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Using Genetic Mouse Models to Gain Insight into Glaucoma: Past Results and Future Possibilities

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Abstract

While all forms of glaucoma are characterized by a specific pattern of retinal ganglion cell death, they are clinically divided into several distinct subclasses, including normal tension glaucoma, primary open angle glaucoma, congenital glaucoma, and secondary glaucoma. For each type of glaucoma there are likely numerous molecular pathways that control susceptibility to the disease. Given this complexity, a single animal model will never precisely model all aspects of all the different types of human glaucoma. Therefore, multiple animal models have been utilized to study glaucoma but more are needed. Because of the powerful genetic tools available to use in the laboratory mouse, it has proven to be a highly useful mammalian system for studying the pathophysiology of human disease. The similarity between human and mouse eyes coupled with the ability to use a combination of advanced cell biological and genetic tools in mice have led to a large increase in the number of studies using mice to model specific glaucoma phenotypes. Over the last decade, numerous new mouse models and genetic tools have emerged, providing important insight into the cell biology and genetics of glaucoma. In this review, we describe available mouse genetic models that can be used to study glaucoma-relevant disease/pathobiology.

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Furthermore, we discuss how these models have been used to gain insights into ocular hypertension (a major risk factor for glaucoma) and glaucomatous retinal ganglion cell death. Finally, the potential for developing new mouse models and using advanced genetic tools and resources for studying glaucoma are discussed.

Keywords

neuroinflammation; IOP; axonal degeneration; trabecular meshwork; mouse genetics; genomics; neurodegeneration; DBA/2J

1. Introduction

Glaucoma is defined and unified by a distinct pattern of retinal ganglion cell (RGC) death. However, glaucoma is a heterogeneous disorder (often termed ‘glaucomas’) with RGCs likely insulted in multiple ways. Even within a patient, different RGCs may experience different stresses that trigger or contribute to cell loss (Casson et al., 2012). Age, elevated intraocular pressure (IOP; or ocular hypertension), and family history are major risk factors for glaucoma. IOP regulation is in itself complex and in most cases where IOP is a factor in glaucoma, it is not known why it becomes pathologically elevated. Given this complexity, multiple models of glaucoma are required to fully understand the disease. A single animal model can never precisely model all aspects of all human glaucomas, and modeling specific aspects of the cell biology and/or the genetics of human glaucoma requires careful consideration. The choice of the animal model should be made based on the precise glaucoma-relevant phenotype(s) being assessed. With this perspective, mice are a powerful tool to unlock the genetic causes, susceptibility factors, and physiological pathways underlying human glaucoma. The similarity between human and mouse eyes, coupled with the ability to use a combination of advanced cell biological and genetic tools in mice, make the mouse an excellent system for unraveling the molecular pathways that control glaucoma pathophysiology (see Figure 1 and 2 for overview).

Several genes that cause glaucoma-relevant phenotypes have recently been identified in mice. Moreover, mouse models with mutations in known human glaucoma genes have been used to study the genetic underpinnings of glaucoma pathobiology. These models provide insight into (i) the cell biology underlying specific phenotypes of glaucoma, (ii) the physiology of glaucoma-relevant tissues, (iii) the pathophysiological response of cells subjected to glaucoma-relevant insults, and (iv) the molecular genetics of glaucoma-relevant phenotypes and endophenotypes. As there are many in depth reviews about mouse models of glaucoma (e.g. Libby et al., 2005b; Lindsey and Weinreb, 2005; Morrison et al., 2011) and about how mouse genetics can be used to study physiology and pathophysiology (Ermann and Glimcher, 2012; Justice et al., 2011; Schofield et al., 2012; van der Weyden et al., 2011), in this review we discuss the strengths and the utility of using genetic mouse models of glaucoma to gain insights into the cell biology and genetics underlying glaucoma-relevant phenotypes (**Section 2**), key aspects of anterior segment disease (**Section 3**) and retinal ganglion cell death (**Section 4**) that have been elucidated through the use of genetic models. Furthermore, we highlight ways that the mouse can be used to address unanswered

questions about glaucoma and how it can be used in the future to help unravel the complexities of human glaucoma.

2. Strengths and utility of mice to model glaucoma-relevant phenotypes

In order to study a disease-relevant phenotype, it is ideal, though not always possible, to have characteristics of the phenotype carefully defined in both humans and the animal model. This is especially critical when studying a heterogeneous disease like glaucoma in which multiple distinct pathological insults are observed in several ocular tissues. Mouse models have in the past both improved our understanding of human disease and have proved to be an invaluable tool for discovering therapeutic targets. Overall, current knowledge of the physiology of the anterior segment, retina, and optic nerve in humans and mice strongly support the utility of the mouse to model key phenotypes associated with glaucoma. However, as our understanding of the key molecular pathogenic events in human glaucoma continues to grow, animal models will need to be refined to include specific aspects of human glaucoma as new information becomes available.

2.1. Glaucoma relevance of the mouse anterior segment

Over the last decade, many studies have found that mouse models can be used to examine glaucoma-relevant aspects of anterior segment physiology. Mouse and human both have similar cyclical IOP patterns (Aihara et al., 2003; Li and Liu, 2008; Liu et al., 2003; Mosaed et al., 2005; Savinova et al., 2001). Also, similar mutations lead to anterior segment disease in both mice and humans (see below). Most importantly, in terms of IOP regulation, key physiological parameters are similar between mice and humans, including: similar mechanisms regulating aqueous humor production and outflow; similar response to IOP lowering drugs; and no detectable washout rate in either species (e.g. Aihara et al., 2003; Boussommier-Calleja et al., 2012; Lei et al., 2011; Lindsey and Weinreb, 2002). Thus, physiological measurements show that the mouse is a good model system for studying glaucoma-relevant anterior segment physiology and pathophysiology. The mouse does however, have a proportionately larger lens than humans and correspondingly, the shape of the anterior chamber is different in mice (Schmucker and Schaeffel, 2004). With respect to glaucoma, consequences of this variation have not been studied in depth, but would likely change the presentation of clinical features associated with secondary forms of glaucoma, including iris-lens rubbing (pigment dispersion syndrome) and lenticular accumulations of exfoliative material (exfoliation syndrome). Overall, the basic physiology and anatomy of glaucoma relevant structures in the mouse and human anterior segment are very similar.

2.2. Glaucoma relevance of the mouse retina and optic nerve head

The utility of the mouse as a model for studying ocular hypertension-induced RGC degeneration has been questioned in the past primarily because of differences in the morphology and structure of the optic nerve head. The mouse optic nerve head does not have collagen beams and instead contains pores created by optic nerve head astrocytes (this has been termed a glial lamina; Howell et al., 2007). Because the optic nerve head is a key site in ocular hypertensive glaucoma, it is reasonable to assume that differences in optic nerve head morphology may limit the utility of the mouse in modeling ocular hypertension-

induced optic neuropathy. However, over the last decade, findings from several groups have validated the usefulness of the mouse as a model for ocular hypertension-induced RGC death. Mouse models exhibit many hallmarks thought to characterize human glaucoma, including segmented axon loss, a defined insult to RGC axons at the optic nerve head, early loss of axonal transport, ocular hypertension-induced abnormalities in RGC physiology, specific loss of RGCs, and reduced glaucomatous damage by interventions that lower IOP (Buckingham et al., 2008; Filippopoulos et al., 2006; Howell et al., 2007; Jakobs et al., 2005; Nagaraju et al., 2007; Schlamp et al., 2006; Schuettauf et al., 2002; Wong and Brown, 2013). Furthermore, many molecular pathways shown to be important in human glaucoma through gene expression studies have also been reported in mouse models of ocular hypertension-induced RGC death. Thus, ocular hypertensive mouse models recapitulate key aspects of human glaucoma.

