Chapter

Retinal Disorders in Humans and Experimental ALS Models

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Abstract

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease that severely impairs the patient's mobility, as it mainly affects the upper and lower motor neurons in the spinal cord. In addition, alterations have also been demonstrated in different parts of the central nervous system (CNS), such as the brain and brainstem. The retina is a projection to the brain and is considered as a "window" to the CNS. Moreover, it is possible to use the retina as a biomarker in several neurodegenerative diseases, even in the absence of major visual impairment. Classically, it was thought that the eyes were not affected in ALS, with respect to extraocular muscles, whereas the remainder of the muscles of the body were distressed. Nevertheless, retinal changes have recently been found in this pathology and could help in diagnosis, follow-up, and even monitoring therapies in this disease.

Keywords: amyotrophic lateral sclerosis, ALS, retina, animal models, SOD1, microglia, protein aggregates, axon pathology, neurodegeneration, neuroinflammation

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common progressive motor neuron disease, accounting for 80–90% of all motor neuron diseases cases [1]. Worldwide, the incidence per 100,000 people ranges from 0.3 to 2.5 cases per year [2–5]. Only 10% of the cases are familial [6], ranging from 2 to 15% depending on the population [7], whereas 90% of the cases are sporadic or seemingly sporadic. Overall, both the incidence [8] and the prevalence [9] of ALS vary according to location and race. ALS is more common in men than in women, with a ratio of 1.5:1 [5].

This neurodegenerative disease is rapidly progressive with a typical combination of symptoms of both upper motor neurons (UMNs) and lower motor neurons (LMNs) in different degrees, causing muscle fiber atrophy, which seriously affects the patient's mobility and quality of life [2, 4, 10]. ALS comprises overactive reflexes, as well as muscle weakness and stiffness, and it also involves the swallowing, speech, and respiratory muscles [10–12]. In fact, patients usually die within 2–3 years from

diagnosis, frequently due to respiratory failure [5, 13]. The disease usually has a spinal onset, beginning in the extremities and spreading to the rest of the body; however, one in every four patients has a bulbar onset, which has a worse prognosis [4]. ALS is a heterogeneous disease with asymmetrical onset and spreading of UMN and LMN dysfunction, which makes its classification very complex [14]. In addition, no single specific test exists for ALS diagnosis; it is a diagnosis of exclusion based on the initial symptoms, the progression of the disease, and tests to eliminate overlapping conditions.

Although ALS has been considered an exclusively motor disease, over the last few years, several studies have focused on assessing the possible participation of nonmotor areas of the central nervous system (CNS) in this illness. Actually, neuroimaging tests have shown an overall reduction in brain volume, with a loss of focal gray matter and regional white-matter alterations [15–20]. The alteration of these areas leads to cognitive and behavioral changes [16, 18]. During the course of the disease, it has been found that 50% of ALS patients have some degree of cognitive impairment, mainly featuring executive dysfunction and mild memory loss [15, 21].

Classically, it was thought that the eyes were not affected in this disease, with respect to the eye motor muscles, whereas the remaining muscles of the body were affected [22]. However, some studies have found abnormal ocular movements in these patients [23–28]. Nevertheless, this classical concept did not refer to the retina or the optic nerve. Actually, these patients have demonstrated not only abnormal evoked potentials [29–33] but also astrogliosis in nonmotor areas, specifically in the occipital area [34]. Even a significant interocular difference of the P100 in ALS patients was demonstrated in a study of visual evoked potentials [33], similar to the existing asymmetry in the CNS of these patients [14]. Some researchers have also analyzed changes in the visual pathway (a nonmotor neuron area) using optical coherence tomography (OCT) in ALS patients [35–44], finding different changes in the retina and optic nerve, some with contradictory results, stressing the importance of classifying patients by both stage and type of ALS, given the high heterogeneity of the disease.

The retina is considered as an open window to the CNS, and it is possible to use it as a biomarker in multiple neurodegenerative diseases, whether or not there is visual impairment. In recent years, many studies have emphasized the importance of the retina in the diagnosis and monitoring of neurodegenerative diseases, with various pieces of evidence highlighting its value as a biomarker [45–57]. However, what was not so evident was the possible involvement of the retina in neuromuscular diseases, which are chronic progressive neurological diseases, such ALS, that predominantly affect the spinal cord, whereby the neurological involvement is far from the visual pathway.

