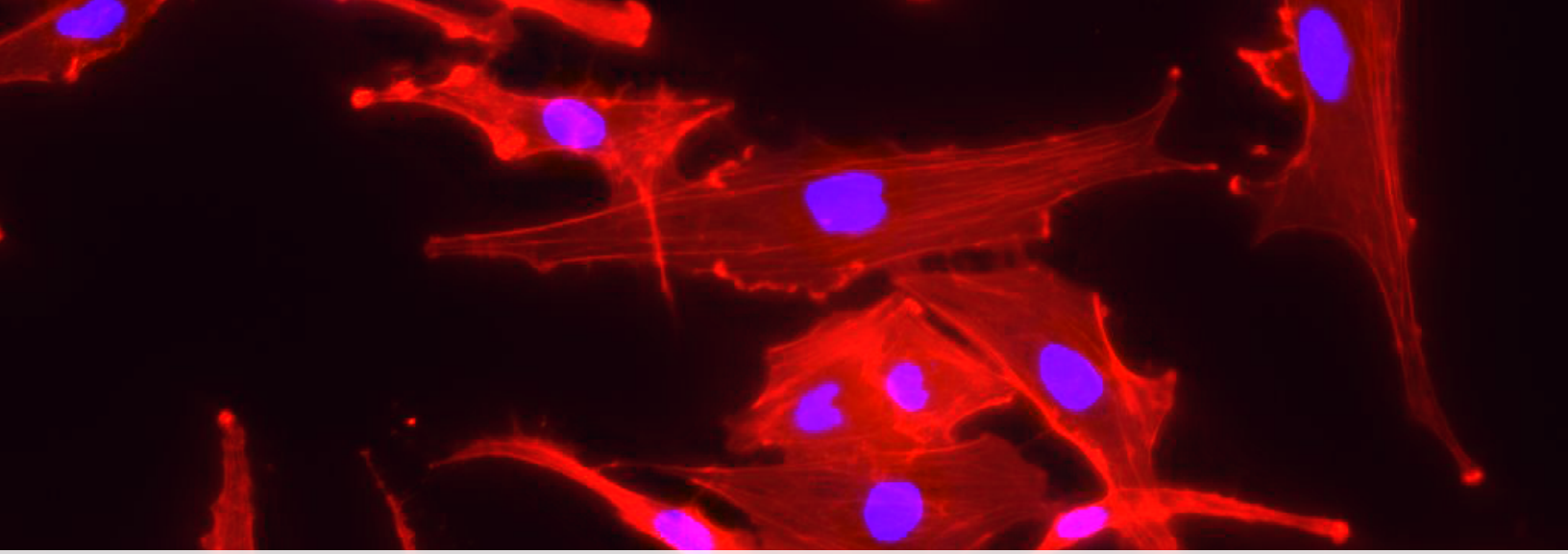

The Burden of Retinal Degenerative Diseases

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CONTENTS

Retinal degenerative diseases, like diabetic retinopathy, glaucoma, and age-related macular degeneration (AMD), are leading causes of vision loss and blindness worldwide[1].

The Burden of Retinal Degenerative Diseases

Vision impairment and blindness have a substantial impact on people's ability to perform everyday tasks and can affect their quality of life. In fact, vision impairment reduces mobility, affects mental wellbeing, exacerbates the risk of dementia, increases the likelihood of falls and road traffic crashes, increases the need for social care, and ultimately leads to higher mortality rates[2].

Globally, at least 2.2 billion people have a vision impairment or blindness, of whom at least 1 billion have a vision impairment that could have been prevented or has yet to be addressed[2].

Moreover, in the “World report on the vision” from World Health Organization (WHO), it was reported that 11.9 million people globally have a moderate or severe vision impairment or blindness due to glaucoma, diabetic retinopathy, and trachoma that could have been prevented[3].

The estimated costs of preventing the vision impairment in these 11.9 million would have been US$5.8 billion[3].

Despite the scarce economic estimates, it was suggested that the annual global productivity loss from vision impairment is approximately US$410.7 billion purchasing power parity[4]. That way the prevention of vision impairment represents a significant opportunity to reduce the substantial personal and societal burden associated with vision impairment and blindness.

The pathophysiology of diabetic retinopathy, glaucoma, and AMD is still not completely understood, and novel biomarkers for diagnosis and disease progression are needed. Early diagnosis and intervention on patients with retinal degenerative disease would be key to improve the outcomes of treatment. Despite the increasing interest of the scientific community in trying to elucidate the mechanisms underlying retinal dysfunction and degeneration, that knowledge is yet to translate into effective therapies for these retinal degenerative diseases. That way, there is a clear unmet clinical need for new and more effective therapeutic strategies[5].