3. Glaucoma-relevant anterior segment disease

Over the last several decades, technical advances have greatly increased the usefulness of the mouse for studying glaucoma-relevant anterior segment physiology. Intraocular pressure and aqueous outflow can now be reliably measured in the mouse (Aihara et al., 2003; Avila et al., 2001; Boussommier-Calleja and Overby, 2013; Cohan and Bohr, 2001; John et al., 1997; Lei et al., 2011; Wang et al., 2005). Also, many imaging technologies used to assess human anterior segment pathology are used in mice, including OCT imaging (Li et al., 2014; Nair et al., 2011), and gonioscopy (Smith et al., 2002; Trantow et al., 2009). Advanced mouse genetic and cell biological tools are also being employed to study anterior segment biology. For example, several groups recently used genetically altered mice (conditional alleles and reporter alleles) to study Schlemm's canal development (Aspelund et al., 2014; Kizhatil et al., 2014; Park et al., 2014; Thomson et al., 2014). Mouse strains also differ in IOP level, outflow rate, susceptibility to steroid-induced ocular hypertension, and susceptibility to ocular hypertension due to other anterior segment diseases. Standard mouse genetic tools can be used to identify the genes responsible for the differences between mouse strains regarding these important glaucoma-relevant phenotypes (Anderson et al., 2006; Boussommier-Calleja and Overby, 2013; John et al., 1997; Savinova et al., 2001; Whitlock et al., 2010). Identifying these genes will likely provide fundamental insight into IOP regulation, opening up new areas of investigation and models for studying how IOP becomes dysregulated. Below, we describe currently available mouse genetic models that are being used by many groups to understand anterior segment development and disease.

3.1 Ocular Hypertensive Mice

3.1.1 DBA/2J mice—DBA/2J mice develop a pigment dispersing iris disease that leads to elevated IOP and RGC loss (Chang et al., 1999; John et al., 1998; Libby et al., 2005a). Mutations in two genes, *Gpnmb* and *Tyrp1* (the mutant alleles are $Gpnmb^{R150X}$ and $Tyrp1^{b}$ respectively) cause an iris disease that has two main components: iris pigment dispersion and iris stromal atrophy. The iris pigment dispersion phenotype resembles pigment dispersion syndrome (PDS) in humans (Anderson et al., 2002). The iris disease and subsequent IOP elevation can be mitigated by reconstituting the bone marrow of DBA/2J mice with bone marrow from DBA/2J-$Gpnmb^{+}$ mice (Anderson et al., 2008; Mo et al.,

2003). Since bone marrow contains progenitor cells of the immune system, it has been suggested that the *Gpnmb*^{R150X} bone marrow-derived cell lineages of standard DBA/2J mice contribute to an abnormal inflammatory response resulting in pigment dispersion and subsequent elevation in IOP (Anderson et al., 2008; Mo et al., 2003). Adaptive immune components (T and B cell mediated) have been ruled out in the propagation of the iris disease (Anderson et al., 2008) and it is likely driven by innate immune responses (Nair et al., 2014). Together, these data highlight the need to further study how ocular immune processes and melanosome function can contribute to glaucoma-relevant anterior segment disease.

DBA/2J mice are also an important resource to study the genetics of ocular hypertension. C57BL/6J mice that are homozygous for the *Gpnmb*^{R150X} and *Tyrp1*^{b} mutations develop a DBA/2J-like iris disease with severe dispersion of iris pigment (Anderson et al., 2006). However, these mice are much less likely to develop ocular hypertension. These results indicate that DBA/2J mice have a greater susceptibility to IOP elevation than C57BL/6J mice. Thus, genetic factors could be key mediators for how the iridocorneal angle responds to dispersed pigment in pigmentary glaucoma and other ocular hypertensive diseases. The differential response of C57BL/6J and DBA/2J mice to pigment dispersion is similar to the differing responses of people with PDS, where only some individuals with PDS develop ocular hypertension (Siddiqui et al., 2003). The discovery of molecules or genetic pathways that segregate between DBA/2J and C57BL/6J may hold clues about human glaucoma pathophysiology and the genetic susceptibility factors that cause/modulate glaucoma in heterogeneous populations.

3.1.2 Myocilin mutant mice—Mutations in the myocilin gene (*MYOC*) cause ocular hypertension and glaucoma (Menaa et al., 2011; Stone et al., 1997). In humans and mice, MYOC is expressed in numerous ocular tissues, most notably in trabecular meshwork (TM) cells (for a review see; Resch and Fautsch, 2009). In cultured human TM cells, *MYOC* is induced by glucocorticoids, oxidative stress, cell stress signaling pathways, and potentially other unidentified factors in the aqueous humor (Fautsch et al., 2005; Polansky et al., 1997; Tamm et al., 1999). TM cells normally secrete MYOC, however mutations in *MYOC* can prevent secretion of mutant and wild type MYOC (Jacobson et al., 2001). These data and other studies raised important questions about whether myocilin had a normal function in IOP regulation and how its level and/or location of expression contribute to ocular hypertension. In a series of experiments, several groups have used mouse genetics to gain a better understanding of the normal and pathological functions of myocilin.

The first generation of genetic models manipulated levels of the mouse *Myoc* gene. Null mutations in mice do not result in ocular hypertension, RGC loss, or other gross ocular abnormalities (Kim et al., 2001). There is a similar absence of glaucoma-related phenotypes observed when *Myoc* is overexpressed in the mouse at levels comparable to the elevated MYOC expression seen in humans with steroid-induced IOP elevation (Gould et al., 2004a). These results helped explain genetic studies of glaucoma in human patients; in humans, a likely null mutation has been identified that is not associated with glaucoma (Pang et al., 2002). Conversely, >90% of glaucoma-related mutations are in a well-conserved functional domain of MYOC, the olfactamedin domain, and likely affect MYOC structure (Resch and

Fautsch, 2009). The results gained from genetic models indicated that MYOC does not have a significant function in normal IOP regulation and that mutant MYOC proteins with abnormal function (gain of function) were likely causing ocular hypertension.

To determine how mutant *MYOC* leads to glaucoma, several groups began testing disease-specific alleles in mice. Gould et al. (Gould et al., 2006) and Senatorov et al. (Senatorov et al., 2006) focused on the Y437H allele, a mutation that causes a severe form of juvenile open angle glaucoma. Results varied, but generally, expression of mouse *Myoc* with a mutation orthologous to Y437H affected secretion of both mutant and wild type *Myoc* without causing either substantially elevated IOP or RGC loss in young or aged mice across multiple mouse strains. Expression of human *MYOC* and $MYOC^{Y437H}$ in the mouse lens also did not cause IOP elevation or RGC loss (Zillig et al., 2005). An important finding was made when Shepard et al. used an adenovirus to express human $MYOC^{Y437H}$ in the iridocorneal angle and saw those eyes develop high IOP (Shepard et al., 2007). Human myocilin contains a targeting signal for peroxisomes that does not exist in mouse myocilin (Shepard et al., 2007). This domain appears to be critical for mutant myocilin-induced toxicity in trabecular meshwork cells. Subsequently, three groups have reported IOP elevation using different strategies for expressing human $MYOC^{Y437H}$ in the mouse eye (McDowell et al., 2012; Zhou et al., 2008; Zode et al., 2011). Most recently, transgenic mice were generated that express $MYOC^{Y437H}$ under control of a CMV promoter in the iridocorneal angle and sclera (Zode et al., 2011). *Tg-*$MYOC^{Y437H}$ mice show reduced secretion of MYOC into the aqueous humor, elevated IOP after 3 months of age and RGC loss after 4 months of age. Other *MYOC* mutations are suggested to have alternative pathogenic mechanisms (McKay et al., 2013). Expressing these other human disease-associated alleles in mice (humanized mice) will likely provide an important method by which to distinguish the pathogenic mechanism and test whether therapies need to be designed based on specific variants. As an excellent example of this, Zode and colleagues showed that treating mice with a drug that reduces ER stress significantly lessened IOP elevation in the *Tg-*$MYOC^{Y437H}$ (Zode et al., 2011). Overall, humanized mice represent a valuable to tool to study the genetics, function, and treatment of myocilin disease variants in ocular hypertension and glaucoma.

