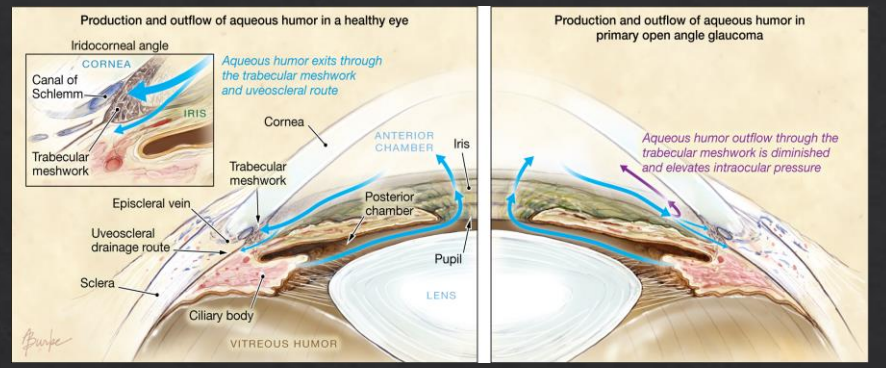
Assessing the integrity of eye-brain communication in a mouse model of pediatric glaucoma

Naseem Amirmokhtari

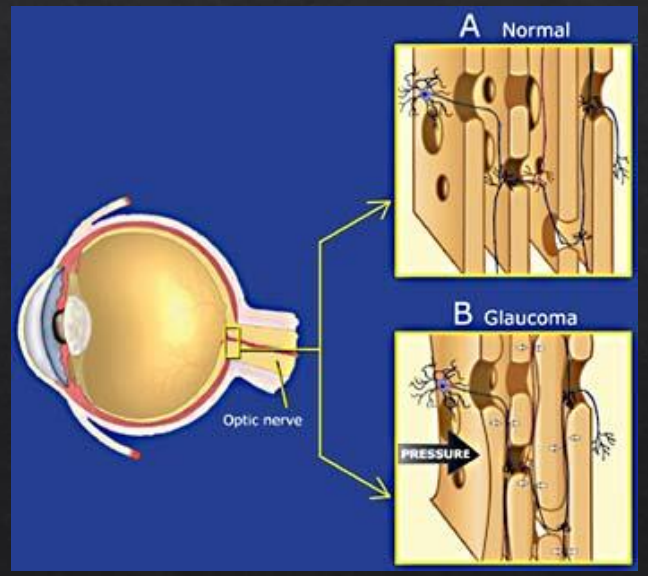
Advisor: Dr. Matthew Smith, PhD

Northeast Ohio Medical University (NEOMED)

Glaucoma



- Group of optic neuropathies—affects both adults and children.
  -i.e. open angle, closed angle, congenital, juvenile etc…
- Global prevalence of 3.54% for 40-80 year old population--projected to afflict 112 million people worldwide by 2040.
- Childhood glaucoma is less common- estimated to be 2.29 per 100,000 patients younger than 20 years (Aponte et. al, 2010).
- Onset and progression primarily attributed to intraocular pressure (IOP) both in adults and kids.
- Regardless of phenotypical nuance, occurring in young or old, glaucoma blinds through damage, dysfunction, and death of retinal ganglion cells (RGCs).



Primary congenital glaucoma (PCG)

- PCG is a specific form of pediatric glaucoma manifesting at birth or within 3-years of age.
- PCG is the most common form of childhood glaucoma in both the US and globally, affecting 1 in 68,254 births in the United States.
- PCG characterization is most often associated with:
  - Increase in intraocular pressure (IOP)
  - Abnormal development anterior eye structures (trabecular meshwork, iridodocorneal angle)
  - Genetic risk factors: congenital glaucoma (CYP1B1 and LTBP2), developmental glaucoma (PITX2, FOXC1, PAX6, and LMX1B), juvenile-onset primary open angle glaucoma (MYOC), and familial normal-tension glaucoma (OPTN and TBK1).

Genetic considerations for PCG

- Two risk factor genes implicated cytochrome P450 1B1 (CYP1B1) (most common) and latent transforming growth factor binding protein 2 (LTBP2)

- Most often recessive---mutations include missense mutations, nonsense mutations, frameshifts, and large-gene deletions (See Wang and Wiggs, 2014 for review).