The purpose of this review is to analyze the retinal changes that have been described in different animal models in this disease, to compare them with each other and to correlate them with the changes described in humans to highlight the possible role of the retina as a biomarker in this disease.

2. Retinal histopathological studies in amyotrophic lateral sclerosis patients and experimental models

ALS is a neurodegenerative disease, which shares some pathophysiological mechanisms common to other diseases of the CNS, such as vascular pathology, glutamate excitotoxicity, fragmentation, aggregation, and functional abnormalities of the mitochondria, impaired retrograde and anterograde axonal structure and transport,

increased free-radical and oxidative stress, protein aggregation, and neuroinflammation [12, 58–61]. However, studies in the retina are scarce and have focused only on four such mechanisms, as described below.

2.1 Histopathological studies in ALS demonstrating intraretinal protein inclusions

(**Table 1**, **Figure 1**) Accumulated and altered proteins can interfere with neuronal traffic or can abduct proteins that are essential for proper neuronal functioning causing neurotoxicity [62]. The ubiquitin proteasome system plays an important role in ALS, with reactive ubiquitin inclusions being characteristic of this pathology [5, 63]. Among them, TDP-43 and p62 proteins are specifically indicative of ALS. These inclusions, which are positive for P62 and negative for TDP-43, have been demonstrated in the brain, hippocampus, and cerebellum in ALS patients [64, 65].

There are scarce studies that have focused on the histopathology of retinal tissue in both ALS patients and animal models of mammals with ALS. Actually, the first histopathological analysis in the retina was performed in 2014 on a patient with the C9orf72 mutation. In this study, protein intracytoplasmic p62-positive and pTDP43-negative perinuclear aggregates, typical of ALS/frontotemporal dementia (FTD), were observed in the inner nuclear layer (INL) of the retina [66]. Both the poly-(GA) n dipeptide repeats and ubiquitin in the retina were positively stained for p62, similar to the perinuclear inclusions localized in the brains, specifically in the dentate gyrus, of patients with this mutation [66]. The authors suggested that most of the p62-positive inclusions found were likely placed within the cones of bipolar cells (OFF bipolar cells) and between amacrine and horizontal cells, because they were also stained with GLT-1 and recoverin; in addition, these retinal deposits could be related to the contrast sensitivity impairment manifested by the patient [66]. Moreover, Volpe et al. [67] analyzed two retinas from ALS patients with C9orf72 mutations and demonstrated (i) specific p62 inclusions mostly in the INL (94.9%) and in a smaller proportion in the retinal ganglion cell layer (GCL) (5.1%) in one patient, and (ii) ganglion cell axonal atrophy specifically in the papillomacular bundle in the second patient. On the other hand, abundant positive ubiquilin 2-positive inclusions were also shown in a transgenic mice experimental model with mutant UBQLN2, mostly in the inner plexiform layer (IPL), with a smaller amount in the outer plexiform layer (OPL) and a scarce amount in the GCL. This ubiquilin 2 aggregation in the layers of the retina with more synapses is associated to the ubiquilin 2 accumulation in the dendritic spines of the hippocampus, and it may also be related to the dementia observed in this experimental model. Furthermore, few ubiquilin 2-positive aggregates were detected between the neurosensorial retina and the retinal pigment epithelium, whose appearance was analogous to that of drusen [67]. Similarly, in patients with FTD and progranulin deficiency, lipofuscin deposits were found, sometimes associated with subretinal drusen-like aggregates [68]. Retinal thinning in these patients was detected by OCT before symptoms, suggesting that the eye is affected in progranulin-deficient frontotemporal dementia disease [69].

