Chapter

An Overview of Glaucoma: Bidirectional Translation between Humans and Pre-Clinical Animal Models

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Abstract

Glaucoma is a multifactorial, polygenetic disease with a shared outcome of loss of retinal ganglion cells and their axons, which ultimately results in blindness. The most common risk factor of this disease is elevated intraocular pressure (IOP), although many glaucoma patients have IOPs within the normal physiological range. Throughout disease progression, glial cells in the optic nerve head respond to glaucomatous changes, resulting in glial scar formation as a reaction to injury. This chapter overviews glaucoma as it affects humans and the quest to generate animal models of glaucoma so that we can better understand the pathophysiology of this disease and develop targeted therapies to slow or reverse glaucomatous damage. This chapter then reviews treatment modalities of glaucoma. Revealed herein is the lack of non-IOP-related modalities in the treatment of glaucoma. This finding supports the use of animal models in understanding the development of glaucoma pathophysiology and treatments.

Keywords: animal models, rodents, preclinical models, glaucoma, IOP, IOP-lowering, optic nerve degeneration, retinal ganglion cells

1. Introduction

Glaucoma is a heterogenous disease with numerous contributing factors including environment, genetics, and epigenetics. It is the primary cause of irreversible blindness in the world and the number of patients diagnosed is only projected to increase in the coming years. The use of preclinical animal models has exponentially progressed our understanding of the underlying pathophysiology of the disease, as well as providing a platform for generating and testing successful therapeutics. This chapter delves into the fundamentals of glaucoma including the pathologies, types, and symptoms with a subsequent description of the numerous preclinical animal models of the disease and what has been garnered from their study. We will then discuss treatments of glaucoma that are either FDA-approved or in development and conclude by summarizing how pre-clinical studies have advanced the development of new glaucoma therapeutics.

1.1 Basics of glaucoma

The eye is an elaborate structure suited to performing its unique function: collecting photons of light and converting that to an electrical signal allowing for vision. Because light needs to enter the eye without impedance, the cornea and lens lack vasculature. However, a source of nutrients and waste efflux is necessary for tissue survival. This necessity is answered by way of the aqueous humor, a water-based filtrate of the blood produced by the ciliary body of the eye. As it flows from the ciliary body through the pupil, the aqueous humor carries and bathes the tissues with sugars, vitamins, and other necessary supplies for cellular survival while carrying the excreted cellular metabolic waste products back to the blood stream for removal. The exit path for the aqueous humor is through the trabecular meshwork, Schlemm's canal and supplementary outflow structures (**Figure 1A**). Any imbalance between aqueous humor production and its elimination can have an impact on intraocular pressure (IOP). As they exit the eye, the axons of retinal ganglion cells (RGCs) converge and they become the nerve fibers of the optic nerve (**Figure 1B**) [1]. RGC axon degeneration can be induced by both elevated IOP-related changes and IOP-independent factors.

Glaucoma, defined as pathological damage to the optic nerve (ON), results in visual field defects due to death of the retinal ganglion cells (RGCs) and damage to their axons. Glaucoma is the leading cause of irreversible blindness globally. Trends predict that by 2040, as many as 111.8 million people worldwide will have this disease [2]. Of the approximately 80 million current cases, 11 million are estimated to result in complete blindness [3]. Glaucoma's primary risk factor is elevated intraocular pressure (IOP), although some forms of this disease do not include this endophenotype.

This disease is classified into the conventional categories of primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG), as well as primary congenital glaucoma, normotensive glaucoma (NTG), and pigmentary dispersion glaucoma. Other rare types exist, such as exfoliation glaucoma and traumatic glaucoma. The most important modifiable risk factor for onset and progression of glaucoma is elevated IOP, although normotensive glaucoma patients suffer from vision loss in spite of their IOP being in the normal physiological range, highlighting the complexity of this disease. In other subtypes of

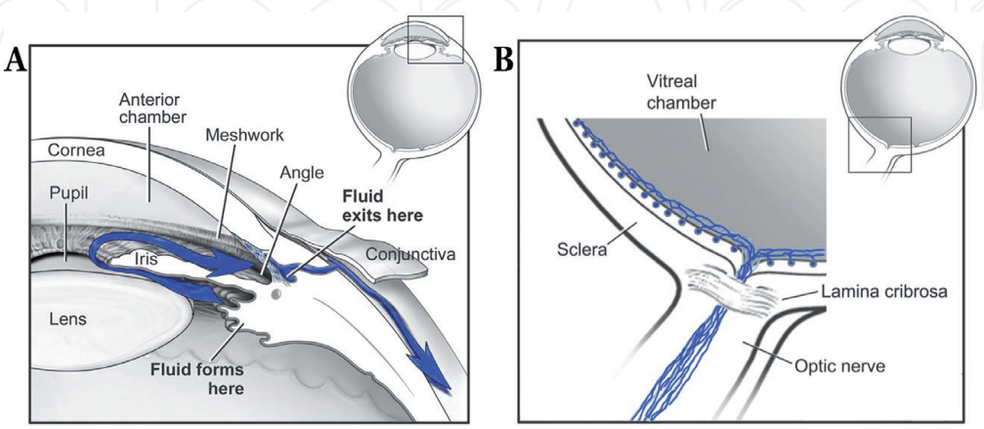

Figure 1.
Aqueous humor dynamics and retinal ganglion cells: two areas of glaucoma-related research. (A) The aqueous humor is produced by the ciliary body and exits through the trabecular meshwork and other outflow structures leaving the eye and entering the blood stream (flow shown in blue). (B) The axons of the retinal ganglion cells (blue) form the optic nerve and run through the lamina cribosa as they exit the eye. In glaucoma, retinal ganglion cells and their axons can be damaged due to IOP-related or IOP-independent mechanisms. Figures modified from Li, et al. 2012. Courtesy: National Eye Institute, National Institutes of Health.

this disease, glaucoma can also result from a variety of IOP-related mechanisms due to structural alterations that inhibit outflow of aqueous humor. These include trabecular meshwork obstruction by foreign material, trabecular endothelial cell loss, loss of phagocytic activity of the trabecular meshwork, loss of giant vacuoles from the endothelium of Schlemm's canal and reduced pore size or density in the wall of Schlemm's canal [4].

