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(71) Applicant: **THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK** [US/US]; 412 Low Memorial Library, 535 West 116th Street, New York, NY 10027 (US).

(72) Inventors: **JOHN, Simon, W., M.**; 7 Holland Avenue, Bar Harbor, ME 04609 (US). **ZHANG, Chi**; 263 Hickory Avenue, Tenafly, NJ 07670 (US).

(74) Agent: **PETRUZZI, Heather** et al.; Wilmer Cutler Pickering Hale and Dorr LLP, 2100 Pennsylvania Avenue, NW, Washington, DC 20037 (US).

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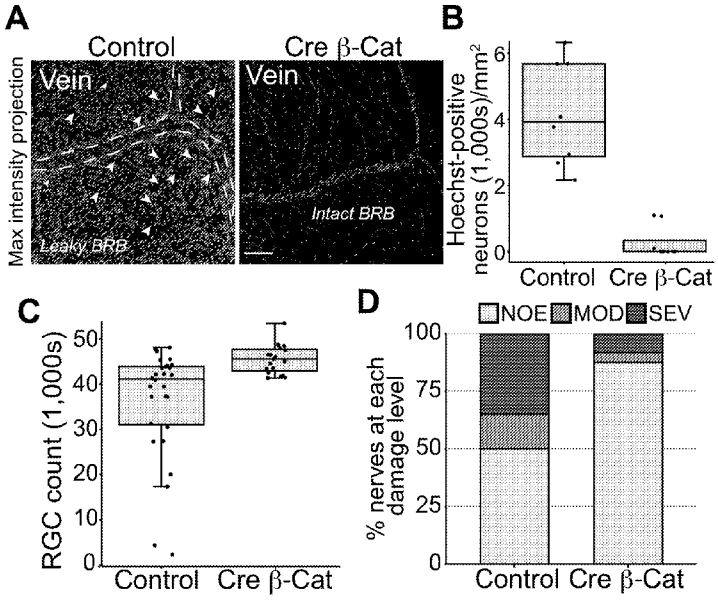

FIGS. 5A-D

(57) Abstract: A method of treating or preventing glaucoma in a subject is disclosed wherein the method includes administering to the subject a therapeutically effective amount of a therapeutic agent comprising MFSD2A, a derivative of MFSD2A or a modulator of MFSD2A, or a pharmaceutical composition thereof.

[Continued on next page]

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SPECIFIC BLOOD RETINAL BARRIER DEFECT IN GLAUCOMA DIRECTS NEW TREATMENTS AND CLINICAL TESTS

CROSS REFERENCE TO RELATED APPLICATION

[0001] The application claims the benefit of and priority to U.S. Provisional Application No. 63/513804, filed on July 14, 2023, the content of which is hereby incorporated by reference its entirety.

INCORPORATION BY REFERENCE

[0002] Any patent, patent publication, journal publication, or other document cited herein is expressly incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0003] This invention was made with government support under grants EY032062, EY032507, and EY018606 awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD

[0004] This application is generally related to methods of treating and/or preventing glaucomatous neurodegeneration in a subject as well as methods for monitoring BRB compromise as part of the treatment process.

BACKGROUND

[0005] Increased intraocular pressure (IOP) is an important risk factor for glaucoma. While topical monotherapy is the preferred treatment for IOP control in accordance with European Glaucoma Society Terminology and Guidelines, most patients require multidrug therapy to achieve their target IOPs. These medications are associated with significant financial costs, especially as patients need to chronically use these therapies until they undergo surgical intervention for IOP lowering.

[0006] In most types of glaucoma, a harmfully high intraocular pressure results in retinal ganglion cell (RGC) death and ultimately vision loss. Multiple insults damage distinct parts of the RGCs in glaucoma. In addition to direct damage to optic nerve head (ONH) axons, there is evidence for various damaging processes within the retina, including perturbed metabolism, dendrite/synapse changes, endothelial cell changes, and altered blood flow.

When developing improved treatments, it is important to understand and target distinct pathogenic events including those in the retina, ONH, and their vasculature. It would be beneficial to have testing capable of providing a useful indicator of patients with high IOP who are/will go on to develop glaucomatous neurodegeneration versus those patients with high IOP who are not progressing. Furthermore, it would be advantageous if treatment methods were available to prevent progression of high IOP to glaucoma.

[0007] Methods for treating or preventing IOP from becoming elevated are useful in glaucoma but are not adequately effective in many patients. There would be an advantage to treatments with different mechanisms of action to IOP lowering treatments, which could be used to augment the effects of existing IOP treatments. It would also be beneficial to have methods other than measuring IOP for monitoring the progress of treatment or of identifying patients at increased risk of progression. Furthermore, it would be useful to have treatments that can be used to treat or prevent blood-retinal barrier (BRB) compromise in a subject.

SUMMARY

[0008] In one aspect, a method of treating or preventing glaucoma in a subject is disclosed. In accordance with some embodiments, the method includes administering to the subject a therapeutically effective amount of a therapeutic agent comprising MFSD2A, a derivative of MFSD2A or a modulator of MFSD2A, or a pharmaceutical composition thereof.

[0009] In some embodiments, the subject exhibits elevated IOP.

[0010] In some embodiments, the modulator of MFSD2A is a MFSD2A agonist.

[0011] In some embodiments, the method includes administering to the subject an additional therapeutic agent.

[0012] In some embodiments, the one or more additional therapeutic agents are selected from the group consisting of a Wnt agonist, an antioxidant agent, an anti-inflammatory agent, an agent that modulates metabolism, an agent that modulates the integrated stress response, an agent that modulates the unfolded protein response, an agent that modulates forms of autophagy, an agent that modulates the expression or activity of genes controlling or mediating antioxidant or other protective responses, a senolytic agent, an agent that modulates the mitochondria or mitophagy, an anti-aging agent, another agent that modulates intraocular pressure, a resilience-boosting agent, an antifibrotic agent, an agent that prevents epithelial mesenchymal transition or endothelial mesenchymal transition, a neuroprotective

agent, a gene therapy agent, growth factor, insulin, endothelin antagonists, complement system inhibitors and combinations thereof.

[0013] In some embodiments, the additional therapeutic agent is a pyruvate compound.

[0014] In some embodiments, the additional therapeutic agent is ethyl pyruvate.

[0015] In some embodiments, the additional therapeutic agent is nicotinamide (NAM).

[0016] In some embodiments, the additional therapeutic agent includes a derivative or analog of nicotinamide, wherein the derivative or analog of nicotinamide is selected from the group consisting of nicotinic acid, nicotinamide riboside, nicotinamide mononucleotide, nicotinamide adenine dinucleotide and combinations thereof.

[0017] In some embodiments, the derivative or analog of nicotinamide is nicotinamide riboside.

[0018] In some embodiments, the additional therapeutic agent includes a pyruvate compound, NAM, a derivative or analog of NAM, a Wnt agonist, an IOP lowering agent, or combinations thereof.

[0019] In some embodiments, the method also includes prescribing a diet rich in fatty acids to the subject.

[0020] In another aspect, a method of treating or preventing blood retina barrier (BRB) compromise in a subject is disclosed. In some embodiments, the subject exhibits or has exhibited elevated IOP, glaucoma or normal tension glaucoma. The method includes determining if there is vascular leakage associated with the subject's retinal veins, optic nerve head (ONH) vessels or both and if such vascular leakage is detected, providing treatment to the subject, wherein the treatment includes administering to the subject a therapeutically effective amount of a therapeutic agent including one or more Wnt signaling pathways activators, MFSD2A, a derivative of MFSD2A or a modulator of MFSD2A, or a pharmaceutical composition of any of the foregoing.

[0021] In some embodiments, the one or more Wnt signaling pathways activators includes a β-catenin signaling modulator.

[0022] In some embodiments, MFSD2A, a derivative of MFSD2A or a modulator of MFSD2A is administered to the subject.

[0023] In some embodiments, the method further includes monitoring any vascular leakage associated with the subject's retinal veins over a period of time and adjusting the treatment to the subject based on a change in vascular leakage.

[0024] In some embodiments, the retinal vein includes a peripheral retinal vein.

[0025] In some embodiments, the step of determining if there is vascular leakage associated with the subject's retina is determined using angiography.

[0026] In some embodiments, the angiography uses either fluorescein or indocyanine green tracers.

[0027] In some embodiments, the treatment further includes administering to the subject an additional therapeutic agent.

[0028] In some embodiments, the one or more additional therapeutic agents are selected from the group consisting of a Wnt agonist, an antioxidant agent, an anti-inflammatory agent, an agent that modulates metabolism, an agent that modulates the integrated stress response, an agent that modulates the unfolded protein response, an agent that modulates forms of autophagy, an agent that modulates the expression or activity of genes controlling or mediating antioxidant or other protective responses, a senolytic agent, an agent that modulates the mitochondria or mitophagy, an anti-aging agent, another agent that modulates intraocular pressure, a resilience-boosting agent, an antifibrotic agent, an agent that prevents epithelial mesenchymal transition or endothelial mesenchymal transition, a neuroprotective agent, a gene therapy agent, growth factor, insulin, endothelin antagonists, complement system inhibitors and combinations thereof.

[0029] In some embodiments, the additional therapeutic agent includes a pyruvate compound.

[0030] In some embodiments, the one or more additional therapeutic agents includes ethyl pyruvate.

[0031] In some embodiments, the additional therapeutic agent includes nicotinamide.

[0032] In some embodiments, the additional therapeutic agent includes a derivative or analog of nicotinamide, wherein the derivative or analog of nicotinamide is selected from the group consisting of nicotinic acid, nicotinamide riboside, nicotinamide mononucleotide, nicotinamide adenine dinucleotide and combinations thereof.

[0033] In some embodiments, the derivative or analog of nicotinamide is nicotinamide riboside.

[0034] In some embodiments, the additional therapeutic agent includes a pyruvate compound, NAM, a derivative or analog of NAM, a Wnt agonist, an IOP lowering agent, or combinations thereof.

[0035] In some embodiments, the therapeutic agent is conjugated directly or indirectly to an agent that targets retinal endothelial cells.

[0036] In accordance with another aspect, a method of monitoring treatment of a subject that has exhibited elevated IOP, glaucoma or normal tension glaucoma and said subject has been prescribed a drug at a dosage level to lower IOP or treat glaucoma or normal tension glaucoma is disclosed. In some embodiments, the method includes determining if there is vascular leakage associated with the subject's retinal veins, optic nerve head (ONH) vessels or both and if such vascular leakage is not present, or is present at a low, clinically acceptable level, then discontinuing the drug or lowering the dosage level.

[0037] Any one of the embodiments disclosed herein may be properly combined with any other embodiment disclosed herein. The combination of any one of the embodiments disclosed herein with any other embodiments disclosed herein is expressly contemplated. Specifically, the selection of one or more embodiments for one substituent group can be properly combined with the selection of one or more particular embodiments for any other substituent group. Such combination can be made in any one or more embodiments of the application described herein or any formula described herein.

DESCRIPTION OF THE DRAWINGS

[0038] The application is described with reference to the following figures, which are presented for the purpose of illustration only and are not intended to be limiting. In the Drawings:

[0039] **FIG. 1A** are magnified images of retinal flat mounts of D2-*Gpnmb*$^{+}$ control and D2 mice, according to one or more embodiments.

[0040] **FIG. 1B** is a graph showing quantification of leakage for D2-*Gpnmb*$^{+}$ control and D2 mice, according to one or more embodiments.

[0041] **FIG. 1C** is a bar chart showing percentage of retinas with leakage over time in D2 and D2-*Gpnmb*$^{+}$ mice, according to one or more embodiments.

[0042] **FIG. 2A** provides images of retinal flat mounts of two models that develop high IOP and glaucoma, B6.*Lmx1b*$^{V265D/+}$ and HAMA, according to one or more embodiments.

[0043] **FIG. 2B** is a graph showing quantification of leakage in B6.*Lmx1b*$^{V265D/+}$ eyes vs. controls, according to one or more embodiments.

[0044] **FIG. 2C** is a graph showing quantification of leakage in HAMA eyes vs. controls, according to one or more embodiments.