Eye degeneration was reported in an ALS *Drosophila* model that expressed C9orf72 repeat expansion. The expansion of a noncoding GGGGCC hexanucleotide repeat of the C9orf72 gene on chromosome 9p21 is the most common point mutation in familiar ALS, which generates dipeptide repeat proteins that aggregate in the brain. It is noteworthy that some synthesized compounds revealed a significant biological effect by blocking the neurodegeneration of fly retina at different efficacy levels and upgrading

Mechanism	Author and year	Retinal tissue	Main retinal findings	Other comments
Protein inclusions	Fawzi et al. 2014	One patient with ALS secondary to a C9orf72 mutation	Protein intracytoplasmic $p62^{+}$/TDP43-perinuclear aggregates in the INL	Most of the p62-positive inclusions found were likely placed within OFF bipolar cells and between amacrine and horizontal cells; they may have been responsible for the contrast sensitivity impairment in this patient
	Volpe et al. 2015	UBQLN2P497H TG mice	$Ubiquilin2^{+}$ inclusions mostly in the IPL, with a smaller amount in the OPL and in the GCL	Drusen-like ubiquilin 2-positive aggregates at the level of the sub-RPE space
		Two patients with ALS secondary to a C9orf72 mutation	First patient: Specific $p62^{+}$ inclusions: 94.9% in the INL and 5.1% in the GCL	Second patient: ganglion cell axonal atrophy specifically in the papillomacular bundle
	Azoulay-Ginsburg et al. 2021	ALS fly *Drosophila* model expressing C9orf72 repeat expansion	Eye neurodegeneration	Compounds 9 and 4 of chemical chaperones blocked and upgraded the eye neurodegeneration
Neuroinflammation	Ringer et al. 2017	TG mouse model SOD1G93A	$hSOD1^{+}$ vacuoles in the dendrites of excitatory retinal neurons in the IPL, with hardly any in the GCL and INL	No signs of activation of either the astroglia or the microglia of the retina
	Cho et al. 2019	Mouse model of ALS devoid of Ranbp2	↑ Amoeboid forms and microglial cells surrounding the RGCs	Hypertrophy in RGCs + ↑ metalloproteinases in RGCs + axonopathy in the optic nerve
	Rojas et al. 2021	TG mouse model SOD1G93A (late stage)	Microglial cells activation in retinal tissue	Loss of RGCs
			Cell thickening in the area occupied by each microglial cell	
			↑ Microglial arborization in the area with hyper-ramifications in the inferior sector of the OPL	M1 phenotype or proinflammatory state of microglia: neurotoxic
			Retractions of cells processes + migration and clustering of cells in some areas of the retina	

Mechanism	Author and year	Retinal tissue	Main retinal findings	Other comments
Retinal spheroids and axon pathology	Sharma et al. 2020	Retinal sections of 10 postmortem eyes from ALS patients	PAS^+ spheroids (> 9.07 μm in diameter) in the RNFL	No significant correlation of retinal spheroids and axon pathology with clinical characteristics of the ALS patients (age at death, gender, disease duration, mode of disease onset, ALSFRS-R, and rate of disease progression)
			$P\text{-}NF^+$ spheroids (8 to 15 μm in diameter) in the peripheral and pRNFL	
			$NP\text{-}NF^+$ spheroids (7 to 10 μm in diameter) in the RNFL	
			↑ NP-NF signal in the RNFL and IPL	
Vasculopathy	Abdelhak et al. 2018	34 ALS patients with clinically diagnosed ALS who underwent an OCT	The outer wall thickness of retinal vessels was thicker in ALS patients than in controls	Thinning of the ONL, suggesting a possible impairment of rod and cone function
			There was also no correlation between the vessel measurements and clinical parameters	The whole retinal thickness was negatively correlated with the ALSFRS-R

ALS: amyotrophic lateral sclerosis; FTD: frontotemporal dementia; INL: inner nuclear layer; UBQLN2P497H: dysfunctional ubiquilin 2; TG: transgenic; IPL: inner plexiform layer; OPL: outer plexiform layer; ONL: outer nuclear layer; INL: inner nuclear layer; GCL: ganglion cell layer; sub-RPE: subretinal pigment epithelium; hSOD1: human superoxide dismutase 1; Ranbp2: RAN-binding protein 2; RGCs: retinal ganglion cells; PAS: periodic acid Schiff; P-NF: phosphorylated form of neurofilament; NP-NF: non-phosphorylated form of neurofilament; RNFL: retinal nerve fiber layer; pRNFL: peripapillary retinal nerve fiber layer; OCT: optic coherence tomography; ALSFRS-R: ALS Functional Rating Scale—Revised.

Table 1.
Retinal findings in ALS animal models and patients.

this eye degeneration. The most active chemical chaperones were compound 9, which is a peptide derivative targeted to the endoplasmic reticulum, and compound 4, which is targeted to the lysosome. Consequently, both might be used as a new class of drug candidates to treat ALS and other protein misfolding disorders [70].

