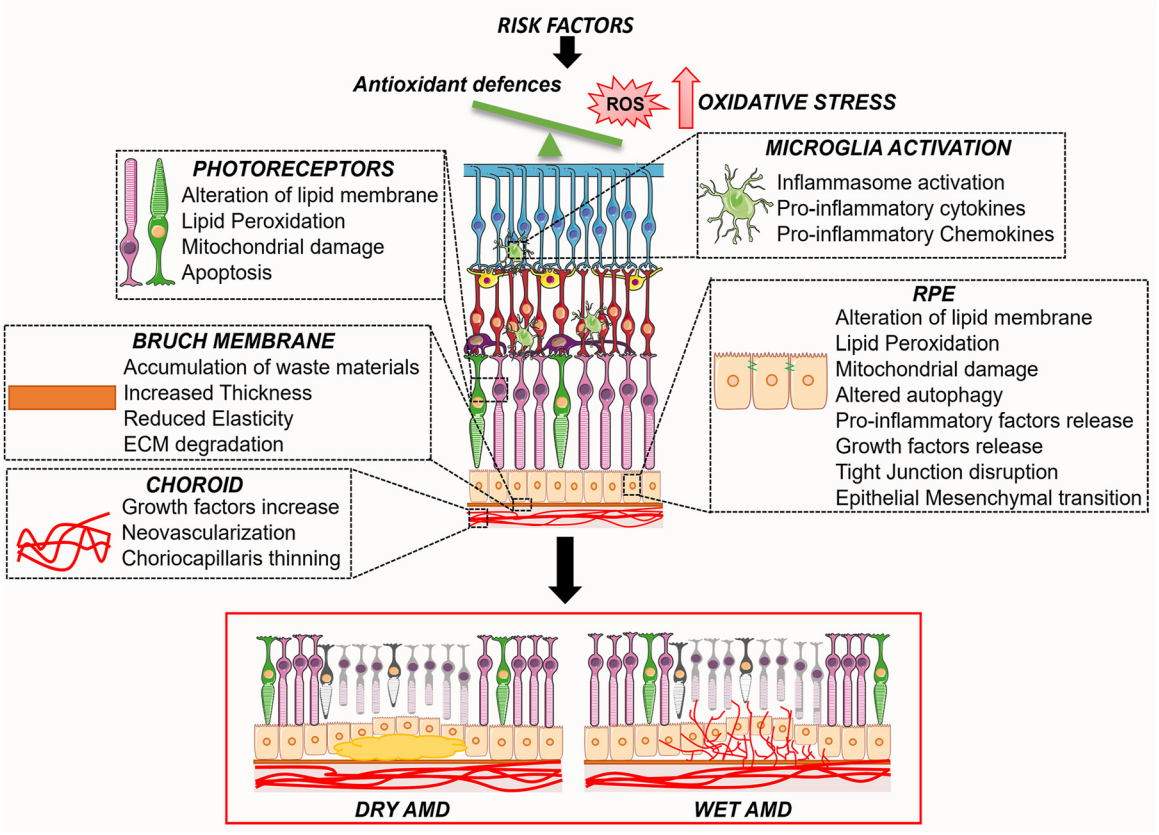

Figure 1: Schematic representation of the main features of DRY and WET AMD. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

cataract surgery, chronic kidney disease, hypertension, hyperthyroidism, diabetes, Parkinson's and Alzheimer's disease, genetic predisposition and racial background are classified as risk factors, as detailed elsewhere (Heesterbeek et al. 2020b). Recently thanks to technological advancements, including the single-cell sequencing (sc-RNAseq), genetic factors had also been identified to be involved in AMD development and progression (Collin et al. 2023; Voigt et al. 2021, 2022). Furthermore, several genetic studies have significantly contributed to the knowledge on AMD pathology. To date, the genomic heritability can be elucidated by both common and rare genetic variants in less than 40 genetic loci, which are primarily involved in the complement system, extracellular matrix remodelling, and lipid metabolism (reviewed by Dietzel et al. 2014). The potential benefit of identifying all risk factors could be the ability to predict the disease progression in individual patients, but mainly to improve the design of future clinical trials for the development of new effective therapeutic strategies. The following two paragraphs will describe the dual role played by light in its interaction with the visual system: the light as a necessary stimulus for vision, but also the light as a stressor to induce retinal degeneration.

1.3 Light as an environmental stimulus for visual perception

Light is fundamental for the correct functioning of the visual sensory system, which is sensitive to a spectrum of wavelength between 400 and 760 nm. The phototransduction is triggered when photons of light reach the retina and are focused on the macula (Figure 2). In the retina, photoreceptors convert light into graduated electrical signals, which become action potentials in the ganglion cells to be transferred to the higher visual cortex. The light triggers specific photochemical reactions which take place in the discs of photoreceptors outer segments. Rhodopsin and opsins are two kinds of photopigments rod and cone respectively. As reported in Figure 3, phototransduction involves six consequential steps: in the Step 1 the light converts the rhodopsin from opsin+11-cis retinal (the vitamin A aldehyde) to opsin+ All-trans retinal. In Step 2, the activated form of rhodopsin, called metarhodopsin, binds the transducin, which is a G-protein composed of three units (α, β, γ). In Step 3 transducin converts GTP (guanosine triphosphate) into GDP (guanosine diphosphate) while its α subunit detaches from β and γ ones. Then, in the Step 4, the α subunit of transducin

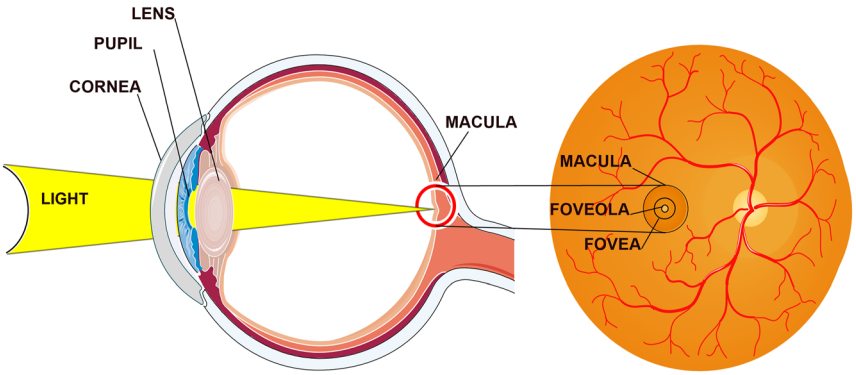

Figure 2: Schematic representation of the interaction between light and eye. The light is able to cross the external components of the eye: the cornea, the pupil and the lens. In the back of the eye the light is focused on the fovea. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

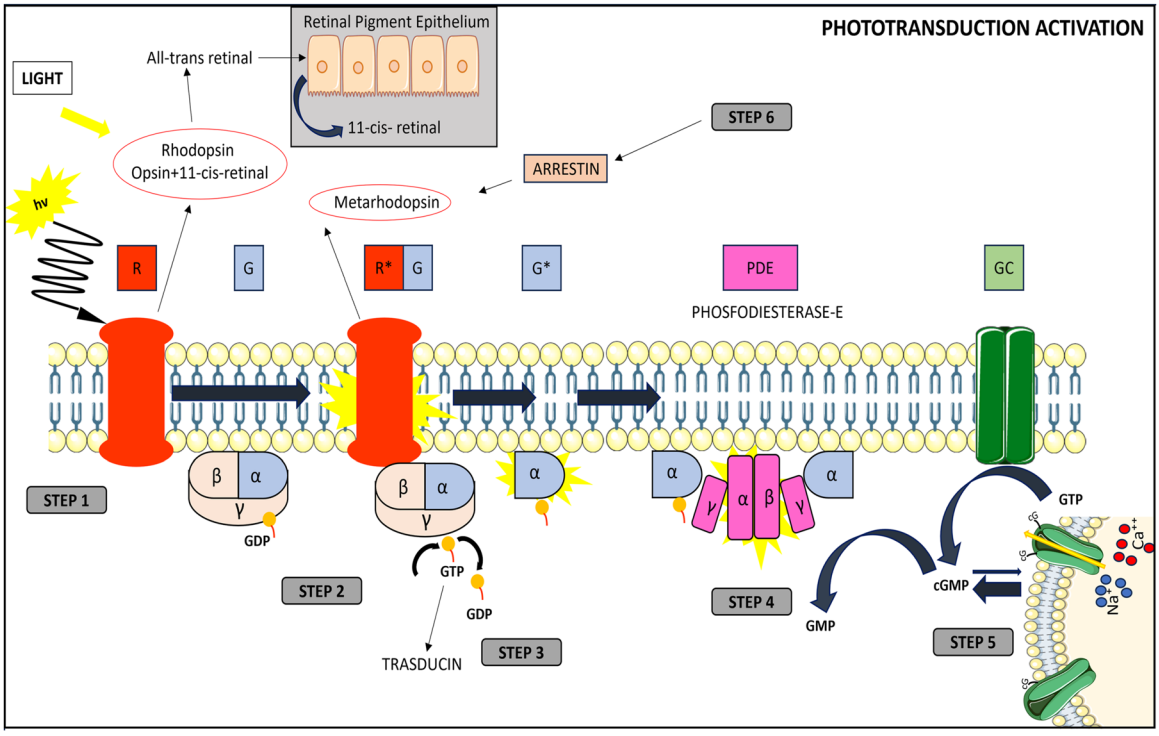

Figure 3: Schematic representation of the phototransduction cascade. Abbreviations: hv: photon; R: rhodopsin; G: trasducin; PDE: phosphodiesterase E; GC: guanylyl cyclase. The asterisk indicates the activated forms of rhodopsin (R*) and trasducin (G*). Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

activates the enzymatic reaction to convert cGMP into GMP through its binding to the phosphodiesterase E (PDE), counteracting the activity of guanylate cyclase (GC), able to convert GTP in cGMP (step 5). The cGMP is a key regulator of ion channels activity; in fact, the cGMP closes Na^{+}/Ca^{2+} ion channels, blocking positive charges flux and causing the hyperpolarization of photoreceptors. In daylight conditions, this cascade of events determines a hyperpolarization of photoreceptors and the consequent decreased release of glutamate by the synaptic terminal. On the contrary, in dark conditions photoreceptors are depolarized and the release of glutamate increases. The subsequent depolarization or hyperpolarization of bipolar cells (classified in ON and OFF) is related to glutamate receptors activated in the postsynaptic membrane (ionotropic/excitatory, metabotropic/inhibitory). The ON bipolar cells, having metabotropic receptors, are depolarized when the light reaches the centre of the retinal receptive field, driving an excitatory stimulus to the ON ganglion cells. Conversely, the OFF bipolar cells, which have ionotropic receptors, are hyperpolarized when the light reaches the centre of the retinal receptive field, determining the inhibition of the OFF ganglion cells. This phenomenon is

peculiar of retinal electrical properties and represents the way through which the retina elaborates the light/dark stimuli and discriminates the response from the centre/periphery visual field. This phenomenon is peculiar of retinal electrical properties and represents the way through which the retina elaborates the light/dark stimuli and discriminates the response from the centre/periphery visual field. During the last step of the visual cycle (step 6), the arrestin converts the metarhodopsin to rhodopsin to restart a new cycle. RPE also plays a pivotal role in the visual cycle participating in converting all-trans retinal to 11-cis and making it available for a new visual cycle and in the discs renewal. Therefore, the dysfunction of RPE causes an unavailability of the retinal-11-cis and an accumulation of waste material, called lipofuscin, in the subretinal space.