3.1.3 Other mouse genetic models for studying IOP—Mouse anterior segment physiology is similar to humans and there is now a full range of tools to interrogate ocular fluid dynamics in the mouse (discussed above). As a result, there are a growing number of reports using both forward and reverse genetic approaches to study anterior segment physiology and pathophysiology in mice. Reverse genetic approaches, specifically altering the genome, are being used to test specific hypotheses about a molecule's role in IOP regulation (Chatterjee et al., 2014; Haddadin et al., 2009; Haddadin et al., 2012; Luo et al., 2014; Zhang et al., 2009). While these mutants generally have only a small increase or decrease in IOP, they do provide valuable information about the molecules and pathways contributing to IOP homeostasis. Furthermore, this approach is likely to become commonplace as more and more mouse mutants with targeted alterations in specific genes are made available. A growing number of mutant mouse alleles are being generated by individual investigators and large consortiums (Schofield et al., 2012; van der Weyden et al.,

2011). Liu and colleagues have made several valuable Cre lines that use the Myocilin promoter to control gene expression (Liu et al., 2011). Furthermore, it is important to develop Cre lines specific for each cell type in the anterior segment that contributes to IOP regulation and to make these Cre lines inducible, so that they can be driven at specific developmental and adult time points.

Forward genetic approaches in the mouse, identifying genes based on a specific phenotype, have generated new mouse models as well. One example is the generation of new mutants from an N-ethyl-N-nitrosourea (ENU) based mutagenesis screen that was designed to find mouse mutants with glaucoma-relevant anterior segment pathology. This approach was used to generate a mutant with cardinal features of human angle closure glaucoma (ACG) (Nair et al., 2011). In ACG, the iris is pushed against the trabecular meshwork (TM), thus obstructing aqueous humor outflow and subsequently leading to elevated IOP (Quigley, 2009). Multiple anatomical and physiological factors are thought to participate in the pathogenesis of ACG. Similar to patients with ACG, the mutant mice have features that predispose them to angle closure and high IOP, including reduced ocular axial length, a relatively large lens, and a shallow angle (Lowe, 1970; Nair et al., 2011; Quigley, 2009). In these mice, the causal mutation was identified in the serine protease, *Prss56* (Nair et al., 2011). Importantly, mutations in *PRSS56* contribute to posterior microphthalmos or nanophthalmos in humans, a condition with severe reduction in ocular axial length (Gal et al., 2011; Nair et al., 2011; Orr et al., 2011). As a result of this severe anatomical predisposition, some individuals go on to develop ACG (Gal et al., 2011; Nair et al., 2011; Orr et al., 2011). In addition, rare variants of *PRSS56* have been reported to be associated with primary angle closure glaucoma (PACG; Jiang et al., 2013). Future studies utilizing the mutant *Prss56* mouse model will provide a better understanding of ACG relevant phenotypes at a mechanistic level. Another mutant strain from an ENU-based mutagenesis screen was found to develop cataracts and high IOP as a result of a mutation in *Tdrd7*, a component of RNA granules (Lachke et al., 2011). Interestingly, despite developing elevated IOP, *Tdrd7* mutants do not exhibit any gross morphological abnormalities in the ocular drainage tissues and their angles are primarily open. It was hypothesized that IOP elevation in *Tdrd7* mutant mice develops as a consequence of abnormalities in the protective stress response in the drainage tissues, in particular, oxidative stress. The *Tdrd7* mutants provide a model to study the role of RNA granules in drainage tissue-specific stress responses.

3.2 Developmental glaucoma models

Primary Congenital Glaucoma (PCG) is a less common, though severe subtype of glaucoma resulting from abnormal development of the anterior segment. Accordingly, PCG falls under the category of Anterior Segment Dysgenesis (ASD), a group of diseases characterized by the improper development of anterior ocular tissues. Aberrant morphogenesis of glaucoma-relevant anterior segment tissues—e.g. trabecular meshwork and Schlemm's canal—often leads to elevated IOP and ultimately, RGC death and vision loss (Figure 1). The cellular and molecular mechanisms underlying development of the anterior segment remain largely undefined. For instance, for the majority of cell types in the iridocorneal angle, including the trabecular meshwork, we do not yet understand the factors required for cell determination

and commitment. Similarly, for many of the genes that cause ASD, we do not know when and where these genes function in normal development, or how mutations in them lead to ASD. Mice are ideally suited for asking basic developmental biological questions and assessing how mutations affect tissue morphogenesis. Due to the availability of many ASD-relevant mutants, the ability to genetically manipulate the genome in a developmentally controlled manner, and the numerous cell biological tools available in the mouse to study development, mice have been used extensively over the last several decades to study the molecular genetics of ASD (recently reviewed in depth; Ito and Walter, 2014; Reis and Semina, 2011).

Fundamental insight into the pathogenicity of human disease variants in ASD has been achieved through a combination of human genetics and the use of mouse models (Gould and John, 2002; Gould et al., 2004b; Liu and Allingham, 2011). Mice and humans with orthologous mutations develop similar phenotypes in many cases. Cloning of ASD mutations in mice has led to identifying ASD genes in humans (Kuo et al., 2012; McKeone et al., 2011). Additionally, many phenotypes characteristic of ASD—ciliary body and trabecular meshwork hypoplasia, peripheral iridocorneal adhesion, buphthalmos, and elevated IOP—have been found in both spontaneous and genetically modified mouse mutants (Chang et al., 2001; Gould et al., 2007; Kroeber et al., 2010; Libby et al., 2003; Mao et al., 2011; Sarode et al., 2014; Smith et al., 2000; Sowden, 2007; Weng et al., 2008; Zhou et al., 2013). Mutations in transcription factors (*PITX2*, *FOXC1*, *FOXF2*, *LMX1B*, *PAX6*) are responsible for numerous cases of ASD and PCG (Gould et al., 2004b) and mouse mutants have been used to study the cell biology of these genes in the eye. Recent work with mice carrying mutations in various cell-cell signaling components is beginning to give us insight into the inductive events that drive anterior segment development. For instance, BMP4, a secreted molecule belonging to the TGFβ superfamily, is believed to be necessary for proper formation of both the ciliary body and the iridocorneal angle (Chang et al., 2001; Zhou et al., 2013). Additionally, mutations in several genes influencing the extracellular matrix (eg. CollagenIVa1, CollagenXVIIIa1, and Peroxidasin) have been shown to lead to ASD-relevant phenotypes in mice (Gould et al., 2007; Mao et al., 2011; Yan et al., 2014; Ylikarppa et al., 2003). Recently, mouse studies demonstrated that Schlemm's canal develops from existing blood vessels by a newly discovered process of vascular development with features of vascuologeneiss, angiogeneisis and lymphangiogenesis (Aspelund et al., 2014; Kizhatil et al., 2014; Park et al., 2014; Thomson et al., 2014). Various vascular receptors are expressed during this process including KDR (VEGFR2) and TEK (TIE2), with KDR function being essential for normal development of Schlemm's canal (Kizhatil et al., 2014). These receptors and their downstream proteins are excellent candidates to contribute to PCG and other developmental glaucomas (Kizhatil et al., 2014; Thomson et al., 2014). Together, these results implicate many different developmental events and signaling pathways in the morphogenesis of anterior segment structures. On an even more basic level, these studies and others have identified factors involved in patterning the mammalian eye as well as the general time line of events in anterior segment morphogenesis (Cvekl and Tamm, 2004; Heavner and Pevny, 2012; Ito and Walter, 2014; Napier and Kidson, 2007; Smith et al., 2001).