[0045] **FIG. 3A** provides representative images of staining with tight junction markers (CLDN5 and ZO-1) in retinal flat mounts of 9-month-old D2-*Gpnmb*$^{+}$ normotensive controls and D2 mice, according to one or more embodiments.

[0046] **FIG. 3B** provides representative images of staining with pericyte markers (Desmin and NG2) in retinal flat mounts of 9-month-old D2-*Gpnmb*$^{+}$ normotensive controls and D2 mice, according to one or more embodiments.

[0047] **FIG. 3C** is a graph showing quantitative analysis of fluorescence by measuring integrated density (IntDen) in Image J for CLDN5 and ZO-1, according to one or more embodiments.

[0048] **FIG. 3D** is a graph showing quantitative analysis of fluorescence by measuring integrated density (IntDen) in Image J for Desmin and NG2, according to one or more embodiments.

[0049] **FIG. 4A** provides representative images of Albumin (staining in retinal flat mounts of 9-month-old D2-*Gpnmb*$^{+}$ and D2 mice and a graph showing quantification of albumin leakage as fluorescence intensity outside of the vessels.

[0050] **FIG. 4B** is a magnified image showing areas with total loss of MFSD2A expression (arrowheads) and reduced expression (arrows), according to one or more embodiments.

[0051] **FIG. 4C** is a magnified image showing that absence of MFSD2A correlates with albumin leakage (arrowheads), according to one or more embodiments.

[0052] **FIG. 4D** is a magnified image showing that if the vein retains some MFSD2A expression, albumin is confined within the vessel (arrows), according to one or more embodiments.

[0053] **FIG. 5A** provides representative images of Hoechst staining in 9-month-old D2.*Ctnnb1*$^{flex3/+}$ (Control) and D2.*Ctnnb1*$^{flex3/+}$; Cdh5-CreERT2 (Cre β-Cat) mice.

[0054] **FIG. 5B** is a graph showing quantitative leakage in Control vs. Cre b-Cat mice, according to one or more embodiments.

[0055] **FIG. 5C** is a graph showing quantitative Retinal Ganglion Cell (RGC) quantification in Cre β-Cat mice vs. Control mice.

[0056] **FIG. 5D** is a frequency distribution of optic nerve damage for Cre β-Cat mice and Control mice. SEV = severe, MOD = moderate, NOE = no/early nerve damage.

[0057] **FIG. 6A** provides representative images of Hoechst staining D2.*Ctnnb1*$^{flex3/+}$ (Control) and AAV-*Mfsd2a* (v*Mfsd2a*) mice.

[0058] **FIG. 6B** is a frequency distribution of optic nerve damage for v*Mfsd2a* mice and Control mice. SEV = severe, MOD = moderate, NOE = no/early nerve damage.

[0059] **FIG. 7** provides representative images of Hoechst staining 129.Lmx1b$^{V265D/+}$ eyes with high IOP.

DETAILED DESCRIPTION

[0060] In one aspect, a method of treating or preventing glaucoma in a subject is disclosed. In one aspect, the method includes administering to the subject a therapeutically effective amount of a therapeutic agent comprising MFSD2A, a derivative of MFSD2A or a modulator of MFSD2A, or a pharmaceutical composition thereof.

[0061] The singular forms “a”, “an” and “the” include plural reference unless the context clearly dictates otherwise. The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

[0062] As used herein the term “about” is used herein to mean approximately, roughly, around, or in the region of. When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 20 percent up or down (higher or lower).

[0063] An "effective amount", “sufficient amount” or “therapeutically effective amount” as used herein is an amount of a compound that is sufficient to effect beneficial or desired results, including clinical results. As such, the effective amount may be sufficient, for example, to reduce or ameliorate the severity and/or duration and/or onset of vascular leakage. An effective amount also includes the amount of the compound that avoids or substantially attenuates undesirable side effects.

[0064] As used herein, the term “glaucoma” refers to an eye disease that results in damage to the retina and optic nerve and visual dysfunction or vision loss. Glaucoma occurs

more commonly among older people. Vision loss from glaucoma is permanent and is irreversible.

[0065] As used herein, the term "normal intraocular pressure" (normal IOP) in humans refers to a human subject having an IOP value of 10 mmHg to 21 mmHg. Some individuals, however, may develop optic nerve damage despite a normal IOP (known as normal-tension glaucoma).

[0066] As used herein, the term "high intraocular pressure" (high IOP) in humans refers to a human subject having an IOP value greater than 21 mmHg (or 2.8 kPa). As used herein, the term high IOP is used interchangeably with ocular hypertension (OHT). High IOP is known to be a risk factor for glaucoma. Some individuals, however, may have high IOP for years and never develop glaucoma or optic nerve damage.

[0067] As used herein, the term "elevated intraocular pressure" (elevated IOP) in humans refers to a human subject having either high IOP or an IOP value that is significantly greater than the subject's baseline IOP. For example, in accordance with one embodiment, a subject having an IOP more than 4 mmHg greater than the subject's baseline IOP would be considered to have an elevated IOP. For example, in accordance with another embodiment, a subject having an IOP more than 5 mmHg greater than the subject's baseline IOP would be considered to have an elevated IOP.

[0068] As used herein, the term "normal tension glaucoma" refers to subjects with glaucoma but with IOP in the normal range.

[0069] As used herein, the term "preventing" or "prevention" with respect to, for example, neuronal dysfunction, damage or death in general refers to the ability of the compounds or agents of the present invention to confer neuroprotection, preferably before such dysfunction, damage, death, or disease occurs. Thus, prevention includes delaying the onset or progression or reducing the severity of neuronal damage/extent of neuronal death/loss among a population of neurons in glaucoma.

[0070] As used herein and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results may include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminution of extent of disease, a stabilized (*i.e.*, not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression,

amelioration or palliation of the disease state and remission (whether partial or total), whether detectable or undetectable.

[0071] The blood-retinal barrier (BRB), like the blood-brain barrier (BBB), controls and restricts the transit of molecules from blood into the surrounding neural tissue. This maintains retinal function and prevents neural damage by immune and other damaging molecules. The BRB restricts entry of molecules into neural tissues by both maintaining paracellular endothelial cell junctions and by actively suppressing transcytosis (transcellular vesicular transport). The Wnt/β-catenin signaling pathway maintains the BBB/BRB by inducing expression of tight junction proteins (e.g., Claudin 5; CLDN5, Zonula occludens-1; ZO-1) and inhibiting transcytosis to a nearly undetectable level. Transcytosis is inhibited by inducing the expression of the major facilitator superfamily domain-containing protein 2A (MFSD2A, a key inhibitor of transcytosis). MFSD2A helps create a lipid-enriched environment that suppresses caveolar vesicle formation and transcytotic transport. The loss of BRB or BBB integrity, either due to the loosening of tight junctions, increased transcytosis, or both, triggers vascular leakage and is damaging in central nervous system diseases.

[0072] In accordance with the present application, the BRB in three models with distinct IOP elevating mechanisms was studied. Two inherited, human-relevant mouse models of high IOP and glaucoma (DBA/2J and B6.*Lmx1b*$^{V265D/+}$ mice) were used. DBA/2J mice develop a chronic form of pigmentary glaucoma caused by genes that affect melanosomal biology and are widely used for glaucoma research. Melanosomal biology also contributes to human pigmentary glaucoma as some patients have mutations in the melanosomal genes *PMEL*. Findings using DBA/2J mice have been translated to clinical trials for primary open-angle glaucoma, (POAG, a common form of glaucoma) with promising initial results. The *Lmx1b* model is directly relevant to human glaucoma as *LMX1B* is an important human glaucoma gene, contributing to both childhood glaucoma and later-onset POAG. Mice with an *Lmx1b* mutation that causes early-onset glaucoma on the C57BL/6J strain (B6.*Lmx1b*$^{V265D/+}$) were used in accordance with the present application. To precisely control the timing of IOP elevation and allow clear determination of the role of IOP, an experimentally induced model of high IOP (HAMA model) was used as a third experimental system. In this model, IOP elevation is induced via injection of a photopolymerizable, hyaluronic acid-based, hydrogel into the drainage angle in front of the ocular drainage

tissues. This stable, biocompatible hydrogel acts as a fluid-flow resistor that elevates IOP without a need to induce tissue damage, contrary to most induced models that use lasers, saline, or beads to block and damage the drainage tissues. In all 3 models, high IOP was shown to induce BRB breakdown and vascular leakage that can be detected by low molecular weight tracers but is subtle and does not involve hemorrhage. Using DBA/2J mice, the leakage could be rescued, which shows that it contributes to neurodegeneration in glaucoma, providing a new target for treatments.

[0073] The present application shows that subtle and partial compromise of the BRB allows low molecular weight molecules to leak from veins into the retinal parenchyma. This partial compromise is shown herein to be a contributor to glaucoma progression. By specifically stabilizing β-catenin within vascular endothelial cells, it is possible to prevent leakage and ameliorate RGC death. Vascular leakage following IOP elevation of distinct etiologies was shown to occur in three models of glaucoma, and on different genetic backgrounds. This suggests that BRB compromise may be a general feature driving glaucoma.

[0074] In all three glaucoma models studied here, BRB leakage was only detected in the ganglion cell layer. In other studies of vascular leakage caused by β-catenin signaling pathway mutations (e.g., LRP-5, Frizzled-4), leakage occurs from both veins and capillaries of all retinal layers. (Chen J, et al. Wnt signaling mediates pathological vascular growth in proliferative retinopathy. *Circulation.* Oct 25 2011;124(17):1871-81. doi:10.1161/CIRCULATIONAHA.111.040337; Ye X, et al., Norrin, frizzled-4, and Lrp5 signaling in endothelial cells controls a genetic program for retinal vascularization. *Cell.* Oct 16 2009;139(2):285-98. doi:10.1016/j.cell.2009.07.047.) This difference is likely related to those mutations profoundly affecting signaling in all of these vascular beds, while for unknown reasons (possibly mechanical) IOP only affects the major veins at the inner retinal surface.

[0075] Although not wishing to be bound by theory, it appears that increased transcytosis due to loss of MFSD2A, but not tight junction (TJ) defects, is the main driver of BRB compromise in D2 mice. The same mechanism appears to be present in the other tested models of IOP elevation. The data disclosed herein for D2 mice shows that experimentally stabilizing β-catenin/Wnt signaling rescues vascular barrier defects in different conditions.

[0076] Therapeutic agents that may be useful herein to reduce vascular leakage include, but are not limited to the following therapeutic agents or methods:

[0077] I. Inhibition of Wnt pathways / β-catenin modulators

a. Lithium chloride prevents degradation of β-catenin. It inhibits the degradation pathway involving GSK-3β and 3β and CK-1α.

b. Indirubins and derivatives - reversible GSK3b inhibitors.

c. Other GSK3b inhibitors including plant and fungal extracts/derived molecules or any class of small molecule with inhibitory properties.

d. Any small molecule that acts by inhibiting molecules that inhibit the Wnt β-catenin pathway.

e. Monoclonal antibodies (mAbs) that interfere with Wnt /B-catenin inhibitors e.g., antiDKKs or antiSFRP mAbs (SFRP and DKKs bind to WNT receptor complex members (FZDs and LRPs,) to inhibit Wnt signaling. Anti-NOTUM Mabs or small molecules (NOTUM inactivates Wnts).

f. Molecules or gene therapies that inhibit GSK-3β and CK-1α or other molecules that act to degrade β-catenin may also stabilize or increase β-catenin.

g. PROTACs (PROteolysis TArgeting Chimera) or molecular glues or any other rationally designed technologies to make therapeutic agents to inhibit, degrade or otherwise interfere with GSK-3β or other molecules that act to promote degradation of β-catenin.

h. Other GSK3b inhibitors including plant and fungal extracts/derived molecules or any class of small molecule that target other molecules which inhibit Wnt or β-catenin signaling.

i. As above using antisense oligonucleotides, antibody drug conjugates etc.