2.2 Histopathological studies in ALS and neuroinflammation in retinal tissue

(**Table 1**, **Figure 1**) Neuroinflammation is a pathophysiological mechanism, which involves the activation of astroglial and microglial cells, and it occurs in many neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, ALS, and glaucoma [62]. Microglial cells are the macrophages of the CNS and have the ability to respond to injury by becoming activated; they can proliferate, migrate, and change shape, acquiring an amoeboid appearance in the most active state [62, 71]. On the one hand, in an attempt to protect against damage, microglial cells can secrete proinflammatory molecules, such as interferon γ or interleukin (IL)-1β [62]. Nonetheless,

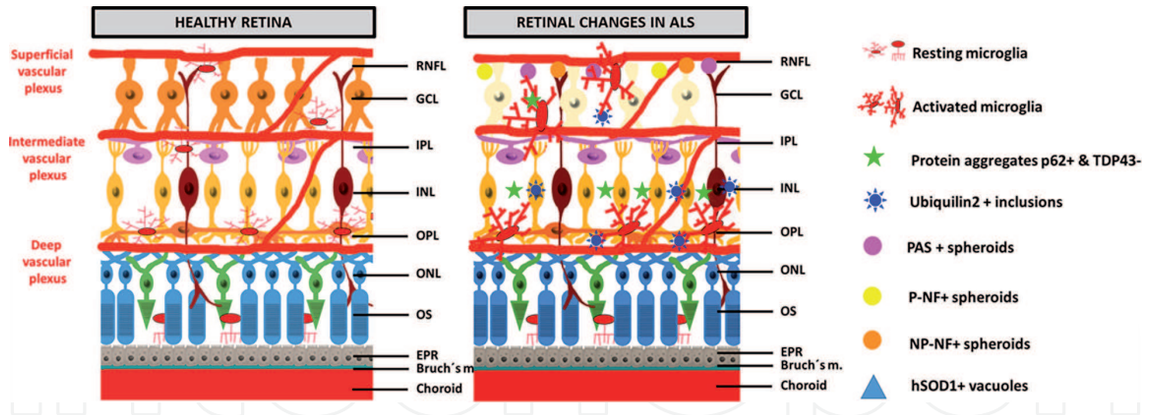

Figure 1.
Summary of retinal changes in amyotrophic lateral sclerosis. (A) Healthy retina. (B) Retinal changes in ALS. Most of retinal changes are mainly detected in the inner layers: (i) ganglion cell loss; (ii) activated microglia in outer plexiform layer (OPL) and inner layer complex (ILC) (constituting an inner plexiform layer and a nerve fiber–ganglion cell layer); (iii) p62-positive and TDP43-negative protein aggregates mostly in the inner nuclear layer with some in the ganglion cell layer (GCL); (iv) ubiquilin 2-positive inclusions mostly in the inner plexiform layer (IPL), with a smaller amount in the outer plexiform layer (OPL) and in the GCL; (v) periodic acid Schiff (PAS)-positive spheroids in the retinal nerve fiber layer (RNFL) and phosphorylated form of neurofilament (P-NF)-positive spheroids in the in the peripheral and peripapillary RNFL; (vi) non-phosphorylated form of neurofilament (NP-NF)-positive spheroids in the RNFL; (vii) hSOD1-positive vacuoles in in the IPL, with hardly any in the GCL and inner nuclear layer (INL).

uncontrolled activation of the M1 phenotype can lead to a state of chronic inflammation, which can induce neuronal death. On the other hand, microglial cells can also secrete anti-inflammatory molecules, such as IL-10 and the enzyme arginase 1 (Arg1), in order to control inflammation, repair tissue, and improve neuronal survival [62, 71–73]. Consequently, activated microglia can acquire two different activation phenotypes: an M1 or proinflammatory phenotype vs. an M2 or anti-inflammatory phenotype, both of which can be influenced by molecules derived from surrounding cells such as astrocytes [62, 74]. Astrocytes are glial cells of ectodermal origin that perform numerous functions for neuronal survival [75], such as maintenance of the volume and composition of the extracellular space, maintenance of the blood-brain barrier, and regulation of synaptic transmission [76], as well as metabolic maintenance and neuronal survival [77, 78]. When astrocytes are damaged and consequently activated, "astrogliosis" occurs [79]. If this astrogliosis is severe, a glial scar may form [75]. Reactive astrocytes can interact with microglia and neurons and can impair the function of neurons after an injury [80].