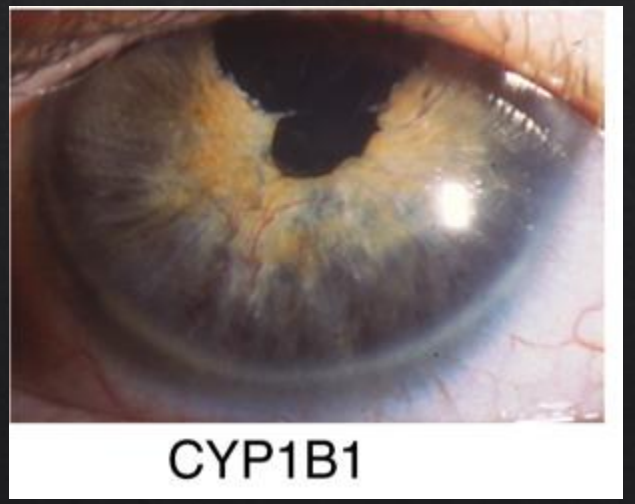
- "Deletions" cause more severe/early onset disease phenotypes

- Primary role of protein in PCG is unclear----may actively play a role in retinoic metabolism which is necessary for proper anterior eye development (trabecular meshwork and iridocorneal angle).

  ---Patients commonly present at birth with anterior segment dysgenesis

- In addition to anterior ocular tissues, CYP1B1 is expressed in retinal bipolar cell and retinal ganglion cells. Concentration increases after birth.

Lui et al., 2013



Wang & Wiggs, 2014

Key Unknowns

- What role does CYP1B1 play (beyond the front of the eye) under normal conditions in the retina?
- What role does it play in the pathophysiological onset and progression in PCG?
- Are retinal ganglion cells affected differentially in pediatric v. adult cases?

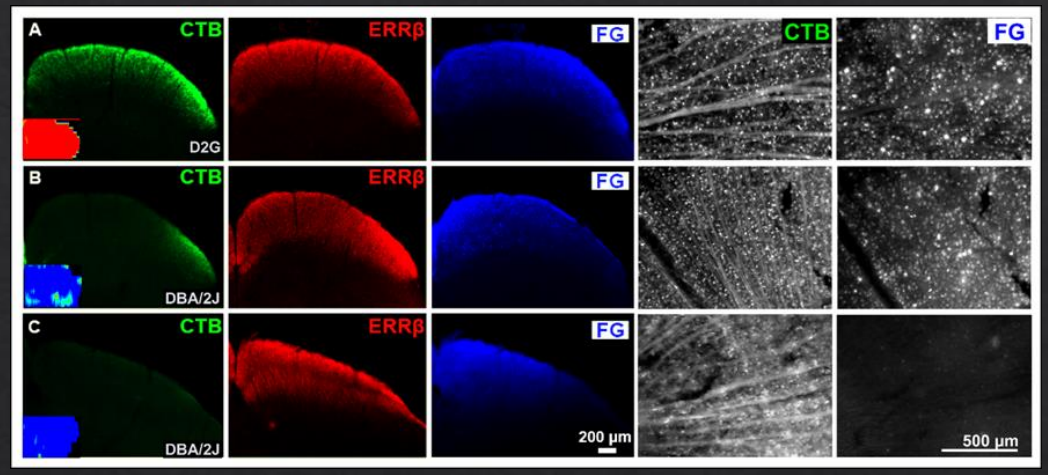
Pathophysiology of Glaucoma
What we know from studying animal models of glaucoma…

- Two principal mouse models;
  - -Naturally occurring =DBA/2J (adult)
  - -Inducible = Microbead occlusion (most done in adult)