[0078] II. Direct activation of Wnt or β-catenin signaling

a. Gene therapies using viruses or other means to express β-catenin or molecules that would promote its activity in endothelial cells.

b. Gene therapies using viruses or other means to activate Wnt signaling in endothelial or other vascular cells.

c. Wnt/ β-catenin pathway activators that can increase pathway signaling. *See, e.g.*, Bonnet, C., et al., Wnt signaling activation: targets and therapeutic opportunities for stem cell therapy and regenerative medicine, RSC Chem. Biol., 2021, 2, 1144.

d. The Wnt pathway agonist F4L5.13 (AntlerA). It selectively binds with FZD4/ LRP5 and activates β-catenin signaling. It does not bind to other vertebrate FZDs.

e. Wnt or Wnt-derived proteins and other mimics.

f. Norrin- a protein that is the endogenous activator of FZD4 and LRP5/6.

g. Natural products e.g., Plant, fungal, bacterial or marine extracts/molecules that activate Wnt/ β-catenin (*see, e.g.*, **Bonnet 2021** review).

h. Antibody drug conjugates to drive activation.

i. enDUBS (engineered deubiquitinases) to prevent degradation of β-catenin or Wnt activating molecules.

j. Wnt7b - Any molecules to activate Wnt7b gene, its activity, prevent its inhibition/degradation or gene therapy with Wnt7b. *See, e.g.*, Chidiac et al., *A Norrin/Wnt surrogate antibody stimulates endothelial cell barrier function and rescues retinopathy,* EMBO Mol Med (2021)13:e13977.

[0079] III. Activation of MFSD2A

a. Gene therapy expressing or otherwise activating MFSD2.

b. enDUB to prevent MFSD2A degradation.

c. Fish oil /fatty acids can increase MFSD2A in brain; a diet high in fatty acids.

[0080] BRB compromise may occur commonly in POAG and other forms of human glaucoma. It could have been previously missed as tracers need to be used for detection and the leakage could be restricted to the peripheral regions of retinal veins. In the clinic, glaucoma patients are not normally evaluated using vascular tracers, and the vasculature in the far retinal periphery is rarely assessed even in research studies. In the *Lmx1b* mouse model, some leakage also occurred from vessels in the optic nerve head. Although subtle vascular leakage (tracer leakage without hemorrhage) has been noted in the ONH of patients with POAG, vascular leakage is rarely considered in glaucoma and has not been directly targeted by treatment.

[0081] Surprisingly leakage through a compromised BRB contributes to progression in glaucoma, which was previously unexpected. The control of transmigration is a part of the BRB that is molecularly distinct to the prevention of fluid flow through cell junctions or inhibition of transcytosis. (Campbell M, et al., The blood-retina barrier: tight junctions and barrier modulation. *Adv Exp Med Biol.* 2012;763:70-84; Klaassen I, et al., Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. *Prog Retin Eye Res.* May 2013;34:19-48. doi:10.1016/j.preteyeres.2013.02.001). BBB breakdown is an established feature of various neurodegenerative diseases, including Alzheimer's disease. In these conditions, the barrier loss results from either loosening of tight junctions, increased transcytosis, or both.

[0082] The BRB disruption in Alzheimer's disease is less specific and more severe than documented here in glaucoma. It disrupts tight junctions and affects the extensive capillary network in the retina. The results herein suggest that specific venous leakage of tracers would be a useful diagnostic test in glaucoma, and a tool in guiding treatment choices. However, it is possible that different patterns of leakage may occur depending on the magnitude of IOP, type of glaucoma, and/or genetic and environmental background. For example, leakage may be venous leakage, transcytosis and/or junctional leakage. The presence, absence, severity and/or location of vascular leakage may be able to distinguish those whose glaucoma is actively progressing from those in a currently stable state, Another possibility includes the use of such tests to differentiate those with ocular hypertension who need treatment because their glaucoma is likely to progress from those with ocular hypertension who are unlikely to progress and do not need treatment. Currently, it is not possible to predictively distinguish these groups. This results in withholding of necessary treatment from some individuals with high IOP but overtreating of others, and unnecessarily

exposing them to the potential side effects of these treatments. Assessing vascular leakage may solve this need and be useful as a rapid test of treatment efficacy when refining treatment options for individual patients with IOP lowering or other medications, or even when developing new therapeutic molecules using animal models. BRB leakage could conceivably occur in individuals with normal tension glaucoma, either for reasons other than IOP, due to a unique sensitivity of their BRB to IOP levels that are not considered elevated, or because higher IOP was missed due to inadequate sampling.

[0083] In some embodiments, the method further comprises administering one or more additional therapeutic agents, or one or more pharmaceutical compositions thereof, to the subject. In some embodiments, the one or more additional therapeutic agents are selected from the group consisting of an antioxidant agent, an anti-inflammatory agent, an agent that modulates metabolism, an agent that modulates the integrated stress response, an agent that modulates the unfolded protein response, an agent that modulates forms of autophagy, an agent that modulates the expression or activity of genes controlling or mediating antioxidant or other protective responses, a senolytic agent, an agent that modulates the mitochondria or mitophagy, an anti-aging agent, another agent that modulates intraocular pressure, a resilience-boosting agent, an antifibrotic agent, an agent that prevents epithelial mesenchymal transition or endothelial mesenchymal transition, a neuroprotective agent, a gene therapy agent, and a combination thereof. In some embodiments, the agent that modulates metabolism reprograms or boosts metabolism. In some embodiments, the antioxidant agent reduces oxidative stress and boosts antioxidant control. In some embodiments, the gene therapy agent results in genome editing, genome reprogramming, epigenetic editing, epigenetic reprogramming, or a combination thereof. In some embodiments, the agent is an anti-transforming growth factor-β ("TGFB") or ligand trap molecule. In some embodiments, the one or more additional therapeutic agents are selected from the group consisting of pyrroloquinoline quinine, *N*-acetyl cysteine, a pyruvate compound and a combination thereof.

[0084] Examples of other agents that may be used in combination with method disclosed herein include, but are not limited to, nicotinamide, saffron, berry extract or powder, antioxidants (e.g., billberry), flavonoids, anthocyanins, carotenoids, polyphenols, folate, xeoxanthine, polyamines (e.g., spermidine), Ginko biloba (glaucoma vitamin supplement (Glaucocetin), Co enzyme Q10, Vitamin A, Vitamin B12, Vitamin B50 complex, Vitamin C, Vitamin D, Vitamin E, betaine, choline, citicholine, Epigallocatechin-3-gallate (EGCG and its derivatives or analogs), compounds that increase activity of Sirts including Sirt3, lutein,

xeozanthine, molecules that induce genes that protect from oxidative damage, pyruvate, dimethylfumarate and other forms of fumarate, and antifibrotic agents (such as anti-TGFBs, curcumin, blueberry, silymarin, coffee, vitamin C, E, and D, resveratrol, quercetin, and epigallocatechin-3-gallate). In accordance with some embodiments, the additional agent may be a metabolism supporting molecule, such as, but not limited to: ketones, ketone bodies-metabolites used in energy metabolism (e.g., hydroxubutarate, Beta-hydroxybutarate (BHB) and its salts, e.g., acetoacetate). The methods disclosed herein may also be combined with diets shown to help in lowering IOP such as, but not limited to, ketogenic diet and low carb diet. The methods disclosed herein may also be combined with glucagon like peptide (GLP-1) agonists including, but not limited to, Exenatide, Liraglutide and Semaglutide. Taurine and creatine can also be used in combination with the methods disclosed herein. Additional examples useful herein also include molecules and gene therapies that increase or induce NMNAT2 (nicotinamide mononucleotide adenosyl transferase) or other NMNATs, molecules or gene therapies that induce or otherwise increase signaling of the TEK/Angiopoietin system, any molecules that increase NAD, or insulin and its derivatives, agents or gene therapies that increase Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) activity/signaling, treatments that increase CNTF, BNDF or other beneficial growth factors. This also includes without limitation encapsulated cells in implants that release the therapeutic agent(s) including these growth factors and/or proteins, peptides, antibodies, agonists, anti-inflammatory molecules, and metabolites. This also includes similar systems for ANPT/TEK, insulin, etc. Additional examples that can be combined with the present treatment include any IOP lowering medications e.g., Latanprost and other prostaglandin analogs. The method disclosed herein could also be combined with laser and/or other surgeries aimed at lowering IOP, with various implanted tubes, shunts and stents that lower IOP or long-term drug formulations and delivery devices. Biotin, hemp seeds/powder, long chain polyunsaturated fatty acids (PUFAS) (e.g., omega 3 fatty acids), spirulina and leafy green powder or extracts can also be combined with the treatment as disclosed herein. Growth factors or insulin can also be combined with the treatment. Insulin is a growth factor and suggested to maintain blood flow in glaucoma. Endothelin antagonists, e.g., Bosentan and Macitentan that promote vasodilation and blood flow can be used. Complement system inhibitors can also be used. Derivatives and analogs of the compounds disclosed herein can also be used.

[0085] In some embodiments, the one or more additional therapeutic agents are administered together in a pharmaceutical composition. In other embodiments, the one or more additional therapeutic agents are administered separately. In still other embodiments, the subject is already being treated with the one or more additional therapeutic agents when the therapeutic agent disclosed herein is administered; or the subject is administered the one or more additional therapeutic agents after being administered the therapeutic agent disclosed herein.

[0086] In some embodiments, the therapeutic agent disclosed herein is administered in an amount sufficient to prevent or reduce vascular leakage, prevent or reduce one or more changes to the structure or function of one or more ocular tissues, prevent or reduce abnormal cell death, or a combination thereof. In some embodiments, the therapeutic agent disclosed herein is administered in an amount sufficient to prevent or reduce malformation or dysfunction of ocular drainage structures, prevent or reduce one or more developmental anomalies, prevent or reduce neural or non-neural cell degeneration, dysfunction, or death, or a combination thereof. In some embodiments, the therapeutic agent disclosed herein is administered in an amount sufficient to prevent or reduce one or more changes in the subject resulting from environmental exposure, disease, aging, metabolic anomaly, mitochondrial anomaly, genetic mutation, or a combination thereof. In some embodiments, the therapeutic agent disclosed herein is administered in an amount sufficient to prevent or reduce one or more developmental anomalies in the subject, such as, but not limited to, changes to anterior chamber depth, pupil abnormalities, iridocorneal adhesions, the ocular drainage tissues or a combination thereof.

[0087] In some embodiments, the amount of therapeutic agent disclosed herein sufficient to produce the effects described in the preceding paragraph will be the same when administered alone or in combination with one or more additional therapeutic agents. In some embodiments, the amount of therapeutic agent disclosed herein sufficient to produce the effects described in the preceding paragraph will be less when administered with one or more additional therapeutic agents than when administered alone.

[0088] In some embodiments, the subject is a mammal. In some embodiments, the subject is a mouse or rat. In some embodiments, the subject is of canine or equine origin. In

some embodiments, the subject is a non-human primate. In some embodiments, the subject is a human.

[0089] In some embodiments, the subject has elevated IOP. In other embodiments, the subject is at risk, including, but not limited to, genetic risk, for developing elevated IOP. In other embodiments, the subject has elevated IOP and is at risk, including, but not limited to, genetic risk, for developing more serious variants or complications of the condition. In some embodiments, the subject has elevated IOP and is at risk, including, but not limited to, genetic risk, for developing additional conditions, which additional conditions may or may not be related to the original condition.

[0090] In any one of the embodiments described herein, the method further comprises administering one or more additional therapeutic agents, or one or more pharmaceutical compositions thereof, to the subject.

[0091] In some embodiments, elevated IOP is a risk factor for a disease or disorder. In some embodiments, elevated IOP is a risk factor for a disease or disorder of the eye. In some embodiments, elevated IOP is a risk factor for a non-neurodegenerative or a neurodegenerative disease or disorder of the eye.

[0092] In some embodiments, elevated IOP is a risk factor for glaucoma. In some embodiments, elevated IOP is a risk factor for age-related macular degeneration. In some embodiments, elevated IOP is a risk factor for dysfunctions, malformations, and/or death of one or more cells, tissues, or structures in the eye.