1.4 Light as a risk factor for human AMD

Throughout our life, eyes are constantly exposed to photons with an increased risk of phototoxic damage to retinal cells. Before going in depth to noxious effects of the light, it is important to note that continuous light exposure can induce different types of damage: photochemical, photothermal and photomechanical; these damages depend on the transparency of optical components of the eye (Glickman 2002; Wu et al. 2006). Each tissue of the eye absorbs specific wavelengths of light (i.e. the cornea below 295 nm, the lens around 300–315 nm) and the degree of absorption is higher during the young age compared to the senile one. Therefore, retinal cells are exposed to light with shorter wavelengths, exposure to both natural and artificial light notably increased, due to the development of technology and longer life expectancy (Shen and Tower 2019; Wong et al. 2014). Noteworthy, in physiological conditions, the visual system has the capability to protect itself from the toxic effect of prolonged light exposure thanks to the activation endogenous repair mechanisms. An age-related failure of retinal self-repair capacities leads to the dysfunction and subsequent degeneration of this tissue, as it happens during AMD. High levels of light exposure induce the production of reactive oxygen species (ROS), the peroxidation of membrane phospholipids, the activation of proinflammatory pathways and the up regulation of cytokines and growth factors (Shang et al. 2017; Zhao et al. 2018). However, it should be noted that not all the wavelengths of light are harmful to the retina; indeed, red and near-infrared lights have been used as a therapeutic approach called photo-biomodulation (PBM) (Kim and Won 2022). On this basis, researchers in the field were able to use light to induce retinal degeneration in animal models, allowing the study of AMD pathogenesis. In the next paragraph we reported an overview of the AMD rodent models, with a particular focus on the light damage paradigm.

2 Animal models of AMD induced by light

The complex aetiology of AMD is reflected in the lack of therapies and the effort put in place to find good animal models which faithfully reproduce the pathology. During the years, several rodent models were developed to study the pathogenesis and possible therapeutic approaches for AMD. We briefly summarize the main *in vivo* models of AMD and the features studied in each of them in Supplementary Table S1. Unfortunately, in most of these models, the degeneration proceeds slowly and takes many weeks to months to reproduce some of the hallmarks of the disease. Thus, the need for more eloquent *in vivo* models able to recapitulate the main aspects of AMD encouraged many researchers in the field to make progress. In this context, several papers reported light damage (LD) as a suitable and reliable model to reach this goal. In our laboratory, we established a rat model of AMD by exposing albino Sprague Dawley (SD) rats to white light for 24 h (h) (1000 lux) (Tisi et al. 2019b, 2020a,b, 2022, 2023). Noell et al. first reported the light damage paradigm in the literature (Noell et al. 1966). They exposed the animals to different wavelengths of light at different time points, demonstrating that high intensity light damaged the rat retina. After light exposure there was the presence of oedematous areas in the light-exposed retinas, together with morphological alterations in the RPE and the outer nuclear layer (ONL). These findings were further corroborated by electroretinogram recordings in which the electrical response of the retina was decreased after light exposure, compared to the control animals (Noell et al. 1966). This pioneering research gave important insights into retinal degeneration processes. Some years later, Marc et al. demonstrated that light-induced retinal damage (LIRD) in albino rats recapitulates many features of human atrophic AMD, more than other murine AMD models (Marc et al. 2008). Since then, many research groups in the field started to develop LD models by using different animal strains or light damage protocols. The effects of light on retinal toxicity could be influenced by many factors: pigmentation, time and intensity of light, acute and chronic exposure, source of light and wavelengths (Youssef et al. 2011). Also, melanin is an endogenous protective factor for the retina, and it is in the iris (the light or dark color of the iris depends on its concentration) and in the RPE. Sanyal and Zeilmaker in 1988

performed an experiment on chimeric mice, with a different proportion of albino and pigmented cells, and demonstrated that following exposure to constant light for five weeks in mice with more pigmented cells there was increased photoreceptor survival (Sanyal and Zeilmaker 1988). Thus, RPE pigmentation is considered a protective strategy of the retina against oxidative stress, and this is reflected in the race-related incidence of AMD (Bressler et al. 2008). Based on this, the first LD models were made on albino strains, because the induction of retinal damage by light is easier than in pigmented animals, since some strains are particularly resistant (Zhong et al. 2016); however, LD paradigms on pigmented animals were also developed (Krigel et al. 2016; Wooff et al. 2023). Another influencing factor is represented by the light sources and wavelengths used to induce retinal degeneration. Fluorescent lamps were the most used at the beginning, but their use was then replaced by light emitting diodes (LED) lights, which facilitated the optimization of parameters, including wavelength and irradiance time. Although the blue component of commercially available LED lights is hazardous for retinal tissue, LEDs are largely used during our daily life (Contín et al. 2016; Ouyang et al. 2020; Van Norren and Gorgels 2011). Therefore, lights with specific wavelengths in the blue spectrum have been recently used to induce retinal degeneration in rodents. We are constantly exposed to LED illumination and, since AMD is a chronic disease, one more discriminant for the development of LD models was the acute or chronic exposure to light. In Table 1 we summarized the main established LD paradigms in the literature, indicating the rodent strains, timing, spectrum, light intensity and acute or chronic exposure. In the next paragraphs we will deeply explore AMD hallmarks reproduced by LD models and molecular markers used for their study (Table 2), underlining benefits of their use to explore AMD pathogenesis and therapeutics.

3 Oxidative stress in human AMD

The retina is characterized by high oxygen consumption, because of its metabolic activity and constant exposure to light and UV radiation. Energy metabolism naturally generates oxidative stress through the production of ROS, which are chemicals containing oxygen (i.e. superoxide anion, hydroxyl radical, hydrogen peroxide and singlet oxygen) (Genestra 2007). ROS are first produced as a byproduct of mitochondrial metabolism, and during the phagocytosis of photoreceptors outer segments for their renewal (Brown et al. 2019; Datta et al. 2017). ROS serves as signalling molecules, regulating protein function and the activation of retinal antioxidant defences (Sies and Jones 2020). To counteract ROS accumulation, RPE synthesizes and releases antioxidant molecules, like glutathione, heme, antioxidant vitamins and redox proteins (i.e. thioredoxin) (part of Age-Related Eye Disease Study (AREDS) supplementation components). However, the retina eliminates ROS accumulation mainly through the nuclear factor erythroid-2-related factor 2 (NRF2)-related antioxidant enzymes, involving superoxide dismutase (SOD), catalase, and peroxidase, in addition to free radical scavengers (Lambros and Plafker 2016). Alongside antioxidants, autophagy also contributes to the redox defence of RPE (Ferguson and Green 2013). During aging, retinal antioxidant machinery is impaired or overwhelmed and neural retina and RPE are extensively exposed to oxidative damage (Tisi et al. 2021; Zhang et al. 2015). Furthermore, genetic polymorphism and lifestyle also exacerbate ROS accumulation (Othman et al. 2015; van Leeuwen et al. 2018).

3.1 Oxidative stress in LD models

In this context, many studies underline how light exposure could cause oxidative stress in LD animal models (Narimatsu et al. 2013; Organisciak et al. 2013; Qi et al. 2015; Tanito et al. 2002; Wang et al. 2021; Yang et al. 2022). Nrf-2 signalling pathway, which is the main antioxidant defence of the human retina, is impaired in LD mice (Yang et al. 2022) as it happens in retinas from AMD donors (Datta et al. 2017). Furthermore, an overall increase in retinal ROS was showed both after an acute (Narimatsu et al. 2013; Tisi et al. 2019b) and a chronic light exposure (Benedetto and Contin 2019), in albino rats. LD causes an up-regulation of heme oxygenase 1 (HO-1) and thioredoxin (TrX) in an attempt to counteract oxidative stress (Organisciak et al. 2013; Qi et al. 2015; Tanito et al. 2002). On the other hand, in LD retinas was showed an increase in CEP and MDA (Organisciak et al. 2013; Qi et al. 2015; Tanito et al. 2002), a clear sign of lipid peroxidation. Systemic alterations of antioxidant defences were also observed; indeed, in albino rats there was an increase in the blood levels of MDA, NO (nitric oxide) and NOS (nitric oxide synthetase) after light damage, while GSH-Px (glutathione peroxidase) and SOD (superoxide dismutase) levels were down-regulated (Qi et al. 2015; Shin et al. 2022; Wang et al. 2021). The increased oxidative stress and the impairment of the antioxidant defences due to light exposure induce the disruption of organelles and macromolecules, as deeply explained throughout this review.

Table 1: Light damage rodent models resembling AMD features.