Astrocyte activation [81], microglial activation [82], and the appearance of lymphocytes [83] have been found in animal models of ALS (with SOD1 mutations) and in ALS patients. In ALS there are reactive microglia and astrocytes, which can result in motor neuron injury and subsequent death [73, 74, 81]. The SOD1G93A mouse model is one of the most suitable and widely used for preclinical studies in ALS, attributable to the animals having an analogous phenotype to patients. These animals develop limb paralysis due to the loss of motor neurons in the spinal cord, with a reduced lifetime of 150 days [84]. Microglial activation also occurs in ALS, as observed in SOD1-mutated mice and in spinal cord samples from ALS patients, which could exacerbate neuronal damage [73, 74, 81, 85]. In fact, it has been shown that exogenous extracellular mutation of SOD1G93A is not directly toxic to motor neurons, but requires microglial activation for toxicity in primary motor neuron and glia cultures [86]. Furthermore, in SOD1 transgenic mice, activated astrocytes and microglia have been shown to contribute to disease progression but not to disease

onset [87–89]. In ALS, microglial activation and proliferation have been observed in areas of significant motor neuron loss, such as the motor cortex, brainstem motor nucleus, corticospinal tract, and ventral horn of the spinal cord [90–94], as well as in areas with mild degeneration [95]. Precisely, in postmortem spinal cord analysis of patients with advanced stages of ALS, reactive astrocytes were found in the dorsal and ventral horn of the spinal cord [96] and in the gray [97] and white matter [34] of the cerebral cortex. Similarly, reactive microglia were found in the motor nucleus of the brainstem, motor cortex, corticospinal tract, and ventral horn of the spinal cord [91]. Reactive microglia were also observed in vivo using the PET imaging technique C-PK11195, finding a close relationship between microglial activation and upper motor neuron damage, but not lower motor neuron injury [98]. Moreover, in the SOD1 model, it was confirmed that overexpression of the SOD1 mutation in glial cells contributes to motor neuron damage, and that the degree of neuronal injury depends on the degree of glial cell pathology [99]. Microglial cells of SOD1-mutated mice suffer different degrees of morphological changes from resting to macrophagic amoeboid forms [91]. Lastly, symptomatic SOD1 transgenic mice also have increased numbers of microglial cells, mainly due to the proliferation of resident microglia [100].

Bearing in mind all of the above, both the microglia and the astrocytes play an important dual role in the progression of the ALS. Nevertheless, most studies about the involvement of microglia in ALS have been conducted in the motor cortex, brainstem motor nucleus, corticospinal tract, and ventral horn of the spinal cord. To our knowledge, there are only three studies that investigated the glial cells of the retina in relation to ALS [101–103].

In the first one, a mouse model of ALS devoid of RAN-binding protein 2 (Ranbp2), microglial activation was confirmed. Ranbp2 is a protein, which plays an important role in nucleocytoplasmic transport and whose regulation is affected in both sporadic and familiar ALS [104]. In this ALS mouse model, there was microglial activation with an increase in the number of microglial cells surrounding retinal ganglion cells (RGCs), as well as a noteworthy increase in amoeboid forms relative to controls. In addition, there was an increase in metalloproteinases in RGCs, and both hypertrophy in RGCs and axonopathy in the optic nerve were found [102].

In the second model, a TG mouse model of ALS SOD1 (SOD1G93A), there was a vacuolization, with hSOD1-positive vacuoles placed in the dendrites of excitatory retinal neurons, which were detected principally in the inner plexiform layer (IPL) and hardly in the GCL and INL; however, no signs of activation of either the astroglia or the microglia of the retina were shown compared with to the wild-type mice [101]. However, the authors did not rule out the possibility that the microglia were undergoing functional changes (in cytokines) related to the inflammatory process. Nevertheless, neuronal changes observed in this SOD1G93A ALS model in the brain at 50 days of age were followed by microglial morphological changes at 60 days [105–107]. Therefore, the authors concluded that, if there is an inflammatory process in the retina, microglia would be in a different, less reactive or even neuroprotective phenotype [101].