[0093] In some embodiments, dosages of from about 50 – 5000, more particularly from about 300-2500, still more particularly from about 500-2000 mg/kg/day NAM lessen the severity of ocular developmental abnormalities and IOP elevation in mice. In some embodiments, combination of NAM and other agents capable of modulating metabolism (e.g., metabolic boosting agents) affords a higher degree of protection against the severity of ocular developmental abnormalities and IOP elevation in mice compared to NAM alone. For example, in some embodiments, combination of NAM and a metabolic boosting agent affords greater protection against developmental pupil abnormalities.

[0094] One of ordinary skill in the art can convert the dosages from one species to another using the teachings in Freireich et al., *Quantitative comparison of toxicity of*

anticancer agents in mouse, rat, dog, monkey and man, Cancer Chemother Rep. 50(4):219-244, 1966 (incorporated herein by reference). This results in an animal equivalent dosage based on the mouse dosage. For example, a mouse dosage of 550mg/kg NAM is equivalent to a dose of about 2.7g/day for 60kg person. In some embodiments, treatment is continued until a certain target IOP is obtained. In some cases, treatment is continued for an extended period of time, such as weeks, months or years to prevent development of elevated IOP.

[0095] In accordance with some embodiments, typical dosing may be once, twice, three or more times a day. Total daily dose may be administered once, or administered as two, three or more separate doses. For multiple dosing, each dose can be the same amount or different amounts. The pharmaceutical composition may be administered in the morning or evening. The pharmaceutical composition may be taken with or without meals.

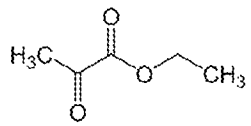
[0096] In some embodiments, the method disclosed herein enables use of lower dosages of other treatment modalities, such as, but not limited to, NAM and pyruvate compounds and other agents capable of modulating metabolism.

[0097] The present disclosure contemplates the use of derivatives or analogs of NAM as well. Derivatives or analogs of NAM may include, but are not limited to, nicotinic acid, nicotinamide riboside, nicotinamide mononucleotide and nicotinamide adenine dinucleotide (NAD^+) and other nicotinoyl ribosides and nicotinamide riboside derivatives that promote the increase of intracellular levels of nicotinamide adenine dinucleotide (NAD+) in cells and tissues. Derivatives or analogs of NAM may also include, but are not limited to, conjugates with imaging agents, macromolecules, biomacromolecules, targeting agents, and isomers and combinations thereof; and pharmaceutically acceptable salts thereof.

[0098] Pyruvate compounds, as used herein, include both the conjugate base pyruvate (CH_3COCOO^-) and pyruvic acid ($CH_3COCOOH$). Pyruvate is the simplest of the alpha-keto acids, with a carboxylic acid and a ketone functional group, and is a key intermediate in several metabolic pathways.

[0099] In some embodiments, the pyruvate compound is a pharmaceutically acceptable salt of pyruvate. Pharmaceutically acceptable salt refers to the relatively non-toxic, inorganic and organic acid addition salts of the compounds. These salts can be prepared in situ during the final isolation and purification of the compound, or by separately reacting pyruvate with a suitable counterion and isolating the salt thus formed. Representative counterions include

potassium, calcium, magnesium, ammonium, arginine, diethylamine, ethylenediamine, and piperazine salts, and the like. For example, in some embodiments, the pyruvate compound is selected from the group consisting of calcium pyruvate, potassium pyruvate, and magnesium pyruvate.

[0100] In some embodiments, the pyruvate compound is a pyruvate alkyl ester derivative. Pyruvate alkyl ester derivatives are forms of pyruvic acid in which an alkyl group is attached to the non-carbonyl oxygen of the carboxylic acid group. Particularly useful alkyl groups include alkyl groups having from 1 to 6 carbon atoms, alkyl groups having 2 carbon atoms are particularly useful (e.g., ethylpyruvate). Ethyl pyruvate has the chemical structure  . The present disclosure contemplates derivatives or analogs of ethyl pyruvate. Derivatives or analogs of ethyl pyruvate may include, but are not limited to, different ester chain lengths or substitutions, different substitutions on the acyl carbon, halogenated and isotopically-derived analogs, and isomers and combinations thereof; and pharmaceutically acceptable salts thereof. Derivatives or analogs of ethyl pyruvate may also include, but are not limited to, conjugates with imaging agents, macromolecules, biomacromolecules, targeting agents, and isomers and combinations thereof; and pharmaceutically acceptable salts thereof. Use of ethyl pyruvate or its derivatives and analogs in a pharmaceutical composition with one or more pharmaceutically acceptable excipients, and/or with one or more drug delivery or targeting vehicles, are also contemplated.

[0101] In some embodiments, the method disclosed herein can also be used in combination with ethyl pyruvate and NAM. In some embodiments, the dosage of ethyl pyruvate is from about 100 – 2000, more particularly from about 250-1000 mg/kg/day in mice and the dosage of NAM is from about 50 – 5000, more particularly from about 300-2500, still more particularly from about 500-2000 mg/kg/day NAM. Human equivalent doses can be calculated as described in the literature.

[0102] In one aspect, a method of treating or preventing blood retina barrier (BRB) compromise in a subject is disclosed. In accordance with one embodiment, the subject exhibits or has exhibited elevated IOP, glaucoma or normal tension glaucoma. The method includes determining if there is vascular leakage associated with the subject's retinal veins,

optic nerve head (ONH) vessels or both and if such vascular leakage is detected, providing treatment to the subject, wherein the treatment comprises administering to the subject a therapeutically effective amount of one or more Wnt signaling pathways activators, MFSD2A, a derivative of MFSD2A or a modulator of MFSD2A, or a pharmaceutical composition of any of the foregoing. Methods for analyzing the eye for vascular leakage include, but are not limited to, angiography. Fluorescein and Indocyanine green (ICG) are two tracers typically used for angiography in a clinical setting. Other tracers can also be used. These tests are well-established clinical tests but they are not typically used in glaucoma and even when looking at other retinal conditions the peripheral retina is not normally included as it is more difficult to view.

[0103] In accordance with one embodiment, the tracer dye is injected intravenously. The tracer molecules fluoresce, emitting light with a longer wavelength. Images are acquired immediately after injection and continued for up to, for example, 30 minutes to monitor mild leakage and excess transcytosis. In accordance with some embodiments, angiography would be used as a routine test for glaucoma. In some embodiments, the emphasis would be more specifically directed to the peripheral retinal veins, which may be uniquely and commonly affected in glaucoma. Other vessels in the optic nerve head (ONH) may also be evaluated. It is surprising that this analysis could be used to assess glaucoma.

[0104] In accordance with some embodiments, human blood test to test for vascular leakage could use a combination of tracers. For example, some combinations that could be used include, but are not limited to, sodium fluorescein or other low MW markers along with labeled Albumin; Sodium Fluorescein or other low MW markers along with labeled ICG/Binds Albumin. This test for vascular leakage could be used as a diagnostic for ongoing glaucoma.

[0105] Furthermore, patient treatment could be guided by the test results obtained by the disclosed testing. For example, the treatment program (e.g., doses, therapeutic agents, etc.) for the patient could be modified to lessen BRB compromise and render/maintain it intact or lessen leakage to as low as possible for that patient by monitoring BRB compromise. This could be modulating the treatment regimen of IOP lowering agents or the Wnt/mfsd2a or other types of therapeutic agents. The target IOP for treatment lowering for any patient is difficult to define. Unsatisfactorily, clinicians have to rely on experience and clinical intuition to set an initial target IOP for each patient. Once that target IOP is reached, time and vision are often lost as it is a wait and see scenario to see if damage gets worse or not. A patient's

susceptibility to damage at a given IOP also changes over time. Assessing the degree and extent/location of BRB leakage could be used to determine when IOP is adequately lowered or when a patient is stable or likely to progress. At the very least it is a measure showing that risk is significantly lowered.

[0106] A combination of lowering IOP to a reasonable clinician decided value along with correction or lessening of BRB compromise would be useful. For normal tension glaucoma, where high IOP is not detected but IOP lowering often helps, monitoring the BRB alone would also be a valuable tool.

[0107] The fact that these tests can be used to make treatment decisions and refine treatment doses and agent combinations is valuable. Importantly, and in addition to refining BRB treating agents, such tests could be used to refine IOP lowering treatments as if IOP is in control the BRB should improve. Metabolism restoring and other glaucoma treatments may also improve the BRB and so it can be an indicator out of their efficacy and used to refine treatment regimens.

Pharmaceutical Compositions

[0108] This application also provides a pharmaceutical composition comprising at least one of the compounds as described herein or a pharmaceutically-acceptable salt thereof, and a pharmaceutically-acceptable carrier.

[0109] The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject pharmaceutical agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and pharmaceutically acceptable for the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as butylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid;

pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0110] As set out above, certain embodiments of the present pharmaceutical agents may be provided in the form of pharmaceutically-acceptable salts. The term "pharmaceutically-acceptable salt", in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present application.

[0111] Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate, magnesium stearate, and polyethylene oxide-polybutylene oxide copolymer as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0112] Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), and/or parenteral administration. Particularly useful formulations include oral and direct to eye or locally around the eye formulations. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated and the particular mode of administration. The amount of active ingredient, which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of 100%, this amount will range from about 1% to about 99% of active ingredient, preferably from about 5% to about 70%, most preferably from about 10% to about 30%.

[0113] Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

[0114] Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

[0115] In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, sodium carbonate, and sodium starch glycolate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and polyethylene oxide-polybutylene oxide copolymer; absorbents, such as kaolin and bentonite clay; lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[0116] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxybutylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets, may be, made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

[0117] The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein.

[0118] Liquid dosage forms for oral administration of the compounds disclosed herein include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isobutyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, butylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Additionally, cyclodextrins, e.g., hydroxybutyl-β-cyclodextrin, may be used to solubilize compounds.

[0119] Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

[0120] Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[0121] Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives or buffers which may be required.

[0122] The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes,

paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

[0123] Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances.

[0124] Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the pharmaceutical agents in the medium. Absorption enhancers can also be used to increase the flux of the pharmaceutical agents of the invention across the skin. The rate of such flux can be controlled, by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

[0125] Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this disclosure. Implants or injectables may also be used to introduce the agent into the eye. Any of the excipients disclosed herein suitable for use in these applications can be incorporated into the pharmaceutical formulations.

[0126] Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds disclosed herein in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[0127] When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1% to 99.5% (more preferably, 0.5% to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

[0128] The representative examples which follow are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those

skilled in the art from the full contents of this document, including the examples which follow and the references to the scientific and patent literature cited herein. It should further be appreciated that the contents of those cited references are incorporated herein by reference to help illustrate the state of the art. The following examples contain important additional information, exemplification, and guidance which can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

EXAMPLES

Methods

Mouse strains, breeding, and husbandry

[0129] All mice were treated in accordance with the Association for Research in Vision and Ophthalmology's statement on the use of animals in ophthalmic research. All animal procedures were performed according to the protocols approved by Columbia University's Institutional Animal Care and Use Committee. DBA/2J mice (strain #000671), DBA/2J-*Gpnmb*$^{+}$/SjJ (strain #007048) and C57BL/6J mice (strain #000664) were purchased from the Jackson Laboratory. Tg(Cdh5-cre/ERT2)1^{Rha} mice were imported from Dr. Carol Troy's lab at Columbia University. Ctnnb1^{tm1Mmt} mice were imported from Dr. Xin Zhang's lab at Columbia University. Both alleles were backcrossed to DBA/2J for >10 generations to produce congenic mice on the DBA/2J background (all experimental mice were ≥N10). B6.*Lmx1b*$^{V265D/+}$ mice were from lab stock. All mice were housed in a 21°C environment with a 14-h light and 10-h dark cycle, fed with a 6% fat diet (PicoLab Rodent Diet 20). Both female and male mice were used for analysis.

Tamoxifen injection

[0130] Tamoxifen (Sigma T5648) solution was prepared at 20 mg/mL in sterile corn oil (Sigma C8267) using a rotator to facilitate dissolution overnight at room temperature, then aliquoted and stored at -80°C. For each mouse, 100 µL of 20 mg/mL tamoxifen in corn oil was administered in 4 intraperitoneal injections spaced 2 days apart.