It is important to note that having any form of ASD puts children at a 50% increased risk for developing glaucoma later in life (Sowden, 2007), however it is unknown precisely why this occurs. It is also possible that clinically undetectable abnormalities in the anterior segment in early childhood could significantly contribute to IOP elevation later in life. Furthermore, clinical severity of ASD does not appear to correlate with the development of glaucoma, suggesting that clinically undetectable changes are critical for the development of ocular hypertension (Ito and Walter, 2014). ASD appears to be highly susceptible to genetic modifiers in both humans and mice (Gould et al., 2007; Gould et al., 2004b; Ito and Walter, 2014; Libby et al., 2003). The ability to perform precise histological analysis and measure glaucoma-relevant physiological parameters (e.g. IOP and outflow measurements) make the mouse ideally suited to study how ASD leads to ocular hypertension and to identify the morphological and genetic factors controlling this progression.

4. Glaucoma-relevant RGC death in mice

Standard genetic tools, such as genetically modified mice, have been used extensively to study glaucoma-relevant RGC loss. Also, other methods have been used in mice to test various hypotheses of glaucoma-relevant RGC death and the effectiveness of particular treatments at stopping RGC loss. Such methods include using viruses to deliver or knockdown genes specifically in RGCs and using microbeads or stem cells to deliver therapeutic substances (reviewed in Harvey et al., 2009; Lebrun-Julien and Di Polo, 2008). Acute injuries in mice, such as mechanical optic nerve crush or intravitreally injecting substances hypothesized to injure RGCs in glaucoma have provided important cell biological information about RGC death; though, it is likely that these acute perturbations do not model key aspects of glaucomatous neurodegeneration. Genetic models offer important advantages over other model systems for studying glaucoma-relevant RGC death as they often show a greater variability and slower progression of IOP elevation (more akin to many human glaucomas), in addition to providing the ability to study the mutation in the context of aging and environmental stressors. Fortunately, there are an increasing number of genetic mouse models being used to gain insight into how RGCs die in both ocular hypertensive and normotensive glaucoma.

4.1 Ocular Hypertensive Mice

4.1.1 DBA/2J mice—In DBA/2J eyes, ocular hypertension is age-related and asynchronous. IOP increases in the majority of eyes from 8 to 12 months of age (Libby et al., 2005a). By 10 months of age, approximately half of DBA/2J eyes have significant optic nerve damage and by 12 months, the majority of eyes have severe glaucomatous damage (Libby et al., 2005a). Reducing IOP in DBA/2J mice pharmaceutically, genetically, or through surgical therapy prevents significant RGC damage (Anderson et al., 2006; Matsubara et al., 2006; Schuettauf et al., 2002; Wong and Brown, 2012, 2013). Thus, RGC loss in DBA/2J mice is dependent upon ocular hypertension. DBA/2J mice have been used to study many cell biological questions relevant to ocular hypertension-induced optic nerve degeneration and RGC death. Below, we describe a few examples where DBA/2J mice were used in combination with mouse genetic tools or pharmaceutical interventions to study glaucoma-relevant RGC death and optic nerve injury (see Figure 3 for examples of genetic

or pharmaceutical manipulation that have been used in DBA/2J mice to dissect the pathological processes involved in ocular hypertension-induced RGC death).

There is ample evidence that RGCs die by apoptosis in human glaucoma and in various mouse models of glaucoma (Guo et al., 2005; Nickells, 1996, 1999; Nickells et al., 2008; Quigley et al., 1995; Whitmore et al., 2005). In DBA/2J mice, several components of the Bcl2 family (major regulators of apoptotic death) are expressed in RGCs during the window of RGC death (Harder et al., 2012; Libby et al., 2005c). Deficiency in the proapoptotic Bcl2 family member *Bax*, prevented RGC somal loss in DBA/2J mice but did not prevent axonal degeneration (Libby et al., 2005c). Interestingly, DBA/2J mice expressing a mutant form of FasL had an earlier onset and more severe loss of RGCs. These data suggest that modulating FasL signaling may reduce RGC apoptosis in glaucoma and indicate that the extrinsic apoptotic pathway is capable of activating ocular hypertension-induced RGC death (Gregory et al., 2011). During the window of RGC death in DBA/2J glaucoma, several pathological insults have been observed including axonal injury at the glial lamina, structural and functional alterations in RGCs, mitochondrial dysfunction, glial dysfunction, neuroinflammation and vascular dysfunction. Along with dissociating the insults that accompany RGC degeneration from the insults that trigger RGC degeneration, determining how these cell intrinsic and cell extrinsic events finally converge on BAX activation leading to the execution of RGC death is an area of ongoing investigation.

Anderson, Hendricks, Quigley, and others suggested that axonal injury is an important early event in human glaucoma and in primate models of glaucoma (Anderson and Hendrickson, 1974; Anderson and Hendrickson, 1977; Quigley and Anderson, 1976; Quigley and Addicks, 1980, 1981). In DBA/2J mice, an early site of axonal injury is at the glial lamina (Buckingham et al., 2008; Howell et al., 2007; Jakobs et al., 2005; Schlamp et al., 2006). Consistent with RGC axons being insulted at the glial lamina, in *Bax* deficient DBA/2J mice, RGC axons survived to the optic nerve head while the axon behind the glial lamina degenerated. Further support for a key role of axonal injury in DBA/2J mice was provided by the finding showing DBA/2J mice that express the Wld^S allele, a naturally occurring mutation that slows axonal degeneration (Fang et al., 2005; Lyon et al., 1993; Mack et al., 2001; Perry et al., 1992), were significantly protected from both axonal and somal degeneration compared to wild type DBA/2J mice (Howell et al., 2007). Thus, axonal injury response and axonal degeneration appear to be key pathological insults for ocular hypertension-induced RGC death in DBA/2J mice. It will be important to identify the genes that control these events, as they could be therapeutic targets for human glaucoma.