Rodent strain	Age (weeks)	Light intensity	Spectrum of light	Exposure time (acute * or chronic +)	AMD features	References
Sprague Dawley rats	8–12	1000 lux	White light	24 h (*)	– Reduced retinal function	Maccarone et al. (2008)
	N\A	1000 lux	White light	24 or 48 h (*)	– RPE alterations	
	8	350 foot-candles	White light	6 h (*)	– Photoreceptors death	Tisi et al. (2020a)
	6–7		White light	24–48 h (*)	– Retinal neovascularization	Tisi et al. (2020b)
	12	1350–1500 lux	White fluorescent	10 days (+)		Riccitelli et al. (2021)
	6		White cool light	Cyclic 12 h (1, 3, 6 months) (+)	– Gliosis	
	12	325 ± 2.5 foot-candles	White and blue LED		– Subretinal deposits	Tisi et al. (2023)
	8		White light	Cyclic 12 h (3, 9, 28 days) (+)	– Oxidative stress	Rutar et al. (2010)
	7	3000 lux		Cyclic 12 h (one week) (+)	– Autophagy alterations	
		750 lux			– RPE disruption	Jiao et al. (2015)
		400 lux			– Photoreceptors death	Marc et al. (2008)
					– Discontinuous BrM	
					– Gliosis	Organisciak et al. (1998)
					– Photoreceptors apoptosis	Benthal et al. (2022)
					– Gliosis	
					– Photoreceptors death	Albert et al. (2010)
					– Gliosis	
					– RPE disruption	Shang et al. (2014)
					– Reduced retinal function	
					– Photoreceptors death	Othman et al. (2015)
					– RPE alterations	
					– Choroidal neovascularization	
					– Anastomosis with retinal vessels	
					– BrM depositions	
					– Reduced retinal function	
					– Photoreceptors death	
					– ROS accumulation	
					– Reduced retinal function	
					– Photoreceptors death	
					– Lipids alterations	
Wistar rats	12	300 lux	White light (32 W, 2950 lumens)	10 days (+)	– Reduced retinal function	Rubin et al. (2022)
	6	2680 cd/m^2		Constant for 4.75, 6, 12, 18 or 24 h (*)	– Photoreceptors loss	
	12	2000 lux	White (LED and fluorescent light)		– RPE degeneration	Jaadane et al. (2017)
	8	3000 lux		Cyclic 12 h (1, 3, 6 months) (+)	– Autophagy alterations	
		500 lux	White cool light		– BRB breakdown	Albert et al. (2010)
		1000 lux	LED (cold-white, blue, green) and cool fluorescent light	24 h constant or cyclic 12 h (one week or one month) (*) (+)	– Oxidative stress	
		1500 lux			– RPE alterations	Krigel et al. (2016)
		6000 lux			– Choroidal neovascularization	
					– Anastomosis with retinal vessels	
					– BrM depositions	
					– Reduced retinal function	
					– RPE dysfunction	
					– Photoreceptors damage	
					– Gliosis	
					– BRB breakdown	
Long Evans rats	8	500 lux	LED (cold-white, blue, green) and cool fluorescent light	24 h constant or cyclic 12 h (one week or one month) (*) (+)	– Reduced retinal function	Krigel et al. (2016)
	N\A	1000 lux			– RPE dysfunction	
		1500 lux			– Photoreceptors damage	Wiegand et al. (1986)
		6000 lux	Constant fluorescent light	2, 3, 4 or 5 days (+)	– Gliosis	
					– BRB breakdown	

Table 1: (continued)

Rodent strain	Age (weeks)	Light intensity	Spectrum of light	Exposure time (acute * or chronic +)	AMD features	References
		10–20 foot-candles			– Photoreceptors death – Lipids alteration – Subretinal deposits	
C57BL/6J	8	100 k lux	White light	1, 3, 5 or 7 days (*) (+)	– Reduced retinal function	Natoli et al.
mice	8	100 k lux	White light	1, 3, 6, 12, 24 h (*)	– Photoreceptors death	(2016)
	N/A	1100 lux	Blue light	3 h constant or cyclic 3 h	– Gliosis	Wooff et al.
	6	7000 lux	White light	(three days) (*) (+)	– Oxidative stress	(2023)
	40	2000 lux	Blue LED light	12 h (*)	– Oxidative stress	Nakamura et al.
	24	800 lux	Blue LED light	4 h (*)	– Photoreceptors death	(2018)
	6	N.S.	White LED light	Two weeks (+)	– Gliosis	Song et al.
	8–12	5000 lux	White fluorescent light	Cyclic 2 h (four weeks) or	– Transient reduction of	(2020)
	8	10,000 lux	White light	cyclic 12 h (39 weeks) (+)	retinal function	Xia et al. (2019)
				1 h (*)	– Deposits accumulation	Wang et al.
				Seven days (+)	– Gliosis	(2023)
					– RPE degeneration	Peng et al.
					– Deposits accumulation	(2012)
					– Photoreceptors death	Cachafeiro et al.
					– Reduced retinal function	(2013)
					– RPE alterations	Gong et al.
					– Oxidative stress	(2022)
					– Autophagy alterations	
					– Photoreceptors death	
					– Oxidative stress	
					– Photoreceptors death	
					– Reduced retinal function	
					– BRB breakdown	
					– RPE degeneration	
					– VEGF up-regulation	
					– Reduced retinal function	
					– RPE degeneration	
					– Oxidative stress	
					– Lipofuscin accumulation	
					– Autophagy alterations	
					– Gliosis	
					– Increased microvessels	
BALB/c mice	8–12	5000 lux	White light	1 h (*)	– Reduced retinal function	Cachafeiro et al.
	N\A	10,000 lux	White fluorescent light	7 h (*)	– BRB breakdown	(2013)
	5	10,000 lux	Blue light	Cyclic 1 h (2 weeks) (+)	– RPE degeneration	Chen et al.
	5	10,000 lux	Blue light	Cyclic 1 h (2 weeks) (+)	– VEGF up-regulation	(2004)
	8–10	4500 lux	Blue light	1 h (*)	– Oxidative stress	Pham et al.
	5	10,000 lux	Blue light	1 h (*)	– Photoreceptors death	(2021)
	8	10,000 lux	Cool fluorescent light	7 h (*)	– Gliosis	Shin et al. (2022)
					– Photoreceptors death	Song et al.
					– RPE degeneration	(2022)
					– Oxidative stress	Xie et al. (2021)
					– Lipofuscin accumulation	Dentchev et al.
					– Photoreceptors death	(2007)
					– RPE degeneration	
					– Photoreceptors death	
					– RPE degeneration	
					– Photoreceptors death	
ddY mice	N\A	5000 lux	White fluorescent light	3 h (*)	– Reduced retinal function	Kuse et al.
					– Photoreceptors death	(2018)
					– RPE degeneration	

LD paradigms were divided in relation to: rodent strains, age, light intensity, spectrum of light, exposure time and the use of acute or chronic stimulus.

Table 2: Molecular hallmarks of LD models related to human AMD phenotype.

AMD features	Marker	Source	Modulation	References
Oxidative stress	Nrf-2	Retina	↓	Yang et al. (2022)
	HO-1	Retina	↑	Organisciak et al. (2013)
	SOD	Retina/blood	↓	Qi et al. (2015) and Shin et al. (2022)
	NO	Blood	↑	Wang et al. (2021)
	NOS	Blood	↑	Wang et al. (2021)
	MDA	Retina/blood	↑	Organisciak et al. (2013), Qi et al. (2015), Tanito et al. (2002), and Wang et al. (2021)
	CEP	Retina	↑	Organisciak et al. (2013), Qi et al. (2015), and Tanito et al. (2002)
	GSH-Px	Blood	↓	Wang et al. (2021)
	Trx	Retina	↑	Tanito et al. (2002)
	8-OHG	Retina	↑	Natoli et al. (2016)
	Acrolein	Retina	↑	Tisi et al. (2019b)
Inflammation	IBA-1	Retina	↑	Rutar et al. (2011b) and Tisi et al. (2019b)
	GFAP	Retina	↑	Rutar et al. (2011b)
	IL-1β	Retina	↑	Yang et al. (2022)
	IL-6	Retina	↑	Wooff et al. (2023)
	IL-18	Retina	↑	Yang et al. (2022)
	Ccl3	Retina	↑	Rutar et al. (2015)
	Ccl4	Retina	↑	Rutar et al. (2015)
	Ccl7	Retina	↑	Rutar et al. (2015)
	Ccl2	Retina	↑	Rutar et al. (2011b)
	CxCl1	Retina	↑	Rutar et al. (2015)
	TNFα	Retina	↑	Rutar et al. (2015)
	CxCl10	Retina	↑	Rutar et al. (2015)
	NRLF3	Retina	↑	Yang et al. (2022)
Lipids alterations	Omega-3	Retina/blood	↓	Othman et al. (2015)
	Omega-6	Retina/blood	↑	Othman et al. (2015)
	Endocannabinoid receptors	Retina	↑	Maccarone et al. (2016)
	RvE1	Retina	↓	Tisi et al. (2023)
		Blood	↑	

Table 2: (continued)

AMD features	Marker	Source	Modulation	References
Blood retinal barrier alterations	(ZO-1)	Retina	Mislocation	Cachafeiro et al. (2013), Narimatsu et al. (2013), and Xie et al. (2021)
	Beta-catenin	Retina	Mislocation	Cachafeiro et al. (2013)
	N-cadherin	Retina	Mislocation	Cachafeiro et al. (2013)
	Serum albumin	Retina	Mislocation	Cachafeiro et al. (2013) and Krigel et al. (2016)
RPE alterations	RPE65	Retina	↓	Song et al. (2020)
	ER stress markers	Retina	↑	Jaadane et al. (2017) and Song et al. (2020)
	OPA-1	Retina	↓	Wang et al. (2023)
	DRP1	Retina	↑	Wang et al. (2023)
	LC3BII	Retina	↑	Jaadane et al. (2017) and Tisi et al. (2020a)
	p62	Retina	↑	Jaadane et al. (2017) and Tisi et al. (2020a)
	Apoptosis markers	Retina	↑	Natoli et al. (2016), Tisi et al. (2019b), and Tisi et al. (2020a)
	RIPK3	Retina	↑	Song et al. (2022)
Vascular alterations	VEGFA	Retina	↑	Tisi et al. (2020b)
	VEGFR2	Retina	↑	Tisi et al. (2020b)
	bFGF	Retina	↑	Tisi et al. (2020b)
	FGFR1	Retina	↑	Tisi et al. (2020b)
	PEDF	Retina	↓	Cachafeiro et al. (2013)

4 Inflammation in human AMD

The term inflammation is usually related to a protective role from harmful stimuli, such as pathogens or injury, hence it is a cellular response against factors that perturbed the homeostasis of cells and tissues (Meizlish et al. 2021). The long-term inflammatory response is detrimental for the retina and is linked to AMD pathogenesis and progression (Ambati et al. 2013; Kauppinen et al. 2016; Nowak 2006). The breakdown of the blood–retinal barrier (BRB), as described in paragraph 7, causes the entry into the retinal space of inflammatory factors like inflammasomes, complement system, and immune cells. RPE and immune cells produce cytokines and chemokines leading to the inflammatory cascades (Wong et al. 2022). One of the first evidences that confirm the link between the inflammatory mechanism and the AMD were described by Green and Key (1977). Notably, local inflammatory events and abnormalities in inflammatory immune responses may contribute to drusen biogenesis (Hageman et al. 2001) and CNV (Kanda et al. 2008). In early AMD, the RPE activates the inflammasome (triggered by oxidative stress, see Section 3) and subsequently recruits macrophages to Bruch membrane, as reviewed elsewhere (Datta et al. 2017). To date it is well known that the acute inflammatory response is linked to dry AMD, rather than chronic inflammation (Tan et al. 2020) which is responsible for AMD progression to the late stages (Donoso et al. 2006; Kauppinen et al. 2016). Several studies investigated the role of chronic systemic inflammation in intermediate and neovascular AMD, discovering elevated plasma levels of cytokines, such as IL-4, IL-13 and IL-33 (Gotfredsen et al. 2023; Rajeswaren et al. 2023). Some of them (e.g. IL-4), were up-regulated in the plasma of GA patients, suggesting its potential role as a marker of dry AMD; on the other hand, the tumour necrosis factor-alpha (TNF-α) was recently correlated to CNV but not with dry AMD lesions (Khan et al. 2022). Thus, these markers might be considered for their use for a differential