Assessment of retinal vascular permeability with tracer

[0131] Mice were restrained and intravenously injected with 200 ul 0.02% Hoechst 33342 (Invitrogen, H1399) in 0.9% saline. In some cases, mice were intravenously injected with 100 mg Isolectin GS-IB$_4$ From *Griffonia simplicifolia*, Alexa Fluor 647 conjugate

(Thermo Fisher Scientific I32450) in 100ul 0.9% Saline 1-hour prior Hoechst injection to label the retinal vasculature. Fifteen minutes after Hoechst injection, mice were euthanized by cervical dislocation, eyes enucleated, and fixed in 4% paraformaldehyde in 0.1M Phosphate Buffer pH 7.4 for 1h at room temperature. Retinas were subsequently dissected, flat-mounted onto slides, and imaged with a Leica SP8 laser scanning confocal microscope.

Immunohistochemistry

[0132] Eyes were enucleated and fixed in 4% paraformaldehyde in 0.1M Phosphate Buffer pH 7.4 on ice for 3h. Retinas were blocked for 4h at room temperature in blocking solution (Phosphate-buffered saline, 0.5% Triton-X100, 5% donkey serum), and incubated overnight at 4 °C with primary antibody in blocking solution. After washing 5 times for 40 min at room temperature with washing solution (Phosphate-buffered saline, 0.5% Triton-X100), retinas were incubated with secondary antibody in blocking solution overnight at 4 °C. Retinas were washed for another 5 times for 40 min at room temperature with washing solution, and flat-mounted. The following primary antibodies and lectins were used for this study: Mouse anti-CLDN5, Alexa Fluor 488 conjugate (Thermo Fisher Scientific 352588, 1:500); Rabbit anti-ZO1 (Thermo Fisher Scientific 40-2200, 1:200); Rabbit anti-Desmin (Cell Signaling 5332, 1:200); Rabbit anti-NG2 (Sigma AB5320, 1:200); Goat anti-Albumin, FITC conjugate (Thermo Fisher Scientific A90-234F, 1:200); Rabbit anti-Mfsd2a (Cell Signaling 80302, 1:200); Rat anti-CD31 (BD Pharmingen 550274, 1:50); Rabbit anti-RBPMS (Novus NBP2-20112, 1:200) and Isolectin GS-IB_4 From *Griffonia simplicifolia*, Alexa Fluor 647 conjugate (Thermo Fisher Scientific I32450, 1:50). After staining, retinas were flat-mounted and imaged by a Leica SP8 laser scanning confocal microscope or a Keyence BZ-X810 microscope.

Image analysis

[0133] Hoechst tracer labeled retinal images were processed with Imaris 10.0 software. Smoothed surfaces were created based on Hoechst signal (blue wavelength) with background subtraction and splitting of touching objects. All created surfaces were then filtered by Imaris' inbuilt AI machine-learning tools to separate endothelial cells from neurons. In the first round of AI training, several examples of each cell type category were manually assigned. After the next round of AI prediction, several incorrect results were manually corrected and used for the following training round. After 5 rounds of training, the trained model was saved and applied to other images for automated cell-type discrimination. For

each new image, an additional 1-2 rounds of training were applied. RBPMS-stained RGCs for the whole retina were counted by Imaris 10.0 software Spots function. High resolution whole retinal flat mount images were used. Each individual RGCs was marked by a spot according to RBPMS signal and total number of spots were counted afterwards.

[0134] To quantify tight-junction and pericyte protein fluorescence signals in FIG. 2, the major veins were selected using the "freeform" selection tool in Image J to obtain the integrated density.

IOP assessment and induction of high IOP

[0135] IOP was assessed using the microneedle method as previously outlined in detail. (John SWM, et al., Intraocular pressure in inbred mouse strains. *Invest Ophthalmol Vis Sci.* Jan 1997;38(1):249-53; Savinova OV, et al., Intraocular pressure in genetically distinct mice: an update and strain survey. *BMC Genet.* 2001;2:12. doi:10.1186/1471-2156-2-12). In brief, mice were anesthetized with an intraperitoneal injection of ketamine (99 mg/kg; Ketlar, Parke-Davis, Paramus, NJ, USA) and xylazine (9 mg/kg; Rompun, Phoenix Pharmaceutical, St Joseph, MO, USA) right before the IOP measurement. All IOP measurements were taken during the same time period each day. Anterior chamber (AC) depth was also used to ensure that all eyes subjected to tracer studies had experienced high IOP, as increased chamber depth is a reliable measure of even modest exposure to high IOP in mice. When IOP becomes elevated, IOP values of the mouse population actually spread in each direction as homeostatic regulation and diurnal cycles are perturbed. This is well established in various mouse models, with some mice having higher IOP during the light-period of the day and others during the dark cycle. Thus, high IOP is not detected in every eye at every measurement time and AC depth serves as a reliable surrogate. Increased AC depth was never observed in control normotensive mice. For the experimentally induced model, IOP was elevated by polymerizing a hydrogel resistor in the path of aqueous humor drainage. Instead of penetrating the cornea with a 32 G needle, a small incision was made with a very sharp sapphire knife to prevent corneal damage from the surgery. One mL of HAMA was delivered into the angle using a 100-micron OD glass microneedle. Using this method, IOP becomes promptly elevated and the higher values are sustained for about 4 weeks.

Statistical analysis

[0136] Integrated density as a measure of fluorescent signals (FIG. 2) was compared using Welch's t-test for each marker. In the β-catenin stabilization experiments (FIG. 5),

leakage (measured by counting the number of Hoechst-positive neuron cells per mm^2) and RGC counts of each group were compared using Welch's t-test. Fisher's Exact test was used to compare the degree of nerve damage between control and stabilized β-catenin-expressing groups. IOPs in the treatment study were compared using Welch's t-test.

Results

Increased IOP-induces BRB compromise that precedes neurodegeneration

[0137] To investigate BRB leakage, Hoechst tracer was injected into the tail vein. Eyes were harvested 15 minutes later, fixed and retinas were flat-mounted. Hoechst binds to DNA and only labels the nuclei of endothelial cells that line the inside of the vasculature when the BRB is intact. When the BRB is compromised, Hoechst leaks into the neural parenchyma and stains neuronal nuclei outside of the retinal vasculature.

[0138] The BRB was initially studied in the DBA/2J (D2) model, a widely used model of chronic glaucoma. BRB compromise was detected in D2 eyes that had developed high IOP but not in any age and strain-matched, normotensive, control eyes (D2-*Gpnmb*$^{+/+}$, hereafter called D2-*Gpnmb*+, which do not develop high IOP) (**FIG. 1A-B**). **FIG. 1B** shows quantification of leakage in D2 vs. D2-Gpnmb+. Hoechst-positive neural cell nuclei were counted across the retina from 0.81mm^2 regions containing peripheral veins. The line inside the box denotes median value (50th percentile) and the box contains 70th to 25th percentiles as determined in R. Whiskers extend 1.5 times the interquartile range. IOP first becomes elevated in D2 eyes at 6 months of age, with almost all eyes having high IOP by 8.5-9.5 months of age. High IOP is well-established to induce corneal stretching and deepening of the anterior chamber in mice. This deep-chamber phenotype confirmed exposure to high IOP in each eye with detected BRB compromise. Leakage of tracer from retinal blood vessels in D2 eyes also increased in a manner consistent with a pressure-induced etiology. Leakage occurred in 0% of D2 eyes at 6 months (despite some having been exposed to high IOP as evidenced by deep anterior chambers), 30% at 7.5 months, 60% at 8 months, and 90% of eyes at 9.5 months of age. **FIG. 1C** is a bar chart showing the percentage of retinas with leakage over time in D2 and D2-Gpnmb+ mice. IOP elevation starts at around 6 months in these mice, clearly preceding BRB leakage. The leakage was always subtle in that it was only evident by tracer monitoring with no detected hemorrhage. At 7.5 months of age, when we first observed BRB leakage, neurodegeneration is not detected in D2 eyes, as was confirmed by sensitive optic nerve analysis in a subset of eyes. Thus, BRB compromise occurs prior to

neurodegeneration raising the possibility that it contributes to glaucomatous neurodegeneration.

Tracer leakage in DBA/2J mice is restricted to the GCL and specific to retinal veins

[0139] Hoechst tracer that leaks from blood vessels stains nuclei adjacent to the leak. This allowed for precise location of sites of BRB compromise. Hoechst staining neurons are first detected next to the peripheral segments of veins, where they branch in a Y shape as they travel around the far periphery of the retina (**FIG. 1A**). With longer exposure to high IOP, the leakage spreads more centrally as is evident in many 8-month-old D2 eyes. No leakage was ever detected from arteries or capillaries. Importantly, leaked tracer was restricted to the ganglion cell layer (GCL) consistent with leakage from the major retinal veins of the inner retina. No leaked tracer was present in the inner or outer plexiform layers even with more extensive aging to 14.5 months of age.

IOP-induced BRB compromise appears to be general feature of glaucoma

[0140] IOP elevation in DBA/2J mice is induced by a pigment-dispersing iris disease that induces a pigmentary form of glaucoma. To determine if IOP-induced BRB compromise occurs in eyes with a very different IOP elevating etiology and to initially assess its relevance to more common human glaucoma, the BRB in mice with a mutation in a human glaucoma gene, *Lmx1b,* which contributes to a spectrum of human glaucoma including POAG was tested (**FIG. 2A, 2B**). B6.*Lmx1b*$^{V265D/+}$ mice develop high IOP beginning at 3 months and first have glaucomatous degeneration at 6.5 months of age. Hoechst tracer again detected BRB compromise in ocular hypertensive *Lmx1b*$^{V265D/+}$ mice but not in normotensive *Lmx1b*$^{+/+}$ control littermates (**FIG. 2A, 2B**). Tracer leakage was evident in 5.5 months old eyes. This BRB compromise again seems to precede neurodegeneration and be venous specific with leaked tracer restricted to the GCL (**FIG. 2A**).

[0141] As a final model with distinct IOP elevating etiology, IOP elevation was induced using a photo-polymerizable HAMA hydrogel. Although not a genetic model with a human counterpart, this model allowed us to definitively control the timing of IOP elevation and assess the effects of high IOP without confounding effects of other ongoing disease processes. Placing this hydrogel, fluid-flow resistor, in front of the ocular drainage tissues reproducibly elevates IOP without requiring tissue-damaging/blocking processes that are typically more variable. This model reproducibly induces a sustained elevation of IOP. To determine the effects of experimentally elevating IOP on the BRB ocular hypertensive

C57BL/6J eyes that had polymerized HAMA were compared to normotensive strain-matched control littermates that were administered HAMA but without photopolymerization (**FIG. 2C**). BRB leakage was detected in ocular hypertensive but not normotensive eyes. Again, BRB compromise precedes neurodegeneration and appears to be venous-specific and restricted to the GCL (**FIG. 2A**). The documented presence of BRB compromise in ocular hypertensive eyes of mice with two distinct types of glaucoma on two genetically distinct strain backgrounds as well as following experimentally elevated IOP suggest that high IOP may generally induce BRB compromise.