The work in DBA/2J mice complements studies in human glaucoma and in other animal models, showing that early changes occur in multiple compartments of an RGC after an ocular hypertensive insult (reviewed in Almasieh et al., 2012; Morquette and Di Polo, 2008; Whitmore et al., 2005; Yu et al., 2013). Along with axonal stress/injury, numerous studies have also shown early changes in other RGC compartments in response to ocular hypertension, including changes in the soma and dendrites. Crish and colleagues (Crish et al., 2010) showed failure of axonal transport begins in the distal axon and progresses toward the soma in DBA/2J mice. Early RGC dendritic remodeling has also been described in retinas of DBA/2J mice (Stevens et al., 2007; Williams et al., 2013). In a series of papers,

Ju, Weinreb and colleagues established the potential importance of mitochondria physiology in regulating RGC loss in DBA/2J mice (Ju et al., 2009; Ju et al., 2010; Ju et al., 2008; Lee et al., 2014). Abnormalities in mitochondrial dynamics correlated with ocular hypertension-induced RGC loss. Importantly, genetically or pharmacologically protecting mitochondrial function also lessened glaucomatous damage in DBA/2J mice. Determining whether the molecular pathways responsible for these structural and functional alterations in distinct RGC compartments interact with each other, and defining which changes are critical for RGC death should be a priority of future research.

Neuroinflammation has been implicated both in human glaucoma (Baltmr et al., 2010; Cheung et al., 2008; Soto and Howell, 2014) and in RGC loss in DBA/2J mice (Anderson et al., 2008; Danesh-Meyer, 2011; Fan et al., 2010; Howell et al., 2011; Howell et al., 2012; Nickells et al., 2012; Zhou et al., 2005). The complement system, a component of the innate immune system, is a key part of the neuroinflammatory response and complement activation has been reported in human glaucoma and in animal models of glaucoma (Brennan et al., 2012; Fan et al., 2010; Harvey and Durant, 2014; Howell et al., 2011; Kuehn et al., 2006; Orsini et al., 2014; Ren and Danias, 2010; Stasi et al., 2006; Stevens et al., 2007; Tezel et al., 2010). DBA/2J mice are naturally deficient in complement component *C5* (a key protein in the formation of the membrane attack complex), and *C5* sufficient DBA/2J show more RGC loss than standard DBA/2J mice, suggesting C5 plays a damaging role after an ocular hypertensive injury. Similarly, *C1qa* mutant DBA/2J mice have less RGC loss, suggesting that the initiating C1 complex of the classical arm of the complement cascade is damaging in glaucoma (Howell et al., 2011; Howell et al., 2014; Howell et al., 2013). Interestingly, C5 seems much less important than C1, as *C1qa* deficiency is much more protective than C5 deficiency, suggesting that substantial damaging effects of C1 are independent of C5 and completion of the classical arm of the complement cascade. It is unclear how decreasing complement activation protects RGCs, though complement has been implicated in various pathological processes thought to be involved in ocular hypertension-induced RGC death in DBA/2J mice, including synaptic remodeling and monocyte recruitment (Figure 3; Howell et al., 2011; Howell et al., 2013; Howell et al., 2012; Stevens et al., 2007).

Neuroinflammation is often linked closely with vascular compromise and changes in vascular tone have been reported in glaucoma (Gerber et al., 2014). Further implicating the vasculature in glaucoma is the suggestion that blood pressure may play a role in glaucoma (Chauhan, 2008; Flammer et al., 2013; Prasanna et al., 2005; Prasanna et al., 2002; Prasanna et al., 2011; Shoshani et al., 2012). The endothelin system, which controls a variety of processes including blood pressure and astrocyte activation has recently been implicated in neurodegenerative conditions (Jo et al., 2014). Components of the endothelin system are upregulated in human and animal models of glaucoma (Howell et al., 2011; Krishnamoorthy et al., 2008; Prasanna et al., 2005; Prasanna et al., 2002; Rattner and Nathans, 2005). Intravitreally injected EDN1 or END2 peptide kills RGCs in rodents (Howell et al., 2011; Krishnamoorthy et al., 2008; Rattner and Nathans, 2005), especially in the presence of elevated IOP (Howell et al., 2012). Treating DBA/2J mice with endothelin receptor antagonists both slowed and lessened the severity of RGC axonal damage, suggesting that endothelin signaling has a significant role in ocular hypertension-induced RGC death

(Howell et al., 2011; Howell et al., 2014). Interestingly, axonal loss in the DBA/2J mouse has been associated with monocyte entry into the optic nerve head (Howell et al., 2012), further implicating neuroinflammatory responses. An important future direction will be to mechanistically explore the roles of neuroinflammation and vascular dysfunction in glaucoma. However, this work is confounded by the fact that components of these systems are activated in both aging, a major risk factor for glaucoma, and multiple cell types, including astrocytes, RGCs, microglia and cells associated with the vasculature. Here, the mouse can play a major role in determining the beneficial and/or damaging roles of specific genes and cell types in aging and glaucoma using conditional alleles whereby genes are ablated in particular cell types. Importantly, as anti-inflammatory therapies are already commonplace, the inhibition of glaucomatous damage using anti-inflammatory approaches poses a tangible therapeutic option.

Glia are involved in many different processes that can contribute to neurodegeneration, including neuroinflammation (Maragakis and Rothstein, 2006; Mrak and Griffin, 2005; Verkhratsky et al., 2014). Microglia, Müller glia and optic nerve head astrocytes have been hypothesized to play critical roles in glaucomatous neurodegeneration (Chong and Martin, 2015; Seitz et al., 2013). Both retinal and optic nerve head glia have been implicated in ocular hypertensive RGC death in DBA/2J mice. Astrocytes at the optic nerve head have been shown to undergo morphological changes prior to RGC death (Lye-Barthel et al., 2013). Also, gene expression studies have suggested that molecular changes in astrocytes could be involved in the pathogenesis of RGC death in DBA/2J mice (Howell et al., 2011; Howell et al., 2014; Nguyen et al., 2011). Microglial activation has been correlated with RGC death (Bosco et al., 2015) and reducing microglial activation has been suggested to increase RGC viability in DBA/2J mice (Bosco et al., 2008; Bosco et al., 2015; Inman and Horner, 2007). It will be important to functionally test specific aspects of glial activation in DBA/2J mice to precisely define the role of glia in the disease process.

In summary, multiple pathways, both intrinsic and extrinsic to RGCs, have been suggested to contribute to ocular hypertension-induced RGC death in DBA/2J mice (Figure 3). However, the initial molecular changes that trigger neuronal injury remain unclear. Highlighting the complexity of glaucoma, numerous cell biological events are suggested as key players in the disease, including axonal injury, inflammation, glial activation, growth factor deficiency, mitochondrial dysfunction, and synapse withdrawal. Thus, there is still much to be learned about RGC loss in DBA/2J mice. It will be important to continue to critically test molecules and pathways hypothesized to be important through functional tests, including assessing their roles in specific cell types and determining critical molecular events. Careful functional testing of any specific pathway in multiple models (DBA/2J as well as others) will identify potential targets for therapies to prevent glaucomatous neurodegeneration.

4.1.2 Other Models for Studying Ocular Hypertension-Induced RGC death—

Along with genetic models, there are inducible models of IOP elevation that are being used to study ocular hypertension-induced RGC death, including, microbead injections (Morgan Chapter **REF**, this issue), episcleral vein and aqueous outflow sclerosis (Morrison et al., 2015, this issue), acute IOP elevation (Crowston et al., 2015, this issue), glucocorticoid-

induced IOP elevation (Overby and Clark, 2015, this issue), and viral gene transfer to generate TM cell dysfunction and resulting IOP elevation (Pang et al., 2015, this issue). For instance, the microbead model has been used to test the importance of a variety of molecular pathways in ocular hypertension induced RGC death (Hu et al., 2012; Huang et al., 2011; Ward et al., 2014) and to investigate susceptibility factors involved in response to IOP elevation (Cone et al., 2010; Steinhart et al., 2012). The large number of models being used and developed will allow researchers to validate results in multiple diverse models, species, and strains within species.