1.2 Pathophysiology of glaucoma

In the early stages of glaucoma, glial cells in the optic nerve head respond to glaucomatous change. IOP-related mechanisms of damage generally include obstruction of the trabecular meshwork, morphological changes in astrocytes such as enlargement of cell body and processes, as well as upregulation of cytoskeletal and extracellular matrix proteins. Studies have demonstrated that remodeling of astrocytes and increased deposition of extracellular matrix can occur in some forms of experimental glaucoma. Moreover, even a short period of IOP elevation can cause hypertrophy, process retraction, and simplification of the shape of astrocytes in the optic nerve without changes in gene expression. In addition, the accompanying extracellular matrix deposition is believed to be an early defense mechanism to repair or prevent damage to the blood retina barrier [5]. In later stages of glaucoma, glial scar formation can occur as a reaction to injury as a method of protection and healing. Glial cells recruit immune cells, increasing extracellular matrix deposition and inflammatory factors that prevent axonal regeneration [6].

Interestingly, different compartments of RGCs and the optic nerve die by different cellular mechanisms. Specifically, if the RGC axon is cut, the axon distal to the lesion degenerates via Wallerian degeneration, while the axon proximal to the lesion is removed by a process called dying back. Lastly, the cell body of the axon dies via apoptosis [7, 8]. All of which occur among glial scar formation. This process of RGC axon loss can be observed *in situ* during a dilated pupil fundus examination. This area of the posterior globe is termed the optic disc with its center termed the optic cup. Damage from glaucoma causes destruction of the nerve fibers around the rim of this structure and increases the size of the "cup"; an increase in the cup-to-disc ratio is one of the clinical phenotypes that is used to clinically monitor disease progression.

1.3 Classification of glaucoma and early diagnosis in humans

POAG is the most common form of glaucoma and is associated with increased IOP [9]. In broad contrast, PACG has a functional trabecular meshwork with obstructed access, while the trabecular meshwork in POAG is pathological, but open and unobstructed. The initiatory event of PACG is thought to be a blockage between the pupillary portion of the iris and the anterior lens surface, which is correlated with mid-dilation of the pupil [10]. Primary congenital glaucoma, often associated with autosomal recessive disease heritability, results from an abnormal development of the anterior segment and angle of the anterior chamber [11]. NTG is a disease that is nearly identical to POAG but lacks the increased IOP. Pigmentary glaucoma is characterized by accumulation of pigment in the trabecular meshwork and corneal endothelium [12].

Because IOP is the only known modifiable risk factor, it is the target of the majority of current treatment modalities. Increased age has also demonstrated increased risk of ocular hypertension resulting in elevation of IOP and onset of POAG [9]. Damage can also ensue from non-IOP-related mechanisms such as reduced ocular perfusion pressure, excitotoxicity from excessive glutamate,

autoimmune-mediated nerve damage, loss of neurotrophic factors, failure of cellular repair mechanisms, and abnormal autoregulation of retinal and choroidal vasculature [4]. African-Caribbean descent, near-sightedness, decreased thickness of the central cornea, first-degree family history, low ocular perfusion pressure, and diabetes are other associated risk factors [13].

The clinical diagnosis of glaucoma relies on recognition of signs of optic nerve damage via slit lamp biomicroscopy and examination of the optic nerve head, followed by measurement of IOP, assessment of the angle via gonioscope and measurement of visual fields. The only directly observable pathology of the optic nerve is in the intrascleral portion which can be observed as an increased cup-to-disc ratio [14]. Glaucoma can progress over decades if it is not appropriately treated. Because it is not painful unless IOP becomes extremely elevated, the early stages of glaucoma often progress undetected. It manifests clinically in advanced stages, where it first affects the peripheral vision of the affected eye. Unfortunately, the other eye, if unaffected, often compensates for changes in the visual field, making most patients unaware of the development and slow loss of vision. Early characteristics might be identified as difficulty reading in dim light. Due to its asymptomatic and silent onset, assessment of family history and frequent clinical assessment is vital. Additionally, assessment of secondary causes of IOP elevation can be beneficial in devising a treatment strategy that can include medical, laser, and surgical modalities [5].

2. Pre-clinical models of glaucoma

While progress has been made in understanding the genetic pathophysiology of glaucoma in humans using genome-wide association studies (GWAS), pre-clinical animal models provide an extremely valuable resource for identifying and understanding cellular mechanisms of action underlying specific mutations, genetic interactions, and ultimately, a knowledge of the disease pathogenesis to better treat the condition. They have been used to observe and modify the interplay of genetic and environmental factors in complex diseases such as glaucoma. They have also been used to develop and evaluate novel therapeutics. This cycle of observation of a disease phenotype and therapeutics response in humans, followed by replication and in-depth examination in pre-clinical models, with subsequent verification in humans, is a bidirectional translation model that is essential to continual forward progression of disease and treatment studies (**Figure 2**). Although there is great value to the models that have been created, there remain unmet needs.

There are many factors to consider when selecting an animal species population for modeling disease. One is the reproduction of the experimental procedure in both the pre-clinical models and humans [15]. For example, in performing an electroretinogram (ERG), a measurement of the electrical activity of the retina in response to light stimulus, employing the use of anesthetics can affect neurotransmission and affect test results [16]. This can become a confounding issue in smaller animals which must be anesthetized. Other factors to consider include the animal's body temperature and age. If body temperature decreases enough, metabolic processes that affect the chemical reactions necessary for an ERG will be decreased, suppressing the amplitude of the ERG, mainly in mice, rats, and rabbits due to their body weight to surface area ratio [17]. Age should also be considered as it influences amplitudes as well. This has been observed in rabbits with younger rabbits exhibiting smaller amplitudes and older rabbits displaying larger amplitudes [18]. Obtaining IOP values can also be difficult and the method