diagnosis of the two AMD forms. All the above-mentioned cytokines (IL-4, IL-13 and IL-33) are related to both types of macrophages, recruited to damaged tissues that polarize to functional phenotypes like M1 classified as pro-inflammatory and the M2 classified as anti-inflammatory (Zhou et al. 2017). Interestingly, both types of macrophages are present in AMD (dry and wet forms) human eyes as demonstrated for the first time by Cao et al. (2011). Furthermore, it should be mentioned the role of microglia cells (MG), the immunocompetent resident macrophages normally localized nearly to retinal blood vessels in the inner layers of the neural retina (Provis et al. 1995). Resting (or inactive) microglia, which display a ramified shape, lays in the inner layers of normal human retinas (Gupta et al. 2003); while the activated MG cells (balloon shaped, ameboid) start to invade the outer retina and were found in the photoreceptor layer and phagocytosed rod debris (Gupta et al. 2003). As discussed by the authors, the MG cells are activated in response to photoreceptors apoptosis, releasing cytotoxic factors (Gupta et al. 2003) and different inflammatory mediators such as: chemokines, ROS and NO, that all together provide a chronic neuroinflammatory environment (Cuenca et al. 2014). In addition, microglia infiltration contributes to the choroidal neovascularization in wet AMD. The role of the complement system is defending the human body from dangerous stimuli and participating in the tissue homeostasis. Under abnormal circumstances, it can lead to uncontrolled inflammatory response, resulting in disease phenotypes, as happens in AMD. The activation of the complement system is related to the proteolytic cascade triggered by different pathways (classic, lectin and alternative) resulting in the formation of several complement proteins, as reviewed elsewhere (Walport 2001). A multicenter study conducted by Heesterbeek et al. (2020a) on AMD patients at different stages and control subjects showed a correlation between the complement activation levels and the severity of AMD. Furthermore, mRNA levels of complement proteins were studied in patients with late AMD (Demirs et al. 2021). The use of RNA-scope followed by immunohistochemical analysis, showed that the microglia\macrophages cells produce C3 mRNA with the highest overlapping around the GA lesion.

4.1 Inflammation in LD models

The LD model represents a reliable *in vivo* approach to study the role of inflammation in AMD. For instance, several studies used the Albino rat exposed to bright light for 24 h to mimic AMD, demonstrating an increase in chemokine response, related with microglia and complement activation (Rutar et al. 2010, 2011a,b, 2015; Tisi et al. 2019b). In this scenario, Rutar et al. demonstrated that bright light exposure is the trigger event determining the upregulation of cytokines mediators and regulators through a strict correlation with the apoptotic cells. All these events are finely orchestrated through the interaction of cells, RPE and activated microglia cells (Rutar et al. 2015). Recently, Wooff and colleagues evaluated the retinal inflammation level and the microglia\macrophage recruitment in C57BL/6J mice exposed to high light for up to five days (Wooff et al. 2023). Although these mice are naturally more resistant to light damage, there was an up-regulation of key inflammatory markers, such IL-1β, IL-6 and Ccl-2 when they are exposed for 12 h of LD. This evidence was also correlated with an increased recruitment and activation of microglia cells and to the morphological shift from resting to activated state, from 12 h and up to five days of LD. Overall multiple studies, as previously mentioned, support the use of short exposure to photo-oxidative damage as a valid model that closely mimics the main retinal alterations of human AMD. Results obtained from LD rodent models might be applied to uncover early inflammatory events, addressing their involvement in the onset and role of chronic inflammation in late stages of AMD.

5 Lipid alterations in human AMD

Lipids play multiple functions in the human body and are involved in the composition of cell membranes, serve as energy storage, and take part in the network of cellular signalling (these lipids are known as “bioactive lipids”). Accumulated evidence from human studies indicates that lipid metabolism is involved in the pathogenesis of AMD (van Leeuwen et al. 2018). The ratio of plasma omega-6/omega-3 was found to be increased in patients with AMD (Leung et al. 2019). It should be considered that the levels of omega-3 and -6 may also depend on their intake through food (for instance fish is rich in omega-3) and therefore data on their quantification should be carefully interpreted. Moreover, dietary intake of lipids may influence the development of AMD or prevent its progression. Indeed, the intake of omega-3 polyunsaturated fatty acids (PUFAs) (like alpha linolenic acid, ALA; eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA) has been shown to prevent AMD development (Age-Related Eye Disease Study 2 Research Group, 2013. Lutein +zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2) (AREDS2) randomized clinical trial (Chew et al. 2013). Consistently, epidemiological studies indicate that

patients suffering from AMD used to have lower fish intake (Augood et al. 2008; Christen et al. 2011). Moreover, DHA was found to be decreased in the serum of AMD patients (Orban et al. 2015). Omega-3 fatty acids are also the source of other bioactive lipids, known as "specialized pro-resolving lipid mediators" (SPMs), which are emerging as key regulators of the inflammatory processes by actively promoting the resolution of acute inflammation. SPMs in AMD have been poorly investigated, and there is a lack of data from humans. However, based on the protective effects exerted by omega-3, it is expected that their derivatives may help in the retinal protection of those patients. Differently, the intake of omega-6 fatty acids (like linoleic acid) is generally associated with an increased risk of AMD due their pro-inflammatory properties, although few studies demonstrated a significant correlation between those two (Christen et al. 2011; Seddon et al. 2001; Tan et al. 2009). Yet, the omega-6 arachidonic acid (AA) was found to be increased in the serum of AMD patients (Orban et al. 2015). Omega-6 are also the source of eicosanoids (EICs), a class of bioactive lipids (i.e. prostaglandins, PGs; prostacyclins, PGIs; thromboxanes, TXs; leukotriens, LTs), which exerts mainly a pro-inflammatory function (Leuti et al. 2020). Consistently, dysregulation of EICs has been shown in AMD patients, although the studies are still limited to few pieces of evidence. For instance, increased serum levels of PGE_2 have been identified in AMD patients (Orban et al. 2015). Alongside lipids, also lipoproteins, involved in lipid transportation through the circulation, have been associated with AMD (van Leeuwen et al. 2018). Specifically, epidemiological data showed that high levels of high-density lipoprotein cholesterol (HDL-C) in the blood of patients suffering from AMD correlate with an increased risk of the disease, while circulating oxidized low-density lipoproteins (ox-LDL) have not been associated with AMD development/worsening (Klein et al. 2019). Yet, increased levels of triglycerides seem to be negatively correlated with the risk of AMD (Colijn et al. 2019; Wang et al. 2016). Importantly, a large genome-wide association study has identified multiple genetic variants of genes involved in the lipid metabolism in AMD patients, including the ATP binding cassette subfamily A member 1 (ABCA1), APOE, cholesteryl ester transfer protein (CETP) and hepatic lipase C (LIPC) genes (Fritsche et al. 2016), further supporting the involvement of lipids in the pathogenesis of AMD.

5.1 Lipid alterations in LD models

Consistently with the findings observed in humans, also the studies on the LD model demonstrated the involvement of lipids in the retinal degenerative processes induced by light similar to AMD (Dentchev et al. 2007; Othman et al. 2015; Palacios-Pelaez et al. 2010; Tisi et al. 2023; Wiegand et al. 1986). First, supplementation of a dietary formulation based on fish oil, similar to that used for patients with AMD in the AREDS studies, resulted to be protective in the LD model as well (Othman et al. 2015). In the same study, it was also demonstrated that the supplemented animals had an increase in omega-3 and a decrease of omega-6 PUFAs in plasma and retina (Othman et al. 2015), further supporting the protective role of omega-3 PUFAs in AMD. Moreover, continuous light exposure in Long–Evans (pigmented) rats resulted in reduced levels of DHA in rod outer segments (Wiegand et al. 1986), which is in agreement with the decreased DHA levels observed in the plasma of AMD patients (Orban et al. 2015). Compared to humans, more studies have been conducted in *in vitro* and *in vivo* LD models to investigate SPMs. For instance, it has been suggested that neuroprotectin D1 (NPD1) (a DHA-derived mediator) may prevent retinal damage induced by light (Palacios-Pelaez et al. 2010). Moreover, our group has recently demonstrated that the metabolism and signalling of another SPM, Resolvin E1 (RvE1) (an EPA-derived mediator), are altered in the albino rat retina after LD (Tisi et al. 2023). Also, alterations of another class of endogenous bioactive lipids, known as endocannabinoids (eCBs), were highlighted in the albino rat retina following photo-damage, with a major role of the endocannabinoid receptors 1 and 2 (CB_1 and CB_2) (Maccarone et al. 2016). Some eCBs alterations have also been identified in the ciliary body of AMD patients, although specific studies on the endocannabinoid receptors in AMD patients are still lacking (Matias et al. 2006). Finally, to our knowledge, studies on other pro-inflammatory lipids, like the EICs, are still missing in the LD model and would be useful in the future to further support the similarities of lipid alterations with AMD in humans.

6 Drusen and lipofuscin in human dry AMD

One of the main features of dry AMD is the presence of drusen, a yellowish compound rich lipids and proteins, detectable in correspondence of the macular region of the retina, between RPE and BrM membrane. In a first classification, drusen were divided in two types: soft indistinct drusen or reticular pseudodrusen, and soft distinct drusen associated with RPE abnormalities; this classification was proposed by several epidemiological studies including the Blue Mountain Eye Study, Beaver Dam Eye Study and