4.2 Normal tension glaucoma models

Normal tension glaucoma (NTG) is a subset of primary open angle glaucoma that is characterized by RGC loss and optic neuropathy in the absence of detecting elevated IOP. NTG is an interesting type of glaucoma because it suggests that factors intrinsic to retina and/or optic nerve are a primary site of pathophysiology. Below we highlight three different mouse models of NTG, where mice have been used to gain insight into mechanisms that lead to RGC loss.

4.2.1 Optineurin (OPTN)—The OPTN E50K mutation was the first mutation identified in familial normal tension glaucoma (Rezaie et al., 2002). Transgenic mice overexpressing the optineurin E50K mutation ($E50K^{tg}$) were made to investigate the biology of this specific mutation (Chi et al., 2010a). $E50K^{tg}$ mice did not have elevated IOP but did have loss of RGCs (~25% loss by 16 months of age). A caveat of the $E50K^{tg}$ model is the significant thinning of the ONL and INL and loss of other retinal neurons, including amacrine cells, bipolar cells, and photoreceptors (Chi et al., 2010a), cell loss that is not generally observed in glaucoma patients. The high expression levels of E50K OPTN in the $E50K^{tg}$ mice were hypothesized to result in non-specific loss of other retinal neurons through a toxic gain of function mechanism. To test this hypothesis a BAC transgenic mouse expressing human E50K OPTN (BAC-$hOPTN^{E50K}$) close to physiological levels was made (Tseng et al., 2015). Interestingly, in these mice there was age-related loss of RGCs without apparent loss of other retinal cell types. Thus, BAC-$hOPTN^{E50K}$ mice are a valuable resource for gaining insight into the molecular mechanisms of RGC death in NTG. In the mouse eye, OPTN is expressed in RGCs, as well as in the cornea, trabecular meshwork, ciliary epithelium and iris (Kroeber et al., 2006) and it remains unclear why mutations in OPTN lead to RGC loss. Optineurin functions in several physiological processes, most prominently in secretory vesicle transport (Bond et al., 2011) and autophagy (Wild et al., 2011). Determining which, if any, of these processes is critical for RGC death in NTG is an important future direction.

4.2.2 WD-repeat-containing protein 36 (WDR36)—Several studies have reported an association between *WDR36* and both NTG and POAG. Interestingly, these studies have shown that *WDR36* can be causative for glaucoma and be a susceptibility factor for developing glaucoma (Allingham et al., 2009; Hauser et al., 2006; Monemi et al., 2005). WDR36 is expressed throughout the eye, including in tissues important in glaucoma pathogenesis (sclera, iris, ciliary muscle, ciliary body, retina, and optic nerve ; Monemi et al., 2005). We have included *WDR36* in the NTG section because of the data generated from mice (discussed below); however, we realize that it could potentially be included in the

ocular hypertension discussion or in a section focusing on general susceptibility factors. Several mouse mutant alleles have been generated to study the role of WDR36 in ocular biology and disease. Unfortunately, mice homozygous for a null allele of *Wdr36* (*Wdr36*$^{-/-}$ mice) die preimplantation (Gallenberger et al., 2011). *Wdr36*$^{+/-}$ mice do not have RGC loss at 12 months, nor do RGCs in these mice show increased susceptibility to death when exposed to an excitotoxic or ocular hypertensive insult (Gallenberger et al., 2014). There have been 3 mouse alleles generated that have been designed to either create an orthologous mutation to that found in human glaucoma patients or to disrupt molecular function in a region of the gene with an orthologous mutation (Chi et al., 2010b). Transgenic mice overexpressing one of these alleles, *Wdr36* (Del605-607), had a dramatic reduction in peripheral retinal thickness and loss of RGCs as well as amacrine cells and bipolar cells at 16 months of age (Chi et al., 2010b). *Wdr36* (Del605-607) mutant mice do not have elevated IOP and the lens, cornea, and anterior segment of these mice appear to be histologically normal. In the future, it will be important to make a conditional allele of *Wdr36* so that it can be deleted selectively in adult trabecular meshwork, RGCs, or optic nerve head astrocytes to allow assessment of the tissue-specific function of *Wdr36* in the pathogenesis of glaucoma. Also, it will be important to assess the effects of mutant mouse alleles that have orthologous mutations to those found in human glaucoma patients knocked into the mouse *Wdr36* locus.

4.2.3 Glutamate Transporters—To our knowledge, there are no hereditary studies that implicate alleles involved in glutamate regulation in human NTG or in any other form of glaucoma. However, elevated levels of extracellular glutamate cause RGC death and mice deficient in the glutamate transporters, GLAST or EAAC1, exhibit RGC degeneration with varying time courses. GLAST deficient mice had a progressive attrition of RGCs with as much as a 50% loss by 8 months (Harada et al., 2007). These mice were also suggested to have other hallmarks of glaucomatous neurodegeneration including optic nerve cupping and degenerating axons in the optic nerve. EAAC1 deficient mice had RGC degeneration as early as 8 weeks of age, but also have thinning of the inner retina (Harada et al., 2007). At 8 weeks of age, there is a 20% reduction in the number of cells in the ganglion cell layer in EAAC1 deficient mice. Both GLAST and EAAC1 deficient mice have normal IOP and normal iridocorneal angle morphology, suggesting IOP does not directly cause RGC death in these mice (Harada et al., 2007). Both glutamate toxicity and oxidative stress have been hypothesized to contribute to RGC loss in GLAST and EAAC1 deficient mice (Kimura et al., 2015; Namekata et al., 2013; Semba et al., 2014). Glutamate receptor antagonists lessened RGC death at early time points in GLAST deficient mice, indicating that glutamate neurotoxicity contributes to RGC degeneration in these mice (Harada et al., 2007; Namekata et al., 2013). Similarly, attenuating oxidative stress was shown to reduce RGC loss in GLAST knockout mice (Kimura et al., 2015). Collectively, the data from animal models of glutamate transporter loss show that both RGC intrinsic as well as extrinsic mechanisms contribute to RGC degeneration. It will be important in the future to determine if this system is involved in RGC loss in NTG in humans.

4.2.4 Summary of NTG Models—Thus far, mouse models of NTG have revealed a diversity of physiological processes that when disrupted in the retina can lead to RGC degeneration independent of IOP elevation. An important caveat with some of the NTG

mouse models is that they appear to have loss of other retinal neurons, which is generally thought to be inconsistent with glaucomatous neurodegeneration. Furthermore, there are other genetic causes of NTG that remain to be modeled. For instance, copy number variants of *TBK1* cause NTG (Awadalla et al., 2015; Ritch et al., 2014). The normal physiology and pathophysiology of altered TBK1 expression remain to be determined. Using induced pluripotent stem cells from patients with TBK1 copy number variations, evidence is emerging that autophagy is enhanced in these cells (Tucker et al., 2014). Mice are routinely used for studying the effects of copy number variation and can easily be made to express different levels of specific human or mouse alleles (Le et al., 2003; Smithies and Kim, 1994; van der Weyden and Bradley, 2006). Modeling TBK1 expression level variants should provide a helpful addition to the toolbox for studying NTG, especially if the pathophysiology involves an aging component or requires the interaction between different cell types. As new genes are found to cause NTG, regardless of the nature of the mutation, mouse mutants will be important tools for determining the pathophysiological mechanisms resulting from the mutations.