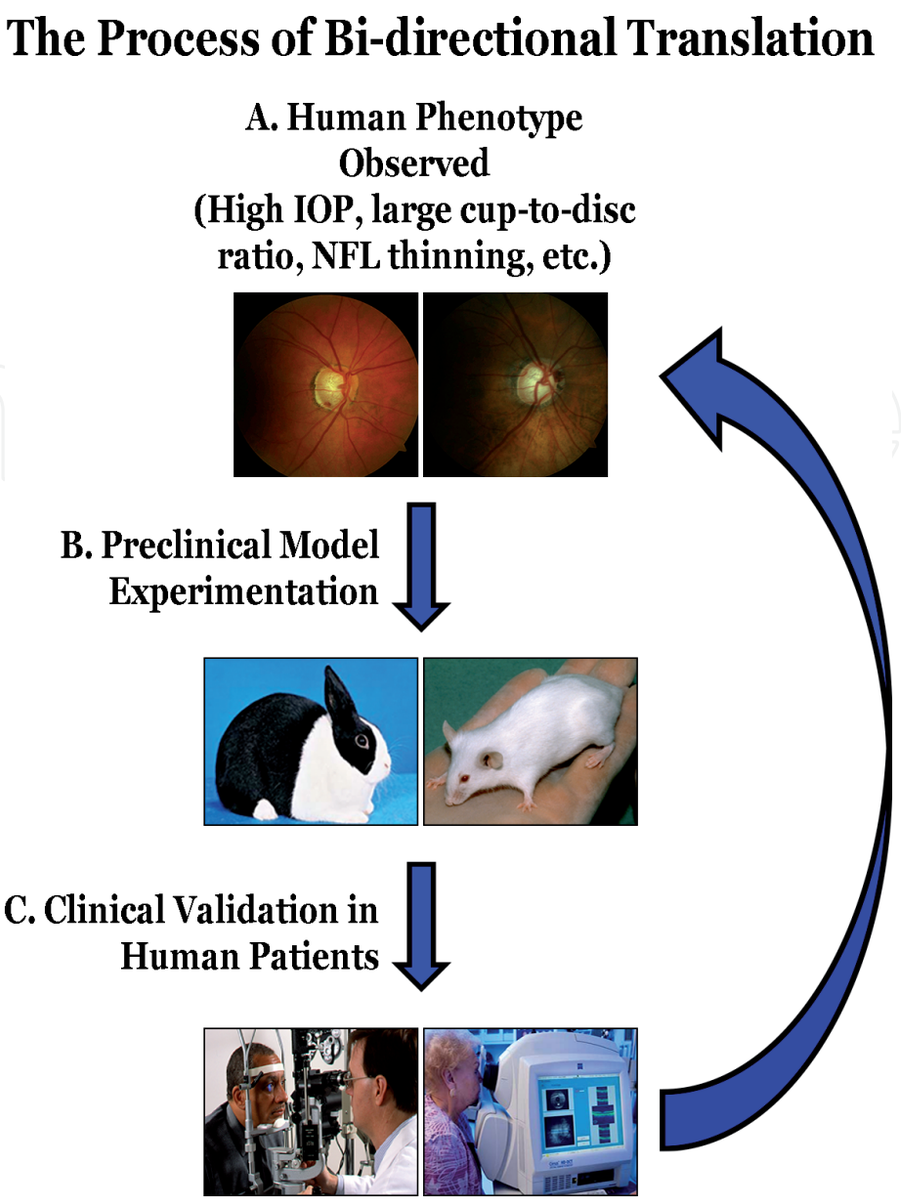

Figure 2.
The progression of bi-directional translation. (A) A disease phenotype is observed in human patients. (B) The observed phenotype is then modeled in a preclinical animal model with as much fidelity to the human condition as possible. (C) After rigorous testing in animal models, the outcomes of those tests (pharmaceutical, or other therapeutic) are then taken back to the human patients and tested for safety and efficacy. If needed, the process repeats. This cycle gives a better comprehension of the disease and how to treat it most effectively.

varies per animal model. In addition, different animal models are susceptible to variations from blood pressure, pulse, respiration, anxiety, as well as sedation and measurement complications [15].

2.1 Naturally occurring models of primary open angle glaucoma (POAG)

2.1.1 Introduction to naturally occurring animal models of POAG

Many genetic loci containing spontaneous polymorphisms have been identified in both human POAG patients and in animals. A few causative genes have been identified such as *MYOC*, *OPTN*, and *WDR36* [11]. Recent studies also postulate RGC number is a risk factor with lower numbers found with gene polymorphisms in *SIX6* (homeobox protein) and *ATOH7* (atonal basic helix–loop–helix transcription factor 7), both of which are associated with worse outcomes [19–22].

2.1.2 POAG in monkeys

Low and high tension POAG was first described in monkeys in 1993. It was found to be maternally inherited, and greater than 40% of the models showed elevated IOP. They displayed loss of RGCs, degeneration of the optic nerve, and damage to the retinal peripheral field. Though while these models are a closer mimic to the human condition, they have strong drawbacks including excessive expense, care, and more complicated handling procedures [15, 23–25].

2.1.3 POAG in dogs

An autosomal recessive POAG has been studied in beagles. Elevation of IOP was seen at 1–2 years of age due to a reduction in aqueous humor outflow. The disease was biphasic: early in the disease the iridiocorneal angle was open, followed by closure with lens subluxation and displacement from the anterior vitreous patellar fossa. The model was used in a genome wide single nucleotide polymorphism array study in which the metallopeptidase *Adamts10*, a candidate gene for POAG, was identified. One noteworthy anatomical difference between dogs and humans is that dogs have an intrascleral plexus rather than a Schlemm's canal, which may result in minor discrepancies between the two models [11, 15, 26, 27].

2.1.4 POAG in mini-pigs

Mini-pig models have also been used as their retina shows likeness to the human retina with holangiotic retinal vasculature, cone photoreceptors in the external retina, a similar scleral thickness, and three types of RGCs [28, 29]. This model can reproduce IOP elevation and can be used to study ocular regeneration with stem cells. Another benefit of this model is its suitability for OCT, corneal topography imaging, and ERG analyses [30].

2.1.5 POAG in birds

Avian models of POAG via light induction technique have been described. They have shown response to antiglaucoma drugs also, which could be beneficial in drug trials targeting IOP [11].

2.1.6 POAG in rodents

Rodent models are valued for their ease of handling and lower management costs. They also have a relatively comparable genome to humans. Drawbacks of mouse models include the lack of a collagenous lamina cribrosa, although it is replaced by one composed of astrocytes. Because the lamina cribrosa is a major location of the pathology that causes optic nerve damage, species differences must be taken into account [11].

Presently, studies have shown that the mutated human myocilin gene, *MYOC Tyr437His* mutation, causes autosomal dominant severe glaucoma with juvenile onset. Mice with a mutation in this same gene develop elevated IOP and 20% RGC loss at 18 months, as well as axonal degeneration in the optic nerve and detachment of the endothelial cells of the trabecular meshwork [31]. A *Col1a1* (alpha-1 subunit of collagen type 1) mutation abrogates matrix metalloproteinase-related cleavage, necessary for turnover of trabecular meshwork, which leads to aggregation and thus increased IOP and RGC death at 24 weeks. This finding infers a correlation between IOP regulation and the turnover of fibrillar collagen in the trabecular meshwork [32].