Rotterdam Study (Klein et al. 1992; Mitchell et al. 1995; Vingerling et al. 1995). Subsequently, after the 2000s, a new grading system was developed by the Age-Related Eye Disease Study (AREDS) and Beckman Initiative for Macular Research Classification Committee. Drusen deposits were classified into three types depending on their size: small drusen (<63 μm), intermediate/medium drusen (63 ≦ μm, ≦125 μm) and large drusen (>125 μm) (Davis et al. 2005; Ferris et al. 2013). Drusen deposition increased the risk for progression to end-stages of GA and neovascularization (Curcio 2018). Schmitz et al. reported the prevalence of the reticular drusen analysing 458 subjects (418 patients had bilateral GA) thanks to the high-resolution imaging technology using a confocal scanning laser ophthalmoscope (cSLO) (Schmitz-Valckenberg et al. 2011). The prevalence of drusen detection was higher on cSLO images (62 %, at least in one eye) when compared with conventional fundus photography technique (18 %) and these results were similar to those described by Cohen and co-workers (Cohen et al. 2007), hence the reticular drusen can be considered a common phenotypic hallmark in eyes with GA due to AMD. The dimension of drusen has been used to provide a clinical estimation of the pathology progression; indeed, the presence of larger drusen indicates a higher risk for AMD progression to the advanced form. As the disease progresses, these drusen expand (Mitchell et al. 2018) and result in GA, characterized by irreversible damage to retinal cells (paragraph 9). The drusen composition has been well documented, identifying several drusen components (Wang et al. 2010); at least 40 % of drusen content is esterified cholesterol and phosphatidylcholine. Other components are vitronectin, TIMP metallopeptidase inhibitor 3, clusterin, Apolipoprotein E, complement factor H, complement factor 5, 6, 8 and 9. In this study was not confirmed the presence of others drusen components as Apolipoprotein B (Malek et al. 2003), amyloid oligomers (Luibl et al. 2006) complement component 3 (Johnson et al. 2001). Lipofuscin comprises abundant and long-lasting intracellular inclusion bodies, related to lysosomes, which contains mainly bisretinoids (Eidred 1993; Weirer et al. 1986), such as vitamin A derivates and N-retinylidene-N-retinylethanolamine (A2E) firstly described by Sakai and coworkers in 1996 (Sakai et al. 1996). Furthermore, the presence of the Lipofuscin within the RPE cells is responsible for the fundus autofluorescence (AF) in imaging (Delori et al. 1995). The Wisconsin Age-Related Maculopathy grading scheme (Klein et al. 1991), thanks to colour fundus photography, described in 1991 reticular soft drusen, then named reticular pseudodrusen (RPD), and known as subretinal drusenoid deposits (Sakurada et al. 2023). The RPD are composed of lipofuscin and extracellular deposits between the photoreceptor and the RPE and are associated with increasing risk to developed late stage of AMD, in particular GA (Finger et al. 2014; Keenan 2023). RPD are related to several retinal changes such as photoreceptors loss because of their localization in the subretinal space, which extended into the outer nuclear layer, resulting in a thinner retinal thickness in the affected area compared to the RPD-free region (Greferath et al. 2016). Similar results were obtained from a longitudinal perspective study (Chiang et al. 2020) in which was found a detectable retinal thinning in AMD patient, with a decrement about 5 % in the central macular region over four years. Furthermore, several choroidal changes are associated with RPD as reported such as: choroidal thickness (CT), choroidal vascular thickness (CVT) and choroidal vascularity index (CVI), highlighted through optical coherence tomography. In AMD choroidal vascular thickness is lost and CVI is significantly reduced (Laíns et al. 2017). Unlike drusen, to date RPD is incompletely characterized and needs further investigation to highlight a clear association with late AMD.

6.1 Drusen and lipofuscin in LD models

It has been already demonstrated that the exposure to light is related to the rate of A2E formation and consequently to lipofuscin accumulation (Ben-Shabat et al. 2002), causing fundus autofluorescence (Delori et al. 1995). Lipofuscin autofluorescence, as well as the lipofuscin granules, can be visible with a large excitation spectrum, ranging from 360 to 647 nm. A recent study conducted by Jeong's lab detected through fundus camera, drusen-like formations in BALB\c mice exposed to blue light (10,000 lux over two weeks for 1 h\day) (Shin et al. 2022). Lipofuscin granules were also detected (Nakamura et al. 2018) in a non-exudative AMD model, by exposing pigmented mice (C57BL/6J) to blue LED light (1100 lux) for 3 h once or for three consecutive days. They detected the drusen-like formations through the OCT and subsequently the retinas were stained with PAS staining, indicates that drusen components were fatty acid peroxides. A visible accumulation, as white spots (lipofuscin deposits), was found in the subretinal space, starting from 1 day till 60 days after the light stimulus. The presence of extracellular material (reticular pseudodrusen-like materials) was also present around the junction of photoreceptors inner and outer segments. Also, in our and other laboratories was used a well-established LD model of AMD based on the exposure of Sprague Dawley rats to white light (Jiao et al. 2015; Rutar et al. 2010; Tisi et al. 2020c, 2023). Tisi and colleagues demonstrated the presence of AF under the retina of exposed rats, especially near the "hot spot" (Tisi et al. 2020c). The Lipofuscin autofluorescence was detected through a confocal microscopy with an excitation

wavelength of 488 nm and emission wavelengths from 488 to 684 nm. The deposits were found in both whole mounted retinas and cryosections, confirming their localization in the subretinal space, in the outer nuclear layer (seven days after the LD) and in the INL (60 days after the photooxidative damage). Furthermore, they also quantified the AF deposit area occupied, revealing that deposits accumulation started seven days after LD but decreased over the time till after 60 days of recovery. Additionally, the accumulation of lipofuscin-like granules correlates with robust recruitment of subretinal microglia\macrophages, and this evidence was correlated with the lower AF deposits found at 60 days after the LD compared to the analysis at seven days (Tisi et al. 2020c).

7 Blood-retinal barrier and its alterations in human AMD

Retinal microenvironment is finely controlled by the presence of the blood–retinal barrier (BRB), which regulates the transport of ions, water and proteins from the bloodstream to the retina and the translocation of waste material from the retina to the subretinal space and then to the systemic circulation. The BRB could be divided into inner BRB (iBRB) and oBRB and both contribute to the fine regulation of the retinal environment (O'Leary and Campbell 2021). The oBRB is the results of the interaction between the CC, the BrM and RPE. The structure of the choriocapillaris is characterized by extensive fenestrations and for this reason does not carry out a selective function but contributes to the BRB allowing the exchange of nutrients between the systemic circulations and the retina (Guymer et al. 2004). The RPE actively contributes to the formation of the BRB (and for this reason detailed in paragraph 8); indeed, the presence of Tight Junctions (TJ) at its apical surface ensures the maintenance of BRB integrity, allowing the controlled transport of substances to the neuroretina (Naylor et al. 2019). TJs are the regulators of the barrier functions and are composed by transmembrane proteins (such as the claudins, the MARVEL family and the JAMs family) and cytoplasmic proteins (including the members of the Zonula Occludens family) (Naylor et al. 2019). RPE also secretes numerous growth factors from its basolateral surface, such as VEGF, contributing to the homeostasis of CC (Caceres and Rodriguez-Boulan 2020). RPE cells also contribute to the synthesis of BrM components. In particular, the BrM is composed of five layers divided in collagenous layers and elastic layers. The main roles of the BrM in the functioning of BRB is allowing the passive diffusion of molecules through its layers and blocking the diffusion of big molecules (Booij et al. 2010). The other part of the BRB is the iBRB, consisting of the retinal vasculature, which originates from the retinal artery and supplies the retinal layers. The blood vessels that take part in the iBRB display a different structure compared to that of the CC, with no fenestration and a high presence of TJs which ensure molecules exchange mainly through a trans-cellular transport. Furthermore, retinal vessels are surrounded by pericytes and glial cells, which contribute to the formation of the so-called neovascular unit (NVU) (O'Leary and Campbell 2021). AMD is characterized by extensive structural and functional changes in both inner and outer BRB, with the consequent loss of its function. BRB alterations occurring during AMD could be different in presence of the dry or wet form. For instance, during the onset and progression of dry AMD, the choroid and choriocapillaris undergo a progressive thinning, which causes reduced nutrient's supplementation (Tisi et al. 2021). On the other hand, during exudative AMD, the over expression of VEGF causes a dysregulated proliferation of the CC and the resultant invasion of the neuroretina, known as CNV (Cabral et al. 2017). More information about vascular changes occurring during wet AMD could be found in the dedicated paragraph (Section 9). The BrM, as a structure with a dynamic nature, is subjected to numerous changes during AMD pathogenesis. Notably, there is the accumulation of metabolism by products which cannot be eliminated and therefore contribute to drusen and basal deposits formation. Alterations in the extracellular matrix, including the imbalance between the secretion of metallopeptidases (MMP) and their inhibitors (TIMPs), also contribute to the formation of drusen and basal deposits in the BrM (Garcia-Garcia et al. 2022). Moreover, other structural changes involved the increased thickness of BrM and the reduction of the elastic properties. In terms of function, these changes imply a loss of the BrM barrier function with altered transport through the BRB (Booij et al. 2010). As mentioned in Section 8, RPE functions start to deteriorate with the accumulation of waste materials together with an auto-fluorescent pigment called lipofuscin (see paragraph 6). Late RPE contribution to the breakdown of BRB during AMD could be ascribed to the morphological and functional alterations, in a process known as epithelial-mesenchymal transition (EMT) (Shu et al. 2020). During EMT, retinal pigment epithelial cells lose their polarity and start to dedifferentiate with the subsequent disruption of the junctional complex characteristic of this structure, and the uncontrolled exchange through the BRB (O'Leary and Campbell 2021). During AMD, multiple alterations also occur in the iBRB with differences between the dry and wet forms. In the past years, it was commonly accepted that in non-exudative AMD, the BRB integrity was not affected because there were

no signs from the clinical analysis in patients with an intermediate form. Nevertheless, analysis of eyes obtained from AMD donors showed the presence of elevated iron levels and proteins of the systemic circulations in the eyecup, indicating a subclinical sign of BRB alterations in these patients. Conversely, during exudative AMD the signs of BRB breakdown are clearly and easily detectable with clinical imaging methods as fluorescein angiography (FA) (Schultz et al. 2019). Alterations of the retinal vasculature are deeply discussed in the dedicated paragraph (Section 9).