5 The future of genetic models of glaucoma

The genomics revolution over the last twenty years is beginning to bear fruit for glaucoma research and a steady stream of genes and genetic loci are being discovered that are linked or associated with glaucoma. Recent large-scale genome wide association studies (GWAS; Hysi et al., 2014; Springelkamp et al., 2014) have identified genes/loci associated with glaucoma-relevant phenotypes. However, in some cases, it is not clear how a gene or a specific allelic variant impacts the disease. For instance, while *CDKN2B-AS1*, an anti-sense transcript that is transcribed on the opposite strand to *CDKN2A* and *CDKN2B*, is strongly associated with glaucoma, the function of *CDKN2B-AS1* remains unclear (Burdon et al., 2012; Burdon et al., 2011; Ng et al., 2014). In a second example, copy number variations in *TBK1* are associated with glaucoma, and although the function of the gene is better understood (Helgason et al., 2013; Minegishi et al., 2013; Tucker et al., 2014; Weidberg and Elazar, 2011), the reason for why copy number variations that include *TBK1* impact glaucoma progression is less clear.

A major limitation of GWAS is that it generally implicates genetic loci and not candidate variants. Plus, GWAS analysis cannot find evolutionarily recent or low frequency genetic variants (Manolio et al., 2009). To address this, studies are now beginning to take advantage of next generation sequencing (NGS) of human samples, using targeted exome and whole genome sequencing approaches. However, progress with these approaches will often not be straightforward and complementary animal and human studies will often be necessary. This is due to both the complex nature of glaucoma, where variations in multiple genes contribute to the disease, and to difficulty ascribing causality to any specific variants among the large numbers of variants detected by sequencing. As with GWAS, NGS studies will likely need to involve large numbers of samples coordinated in a consortium-like manner that has proved successful for GWAS (e.g. the Neighbor and Neighborhood studies Burdon et al., 2011; Lu et al., 2013; Springelkamp et al., 2014; Wiggs et al., 2013; Wiggs et al., 2012). The discovery that naturally existing nucleases, first zinc fingers (Urnov et al., 2010) then TALENS (Joung and Sander, 2013) and now CRISPRs (Sander and Joung, 2014) can be

used to alter single bases or a few bases (‘genome editing’), means that genetically engineered mice can be created in months rather than years. Further advances that include incorporating larger stretches of DNA into the mouse genome will enable insertion of human gene sequences into the mouse genome and the generation of conditional alleles and new Cre driver lines to study the temporal and spatial function(s) of genes. Thus, new genomic technologies will allow us to readily assess putative causal variants identified in human genetic studies in mice. An important goal should be to incorporate genetic variants implicated in human glaucoma into mouse models, improving utility for preclinical and translational studies. These experiments can complement genetic manipulation strategies in human tissues/cells, including the ability to assess variants in specific cell types derived using induced pluripotent stem cells (IPSCs; Ding et al., 2014; Tucker et al., 2014).

The mouse can play other key roles in the development of improved therapies for glaucoma. For instance, human genetic and genomic studies are difficult because of the obvious limitations of working with human subjects and samples. Therefore, the mouse can continue to inform us about novel genes and pathways that impact glaucoma. These experiments can include hypothesis driven approaches where the importance of individual genes can be assessed. Alternatively, unbiased approaches should continue to be pursued where genes are mutated at random (for instance, by ethylnitrosourea mutagenesis). This approach has already identified novel genes for glaucoma research (e.g. Cross et al., 2014; Lachke et al., 2011; Nair et al., 2011; Van Agtmael et al., 2005). Given the genetic complexity of glaucoma, sensitized approaches are also being pursued where mutagenesis results in mice carrying genetic mutations that increase susceptibility for glaucoma-relevant phenotypes but do not directly cause glaucoma. Unbiased genetic approaches may be greatly aided by incorporating the latest generation of inbred and outbred mouse crosses, (collaborative cross and diversity outbred cross respectively; Chesler et al., 2008;

Churchill et al., 2004; Churchill et al., 2012). These are currently the most powerful strains for identifying genetic variations that impact complex disease, and are ideal for mapping glaucoma-relevant modifier genes. Diversity outbred mice are also ideal for assessing efficacy and pharmacokinetics in the preclinical space where potential new drugs can be tested in genetically unique mice under controlled environmental conditions. Finally, as the number of mouse strains with unique mutations and engineered manipulations relevant to glaucoma continue to grow, so will the need for efficient sharing of them amongst investigators and institutions. Several commercial and non-profit repositories for sharing mice exist (see Figure 2 for some examples) and it will be critical that our community remains dedicated to using them fully.

Mice can also be used to probe some of the outstanding questions about complex physiological interactions that may be important in glaucoma. For instance, it has been suggested that both blood pressure (He et al., 2014; Moore et al., 2008) and intracranial pressure (Fleischman and Allingham, 2013) help mediate glaucoma susceptibility. It is possible to manipulate these traits in mice along with IOP to determine the importance of these parameters in RGC susceptibility to IOP elevation (Nusbaum et al., 2015; Takahashi and Smithies, 2004; Verouti et al., 2015). Also, recent work is starting to examine endophenotypes of glaucoma, such as corneal thickness and optic disc morphology in mice

(Harder et al., 2012; Lively et al., 2010a; Lively et al., 2010b; Prasov et al., 2012). These studies will open up avenues of research examining how these phenotypes affect the pathophysiology of glaucoma. Overall, mice have provided important cell biological insight into glaucoma-relevant phenotypes. In the future, continually integrating information gained from human glaucoma studies into mouse models will further enhance the power of mice for studying glaucoma pathophysiology.

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Highlights

- Mice can be used to model specific glaucoma-relevant phenotypes.
- Mice are a valuable tool to determine the molecular mechanisms underlying glaucoma phenotypes.
- Mice are a valuable tool to probe the cell biology underlying findings from human genetic studies.
- Mice can be used to find new genes and molecular pathways that contribute to human glaucoma.