Diabetic Retinopathy

Diabetic retinopathy is a significant complication of diabetes mellitus and a leading cause of vision impairment and blindness in working-age adults[6]. Globally, 3.28 millions of people present visual impairment and 1.07 million are blind due to diabetic retinopathy [4] (4). A major hallmark of the disease is the blood-retinal barrier breakdown. The current available

Therapeutic strategies mainly target neovascularization through the use of anti-vascular endothelial growth factor (VEGF) therapies, laser treatment, and surgery[7]. Nevertheless, diabetic retinopathy is now considered a neuro-vascular disease in which a low-grade chronic inflammatory environment contributes to blood-retinal barrier breakdown and retinal neural dysfunction[8,9]. In fact, the inducible nitric oxide synthase isoform, which is highly involved in inflammation processes, is a key mediator of blood-retinal barrier breakdown triggered by diabetes[10].

Glaucoma

Glaucoma is a leading cause of irreversible blindness and is characterized by optic nerve damage and retinal ganglion cell death[11]. It was calculated that glaucoma led to visual impairment and blindness of 4.13 and 3.61 million people in 2020, respectively[4]. Elevated intraocular pressure (IOP) is an important risk factor in glaucoma and the only one that is modifiable[12]. Indeed, current treatments are directed towards IOP lowering. However, many patients continue to lose vision despite successful

IOP control[13]. Therefore, new and more effective treatments are an emergent clinical need, and retinal neuroprotection has been considered to be an additional therapy[14]. Moreover, it has been described that microglia-mediated neuroinflammation further contributes to retinal neurodegeneration and the underlying mechanisms have been studied to devise novel neuroprotective strategies for glaucoma[15]. That way a new therapeutic approach to glaucoma should be not only focused on IOP lowering but also on protecting retinal ganglion cell from glaucomatous damage[16,17]. The control of microglia-mediated neuroinflammation was demonstrated to confer neuroprotection to the retina[18,19].

Age-related macular degeneration (AMD)

AMD is a major cause of vision loss worldwide and the leading cause of blindness in the elderly[20]. It is responsible for visual impairment in 6.23 million people and blindness in 1.85 million people[4].

AMD is classified in two forms[21]:

- "Early/dry" form: characterized by the accumulation of cellular debris in the subretinal space, beneath the retinal pigment epithelium (RPE), called drusens. The disease can then progress to RPE and photoreceptor loss;
- "Advanced dry/wet" form: characterized by the presence of geographic atrophy (dry) due to RPE and photoreceptor cell death and/or choroidal neovascularization (wet), with new blood vessels arising from the choroid through the RPE layer into the outer retina, leading to photoreceptor dysfunction and degeneration

The dry form of AMD and geographic atrophy are currently untreatable, while VEGF inhibition therapy is the best available treatment for wet AMD[22]. The anti-VEGF therapies present a significant burden for the patients due to the need for repeated intravitreal injections[23]. That way alternative treatment strategies have been proposed in order to ease the treatment burden by reducing the number of injections needed [24,25].

In Vivo Models

The development of several animal models of disease mimicking some features present in retinal degenerative diseases have been significantly contributing to unraveling