2.2 Naturally occurring models of PACG

Spontaneous mutations in *B10-Sh3pxd2b*^{nee}, a rare mutation seen in Frank-ter Haar Syndrome, results in glaucoma alongside skeletal and cardiovascular abnormalities. The formation of podosomes, which degrade the extracellular matrix, is impaired, causing the proliferation of the trabecular meshwork and development of iridiocorneal adhesions due to outflow blockage and high IOP resulting in RGC loss by 3–4 months in mice harboring the mutation [5]. Spontaneous mutations in another gene, the serine protease *Prss56*, mimic angle-closure glaucoma as the ocular axial length is reduced, the lens is large, and thus the angle is narrow [33].

2.3 Primary congenital glaucoma

Congenital glaucoma types include autosomal recessive mutations in *CYP1B1* (cytochrome P450 family 1 subfamily B polypeptide 1) and *LTBP2* (Latent-transforming growth factor beta-binding protein), and autosomal dominant mutations in *MYOC* (myocilin), *OPTN* (optineurin), and *WDR36* (WD repeat-containing protein 36) [5]. Another study adds that the transcription factors FOXC1 (Forkhead box C1), FOXC2 (Forkhead box C2), PITX2 (Paired Like Homeodomain 2), LMX1B (LIM homeobox transcription factor 1 beta), and PAX6 (Paired box protein) contribute to congenital glaucoma [34].

2.3.1 Congenital PACG in rabbits, rats, and cats

Naturally occurring congenital glaucoma was first observed in rabbits in 1886 [11]. It was also documented in albino New Zealand white rabbits in the 1960s that presented with anomalies in the anterior chamber [35–37]. In rats, spontaneous congenital glaucoma was seen in 1926 in an inbred family of Wistar-Albino-Glaxo rats [38]. This population presents with enlargement of the globe, elevated IOP, decreased number of RGCs, and degeneration of the optic nerve head. It has also been used in other studies [11]. Feline glaucoma has also been observed with buphthalmia and similar phenotypes to human primary congenital glaucoma, though its occurrence is rare [39].

2.3.2 Congenital PACG in dogs

Evangelho *et al*. document that the anatomy of dogs suits the development of angle closure because they have reduced ocular axial length, an enlarged lens, and a narrow angle [15]. Congenital PACG has been observed in dogs, but it is very rare, and has not been used widely for studies of this subtype of glaucoma [11, 40].

2.3.3 Congenital PACG in turkeys

In turkeys, secondary angle closure glaucoma has been observed, presenting with buphthalmia, low-grade aqueous cells and flare associated with posterior synechiae formation, resulting in pupillary block and iris bombe [41]. It provides a functional model for angle closure glaucoma, but it is also rare.

2.4 Pigmentary dispersion glaucoma

Pigmentary dispersion glaucoma, first described in the DBA/2 J mouse in 1978 presents with a continual increase of IOP until approximately 9 months coupled with early onset iris depigmentation. It is caused by spontaneous mutations in

tyrosinase-related protein 1 (*Tyrp1*^{b}) and glycosylated protein nmb (*Gpnmb*^{R150X}) [15, 42]. Co-existing mutations in these two genes, leads to pigment dispersion, iris atrophy, anterior synechiae, and increased IOP, as well as loss of RGCs and optic nerve atrophy that is progressive, even after the IOP returns to lower IOP levels in older DBA/2 J mice [43–45]. Similar to humans, it progresses in severity with increasing age. Although pigmentary dispersion glaucoma can affect humans, it is not linked to mutations in either *Tyrp1* or *Gpnmb*.

2.5 Normotensive glaucoma

Normotensive glaucoma presents with the same characteristics as POAG, but it lacks an elevated IOP above the normal physiological range. Speculation concerning the mechanism includes lack of blood flow to the optic nerve, vascular spasm, and multiple mutations [11]. Recently, some investigators have used the marmoset, a small primate, as an experimental glaucoma model. Its advantages include a high reproduction rate and a short time to sexual maturation (12–18 months), as well as their ease of management and breeding [46]. The transgenic marmoset was reported in 2009 as well. Moreover, in 2019 it was documented that aged marmosets showed glaucomatous retinal and brain degeneration as well as thinning of the lamina cribrosa [46]. They did not have accompanying mutations in glaucoma-associated genes nor elevation of IOP, which suggest a normotensive glaucoma phenotype. It was noted that there was increased oxidative stress and reduced brain-derived neurotrophic factor levels, which is neuroprotective for RGCs and also reduced in human glaucoma patients [46].

3. Experimentally induced models of glaucoma

To aid in the study of glaucoma-associated optic nerve damage, models have been created by directly damaging RGC axons or indirectly through elevation of IOP. The optic nerve can be crushed with self-closing fine forceps to cause temporally synchronous injury to the RGC axons. This experimental model is useful to study the effects of RGC damage, but does not replicate the spontaneous, continuous development seen in acute or chronic glaucoma. Kimura *et al*. advocate for experimentally inducible models due to the fact that wild-type animals can be used and the experimental condition can be meticulously monitored, as well as the progression of disease [46].

3.1 POAG models

IOP can be elevated through direct or indirect blockage of the trabecular meshwork via injection of hypertonic saline into episcleral veins, cauterization of the episcleral veins, or photocoagulation with an argon laser, which all block outflow and induce an elevation of IOP [20].

3.1.1 Laser photocoagulation

Laser photocoagulation has been used as an experimental model in non-human primates. This technique damages the trabecular meshwork, which blocks outflow of aqueous humor and elevates IOP [47]. It requires three lasers in a 7-day interval to increase IOP by 60%. This method is expensive and can cause anterior peripheral synechiae, hyphema, and corneal edema. In animals subjected to this procedure, it caused loss of RGCs and thinning of the nerve fiber layer, both phenotypes

associated with glaucomatous damage in humans [17, 48]. An argon laser with the slit lamp can also be used, but only for certain strains of rodents because it can cause pigment affinity and variation in elevation of IOP [49, 50]. If laser photocoagulation is combined with temporary narrowing of the iridiocorneal angle via paracentesis, this results in a sudden, 3-week increase in IOP that is not reflective of glaucoma progression in humans, although more acute changes can be evaluated. It can also cause ischemia and opacity of the central cornea [51]. To create a more chronic elevation of IOP condition, 50 microliters of hypertonic saline solution can be injected into the collecting veins of a rat eye to cause sclerotic damage to the trabecular meshwork and optic nerve. This is accomplished with specialized microneedles and a complex procedure both difficult to execute and to teach. A moderate increase in IOP is seen 7–10 days after the procedure and is recommended for use in studies exploring neuroprotection [52]. In addition, a nylon suture can be used to occlude the vein and cause an increase in IOP [15, 53].