Tight junctions and pericytes appear normal

[0142] Since they are a widely used model of chronic glaucoma with findings that have translated to human glaucoma, ocular hypertensive D2 mice were used to investigate the mechanisms of BRB compromise. The BRB is maintained by the neurovascular unit, including pericytes and endothelial cells, with a key component being tight junctions between endothelial cells. Pericytes are key regulators of the BRB by acting to limit transcytosis and modulate tight junction formation. BRB breakdown can result from compromised tight junctions or loss/dysfunction of pericytes. The integrity of tight junctions (TJs) and the distribution and morphology of pericytes was investigated by immunofluorescence in retinal vessels of D2 and D2-*Gpnmb*$^{+}$ mice. (**FIG. 3A – 3D**). **FIG. 3A and 3B** provide representative images of staining with TJ (A, CLDN5 and ZO-1) and pericyte markers (B, Desmin and NG2) in retinal flat mounts of 9-month-old D2-Gpnmb+ normotensive controls and D2 mice. No differences in TJ components or pericytes were detected between ocular hypertensive D2 and normotensive control mice. **FIG. 3C and 3D** summarize quantitative analysis of fluorescence by measuring integrated density (IntDen) in Image J for CLDN5 and ZO-1 (C), and Desmin and NG2 (D). The fluorescent signal is not significantly different between D2-Gpnmb+ and D2 mice for any of the markers analyzed (all $P > 0.5$, Welch's t-test for each marker. $N = 4$-9 per group). The major TJ protein Claudin-5 was also unchanged in retinal flat mounts of HAMA and B6.*Lmx1b*$^{V265D/+}$ mice, and when assessing whole retinal lysates by Western blotting in D2 mice. These findings suggest that neither TJs nor pericyte loss are the main drivers of BRB disruption after IOP elevation.

Abnormally active transcytosis may underlie IOP-induced BRB compromise

[0143] The lack of detected changes to tight junctions led to investigation of a transcytotic mechanism of BRB disruption. Albumin transport is generally considered a

marker of transcytosis. Thus, albumin transport across the vascular endothelium in D2 and normotensive control mice was compared. **FIG. 4A** provides an image showing albumin staining in retinal flat mounts of 9-month-old D2-Gpnmb+ and D2 mice. Albumin is constrained inside the vasculature in D2-Gpnmb+ but leaks into the retinal parenchyma in D2 mice. **FIG. 4A** also provides a chart showing quantification of albumin leakage as fluorescence intensity outside of the vessels. Albumin remained confined within retinal vessels in age- and sex-matched normotensive D2-*Gpnmb*$^{+}$ mice reflecting an intact BRB with strongly repressed transcytosis. In contrast, albumin leakage was readily evident from retinal veins in ocular hypertensive D2 mice of both sexes, consistent with excessive transcytosis due to deficient repression of vesicular transport. This suggests that abnormally active transcytosis contributes to IOP-induced BRB compromise.

[0144] Given the role of MFSD2A as a key transcytosis inhibitor in CNS veins and capillaries (but not arteries), it was investigated to determine if it is affected by IOP (**FIG. 4B-4D**). Further supporting a transcytotic mechanism, MFSD2A protein assessed by IF was decreased in endothelial cells of peripheral retinal veins of ocular hypertensive mice. In contrast, MFSD2A was not decreased in capillaries of the same hypertensive eyes or in any vessels of normotensive control eyes. Furthermore, the regional loss of MFSD2A matched the locations of BRB compromise (**FIG. 4B-4D**). For example, albumin leaked at sites with no detectable MFSD2A, but was constrained to the vascular lumen in regions where endothelial cells still expressed MFSD2A, even if that MFSD2A expression was at lower-than-normal levels. **FIG. 4B** shows areas with total loss of MFSD2A expression (arrowheads) and reduced expression (arrows). **FIG. 4C and 4D** show that absence of MFSD2A correlates with albumin leakage (arrowheads, **FIG. 4C**). Meanwhile, if the vein retains some MFSD2A expression, albumin is confined within the vessel (arrows, **FIG. 4D**). These data argue that excessive transcytosis due to loss of MFSD2A may be the cause of BRB dysfunction following IOP elevation in D2 mice.

Stabilization of β-catenin prevents BRB breakdown and ameliorates glaucoma

[0145] To test if the BRB leakage damages RGCs in glaucoma, specifically stabilizing β-catenin in endothelial cells was studied to see if it rescues the BRB and modulates glaucoma development. An endothelial cell-specific Cdh5-Cre/ERT2 was used to conditionally activate the expression of a stabilized allele of β-catenin (*Ctnnb1*^{flex3}) (**FIG. 5A-5D**). This allele encodes a form of β-catenin that cannot undergo normal phosphorylation and so is degraded at a decreased rate. Tamoxifen was used to activate the Cre and stabilized β-catenin allele in

7 months old D2 mice (D2.*Ctnnb1*$^{flex3/+}$; Cdh5-Cre/ERT2), **FIG. 5A** provides an image showing Hoechst staining in 9-month-old D2. Ctnnb1flex3/+ (Control) and D2.Ctnnb1flex3/+; Cdh5-CreERT2 (Cre β-Cat) mice. FIG. 5B is a graph showing quantification of leakage in Control vs. Cre β-Cat mice. Hoechst positive neural cell nuclei were counted across the retina from representative regions over the peripheral veins in each eye. Leakage was significantly reduced in the stabilized β-catenin-expressing group (Welch's t-test, p = 0.00012). n = 8/group. The line inside the box denotes median value (50th percentile) and the box contains 70th to 25th percentiles as determined in R. Whiskers extend 1.5 times the interquartile range. FIG. 5C shows that RGC counts are significantly elevated after β-catenin stabilization in Cre β-Cat mice vs. Control mice (Welch's t-test, P = 0.00054, n = 28 (Control), 19 (Rescue). FIG. 5D provides a frequency distribution of optic nerve damage for both groups. Nerve damage was significantly reduced in Cre β-Cat mice at 9 months (Fisher's exact test, P = 0.0072). SEV = severe, MOD = moderate, NOE = no/early nerve damage. N = 40 (Control), 24 (Cre -Cat). Vascular endothelial β-catenin stabilization did not alter the IOP-elevating iris disease or IOP itself at the studied ages. However, the stabilized β-catenin ameliorated vascular leakage (**FIG. 5A, 5B**) and decreased glaucomatous retinal and optic nerve damage (**FIG. 5D**), compared to control mice (D2.*Ctnnb1*$^{flex3/+}$, injected with tamoxifen but lacked *Cre* and so did not express the stabilized allele). Thus, β-catenin stabilization restored BRB function and significantly lessened glaucoma in D2 mice.

[0146] **FIG. 6A** provides an image showing staining in vMfsd2a mice and D2 control mice. BRB is intact in the AAV-Mfd2a mice and leaky in the D2 control mice. **FIG. 6B** provides a frequency distribution of optic nerve damage for both groups. Nerve damage was significantly reduced in the vMfsd2a mice.

[0147] **FIG. 7** shows that high IOP does not induce vascular leakage in 129.Lmx1b$^{V265D/+}$ mice and they do not develop glaucoma. This shows that active leakage reflects glaucoma progression while eyes with elevated IOP but no leakage are stable.

CLAIMS

What is claimed is:

1. A method of treating or preventing glaucoma in a subject comprising administering to the subject a therapeutically effective amount of a therapeutic agent comprising MFSD2A, a derivative of MFSD2A or a modulator of MFSD2A, or a pharmaceutical composition thereof.

2. The method of claim 1, wherein said subject exhibits elevated IOP.

3. The method of claim 1, wherein the modulator of MFSD2A comprises a MFSD2A agonist.

4. The method of any one of claims 1-3, further comprising administering to the subject an additional therapeutic agent.

5. The method of claim 4, wherein the one or more additional therapeutic agents are selected from the group consisting of a Wnt agonist, an antioxidant agent, an anti-inflammatory agent, an agent that modulates metabolism, an agent that modulates the integrated stress response, an agent that modulates the unfolded protein response, an agent that modulates forms of autophagy, an agent that modulates the expression or activity of genes controlling or mediating antioxidant or other protective responses, a senolytic agent, an agent that modulates the mitochondria or mitophagy, an anti-aging agent, another agent that modulates intraocular pressure, a resilience-boosting agent, an antifibrotic agent, an agent that prevents epithelial mesenchymal transition or endothelial mesenchymal transition, a neuroprotective agent, a gene therapy agent, growth factor, insulin, endothelin antagonists, complement system inhibitors and combinations thereof.

6. The method of claim 4, wherein the additional therapeutic agent comprises a pyruvate compound.

7. The method of claim 4, wherein the additional therapeutic agent comprises ethyl pyruvate.

8. The method of claim 4, wherein the additional therapeutic agent comprises nicotinamide (NAM).

9. The method of claim 4, wherein the additional therapeutic agent is selected from the group consisting of a derivative or analog of nicotinamide, wherein the derivative or analog of nicotinamide is selected from the group consisting of nicotinic acid, nicotinamide riboside, nicotinamide mononucleotide, nicotinamide adenine dinucleotide and combinations thereof.

10. The method of claim 9, wherein the derivative or analog of nicotinamide is nicotinamide riboside.

11. The method of claim 4, wherein the additional therapeutic agent comprises a pyruvate compound, NAM, a derivative or analog of NAM, a Wnt agonist, an IOP lowering agent, or combinations thereof.

12. The method of any one of claims 1-11 further comprising prescribing a diet rich in fatty acids to the subject.

13. A method of treating or preventing blood retina barrier (BRB) compromise in a subject, wherein the subject exhibits or has exhibited elevated IOP, glaucoma or normal tension glaucoma, comprising:

determining if there is vascular leakage associated with the subject's retinal veins, optic nerve head (ONH) vessels or both and

if such vascular leakage is detected, providing treatment to the subject, wherein the treatment comprises administering to the subject a therapeutically effective amount of a therapeutic agent comprising one or more Wnt signaling pathways activators, MFSD2A, a derivative of MFSD2A or a modulator of MFSD2A, or a pharmaceutical composition of any of the foregoing.

14. The method of claim 13, wherein the one or more Wnt signaling pathways activators comprises a β-catenin signaling modulator.

15. The method of claim 13, wherein MFSD2A, a derivative of MFSD2A or a modulator of MFSD2A is administered to the subject.

16. The method of claim 13, further comprising monitoring any vascular leakage associated with the subject's retinal veins over a period of time and adjusting the treatment to the subject based on a change in vascular leakage.

17. The method of claim 13, wherein the vascular leakage is associated with the subject's retinal veins.

18. The method of claim 13, wherein the step of determining if there is vascular leakage associated with the subject's retina is determined using angiography.

19. The method of claim 18, wherein the angiography uses either fluorescein or indocyanine green tracers.

20. The method of any one of claims 13-19, wherein the treatment further comprises administering to the subject an additional therapeutic agent.

21. The method of claim 20, wherein the one or more additional therapeutic agents are selected from the group consisting of a Wnt agonist, an antioxidant agent, an anti-inflammatory agent, an agent that modulates metabolism, an agent that modulates the integrated stress response, an agent that modulates the unfolded protein response, an agent that modulates forms of autophagy, an agent that modulates the expression or activity of genes controlling or mediating antioxidant or other protective responses, a senolytic agent, an agent that modulates the mitochondria or mitophagy, an anti-aging agent, another agent that modulates intraocular pressure, a resilience-boosting agent, an antifibrotic agent, an agent that prevents epithelial mesenchymal transition or endothelial mesenchymal transition, a neuroprotective agent, a gene therapy agent, growth factor, insulin, endothelin antagonists, complement system inhibitors and combinations thereof.

22. The method of claim 20, wherein the additional therapeutic agent comprises a pyruvate compound.

23. The method of claim 20, wherein one or more additional therapeutic agents comprises ethyl pyruvate.

24. The method of claim 20, wherein the additional therapeutic agent comprises nicotinamide.

25. The method of claim 20, wherein the additional therapeutic agent comprises a derivative or analog of nicotinamide, wherein the derivative or analog of nicotinamide is selected from the group consisting of nicotinic acid, nicotinamide riboside, nicotinamide mononucleotide, nicotinamide adenine dinucleotide and combinations thereof.