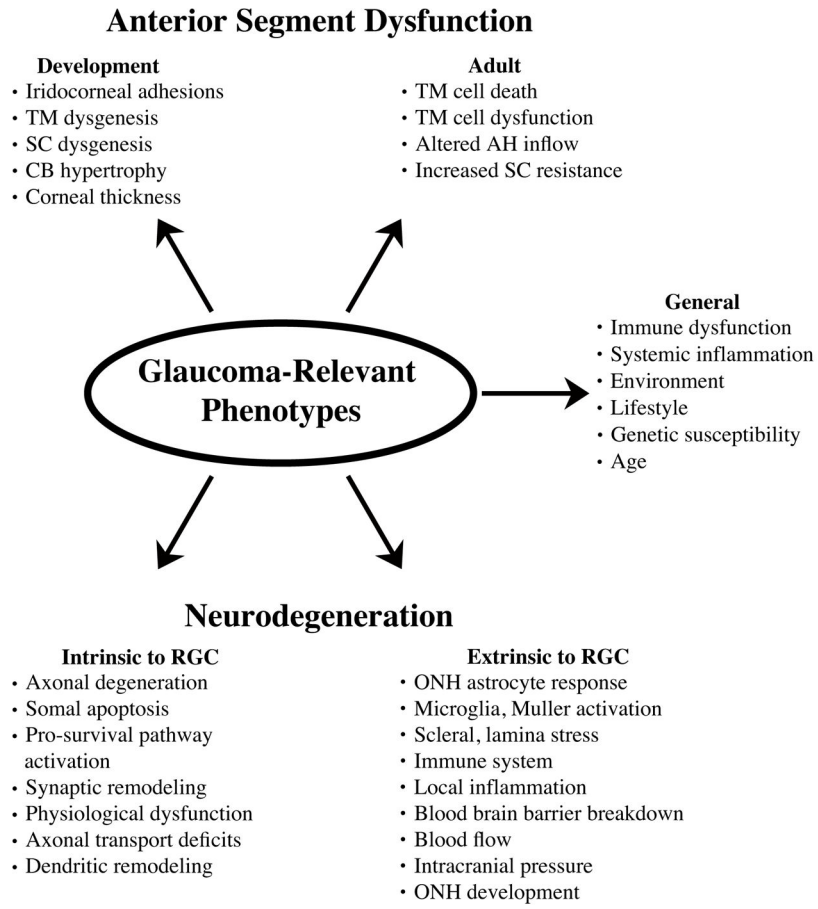

Figure 1. Glaucoma-relevant phenotypes that can be modeled and studied in mice

To study the cell biology of glaucoma, it is helpful to breakdown the disease into the individual events and/or phenotypes that can contribute to the disease. TM, trabecular meshwork; SC, Schlemm's canal; CB, ciliary body; ONH, optic nerve head; RGC, retinal ganglion cell.

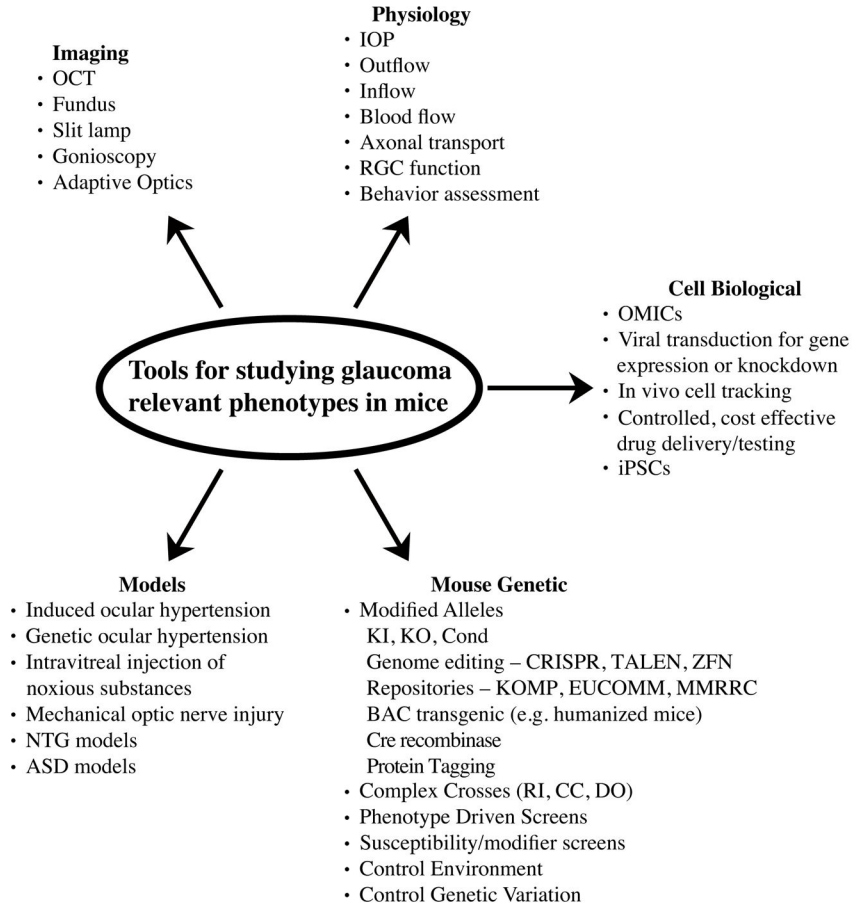

Figure 2. Tools available to study ocular disease in mice

There are many tools available to researchers using mice to study glaucoma-relevant phenotypes including: various imaging technologies, both advanced technologies which can only be used in model organisms and standard clinical imaging tools; physiological measurements to assess both anterior and posterior function; advanced cell biological analysis that can be used to probe the molecular mechanisms of glaucoma; mouse genetics, which can be used to discover new genes and molecular pathways underlying glaucoma and to critically test predicted pathways involved in glaucomatous pathophysiology; and numerous mouse models with glaucoma-relevant phenotypes that can be used to gain insight into human glaucoma. OCT, optical coherence tomography; IOP, intraocular pressure; RGC, retinal ganglion cell OMICs, genomics, proteomics and metabolomics; iPSCs, induced pluripotent stem cells; NTG, normal tension glaucoma; ASD, anterior segment dysgenesis; KI, knock in; KO, knock out; Cond, conditional allele; CRISPR, clustered regularly interspaced short palindromic repeats; TALEN, transcription activator-like effector nucleases; ZFN, xinc-finger nucleases; KOMP, knock out mouse project; EUCOMM, European Conditional Mouse Mutagenesis Program; MMRRC, Mutant Mouse Regional Resource Center; RI, recombinant inbred; CC, collaborative cross; DO, diversity outbred cross.

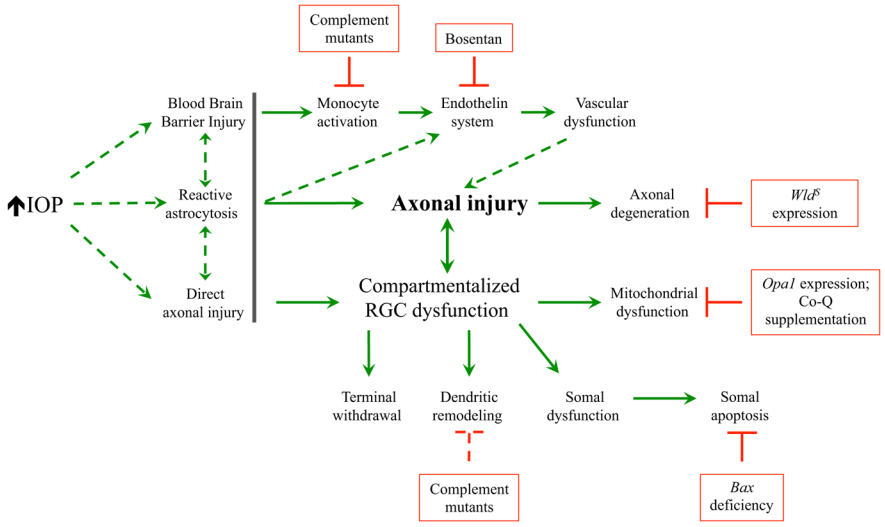

Figure 3. Ocular hypertension leads to numerous events in distinct subcompartments of RGCs that contribute to RGC loss in DBA/2J mice

DBA/2J mice have been used extensively to study the pathogenesis of ocular hypertension-induced RGC death. These studies have led to the idea that different cellular compartments of RGCs respond to ocular hypertension and that targeting these events can lessen RGC loss. This diagram assumes a critical early injury occurs to RGCs axons (axonal injury) in the optic nerve head. However, it is unclear (dashed lines) whether the initial driving event is an insult directly to the axons or through extrinsic means (the gray line is used to separate these potential early events to the later important event of axonal injury). Red boxes represent either pharmaceutical or genetic interventions that blocked a specific event in the degeneration cascade and have lessened RGC loss in DBA/2J mice.