3.1.2 Glaucoma induced by modifying the trabecular meshwork

Topical application or injection of steroids as IOP modulators has also been studied in a variety of animals as a mechanism to elevate IOP and mimic POAG [20]. Its mechanism of action is not fully delineated but is believed to function by stabilization of the lysosomal membrane and the accumulation of glycosaminoglycans polymerized in the trabecular meshwork, which creates resistance to outflow of aqueous humor. Outflow resistance is further increased by elevated expression of fibronectin, elastin, and laminin, which also hinder outflow [15]. Glaucoma induced via this mechanism has been studied in a New Zealand rabbit breed [54], which demonstrated that the angle of the anterior chamber is an important regulatory mechanism of outflow of aqueous humor. It has been used to further understand the composition of the trabecular meshwork, as well as identifying its components such as glycosaminoglycans, hyaluronic acid, keratan sulfate, heparan sulfate, and sulfate-chondroitin [55, 56].

Based on the increased presence of hyaluronic acid in the trabecular meshwork induced by steroids, it was demonstrated that a single application of 1% hyaluronic acid in rat models induced an elevation of IOP for up to 8 days, proving a useful acute model [15]. Though not well understood, this injection causes an accumulation of glycosaminoglycans in the trabecular meshwork, which obstructs outflow. This model is noteworthy for its low cost, ease of execution, and sustainability as it induces a more chronic hypertensive state and could be used for pharmacological studies [15, 57, 58]. As a candidate for animal models, rats have similar benefits to mice. There is a comparable elevation of IOP and corresponding optic nerve changes as seen in humans [11]. As such, a second steroidal approach involves topical dexamethasone used in rats to elevate IOP and study the regulation of myocilin, previously identified as a causative gene of POAG in humans. Though the model demonstrated IOP elevation, mRNA levels of myocilin in the trabecular meshwork and in proximity to Schlemm's canal were not different from the control [59].

3.1.3 Reperfusion ischemia

An additional model to mimic open angle glaucoma is via induction of reperfusion ischemia by paracentesis in the anterior chamber. In this method, the cannulation of the anterior chamber with a microneedle allows precise control of the IOP and suppression of blood flow through the retinal and uveal vasculature. This causes IOP-induced damage to the RGCs [15, 47].

3.2 PACG models

Microspheres have been injected to block the trabecular meshwork and emulate PACG. The microspheres do not block the pupil, which allows observation of the fundus, which makes this a suitable model. However, fluctuations in IOP can limit the efficacy as well as location of the microspheres. Yet, elevation of nitric oxide in the aqueous humor was detected in neovascular and angle closure subtypes, thought to be due to the inflammatory mechanism [15, 60]. Bouhenni *et al*. also writes that there are various rat, mice, and rabbit models of PACG to study the effects of elevated IOP on RGCs and the optic nerve. These models have been created with techniques such as hypertonic saline injection into the episcleral veins, cauterization or ligation of the episcleral veins, or laser photocoagulation as discussed prior [11].

4. Genetically modified models

Genetically modified models allow for a careful examination of the influence of typically a single gene mutation on the onset of elevated IOP, RGC damage, and the progression of glaucoma. They allow for manipulation of loci that contribute to this multifactorial mechanism but require a longer timeframe of study than mechanically induced models [15]. An ideal model should be easily reproducible, economical, and closely emulate human pathology [15]. Of all the species, the mouse is a very common model due to its low cost, ease of breeding and the vast genetic resources that are available. Examples of rodent glaucoma models include but are not limited to the following: intraocular injection of RGC toxins; crush or transection of the ON; manual elevation of IOP using microneedle injection into the anterior chamber; laser photocoagulation of the trabecular meshwork or episcleral veins; transgenic mice with specific mutations (reviewed in [11, 61]); and bead injection into the anterior chamber. While all of these methods have merit, none of them completely mimics the array of human disease presentation, and therefore additional models of glaucoma are still an unmet need of the vision research community.

4.1 POAG models

Transgenic models have been developed such as the *bug eye* mutant, the *lrp2* (lipoprotein receptor-related protein 2) mutant, and the *wdr36* mutant in zebrafish. The *bug eye* shows RGC death and high IOP and was used to identify *lrp2* as the gene responsible for the endophenotypes of elevated IOP, large eyes, decreased retinal neurons, activation of RGC stress genes, and optic nerve pathology [62–64]. Kimura *et al*. also reports a $P2Y_6$ (pyrimidinergic receptor) knockout (KO) mouse that presents with age-dependent optic nerve and RGC degeneration and impaired visual function due to excess production of aqueous humor [46].

4.2 PACG models

Vav2/Vav3 (guanine nucleotide exchange factors) KO mice mimic human spontaneous development of glaucoma, but there is poor understanding of the pathophysiology. These models develop buphthalmia and the iridiocorneal angle changes before 6 months of age and ultimately chronic angle closure glaucoma occurs [65]. They have additionally been identified as candidate genes for a sub-group of open-angle glaucoma in the Japanese [20]. Bouhenni *et al.* find three notable features of

this model: first, elevated IOP occurs naturally in this model; second, the incidence of this phenotype is high and occurs at a reasonably young age; third, human hypotensive glaucoma treatments have shown IOP-lowering effects in this model [11]. This makes this model useful for targeting molecular mechanisms in developing understanding of the pathophysiology.

4.3 Primary congenital glaucoma models

Knockout of *Foxc1* and *Foxc2*, genes involved in embryonic and ocular development, in mice causes death during the embryonic or neonatal periods [66]. Heterozygous *Foxc1*$^{+/-}$ mice have defects in ocular drainage structures, but do not see elevation of IOP. Moreover, *Cyp1b1* KO animals develop ocular defects comparable to human congenital glaucoma, presenting with rudimentary Schlemm's canal, abnormalities in the trabecular meshwork, adhesion of the iris to the trabecular meshwork and peripheral cornea [15]. Another model was described in an albino quail population with a similar phenotype, but due to small size, limited availability, and difficult clinical translation, it is rarely used [11].