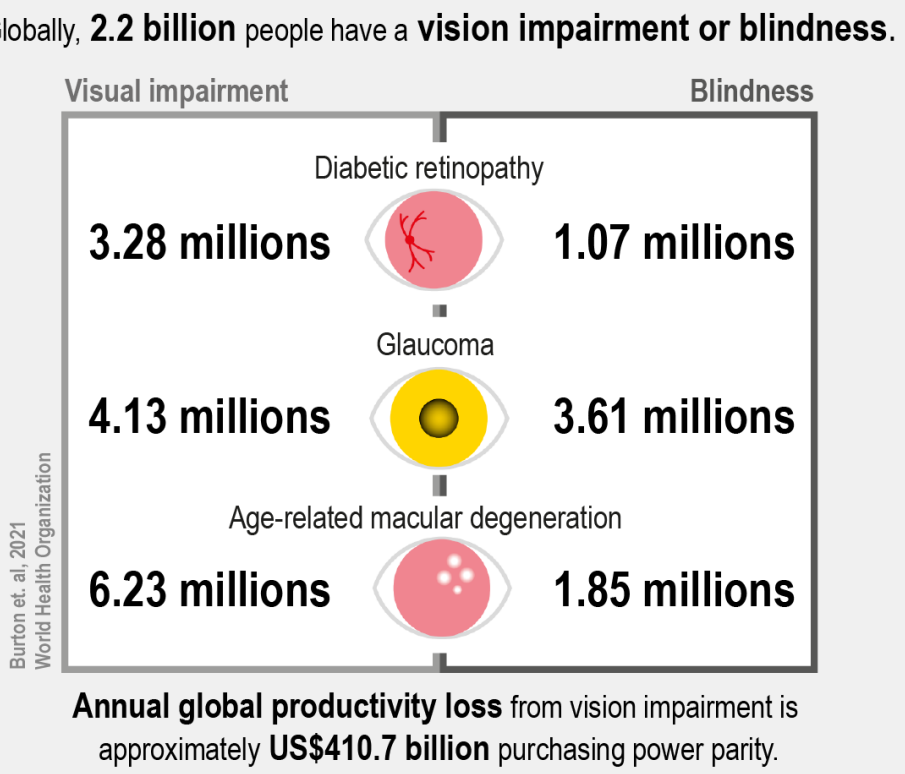

FIGURE 1:
Global estimates of number of people affected by retinal degenerative diseases, namely diabetic retinopathy, glaucoma and age-related macular degeneration, that cause vision impairment and blindness.

important aspects in the understanding of the pathogenesis of the retinal disease. The development of in vivo models also allows testing new and more efficacious possible therapeutic strategies for retinal degenerative diseases[26].

In diabetic retinopathy research, there are several animal models that rely on the induction of both type I diabetes and type 2 diabetes. One of the commonly used animal models of diabetic retinopathy is the streptozotocin (STZ)-induced type 1 diabetes. This model has been used to give insight into the action mechanisms of new and already used treatments in the clinics [27,28]. Goto-Kakizaki (GK) animals are a type 2 diabetes animal model characterized by early and relatively stable mild hyperglycemia, hyperinsulinemia, and insulin resistance [29]. This model mimics some features present in diabetic retinopathy as increased nitric oxide production, early inner blood-retinal barrier breakdown and migration of activated microglial cells from the retina to the choroid [30,31].

Regarding glaucoma, since elevated IOP is the main risk factor, relevant animal

models for glaucoma have been developed around the challenge of producing experimental IOP elevation. However, other non-related IOP animal models have been developed[32].

Glaucoma animal models lead to retinal ganglion cells (RGCs) dysfunction and loss, and optic nerve axonal transport impairment, mimicking some features of the disease. Ocular hypertension (OHT) model, based on laser photocoagulation of the trabecular meshwork and perilimbal and episcleral veins[33], episcleral vein cauterization model[34], and microbeads injection in the anterior chamber[35] are animal models based on the blockage of aqueous humor drainage, increasing IOP, and ultimately leading to RGC loss and damage of the optic nerve. Moreover, pressure-independent animal models, like optic nerve crush, optic nerve transection, and retinal ischemia-reperfusion (I-R) injury[32], have been used to model normal-tension glaucoma, and they have provided insights into the neurodegenerative mechanisms of RGC loss.

The animal models that mimic some features of AMD are focused on both forms of the disease, “dry” and “wet”. Complement factor pathway genetic models (e.g. Ccl2-/- and Ccr2 mice)[36] and light-induced retinal degeneration model[37] have being used to study the advanced dry geographic form of AMD, inducing photoreceptor death, subretinal drusen-like accumulations, thickening of Bruch's membrane and immune activation. The laser-induced choroidal neovascularization animal model is the most used model for “wet” AMD, which induces Bruch’s membrane rupture, inducing the growth of new choroidal vessels into the subretinal area[38].

Monitoring of Retinal Degenerative Diseases

Accurate monitoring of retinal degenerative diseases in patients is vital to preserve their visual function.

Likewise, disease progression can be assessed using similar but adapted methods in animal models.

Monitoring of Retinal Degenerative Diseases

- The leakage of the blood-retinal barrier can be assessed in vivo by **fluorescein angiography**, which is widely used to assess alterations in the retinal vasculature in diabetic retinopathy and age-related macular degeneration patients[39].
- Retinal function and structure can be easily assessed in vivo in animal models of disease by **electroretinography (ERG)** and **optical coherence tomography (OCT)**, respectively. Significant progress have been achieved in retinal imaging techniques that have enabled clinicians to detect structural changes in patients[40]. OCT is a non-invasive procedure used to visualize the anterior and posterior segments of the eye at high resolution that likewise is used in animals[41,42].
- Assessment of visual function, used in the clinic has also been adapted and can be performed in animal models using **quantitative optomotor response** [43].