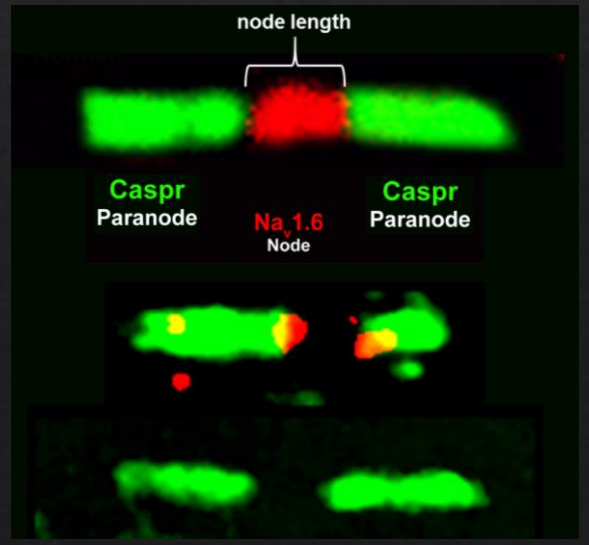
- Semifunctional axons persist after the onset of pathology
  - - Cytoskeletal disruption (Wilson et al., 2016)
  - - Axonal transport deficits (Dengler-Crish et al., 2014)
  - - Gliosis (Cooper et al., 2016)

- Axons remain connected to an extent to brain targets until late in the disease---early synapse metabolic and morphology changes present early on (Smith et al., 2016)

- Early axon functional decline---driven by alteration in node of Ranvier and axon structure and molecular composition
  - ---BUT only in the case of naturally occurring models