4.4 Normotensive glaucoma models

Genetically modified mice with normal IOP have been used to further study normotensive glaucoma. Specifically, mice lacking the glutamate transporter genes, *Glast* or *Eaac1* (Excitatory amino acid transporter 3), develop RGC damage and optic nerve degeneration without the IOP elevation. *Glast* is expressed in Müller glia in the retina and aids in the removal of glutamate, sparing RGCs from the neuroexcitotoxicity due to its accumulation [46]. *Eaac1* is expressed in neurons and assists with the uptake of both cysteine and glutamate [46]. This evidence supports a protective role of these genes in preventing RGC damage.

A deficiency in apoptosis signal-regulating kinase 1 (*Ask1*) also known as mitogen-activated protein kinase 5 (Map3k5) had no neurotoxic effects or effects on IOP. It was also demonstrated that the activation of p38 MAPK and the production of inducible nitric oxide synthase was suppressed in glial cells and RGCs, implying that ASK1 activation could be involved in normotensive glaucoma [67]. Additionally, this model was used to study the neuroprotective effects of *N*-acetylcysteine, which was demonstrated to be protective in the *Eaac1* KO mice. Kimura *et al*. also added that *Glast* or *Eaac1* mutations produce an early onset phenotype, which increases the utility of this model, particularly in studying efficacy of treatments [46]. This is contrasted with the other models of normotensive glaucoma, which are comprised of overexpression of mutated optineurin E50K and tank-binding protein 1, the mutated genes associated with human normotensive glaucoma [46]. These models have a later onset, which makes them less suitable experimentally. In addition, using marmosets mentioned prior, a model is in the works targeting the *Glast* gene, which is speculated to produce a functional model with early onset glaucoma [46].

4.5 Immune response models

In the Wallerian degeneration slow (W1dS) mouse, transfection with a fusion gene of *Nmnat1* and *Ube4b* protect RGC axons by maintaining mitochondrial function. An influx of calcium was documented prior to RGC axon degeneration [68, 69]. This finding was significant, as it proves there are specific molecular mechanisms that modulate axon degeneration. Struebing *et. al* review other molecular mechanisms of RGC degeneration. For example, knocking out *Bax* promotes survival of

RGC bodies, but does not rescue axons. The JNK (c-Jun, N-terminal kinase) pathway is involved in both axonal and soma degradation as deficiency in both *Jnk2* and *Jnk3* results in protection from RGC death after optic nerve crush. Lastly, knocking out *Dlk* (dual leucine zipper kinase) causes cell death in soma but does not affect degeneration of axons [20, 70–72].

Within an investigation of an autoimmune response as a plausible mechanism of RGC damage, it was determined that serum samples of glaucoma patients have increased levels of heat shock protein 27 (HSP27) and heat shock protein 60 (HSP60). Immunization of these proteins in the Lewis rat population resulted in RGC degeneration and axonal loss 1–4 months later, supporting a role of these proteins in glaucoma [11, 73].

4.6 The BXD murine family of recombinant inbred lines of mice

Because most human glaucoma cases are not due to polymorphisms in single genes, it is not surprising that pre-clinical models that examine the effects of mutations in one gene at a time do not fully recapitulate all endophenotypes of human disease. In contrast, a polygenetic approach may advance the field of glaucoma. One approach to this is the involvement of the BXD murine family. BXD mice are a family of recombinant inbred strains that were derived by mating C57/Bl6 (B6) and DBA/2 J (D2) mice and inbreeding the F2 progeny for >20 generations, allowing for natural recombination of genes throughout the entire genome.

Each fully inbred BXD strain is genetically distinct and fixed at each locus of the genome. This family of mice is an outstanding resource as data on >1000 phenotypes and nearly 100 expression data sets (gene, protein, and metabolites) have been collected and are available on GeneNetwork (www.genenetwork.org), an open access resource for the scientific community. Moreover, all BXD and their parent strains have been fully sequenced. There are ~5.2 million single nucleotide polymorphisms, >400,000 insertions/deletions and multiple copy number variants between the progenitors which segregate among the BXD strains. Multiple data sets of the eye, retina, optic nerve phenotypes and expression data are also available on GeneNetwork to facilitate systems genetics analyses at multiple levels from gene to metabolome [74].

The BXD family has become a valuable resource for modeling human disease, including glaucoma. B6 has no glaucoma-associated pathologies with fairly constant IOP until >13 months of age. The optic nerve has little damage throughout the life of B6. In contrast, D2 parents develop pigmentary dispersion glaucoma and have an elevated IOP that reaches its maximum at ~9 months with a subsequent IOP reduction. ON damage increases throughout the lifetime of D2 mice. The influence of pigmentary dispersion on glaucoma can be studied among the afflicted progeny or be eliminated by excluding those strains that harbor mutations in *Tyrp1* and *Gpnmb* from the study plan. By quantifying specific endophenotypes across the BXD family along with gene expression and polymorphism profiles, new and informative insights regarding novel modulating genes can be revealed. Specific to this pre-clinical murine family, several BXD strains spontaneously develop elevated IOP and/or optic nerve damage due to the particular set of polymorphisms in the genome of each individual strain, similar to the human condition. Importantly, the phenotype of each BXD strain is highly reproducible because each strain is fully inbred with a fixed genome and the supply of mice from each strain is essentially unlimited.

As a testament to the power of the BXD family and the genetic/genomic information gathered from these mice, approximately 20 disease-associated strains have already been cloned from the BXD family [74]. One example of the power of using BXD mice in the virtuous cycle of bi-directional translation is the identification of

the novel IOP-modulating gene, *Cacna2d1*, which has demonstrated potent IOP-lowering effects with therapeutic blocking of the function of its gene product with pregabalin in a dose-dependent and haplotype-specific manner [74].