Lastly, the third transgenic murine SOD1G93A model of ALS in an advanced stage of the disease (120 days) showed a loss of the number of $Brn3a^+$ RGCs and a microglial activation in retinal tissue [103]. Signs of microglial activation were found in different retinal sectors (superior, inferior, nasal, and temporal) of different retinal layers: outer plexiform layer (OPL) and inner layer complex (ILC) (constituted by an inner plexiform layer and a nerve fiber–ganglion cell layer). In addition, the microglial activation in this SOD1G93A model of ALS showed a cell thickening in the area occupied by each microglial cell, a significant increase in the area of microglial

arborization with hyper-ramifications in the inferior sector of the OPL, retractions of cell processes, and migration and clustering of cells in some areas of the retina, but no increase in the number of microglial cells [103]. Moreover, phenotypic analysis of the microglia showed an M1 phenotype or proinflammatory state of microglia, as the cells were intensely labeled with anti-IFNγ and anti-IL-1β but did not stain with the characteristic M2 markers (anti-arginase 1 and anti-IL-10) [103]. The significant decrease in the total number of $Brn3a^+$ RGCs at 120 days of illness would be consistent with the damage observed in the RGCs of the ALS models discussed above [37, 101, 102], as well as with the thinning of the peripapillary retinal nerve fiber layer (pRNFL), observed by OCT, in ALS patients compared with controls [36–44]. Consequently, these data would support that, in ALS, not only are motor neurons affected but also RGC loss occurs, considering this disease as a multisystemic disease [103].

In none of the abovementioned models were changes in the outer segments of the photoreceptors found. This could indicate that neither this layer of the retina nor the outer blood-retinal barrier (BRB) would be compromised in these animals. Because, when the outer BRB is disrupted, as in a glaucoma model of laser-induced ocular hypertension, there are morphological changes and an increase in the number of microglial cells in the photoreceptor outer segment layer [108–112]. Moreover, no changes in the number of microglial cells were found in either the OPL or the ILC [101, 103]; however, the group of Rojas et al. described signs of microglial activation [103]. This difference in results in the same experimental model could be due to the fact that Ringer et al. [101] used retinal sections, while Rojas et al. [103] used retinal whole mounts.

As mentioned above, microglial cells have two distinct phenotypic states that can exert neurotoxic or neuroprotective responses depending on the physiological conditions in which they are found. During ALS progression, activated microglia represent a continuum between the neuroprotective M2 phenotype and the neurotoxic M1 phenotype [113]. In SOD1 ALS animal models, in early stages of the illness, microglia in the lumbar spinal cord expressed markers related to the M2 neuroprotective phenotype (Ym1 and CD206); however, in the late stages of the disease, microglia in the lumbar spinal cord expressed markers related to the M1 neurotoxic phenotype (high levels of NADPH oxidase 2 (NOX2)) [74], suggesting that there is a polarization from a neuroprotective phenotype to a cytotoxic phenotype that induces motor neuron damage. In the retina, there is only one study that analyzed whether microglia are in an M1 or M2 activation phenotype [103]. The results of this study showed that, in 120-day-old SOD1G93A mice, microglia were strongly labeled with antibodies against M1 inflammatory cytokines (IFNγ and IL-1β), but not with those against M2 anti-inflammatory cytokines (arginase-1 and IL-10), suggesting that at an advanced stage of the disease retinal microglial cells are in an M1 activation phenotype or in a pro-inflammatory state that could be neurotoxic to RGCs, as demonstrated by the loss of these neurons. These results are consistent with the findings in spinal cords of the same animal model, where microglia in an advanced stage of the disease showed a neurotoxic M1 phenotype, demonstrating the dual role (neuroprotective/neurodegenerative) of microglial cells during the ALS process [74]. Therapeutic approaches that target microglia polarization and result in the induction of the M2 phenotype are promising strategies to ameliorate local neurodegeneration and clinical outcome of the disease [114].

2.3 Histopathological studies in ALS and retinal spheroids and axon pathology

(**Table 1**, **Figure 1**) Alterations in axonal transport (retrograde and anterograde) are a hallmark of ALS, being impaired both in ALS patients and in mutant SOD1

mice. In the spinal motor neuron axons, an accumulation of altered mitochondria, neurofilaments, and autophagosomes [12, 58] was demonstrated. On the one hand, mutated dyneins in ALS mice cause this accumulation in the axons of mitochondria and autophagosomes [58]. On the other hand, altered autophagosomes do not eliminate either altered mitochondria or dilated endoplasmic reticules, which accumulate in the axons of motor neurons and cause them to malfunction [12].