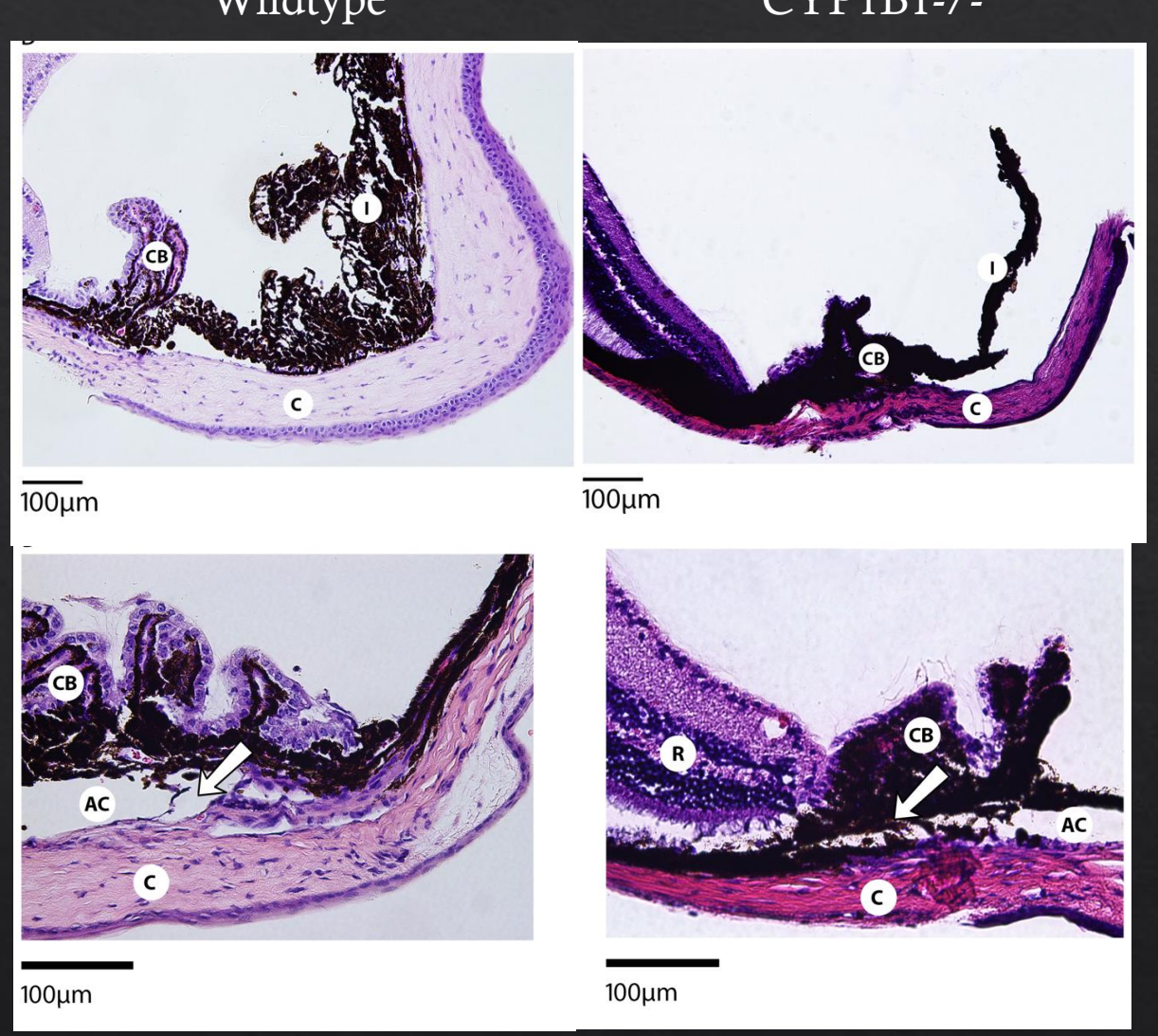
Dengler-Crish et al., 2014



Smith et al., 2018

Animals models for PCG

- CYP1B1-/- mice have been used to study PGC
- CYP1B1-/- mice exhibit anterior eye abnormalities specifically in their ocular drainage structures that are similar to those reported in human PCG patients.
- Controversial, whether mice develop a glaucomatous phenotype.
- Mice have predominantly been used to assess CYP1B1 role in the developing anterior eye structures.
- Unclear how the retina and rest of visual projection are influenced by CYP1B1 absence.



Project Aim

To assess the structural and physiological integrity of retinal ganglion cells in the CYP1B1-/- mouse

Methods

For function

Quantitative Optomotor Response to Measure Visual Acuity

- The optomotor response (OMR) is a reflex used to assess visual function. We used the PhenoSys qOMR system that automatically uses a virtual stimulation cylinder that continuously aligns with the animal´s head position. Based on real-time head tracking, quantitative OMR measurements run fully automatically and objectively.

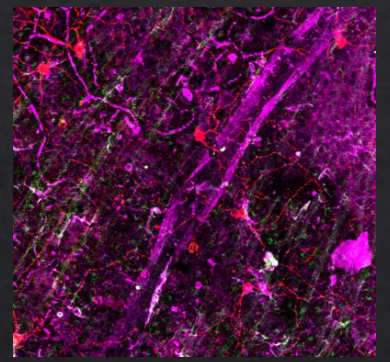
Pattern Electroretinogram (PERG) to Assess Ganglion Cell Function

- Used clinically and in the research setting to directly assess retinal ganglion cell activity.
- Obtained in response to contrast modulation of patterned visual stimuli at constant luminance, i.e. contrast-reversing black and white bars.

For Structure

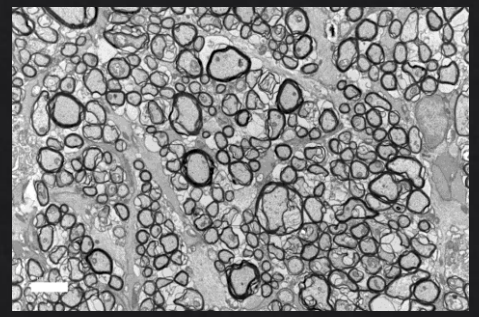
Immunohistochemistry with Epifluorescent Microscopy

- To assess retinal ganglion cell (RGC) soma, synapse, axonal transport and axons structural integrity
- Use of specific antibodies chemically conjugated to fluorescent dyes that bind directly to cellular antigens. Allows visualization of proteins/biomolecules in post-mortem fixed tissue