In other studies, by examining the immune network and the response of the optic nerve to RGC damage, and comparing the two, it was determined that an innate immune network was activated by the optic nerve crush [75]. The importance of the complement cascade or innate immune network as mentioned above, has been demonstrated by knocking out *C1qa* in D2 mice. Both pigmentary dispersion glaucoma and elevated IOP are noted, however, the loss of axons in the optic nerve is lessened, which points to the role of *C1qa* in the degeneration of axons in the optic nerve [76]. A gene network of regulatory mechanisms in the retina that are activated by injury was mapped, and it was determined there are common regulatory mechanisms. In addition, there was a significant quantitative trait locus (QTL) found on chromosome 16 from 80 to 95 Mb that correlates with the expression of *C4b* in the normal retina [77]. In this region, two *cis*-acting QTLs were identified as potential modulatory genes of the innate immunity network, although the modulating gene has not yet been identified [20]. These data suggest possible shared disease mechanisms with age-related macular degeneration (AMD), in which complement proteins are found in the drusen deposits that characterize AMD [45, 78–80].

5. Therapeutics for glaucoma

5.1 Current FDA-approved therapeutics

At baseline, the goal of current glaucoma treatments is to decrease IOP, which is presently the only identified modifiable risk factor. There are a variety of medications that modify IOP by either decreasing production or increasing the outflow of aqueous humor. Factors such as patient compliance, costs, drug penetration, variations in drug metabolism and bioavailability, and even other factors such as systemic diseases, diet, alcohol, etc., all play a role in medical management. Additionally, glaucoma patients require long-term treatment to keep pressures low. Years of treatment can exacerbate ocular surface disease and cause chronic conjunctival inflammation, which may threaten the efficacy of future surgical procedures (such as trabeculectomy). IOP fluctuation can worsen disease progression, seen in the advanced glaucoma interventional study [81].

5.1.1 Modulation of aqueous production

Drugs that decrease the production of aqueous, such as beta blockers and carbonic anhydrase inhibitors, have been the mainstay in glaucoma treatment for years. However, these drugs can have systemic adverse side effects such as bradycardia, impotence, exacerbation of asthma, and also may demonstrate tachyphylaxis over time. The beta blockers, such as timolol, modulate aqueous inflow. The beta-1 receptor increases production of aqueous, and the beta blockers inhibit the production of cyclic AMP by this receptor which reduces aqueous production [13]. Carbonic anhydrase inhibitors, such as brinzolamide, decrease the production of aqueous via suppression of aqueous ducts [13], but can produce a renal metabolic acidosis as they inhibit acid secretion in the proximal tubule of the kidney as well [13]. Other drugs, such as brimonidine, modulate the production of aqueous via the alpha-2 receptor. Adverse effects to this drug can include a delayed hypersensitivity reaction and, due to their ability to cross the blood–brain barrier, they are contraindicated in children under two [13].

5.1.2 Modulation of aqueous humor outflow

Parasympathomimetic drugs, such as pilocarpine, have been used to decrease outflow. They cause contraction of the longitudinal ciliary muscles, which increases aqueous humor outflow. However, due to its three-to-four times daily dosing paradigm, myopic shift, and risk of retinal detachment, along with decreased patient compliance, it is no longer a first-line treatment [13]. Presently, prostaglandin analogs, such as latanoprost (Xalatan), have become the mainstay first-line treatment. They function by increasing outflow via modulation of uveoscleral pores, opening them as a secondary outflow mechanism that decreases outflow resistance. Most recently, Rho kinase inhibitors, such as Rhopressa, inhibit actin/myosin contraction of these muscles and improve trabecular meshwork outflow by decreasing the episcleral venous pressure. In the acute setting, hyperosmotic agents such as glycerol and mannitol can be used to lower the IOP, given intravenously, increasing the tonicity of the plasma and drawing aqueous out of the eye [13].

5.1.3 Sustained-release devices

To combat challenges of drug delivery, different models of sustained release implants have been developed. One intracameral implant called Durysta is an FDA-approved device that delivers bimatoprost into the anterior chamber over a period of four months [82]. Unfortunately, complications of Durysta use include chronic inflammation and corneal endothelial cell loss.

Other sustained-release implants in development include intracanalicular devices, subconjunctival injections, and collagen shields [83]. Examples include latanoprost micro-dose delivery with Optejet (Microprost, Eyenovia), latanoprost delivery via an intracanalicular insert (OTX-TIC, Ocular Therapeutix), and latanoprost or travoprost delivery via a punctal plug (L-evolute and T-evolute, Mati Therapeutics). The latter includes two travoprost delivery platforms, ENV515 (Envisia Therapeutics) and iDose (Glaukos) [83–85]. iDose is a titanium implant that releases a unique formulation of travoprost into the anterior chamber [84]. It releases micro-amounts of the drug over a year and eliminates the barrier of the cornea. It is composed of three parts that titrates travoprost release [84, 86]. It was reported in a randomized, double-blind phase II trial, iDose achieved a 30% reduction in IOP at 12 months, lowering the average number of glaucoma medications per patient compared to the control group of 0.5% timolol, demonstrating non-inferiority with minimal adverse effects [84]. At each stage of development of these FDA-approved therapeutics, multiple pre-clinical models were used to demonstrate safety and efficacy, making their inclusion in the drug pipeline invaluable.

5.2 Therapeutics in development

Treatments that mitigate risk factors other than IOP are an identified gap in treatment modalities [46]. These are especially needed for populations that do not respond to current IOP-lowering medications, when a lowered IOP is insufficient to halt visual field loss, and in normotensive glaucoma cases. A particular need exists for the identification of neuroprotective molecules that act directly on the optic nerve and RGC axons. Although several therapies are being evaluated in multiple laboratories across the world, there remains no FDA-approved neuroprotective option for glaucoma patients. In line with this treatment strategy, Kimura *et al*. disclosed treatment trials exploring the delivery of ciliary neurotrophic factor into the eye via modified human cells that secrete it. They postulate this mechanism has potential for neuroprotective effects in the treatment of normotensive glaucoma [46]. In addition,

on the progressive end of molecular modifying, it may be possible to use stem cell therapy with CRISPR-Cas 9 technology to neutralize mutations causing glaucoma phenotypes. This capability is currently being explored [15], but will take thoughtful risk/benefit analysis.