7.1 BRB breakdown in LD models

Many articles in the literature evaluated oBRB alterations after light exposure in both albino and pigmented rodents. Many papers reported light damage as a useful tool to induce the accumulation of waste material between RPE and BrM in rodents (as detailed in paragraph 6.1). Another alteration commonly found in light damage models of AMD is the disruption of RPE junctional complex. These alterations were mainly studied through the analysis of the Zonula Occludens protein family and changes in the RPE cytoskeleton. In particular, the most used marker is the protein Zonula Occludens-1 which is typically expressed in the cellular membrane of RPE cells. Different research groups showed that the exposure of albino Balb/c mice to white and blue light caused a marked disruption of the TJ. This alteration might be independent of the type of light damage and was found in both acute (Cachafeiro et al. 2013; Xie et al. 2021) and chronic models (Narimatsu et al. 2013). In the literature, there is also evidence of TJ alterations in pigmented mice (Nakamura et al. 2018; Natoli et al. 2016). In 2018 Nakamura et al. settled a light damage model using C57BL/6J mice exposed to blue light (1100 lux 3 h or 1100 lux 3 h for three days) and studied until seven days of recovery. They found that after three days of blue light exposure, the RPE was thicker around the optic nerve, as happens in AMD patients. Furthermore, there is a disruption of cell-cell junction in the RPE of exposed mice one day after light exposure and ZO-1 is not clearly detectable in the cell membrane anymore. Surprisingly, seven days after light exposure the distribution of ZO-1 is comparable to that of the control, probably because of the scavenging role of RPE and choroidal pigments (Nakamura et al. 2018). This example explains the complexity to recapitulate BRB alterations in pigmented rodents; indeed, in this case must be considered the presence of pigments and the necessity of a different stimulus to induce the degeneration. For instance, De Imperial-Ollero et al. used a focal Light Induced damage in C57BL/6 mice. They exposed the animals to 500 lux of LED light for 45 s by positioning the light source 1 mm apart from the corneal apex of the eye. After three days from the light exposure, they have already obtained alterations of ZO-1 localization. Furthermore, RPE cells are enlarged and pleomorphic (Miralles de Imperial-Ollero et al. 2021). These features have been associated with the EMT, which has been shown also in other light damage models (Cachafeiro et al. 2013; Kuse et al. 2018; Tisi et al. 2020a). Cachafeiro et al. showed that 24 h after light exposure (1 h, 5000 lux), in the RPE of Balb/c mice, proteins of adherent junctions, (such as beta-catenin and N-cadherin) completely left their localization (Cachafeiro et al. 2013). These proteins are known to be involved in the EMT process occurring in RPE cells during AMD (Shu et al. 2020). Features of EMT could also be found in albino rats exposed to white light, as shown by Tisi et al. In this study, they showed that seven days after light exposure, the architecture of RPE cells was completely altered in the “hot spot”. For instance, (i) the actin of cytoskeleton is dispersed into the cell cytoplasm and (ii) some cells are enlarged and multinucleated, a classical sign of EMT (Tisi et al. 2020a). Similar signs were found also in albino ddY mice five days after light exposure (8000 lux, 3 h) (Kuse et al. 2018). As explained before, alterations of the oBRB lead to the leakage of systemic circulation proteins to the neural retina (Schultz et al. 2019). To the best of our knowledge, little is known about the leakage of serum protein into the neuroretina in light damage animal models. One evidence was found Balb/c mice, in which light damage provoked the diffusion of albumin from the CC to the outer segment of photoreceptors, demonstrating the presence of an altered transport system throughout the retina (Cachafeiro et al. 2013). The same event was also seen in both Long–Evans and Wistar 24 h after light exposure (Krigel et al. 2016).

8 Retinal pigment epithelium in human AMD

RPE is a monolayer of post-mitotic, pigmented, polygonal and polarized cells placed behind the retina. RPE is one of the main components of the outer blood retinal barrier (oBRB), together with the choriocapillaris and the Bruch’s membrane, it is closely interconnected with the outer segments of photoreceptors, playing a key role in the proper functioning of the visual cycle. In addition, RPE ensures retinal homeostasis through its multiple functions; indeed, RPE cells corroborates photoreceptors functions through the absorption of scattered light, the shedding of photoreceptors outer segments, the renewal of photosensitive pigments and the secretion of growth factors and antioxidant molecules (Strauss 2005). RPE dysfunction and

degeneration is considered one of the first events occurring in AMD (Kim et al. 2021) and, together with other outer retinal layers, is more subjected to light damage (Behar-Cohen et al. 2011). In particular, in RPE cells, light exposure induces an excessive production of ROS because of the high metabolic rate and oxygen consumption of this tissue; due to these features, under physiological conditions RPE is able to counteract ROS accumulation through its antioxidant machinery, which also involved the nuclear factor erythroid-derived factor 2/antioxidant response element (NRF-2/ARE) and peroxisome proliferator-activated receptor co-activator protein 1 (PGC-1) (Garcia-Garcia et al. 2022). Nevertheless, with aging the antioxidant system of RPE cells is less responsive, leading to ROS accumulation and oxidative stress burden. Moreover, oxidative stress and the accumulation of misfolded proteins, together with the impairment of RPE antioxidant machinery, lead to a "metabolic crisis" also associated with mitochondrial damage (Tong et al. 2022). Fusion and fission processes of mitochondria allow the maintenance of ROS levels into a physiological range, and an impaired balance between these two processes is the key to the establishment of the vicious cycle which leads to RPE/photoreceptors degeneration. This hypothesis was supported by numerous studies in which the analysis of mitochondrial function on AMD donor tissues showed fewer mitochondrial, reduced surface area and membrane potential, disrupted cristae together with altered mitochondrial proteome including the dysregulated expression of fusion/fission associated protein (Fisher et al. 2022). Furthermore, Ferrington et al. showed that RPE from AMD donors presents less ATP production and respiration, as evaluated by the Mito Stress assay, signs of a declined mitochondrial function (Ferrington et al. 2017). Oxidative stress could also compromise the correct function of the endoplasmic reticulum (ER), which is responsible for the protein synthesis and folding. During AMD, the dysregulation of these processes leads to the accumulation of misfolded proteins and waste materials (McLaughlin et al. 2022). RPE cells are also specialized in the renewal of photoreceptors' outer segments by its digestion and recycling through the autophagy process. This process is also one of the key mechanisms involved in mitochondrial control, and it is responsible for the digestion of damaged mitochondria; in addition, this process also plays a fundamental role in the removal of misfolded proteins and waste materials accumulated following oxidative damage and ER stress. Recently, was assessed the role of impaired autophagy in the degeneration of RPE cells during AMD pathogenesis (Intartaglia et al. 2021). In the RPE of AMD donors, the content of Ubiquitin and sequestosome-1 (p62/SQSTM1) was up-regulated compared to healthy donors (Ferrington et al. 2016), suggesting an impairment of autophagy. Furthermore, there is evidence of an ER-mitochondria-autophagy axis in RPE cells, involved in AMD progression (Kaarniranta et al. 2023). The late stage of dry AMD is the GA (Zarbin et al. 2014) which is related to the occurrence of incomplete/complete RPE and outer retinal atrophy (iRORA/cRORA) (Garcia-Garcia et al. 2022; Savastano et al. 2020). Light induced animal models reproducing RPE dysfunction/alteration during AMD will be elucidated in the next paragraph.

8.1 LD models reproducing RPE alterations

The RPE dysfunction is one of the events triggering the pathogenesis of AMD, and the effects of light on RPE were assessed in different rodent models, exploring the reproducibility of AMD degeneration processes in both albino and pigmented mice/rats. Light induced oxidative stress process that is able to activate the mechanisms involved in ER stress and misfolded protein accumulation; indeed, the exposure of C57BL/6 mice to visible light resulted in ER stress and the accumulation of waste materials; indeed, after 12 h of exposure to 7000 lux of visible light it was found a significant increase of ER stress markers in the RPE of exposed mice (Song et al. 2020). The same feature was also highlighted in Wistar rats 4.75 h after white LED light exposure. In this work they studied the impact of light exposure in a time-dependent manner (4.75, 6, 12, 18 and 24 h) (Jaadane et al. 2017). Among RPE alterations, also the dysregulation of mitochondrial function was reported in photo-oxidative animal models of AMD. Some papers showed that long-term exposure to blue light caused a significant reduction of RPE thickness in C57BL/6 pigmented mice; in addition, blue light exposure induced the disruption of mitochondrial membranes and cristae (Wang et al. 2023; Xia et al. 2019) and dysregulated expression of mitochondrial dynamics-related proteins, OPA1 (GTPase optic atrophy1) and DRP1 (dynamin-related protein 1), which regulate mitochondrial fusion and fission respectively (Wang et al. 2023). The impairment of mitochondrial function was also observed in Wistar albino rats exposed to white light, which causes the permeabilization of mitochondrial membranes due to the activation of NF-kB which in turn is caused by ER stress (Jaadane et al. 2017). The impairment of the autophagic process was reported for the first time in albino rats by Remè et al. who showed that exposure to white light displayed an excessive activation of autophagic process as a compensatory mechanism to counteract the accumulation of misfolded proteins and damaged organelles caused by light (Remé et al. 1999). The involvement of the autophagic process in light induced

RPE damage was also shown in recent. It has been shown in two papers that the exposure of albino rats to white LED light leads to the block of the autophagic process in a time-dependent fashion after light exposure. It was observed a dysregulation in the expression of LC3BII and p62, two important and well accepted markers of autophagic flux (Jaadane et al. 2017; Tisi et al. 2020a). The dysregulation of autophagy was observed also in pigmented C57BL/6 mice, in which the exposure to blue light exposure was performed; in this case the data obtained by Xia et al. suggest autophagy as a protective mechanism in the early phases after light damage (Xia et al. 2019). Noteworthy, in AMD the autophagy process could play different roles; during the early phases of the pathology there is an enhanced activation of this process as a response to oxidative stress and the necessity of eliminating misfolded proteins and dysfunctional organelles. For instance, some studies highlighted a crosstalk between autophagy and cell death, underlining the potential contribution of autophagy to RPE degeneration (Chen et al. 2016). Recent studies have hypothesized the involvement of other pathways, apart from the canonical apoptosis, involved in RPE cell death such as pyroptosis, necroptosis and ferroptosis (reviewed by Yang et al. 2020). In light induced animal models of AMD different pathways of RPE cell death have been reported (Song et al. 2022; Tisi et al. 2020a). For instance, the exposure of albino Balb/c mice to white LED light for 1 h leads to the activation of necroptosis, which is a controlled type of cell death with necrotic manifestations which involve inflammatory events. During necroptosis there is the formation of the necrosome which requires the recruitment of RIPK3 which is increased after light damage (Song et al. 2022). Nevertheless, Tisi et al. showed an increase of apoptotic cells induced by light damage in the RPE cells of SD rats (Tisi et al. 2020a). The presence of fragmented DNA in the nuclei of RPE cells was also associated with nuclear localization of LC3BII in light damaged retina, suggesting a crosstalk between autophagy and apoptosis in cell death mechanisms (Tisi et al. 2020a). As mentioned above, in the late stages of the pathology there is an extensive loss of RPE cells and photoreceptors in the process called GA (Fleckenstein et al. 2018). Although light damage recapitulates many features of early and late human AMD, the term “geographic atrophy” is rarely used to describe extensive retinal changes in post-LD animals. Nevertheless, Marc et al. in 2008 showed that retinal alterations occurring in light damaged albino rats closely also mimic the features of human GA (Marc et al. 2008). The studies performed on LD animal models confirmed the relevance of RPE homeostasis in the pathogenesis of AMD, making it a good therapeutic target.