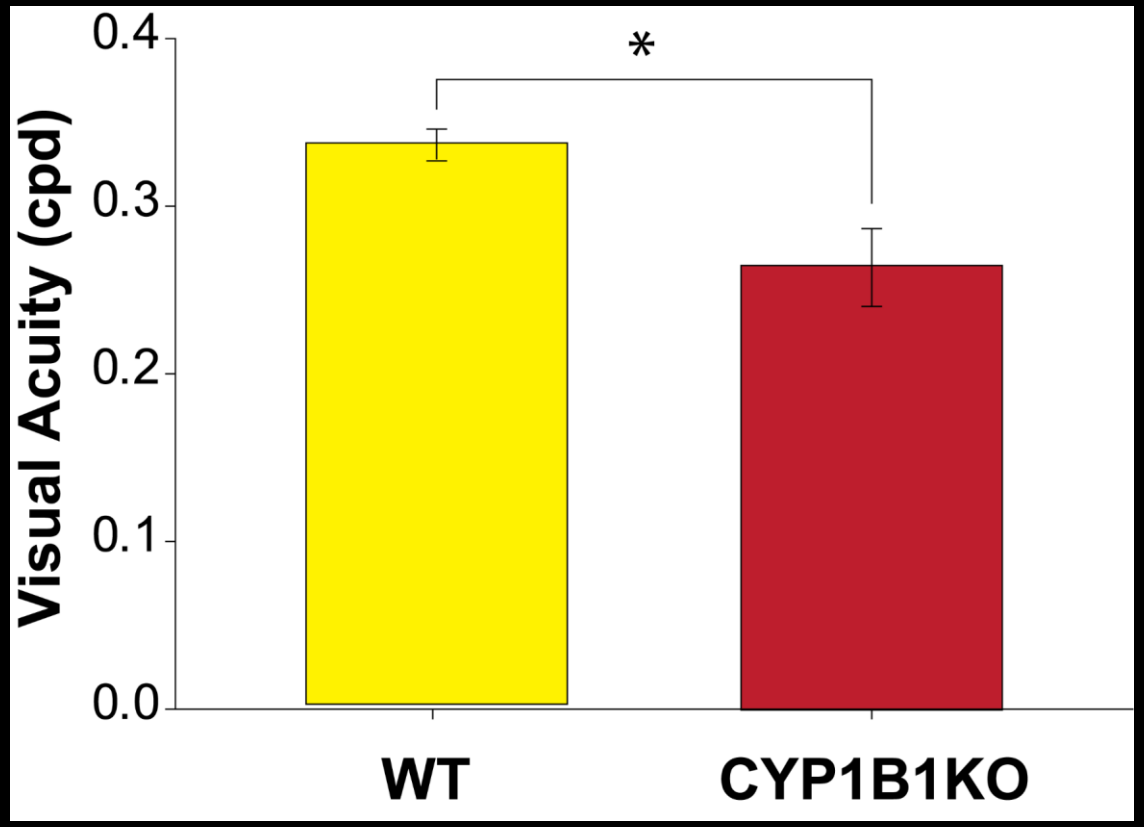
Electron Microscopy

- To assess the ultrastructural composition of RGC axons.



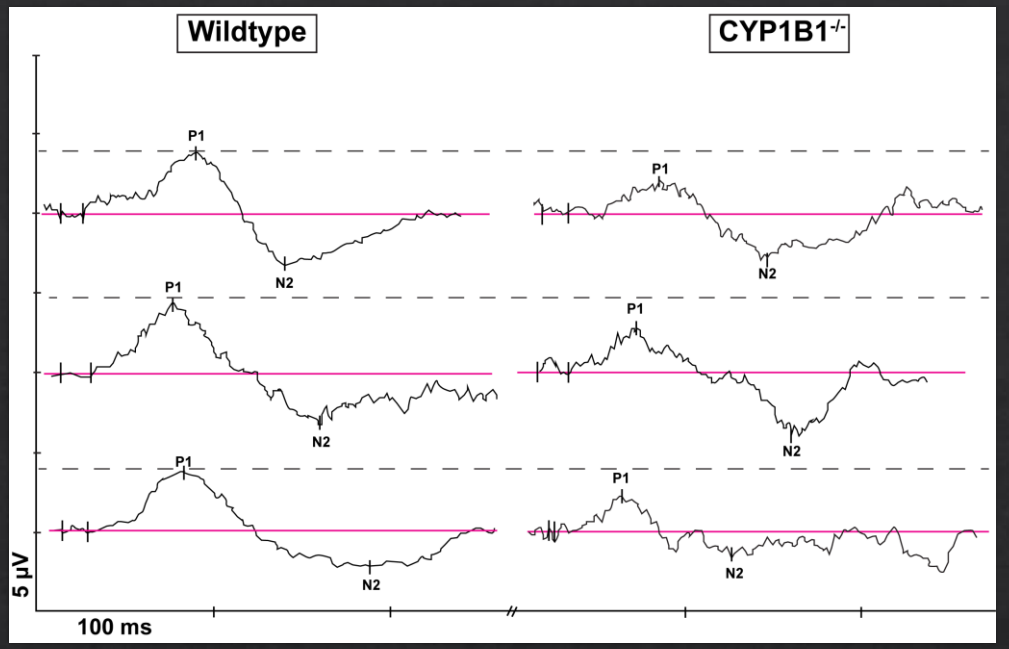
Results: Cyp1b1-/- show poorer visual acuity