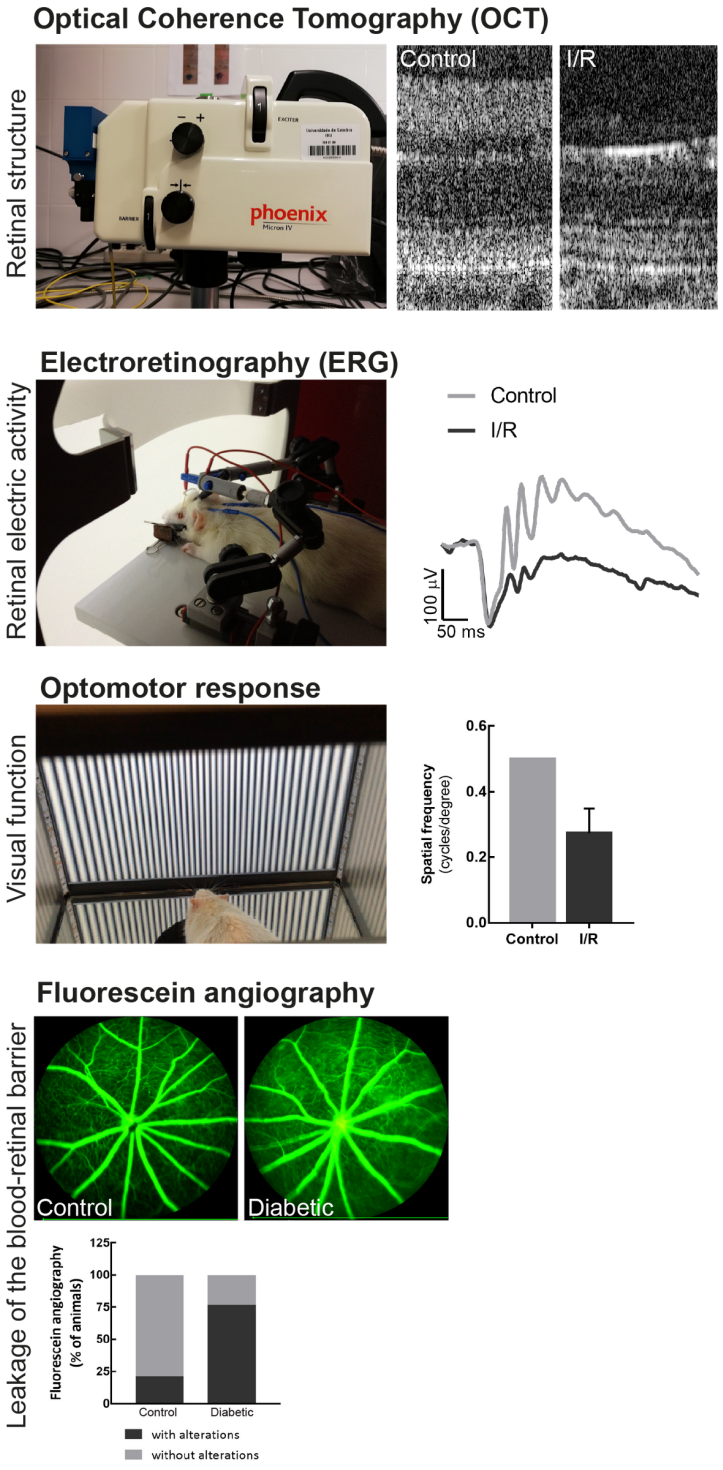

FIGURE 2:
Disease progression in animal models of disease can be performed by optical coherence tomography (OCT, to assess retinal structure), electroretinography (ERG, to assess retinal function), and quantitative optomotor response (OMR, to assess visual function) and fluorescein angiography (to assess leakage of the blood-retinal barrier).

The retina as a Window to the Brain

The concept of "the retina as a window to the brain" arose several years ago since eye examination, and namely the retina, with non-invasive approaches has been considered an additional tool for the early diagnosis of neurodegenerative diseases[44], such as Alzheimer's disease[45,46] and Parkinson's disease[47]. In fact, several studies in Alzheimer's disease animal models[48] and patients[49,50] demonstrated a thinning of the retina assessed by OCT. These studies suggest that the assessment of retinal thickness by OCT may be useful for an early diagnosis of Alzheimer's disease. Similar findings have been reported in Parkinson's disease patients[51,52].

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