9 Vascular alterations in human AMD

Retinal tissue needs a well-organized blood supply system; in particular, the outer retina is irrorated by the choroid, composed of different layers: the suprachoroidal, large and medium vessels layers and the choriocapillaris. The choroid is the structure responsible for nutrient exchange between the neural retina and the systemic circulation (Guymer et al. 2004). The retinal artery, one of the iBRB components (see paragraph 7), branches out into a deep, inner and superficial vascular plexuses placed in the outer plexiform layer, inner plexiform layer and in the ganglion cell layers respectively (Coorey et al. 2012). Alterations in the retinal supply system are mainly involved in the pathogenesis of wet AMD. As described in the paragraph 1.2, choroid could undergo different changes during AMD pathogenesis leading to the occurrence of CNV. It has widely been shown that isoform A of vascular endothelial growth factor (VEGFA) plays a key role in this pathology; it is a prominent angiogenic factor that has been the subject of intense research since its discovery. Other angiogenic factors include insulin like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), TNF-α, angiopoietin-2 and MMPs (Paraoan et al. 2020). The stimulus for retinal neovascularization is often local hypoxia induced by oxidative stress, with blood vessel generation as an attempt to re-oxygenate the ocular tissue (Coorey et al. 2012). Retinal and choroidal hypoxia may induce an upregulation of VEGF production by RPE cells (Paraoan et al. 2020). Unfortunately, the newly formed blood vessels are leaky and prone to haemorrhage, allowing the infiltration of potentially detrimental factors. As mentioned in paragraph 7 (iBRB), retinal vessels are surrounded by pericytes and glial cells, which contribute to the correct functioning of the iBRB. During CNV, glial cells also participate in these pathogenic events. Indeed, CNV occurring in wet AMD is a response to RPE damage and the activation of an immune activation (see paragraph 4) (Ricci et al. 2020). Activated microglial cells are capable to express many pro-angiogenic factors, including VEGF, promoting angiogenesis and affecting retinal vasculature structure, thus promoting BRB breakdown (Coorey et al. 2012).

9.1 Vascular alterations in LD models

There are few light-induced animal models in which typical vascular alterations of AMD were examined. Two of the examined models are based on albino rats, subjected to two different paradigms of light damage. Albert et al. compared

the effects of light damage (12 h, 3000 lux) on the retinas of two albino rat strains: Wistar and Sprague Dawley albino rats. The animals were cyclically exposed for 1, 3 and 6 months to evaluate the progression of the pathology. They showed that in Wistar rats, starting from three months of cyclic light exposure, there is the growth of new vessels from the choroid, which penetrate the BrM and RPE, invading the neuroretina. After six months, the abnormally grown choroidal vessels were anastomosed with retinal capillaries (Albert et al. 2010). These features are similar to those reported in the retinas of patients with wet AMD (Spaide et al. 2020). This model reproduced retinal vascular alteration through a chronic exposition to light (Albert et al. 2010). On the other hand, Tisi et al. used an acute light damage animal model based on the exposure of SD rats to white light (24 h, 1000 lux). The illuminated retinas were analysed immediately and 7, 60 and 120 days thereafter. They demonstrated an up-regulation of VEGFA, bFGF and their receptors, starting seven days after light damage. The up regulation of pro-angiogenic factors was also associated with increased vascularization of the retinal vascular plexuses. They found an increased number of tufts of neovascularization in all retinal plexuses starting from seven days of recovery. Conversely, an up-regulation of the vessels percentage area was only found in the deep plexus after seven days, with a subsequent disruption of the vessels after 60 days and their partial rescue after 120 days. Interestingly, 120 days after light damage microglial cells were still found in the damaged retinas, surrounding the newly formed vessels (Tisi et al. 2020b). This finding is in accordance with the known pivotal role played by microglial cells in neovascularization processes (Coorey et al. 2012). Microvascular alterations were also observed in pigmented C57BL/6J mice after a chronic light damage (4 h per day for seven days, 10,000 lux). Through Fluorescein Fundus Angiography (FFA) they showed a significant increase in microvessels production after LD, probably related to neovascularization phenomenon (Gong et al. 2022). To the best of our knowledge, these are the only currently available light-induced murine models displaying retinal neovascular alterations.

10 Diagnostic techniques: morphological and functional analysis for AMD

The diagnosis of AMD is mainly based on ophthalmoscopy (also known as fundoscopy). Colour fundus photography, optical coherence tomography (OCT), and fluorescein angiography are some of the clinical approaches used for the identification of morphological and structural changes in patients (Jaadane et al. 2023; Zarbin et al. 2014). These analyses are often related to a functional assessment of the retina through the electroretinogram apparatus (Forshaw et al. 2021; Robson et al. 2018) (see paragraph 11). OCT is a non-invasive, rapid technique used in order to obtain cross-sectional and 3-D images of the retina. Conventional OCT provides limited insights into retinal and choroidal changes during AMD pathology. Nevertheless, to obtain high resolution images of RPE, retinal and choroidal structures, new OCT-based approaches were developed (as deepened elsewhere (Zarbin et al. 2014)). The alignment of the cross-sectional OCT images with *en face* images and the high resolution (5-μm axial) of spectral-domain optical coherence tomography (SD-OCT) allows to determine the correlation between the histological features and clinical findings of human pathology, including AMD. Recently it has been proposed by Chen et al. (2023), the using of an ultrahigh resolution spectral domain-OCT (UHR SD-OCT) instrument to visualize and measure outer retina alterations at micrometer scale, as a marker for normal aging versus early AMD. FFA is the gold standard technique to investigate AMD-related vascular alterations, but it is an invasive procedure based on intravenous injections of sodium fluorescein dye. Thus, a less invasive OCT-based technique was developed: optical coherence tomography angiography (OCTA), providing a rapid and non-invasive tool for the diagnosis of retinal vascular pathologies (Jaadane et al. 2023). These clinical imaging tools enable the *in-vivo* evaluation of key AMD alterations, which could be otherwise detectable only *ex-vivo*. High-resolution spectral domain OCT is a tool applicable also to the light damaged rodent model to characterize the *in-vivo* morphological changes that will be compared to the functional analysis and the subsequent *ex-vivo* studies. Following this experimental approach Benthal et al. (2022), characterized the aetiology of decreased cone-driven vision in the light damage SD exposed to a chronic LD. They used the OCT for the *in-vivo* measurements of ONL thicknesses correlated with the assessments of visual function though the ERG recordings. Furthermore, FFA is currently used to assess vascular alterations in LD animal models (Gong et al. 2022). Morphological assessment, such as the measurement of the ONL thickness (or counting the photoreceptors layer) can be performed both *in-vivo* with the OCT, or *ex-vivo* through the analysis of the cryosections, as several research groups already performed (Rubin et al. 2022; Rutar et al. 2010; Tisi et al. 2019b). Furthermore, another important evidence obtained exclusively through the cryosections is the localization of selected markers through the visualizing of the entire retinal structure gained by immunohistochemistry

technique, as Benthal et al. did in his study (Benthal et al. 2022).

11 Retinal function in human AMD

Electroretinogram (ERG) is a non-invasive technique performed to evaluate light-evoked electrical activity of retinal cells that can be performed in both preclinical and clinical studies. Thanks to the light-dependent activity of cones and rods and their connections with the inner retinal circuitry that the ERG response is generated. The cones mediate a photopic vision operating in a range of light intensities between 10^{-1} and 10^{5} lux. The cones are highly concentrated in the center of the retina, especially in the fovea, they are characterized by high spatial and temporal frequency and permit to perceive the colours. Contrary, low resolution and high sensitivity are specific for rods, which guarantee a scotopic vision up to 10^{-2} lux. The electroretinogram is mainly composed by three different waves such as: A-, B- and C-waves that represent the electrical response of photoreceptors (negative a-wave), second order neurons located in the inner retinal (positive b-wave) and the epithelial cells (late-onset positive c-wave) respectively (Robson et al. 2018). The International Society for Clinical Electrophysiology of Vision (ISCEV) determined a guideline on how to obtain, for each of the standard tests including the full-field flash electroretinogram (ffERG), the pattern electroretinogram (pattern ERG or PERG), the multifocal electroretinogram (mfERG), the electrooculogram (EOG) and the cortical-derived visual evoked potential (VEP), a clinical response with a standard protocol, thus enabling the comparison of ERG data set from different laboratories (Forshaw et al. 2021; Robson et al. 2018). The ISCEV standard full-field ERG represents a global retinal response to brief flashes of light, in scotopic or photopic conditions (Robson et al. 2018). Furthermore, in the ffERG the oscillatory potentials (OPs) can be also identified and they are characterized by being a high-frequency, low-amplitude response, which is superimposed on the rising phase of the b-wave (Liao et al. 2023); the OPs activity is related to the inner retinal circuitry that involve bipolar cells, amacrine cells, and/or ganglion cells (Liao et al. 2023; Wachtmeister 1998). Among many clinical applications the ERG is commonly used to diagnose and follow-up the AMD. The focal ERG (fERG) is a more specific test to specifically evaluate the electro-functional response of the macular region and it provides information exclusively from the fovea, by using a small flickering spot to elicit a local response (Hogg and Chakravarthy 2006). The fERG were recently used in combination with the visual acuity and OCT analysis from Savastano et al. (2022) for early diagnosis and prognosis of non-exudative AMD. Notably, the authors described with the longitudinal analysis that both fERG amplitudes and outer retinal thickness decrease after a one-year follow-up. Recently, Messenio et al. evaluated the macular function through the fERG in 47 patients with intermediate AMD compared to 65 healthy subjects, with preserved visual acuity (Messenio et al. 2022). Unsurprisingly was found a statistically significant difference in fERG mean amplitude between the two groups confirming the reduction in the macular function even if the visual acuity is preserved. Interestingly, by the fERG modulation sensitivity can be also evaluated for the cone function abnormalities through the temporal cone flicker sensitivity, as a function of flicker modulation depth as described by Falsini et al. (2000). The authors found that in AMD patients the fERG response relating to stimulus modulation depth, compared to the healthy controls, was altered displaying a different pattern of abnormalities correlated to the macular lesions. The early degenerative changes of cone photoreceptors, that their number should be still almost normal, were associated with gain losses and a modulation depth dependent phase delay, with normal thresholds. The authors speculated that the response gain losses and modulation depth dependent phase delays, with normal thresholds, were associated with early lesions, such as early degenerative changes of cone photoreceptors that the number should be still almost normal. Contrariwise, the more advanced stages were correlated to the increased thresholds, in addition to gain and phase abnormalities. Another important approach is the multifocal ERG (mfERG), described firstly by Sutter and Tran (1992), that allows topographically synchronized stimulation of multiple retinal regions, whose electrical response can be correlated to the function of specific retinal area. For this reason, to date mfERG is considered a useful tool for evaluating and monitoring the retinal function in AMD patients. Recently, Parisi et al. (2020), analysed 27 patients with intermediate AMD and 20 age-matched control eyes in order to investigate the macular function through the mfERG. The macular function in AMD patients was clearly impaired only in the fovea and parafovea area, not significant changes were found in more peripheral retinal areas. Similar results were obtained previously by Gin et al. (2011) that analysed the mfERG in 15 patients with intermediate AMD compared with 14 aged similar controls. At the same time another interesting study was published by Gonzàlez-Garcìa et al. (2016), where the authors combined the mfERG with an imaging technique, the optical coherence tomography (OCT) in 30 patients with dry AMD followed-up for two years. Unsurprisingly both techniques show clear changes over the time, such as increased drusen formation together with the functional impairment. The ffERG can therefore investigate

the impact of peripheral retinal lesions leading to a better understanding of overall retinal function in AMD patients (Berrow et al. 2010) where only a few rods seem to remain within the parafoveal area in the late stages due to a preferential vulnerability and loss of rod over cone photoreceptors in both aging and AMD progression, as demonstrated by Curcio et al. (1993, 1996). In addition to the photoreceptors damaging, reactive oxygen species and oxidative damage contribute to modify the ERG response due to the inhibition of the sodium ion channel, thus resulting in the impairment of the rods-mediated phototransduction and prolonged dark adaptation. Regarding the wet AMD functional evaluation, Nishihara et al. (2008) assessed macular function in 157 patients by using focal macular ERG (fmERG). The amplitudes of fmERGs in the AMD patients displayed a reduction compared to the healthy controls. As well, the analysis of the a- and b-waves showed a clear reduction in the amplitudes of the corresponding waves in the healthy controls in addition to the implicit times becoming 3.8 ms (a-wave) and 10.2 ms (b-wave) longer compared to age-similar healthy subjects. This evidence suggests that the retinal function of these patients is severely impaired. The ERG technique has been performed also in animal models of AMD for preclinical studies as described in the next paragraph.