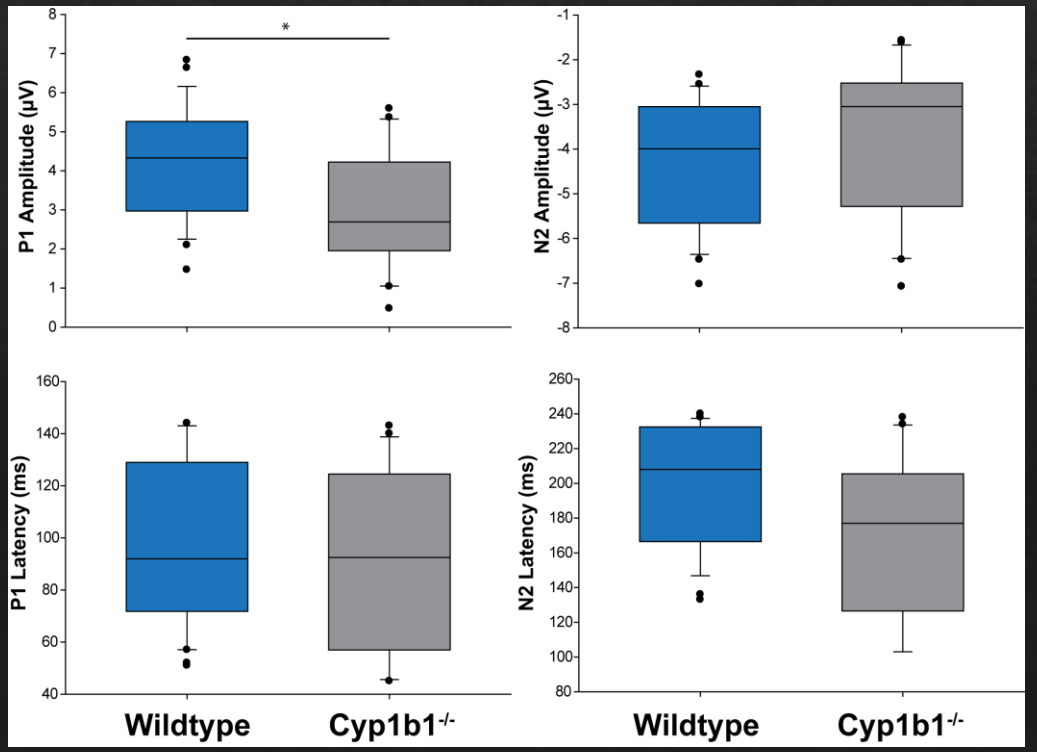
- A score of 0.4 indicated perfect acuity while a score of 0 would be indicative of a completely blind animal.
- Cyp1b1-/- reveal a poorer acuity averaging 0.25 cpd compared to the age matched wildtypes, averaging 0.32 cpd.
- Predicated on the entirety on motor reflex systems being intact, not just visual system.
- Poorer acuity indicates broad defect, but is not sensitive enough to localize it i.e. whether problem is at the level of the retina, the optic nerve, in the brain