26. The method of claim 25, wherein the derivative or analog of nicotinamide is nicotinamide riboside.

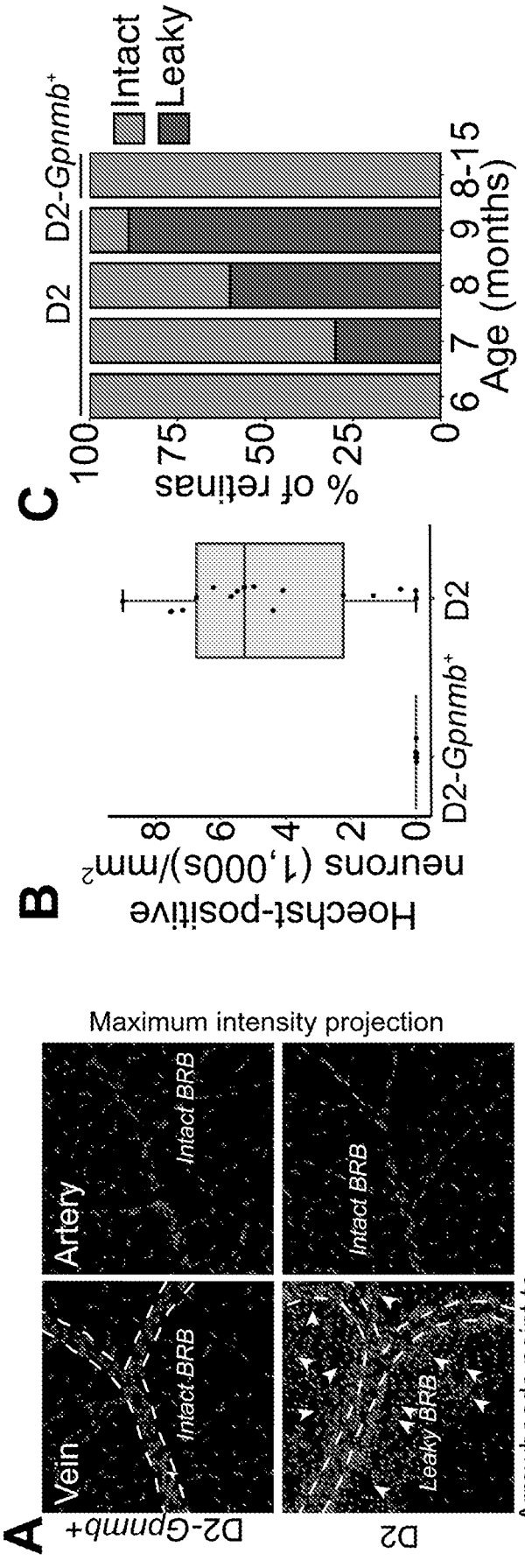
27. The method of claim 20, wherein the additional therapeutic agent comprises a pyruvate compound, NAM, a derivative or analog of NAM, a Wnt agonist, an IOP lowering agent, or combinations thereof

28. The method of claim 1 or 13, wherein the therapeutic agent is conjugated directly or indirectly to an agent that targets retinal endothelial cells.

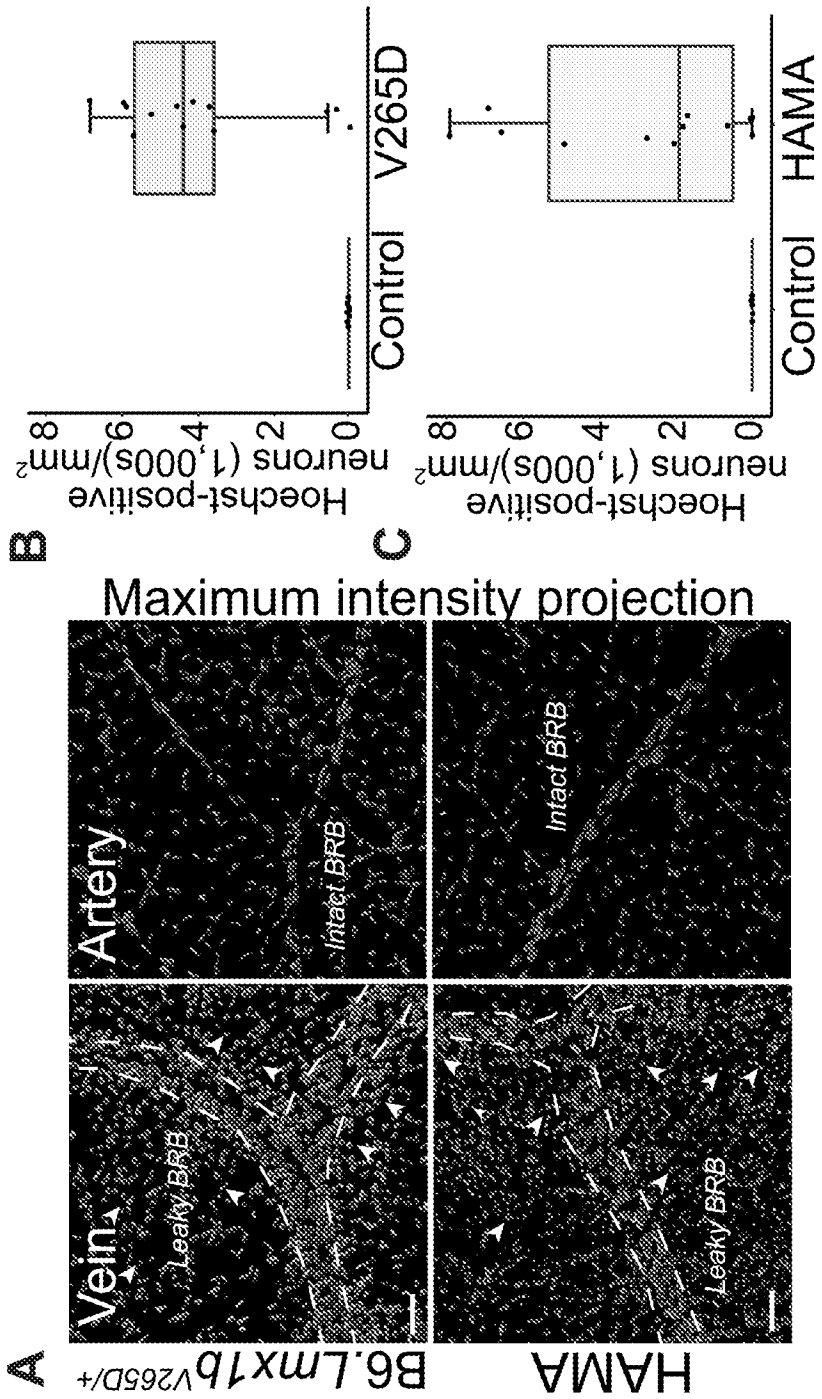
29. A method of monitoring treatment of a subject that has exhibited elevated IOP, glaucoma or normal tension glaucoma and said subject has been prescribed a drug at a dosage level to lower IOP or treat glaucoma or normal tension glaucoma, comprising:

determining if there is vascular leakage associated with the subject's retinal veins, optic nerve head (ONH) vessels or both and

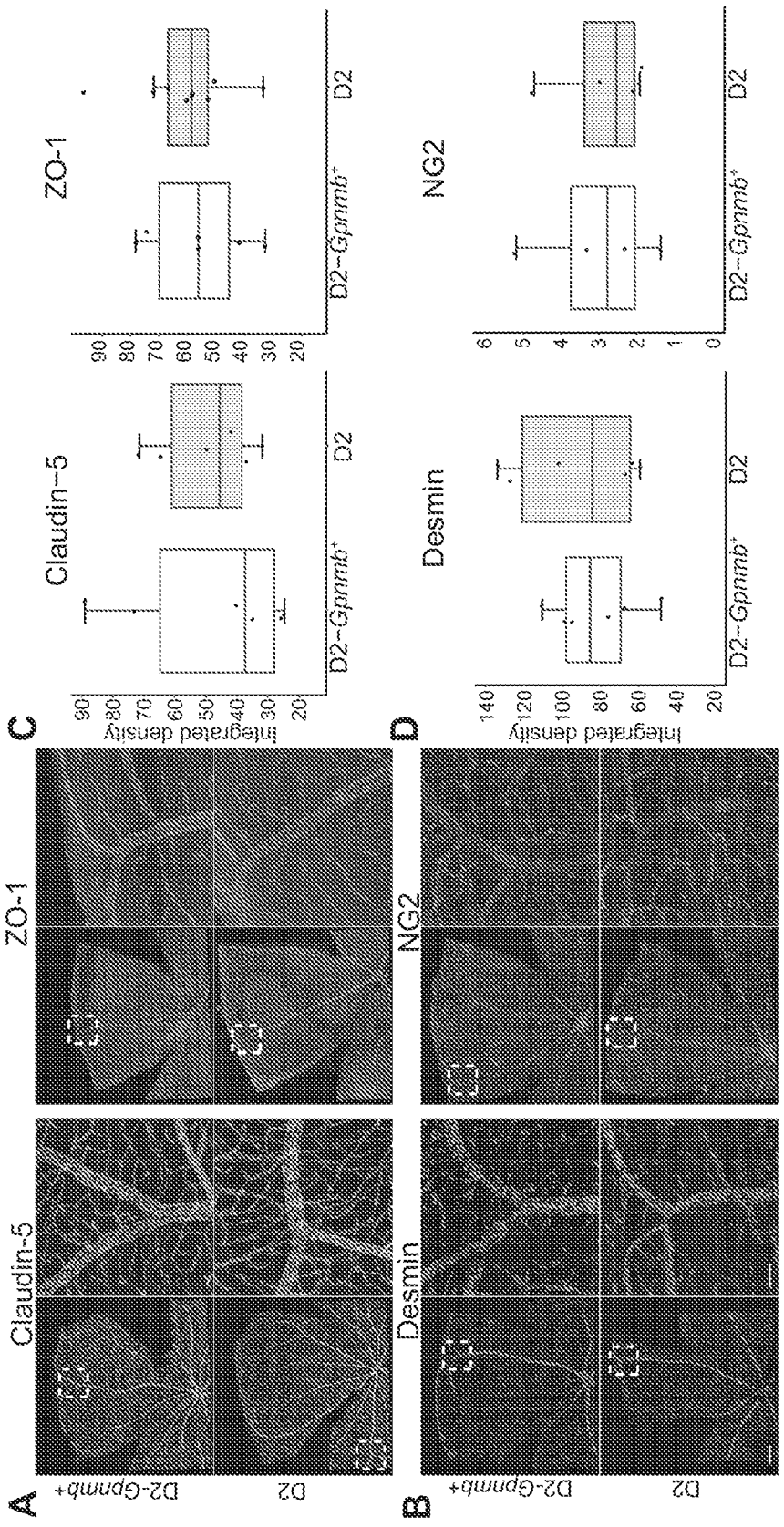
if such vascular leakage is not present, or is present at a low, clinically acceptable level, then discontinuing the drug or lowering the dosage level.