11.1 Electroretinogram in LD models

The functional consequences of the light exposure in rodents are generally assessed using the full-field (or flash) ERG. Many authors (Maccarone et al. 2008, 2016; Tisi et al. 2019a, 2020b) demonstrated the impairment of electrical activity of retinal cells caused by high light exposure. It is well described in our previous papers (Tisi et al. 2020b) that seven days after high light exposure (1000 lux for 24 h) of SD rats is evident a significant decrease of all parameters recorded by ERG, a-, b-waves and oscillatory potentials. The result is the visual function impairment with a similar residual retinal response from seven days up to 120 days, when compared to the unexposed animals as shown in Figure 4. Recently, Riccitelli et al. (2021) analysed the retinal function responses in

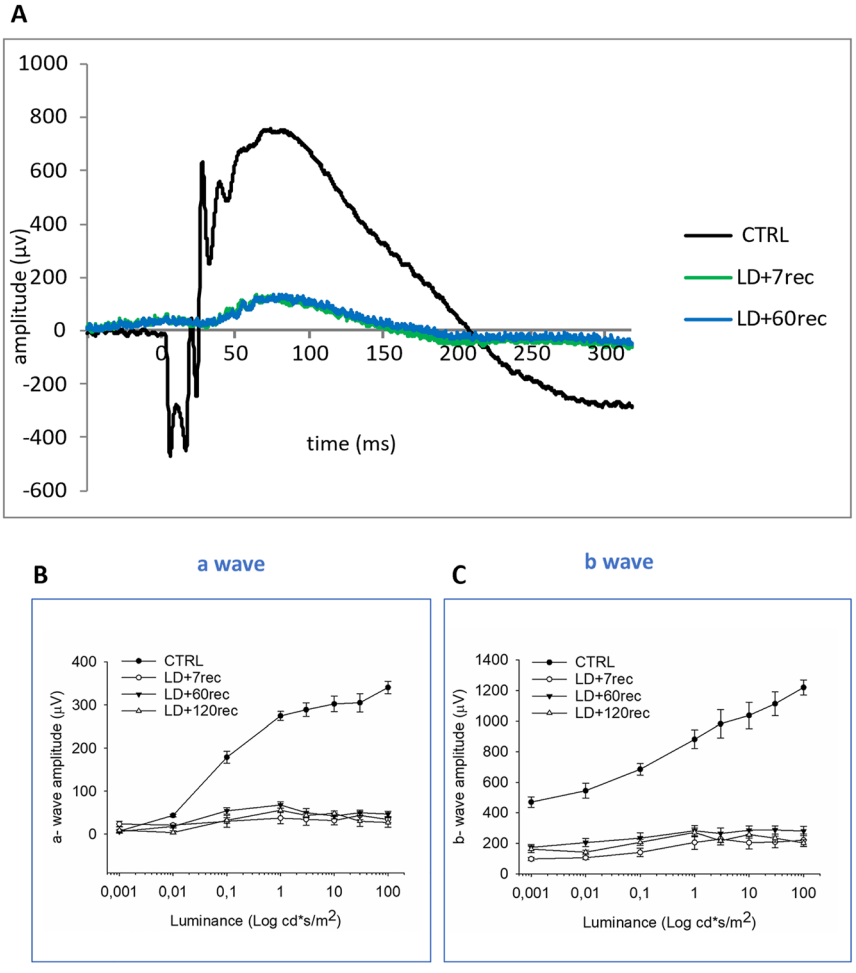

Figure 4: Electroretinogram recordings analysis in a LD model of AMD. (A) Representative fERG recordings of CTRL (control), LD+7rec (LD 24 h+7 recovery days) and LD+60rec (LD 24 h+7 recovery days) experimental groups at 1 cd × s/m^2; fERG recordings (B) a-wave amplitude and (C) b-wave amplitude; adapted with permission from (Fiorani et al. 2015; Tisi et al. 2020a,b).

both scotopic and photopic conditions with the ERG recording at different days of recovery such as 7, 15, 30, 45 and 90 up to seven months after LD (1000 lux for 24 h) in albino SD rats. Interestingly, the authors revealed a partial retinal recovery, maximal after 45 days (34 % for the a-wave, 45 % for the b-wave and 50 % for the OPs) compared to the control group, and after this time it dropped down up to seven months. This evidence was even corroborated with the flicker ERG analysis at frequency domain as the results showed a lower response, mainly after day 90 up to seven months, compared to the control group. In addition, the authors made a comparison between the ERG results performed seven days after different times of light exposure such as 12, 15, 18 or 24 h at 1000 lux. As discussed by the authors the short time of LD is not able to lead to a clearly retinal function deterioration as instead obtained with the 24 h of exposure time. Similar results were obtained by Cachafeiro et al. (2013) that exposed the BALB\c mice at 5000 lux for only 1 h. The authors performed the ERG in scotopic condition 10 days after the LD and both a- and b-waves were compromised due to the photooxidative damage. Despite the known resistance of C57BL/6J mice to light damage, Natoli et al. (2016), demonstrated a clear change of retinal function in response to seven days light damaged (100.000 lux up to seven days), the mice shown a significant reduction in both a- and b-wave (around less 25 % at highest flash intensity) comparable with those elicited in albino rat models. All the mentioned studies highlighted how the exposure to the light leading to a retinal function impairment because of all events triggered by the photooxidative stimulus, as fully explained throughout our review.

12 Discussion

In vivo studies based on animal models are fundamental to investigate biomolecular, genetic and physiological changes responsible for human pathologies. However, a synergic work is necessary, between preclinical and clinical studies, to (i) identify new therapeutic targets, (ii) discover early pathology markers and (iii) test new pharmacological approaches. Having an animal model of pathology could be easier for genetic diseases or disorders with a well-known aetiology, but this is not true for multifactorial/neurodegenerative diseases, including AMD. Indeed, AMD does not have a defined pathogenesis, thus every affected patient might have a unique interplay between risk factors and genetic predisposition, which is not reproducible in animals. Furthermore, anatomical difference between humans and rodents must be also considered: rodents do not have the macula, the region responsible for visual acuity and where the degeneration occurs. Reproduce every single AMD feature in rodents is not possible, however, an ideal animal model should recapitulate the main histological and functional changes of AMD, being inexpensive and with a rapid evolution, as well.

In this review we mainly considered LD paradigms, but we also reported other existing AMD models (Table S1). Many of these are genetic based, even if their development is not an easy task, due to the complex aetiology of AMD. In some cases, AMD features were reproduced in these models through gene defects not involved in the human pathology; on the other hand, their use was fundamental to explore the pathogenic mechanisms related to one or more genes. A critic point is that, in genetic models, desired pathological hallmarks are often slowly developed, making difficult evaluate the disease progression.

LD models has allowed our and other research groups to deep understand novel mechanisms involved in AMD-induced retinal degeneration, using a well-known environmental risk factor for AMD. Light pollution has always been considered harmful for the retina; moreover, there was an increasing concern since LED lights were marketed for everyday use. In this review we summarized the main LD models used, which have been developed starting from different strains, using different light sources and timetable. We discussed the use of mice and rats as models for light-induced retinal degeneration. Although rodents display anatomical difference compared to human retina, rats develop retinal damage in a limited region (known as hotspot), partially resembling macular degeneration. Conversely, LD causes the degeneration in the entire retinal length in mice. Pigmentation also plays a pivotal role during AMD, acting as a ROS scavenger. This role is reflected in a biased incidence of AMD, which mainly affects people with a reduced melanin content. Pigmentation is protective also in rodents; indeed, reproduce AMD features in pigmented rodents is more difficult, requiring more intense light as well as chronic exposure of animals. In these cases, other factors should be considered such as circadian rhythm or excessive heat generated by light sources, which can negatively impact the experimental design. Furthermore, some pigmented strains as C57BL/6J mice are naturally resistant to light exposure due to genetic polymorphisms, not present in humans. Noteworthy, although pigmentation and genetic background are important in light damage paradigms, the age of animals and beginning of light damage are the two main critical factors (Polosa et al. 2016). In our previous studies we used an LD model based on the exposure of albino SD rats to white light (1000 lux, 24 h). We demonstrated that this model reproduces both the atrophic and the

neovascular forms of AMD (Rutar et al. 2010; Tisi et al. 2020b, 2020c). Furthermore, the degeneration process is time-dependent allowing us to characterize different stages of the process. We demonstrated that immediately after light exposure there was a peak of apoptotic cells in the ONL, together with the appearance of subretinal debris in the subretinal space, which are early signs of AMD. Seven days after light damage, the "hotspot" area is clearly visible in a limited region of the rat's retina, with an overall reduction of the ONL layer. At the same timepoint, electroretinogram recordings showed a significant decrease in both a- and b-wave amplitudes, consistent with visual function loss in AMD patients. From seven days after LD, neovascular lesions start to appear, with newly formed vessels invading photoreceptors layer and an up-regulation of pro-angiogenic factors (VEGF and VEGFR2). Notably, 120 days after LD the degeneration involved all superior retina, from dorsal edge to the area close to the optic nerve and affecting also electroretinogram response (Tisi et al. 2020b). Noteworthy, the inferior retina is preserved in our model as it happens in humans.

We can conclude that LD is an eloquent model to explore AMD, which helped our and other research groups to underline several novel mechanisms involved in AMD degeneration (i.e. autophagy, oxidative stress, metabolic lipids pathway and inflammation) (Table 2). We know the criticisms deriving from the use of LD models, but on the other hand these disadvantages lose their relevance, if compared with the advantage of being able to study all the characteristics of such a complex disease within a limited period. Furthermore, LD models actively contribute to the development of an effective strategy to slow down/treat both the early and the advanced forms of AMD. This will be a widespread degenerative disease and, if not properly treated, it will become highly disabling for millions of people worldwide. Expanding the knowledge on AMD is still crucial to discover new early biomarkers useful for a rapid diagnosis and for their use as therapeutic targets as well.

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