There is only one study that focused on this important pathological mechanism in the retina [115]. This study analyzed retinal sections of postmortem eyes from ALS patients with periodic acid Schiff (PAS) and phosphorylated (P-NF) and non-phosphorylated (NP-NF) forms of neurofilament (NF), compared with age-matched controls. Three kinds of spheroids were revealed. First, PAS-positive spheroids with a diameter bigger than 9.07 μm in the retinal nerve fiber layer (RNFL) were observed in most ALS patients (but only in half of controls), most commonly in the pRNFL and the peripheral RNFL, but rarely in the central RNFL in patients with ALS. The density of PAS-positive spheroids was significantly greater in the pRNFL. Second, P-NF-positive spheroids ranging from 8 to 15 μm in diameter were observed in the peripheral and pRNFL only in ALS patients. Additionally, ALS patients showed a stronger P-NF signal intensity in the RNFL in the peripheral, central, and peripapillary regions. Third, NP-NF spheroids ranging from 7 to 10 μm in diameter were observed in the RNFL in some of ALS patients (but not in controls). In addition, in most of the ALS patients, the NP-NF signal was increased in the RNFL and IPL. Nevertheless, there was no significant correlation of these retinal spheroids and axon pathology with the clinical characteristics of the ALS patients (age at death, gender, disease duration, mode of disease onset, revised ALS functional rating scale, and rate of disease progression) [115].

Consequently, patients with ALS show not only hallmark findings in spinal cord motor neurons pointing to disrupted axon transport [116–121] but also retinal spheroids and axon pathology as a shared pathogenesis [115]. Transgenic mice with dysfunctional microtubule-associated motor proteins also display such findings [122–124].

2.4 Retinal vessel pathology and ALS

(**Table 1**, **Figure 1**) Retinal vessels are a reflection of small blood vessels in the brain [125]. Parallel vessel pathology in the retinal and cerebral small blood vessels has been demonstrated in many systemic diseases such as coronary heart disease [126] or stroke [127], as well as in some neurodegenerative diseases such as Alzheimer's disease [128, 129] (even in subjects at high genetic risk of developing Alzheimer's disease [130]) and Parkinson's disease [131].

Some ALS-induced changes have also been described in small blood vessels of the brain, which include a loss of pericytes, endothelial cell degeneration, capillary leakage, downregulation of tight junction proteins, and microhemorrhages in patients with ALS [132, 133]. Moreover, alterations of the structure of small blood vessels of the skin and muscles in ALS patients have been described [134, 135].

There was only one study that analyzed retinal vessel pathology in ALS patients with Spectralis OCT but not with angio-OCT. This study described a thicker outer wall of retinal vessels in ALS patients compared with controls, which may be related to the findings in small blood vessels in skin and muscle biopsies. There were neither significant differences in the vessel diameters between ALS patients with spinal onset and bulbar onset, nor a correlation between the vessel measurements and clinical parameters (disease duration and ALS Functional Rating Scale—Revised (ALSFRS-R)) [136].

3. Conclusions

Much research still remains to be conducted on the retina in both animal models and ALS patients. First, further research should aim to describe the different changes in the retina that occur in all pathogenic mechanisms of the disease. Second, there are several models with different genetic mutations that should also be analyzed. In addition, both the retinal and the choroid changes produced at different times in the evolution of the disease should be studied. It is known that ALS is a heterogeneous disease, with different forms of onset, development, and progression, which may potentially exhibit differences in the retina, as observed in the CNS.

The main findings found in the retina in ALS are summarized in **Figure 1** and **Table 1**.

In conclusion, multiple studies have confirmed that the retina is affected in ALS, mainly in the inner layers, and it could serve as a biomarker in this pathology. These retinal changes can be detected by noninvasive retinal imaging techniques to help in the diagnosis and monitoring of ALS disease. In addition, the retina could be used to evaluate the efficacy of different therapies in ALS in a noninvasive way.

Conflict of interest

"The authors declare no conflict of interest."

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