A
Maximum intensity projection
D2-Gpnmb+
D2
Vein
Artery
Intact BRB
Leaky BRB
Arrowheads point to leakage
B
Hoechst-positive neurons (1,000s)/mm²
D2-Gpnmb+
D2
C
% of retinas
Intact
Leaky
D2
D2-Gpnmb+
Age (months)
6
7
8
9
8-15

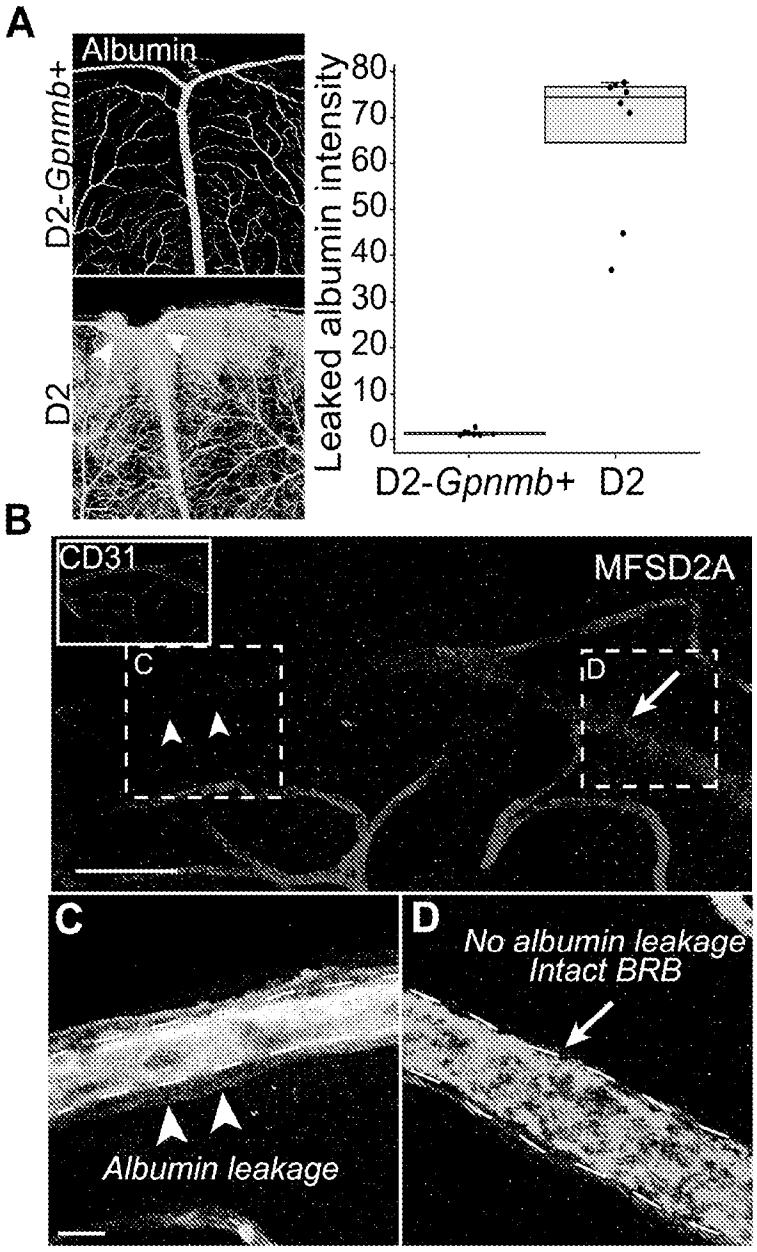
FIGS. 1A-C


A
B
C
B6.Lmx1b V265D/+
HAMA
Maximum intensity projection
Artery
Vein
Intact BRB
Leaky BRB
Intact BRB
Leaky BRB
Hoechst-positive neurons (1,000s)/mm2
Control
V265D
Hoechst-positive neurons (1,000s)/mm2
Control
HAMA

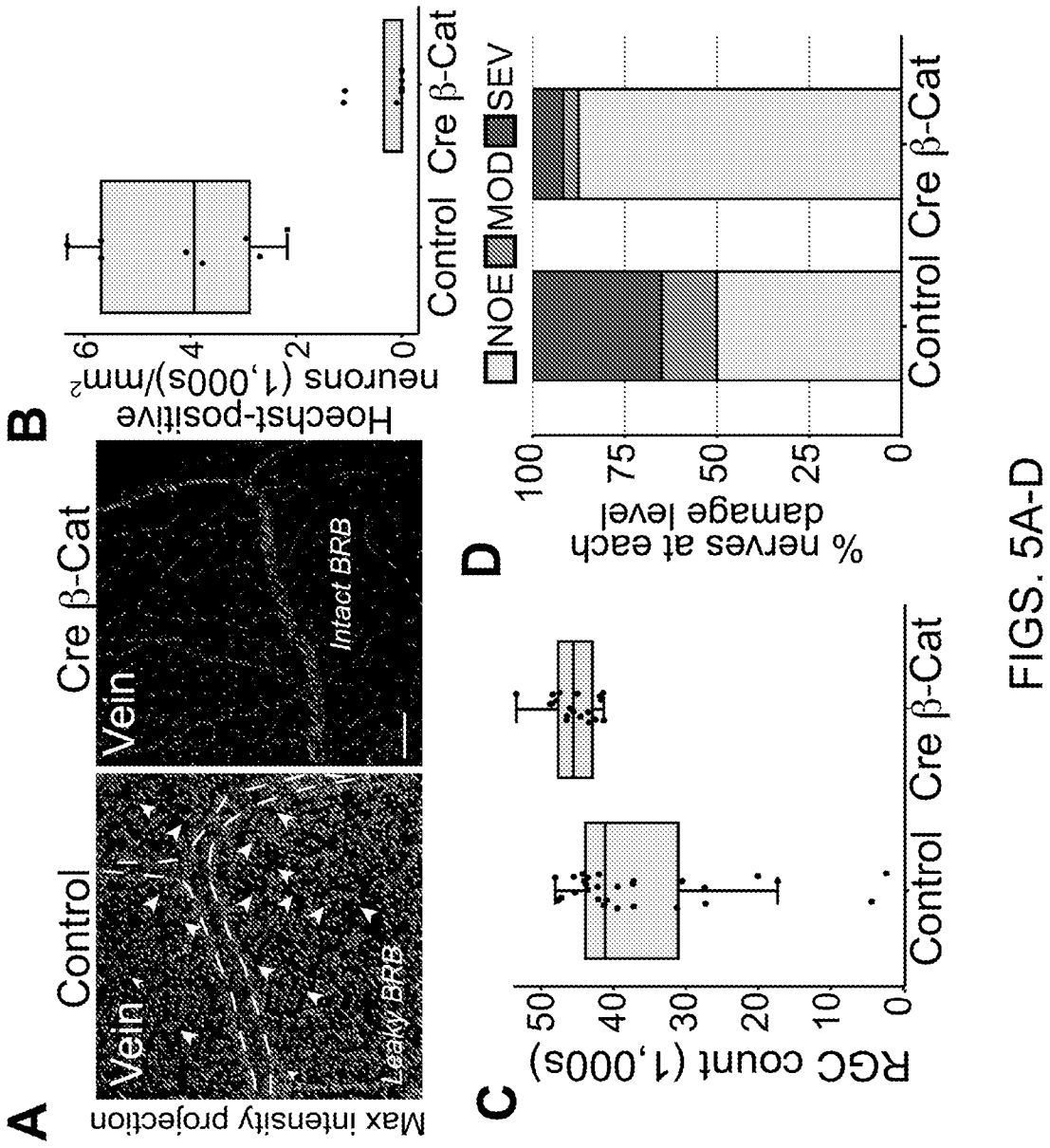
FIGS. 2A-C


A
B
C
D
Claudin-5
ZO-1
Desmin
NG2
D2-Gpnmb+
D2
Integrated density
D2-Gpnmb+
D2

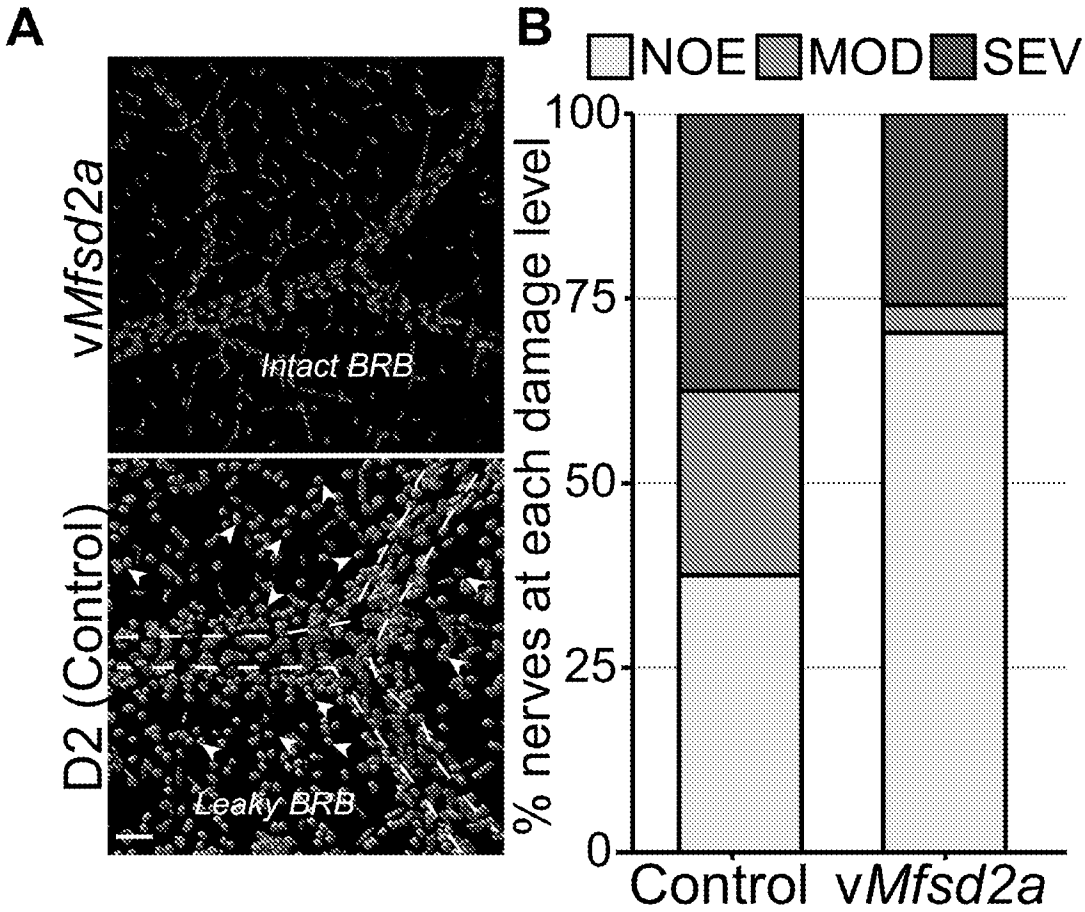
FIGS. 3A-D


A
Albumin
D2-Gpnmb+
D2
Leaked albumin intensity
80
70
60
50
40
30
20
10
0
D2-Gpnmb+
D2
B
CD31
MFSD2A
C
D
C
Albumin leakage
D
No albumin leakage
Intact BRB

FIGS. 4A-D


A
Control
Cre β-Cat
Vein
Vein
Leaky BRB
Intact BRB
Max intensity projection
B
Hoechst-positive neurons (1,000s)/mm²
0
2
4
6
Control
Cre β-Cat
C
RGC count (1,000s)
0
10
20
30
40
50
Control
Cre β-Cat
D
% nerves at each damage level
0
25
50
75
100
NOE
MOD
SEV
Control
Cre β-Cat

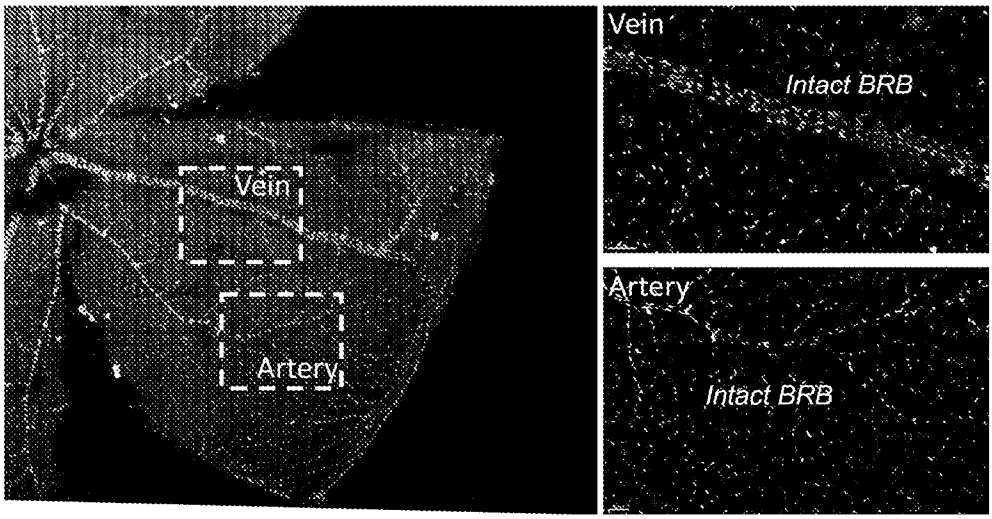
FIGS. 5A-D



Fisher's exact test, $P = 0.03557$

FIGS. 6A-B

BRB is intact in 129.*Lmx1b*$^{V265D/+}$ eyes with high IOP



High IOP does not induce vascular leakage in 129.*Lmx1b*$^{V265D/+}$ mice and they do not develop glaucoma

FIG. 7