Approaching treatment from a different angle, a unique tool to modulate IOP is being explored. The Mercury Multi-Pressure Dial (Equinox) is a pair of pressurized goggles that create a pressure vacuum around the eye, which decreases pressure from gravity and increases ocular blood flow [87]. They operate on the principal that glaucoma is likely a dual-pressure disease due to the balance between IOP and intracranial pressure (ICP) [87]. Thus, the inventors of this device postulate that glaucoma is a result of high IOP and low ICP. By applying a moderate negative pressure in the front of the eye with their goggles, the pressure inside the eye is reduced and the ICP is restored to a physiological level [87]. Moreover, it serves as a point to demonstrate that one direction glaucoma treatment might be headed is 24/7 modulation of pressure where sensors monitor pressure on-the-go, allowing physicians access to IOP data without requiring an office visit and creating a practical opportunity for a telemedicine consultation.

Experimental models of glaucoma are crucial to understanding the mechanism of disease and designing novel treatments. Assessing their axonal degeneration in optic nerve cross sections has proven beneficial in this pursuit. Specifically, the BXD genetic reference panel will be used to detect and study spontaneous models of glaucoma. This will be invaluable to the scientific community, especially in an effort to develop novel treatment options. While the development of these novel modalities takes time, animal models can be used to explore the benefit of existing drugs, a method known as drug repositioning or repurposing [67]. Neuroprotection is one particular facet of interest, and it is postulated available drug modalities for diseases of the central nervous system such as Alzheimer's or Parkinson's might also exhibit neuroprotection in glaucoma patients [88]. Examples of these trials include memantine, valproic acid, edaravone, and niacin (vitamin B3). Unfortunately, memantine was ruled ineffective in 2018 [89]. Valproic acid was demonstrated to be neuroprotective in rodent models of normotensive glaucoma, and one study from India showed improvement of advanced glaucoma cases [90, 91]. Edaravone was also shown to decrease RGC death in mouse models of normotensive glaucoma [92]. Lastly, oral niacin was demonstrated to be protective in mouse models [93]. There is also interest in the potential of dairy products being neuroprotective due to their levels of spermidine, which is a compound that has demonstrated neuroprotection in mouse models [67]. Additionally, using rodent models, Chintalapudi *et al*. have demonstrated IOP-lowering effects of pregabalin, which inhibits calcium channels containing the CACNA2D1 subunit. It is mainly used for neuropathic pain but was developed initially as an antiepileptic [74, 94]. Animal models have clearly demonstrated their necessity in testing for current and future pharmacological treatments.

6. Bidirectional translation from humans to pre-clinical models and back again

The virtuous cycle of bidirectional translation works through the observation of disease phenotypes and processes in human patients, followed by replication and examination of the specific observation in normal and pathological pre-clinical models to discover novel modulators of complex disease mechanisms, and ultimately test those outcomes back in human patients. This process should be repeated until a safe and effective treatment paradigm is obtained or the disease process of better understood. This strategy clearly supports the hypothesis that breakthroughs

in human and experimental models fuel a sustainable model of human observation, pre-clinical model experimentation, and verification in humans. As direct support for this strategy, as discussed above, with the known observation that elevated IOP is the chief modifiable risk factor for POAG, Chintalapudi *et al.* used strains from the BXD family of mice and identified a novel gene modulator of IOP—calcium voltage-gated channel auxiliary subunit alpha2delta 1 (*Cacna2d1*)— that was confirmed in human GWAS investigations [74]. These findings were the platform that allowed for the discovery of pregabalin, an inhibitor of CACNA2D1, as a new IOP-lowering drug with a novel mechanism of action [74]. This, as well as the variety of molecular models discussed above, demonstrates the efficacy of bidirectional translation in driving the diagnosis, treatment, and prevention of complex diseases [95].

The collective generation of large datasets like GWAS have facilitated the identification of gene modulators of complex traits and disease mechanisms. Those tools alone, however, do not account for the complex interplay between inherited and environmental factors. Human limitations are clear; animal models are equipped with the biological resources to support the detailed analyses of disease progression. Pre-clinical models reveal instrumental pathophysiology, which when translated to humans, fuels the evolution of molecular, cellular, developmental, and physiological research [95]. However, glaucoma is a multifaceted disease, which one animal model alone cannot flawlessly emulate. Different animal models display unique features of the pathophysiology of the disease and each help further understanding of glaucoma progression as a whole.

7. Conclusion

In conclusion, the future of glaucoma treatment is in understanding disease mechanisms of glaucoma viewed with an interventional mindset. Increasingly, the safety and efficacy of drug delivery allows better control of IOP. Medical management shortly seeks to improve sustainability through products like Durysta and iDose. Innovation of medical management is in progress with formulations of *N*-acetylcysteine or ciliary neurotrophic factor, as mentioned. It is speculated that glaucoma is headed in a direction of earlier detection, earlier intervention, and chronic modulation, potentially even with tools to adequately assess pressure from home (even 24/7), and to address it with telehealth resources. Technology may permit detection of resistance sites, which allows specification of procedures specific to the individual patient. Largely, it is evident that there is a gap in the treatment of glaucoma with non-IOP-related modalities. This supports the need for animal models in developing further understanding of the pathophysiology of glaucoma, but also in studying the efficacy of present treatment mechanisms (such as IOP-lowering) as well as novel ones (*N*-acetylcysteine, ciliary neurotrophic factor, molecular mapping with animal models, CRISPR-CAS9).

Something to consider with the current information-driven culture, are mechanisms to objectively quantify the degree of pathophysiology present in these animal models, particularly when they are used to examine the efficacy of treatment modalities. Currently, a particular BXD strain which spontaneously develops glaucoma is being used to observe optic nerve pathology (including axonal degeneration and glial scarring) and objectively quantify glaucomatous degeneration. This, as well as the potential of an automated system to carry out this task, has the ability to revolutionize this field. A deep neural network is being developed to reliably assess the progression of axonal degeneration in glaucoma. It provides an opportunity to automate this process to better understand the mechanism of

glaucoma—etiologically and clinically—and to assess the efficacy of treatment trials. By using a uniform stratification of disease progression, large volume studies can be conducted with greater precision, efficiency, and less bias. This will allow for exponential growth in the understanding and treatment of glaucoma as the knowledge of pathophysiology is enhanced. It showcases the use of artificial intelligence to autonomously convert subjective data to an objective form of precise stratification.

To summarize, this chapter makes one thing clear. The "virtuous cycle" of bidirectional translation is essential to further understanding of the pathophysiology of glaucoma. It is the common tool necessary to further observe disease advancement, design more molecular models, identify disease modulators, and discover new therapeutics. Moreover, this chapter reveals that pre-clinical models illuminate this path forward.

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Conflict of interest

The authors have no conflicts of interest.

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