Results: Cyp1b1-/- show reduced RGC Activity

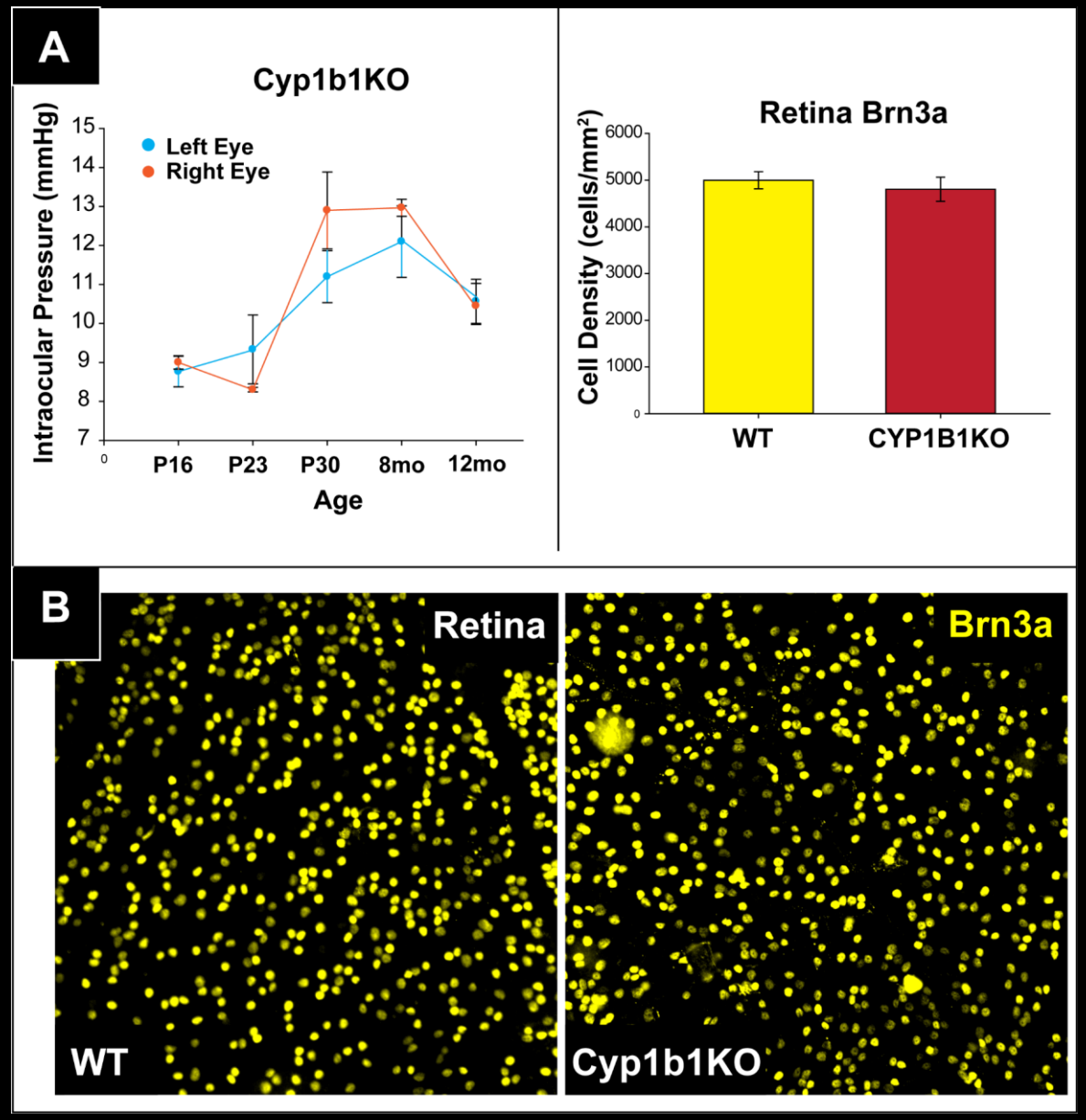
- CYP1B1-/- mice show reduced P1 component amplitude indicative of reduced RGC responsive to visual stimulus.
- P1 effect is similar to what is seen clinically and in in other animal models with glaucoma.
- No significant difference was seen in N2 amplitude nor with regard to the peak onset latency of both the P1 and N2 components.
- Maintained N2 amplitude unusual
- P1 and N2 amplitudes typically decrease with onset of RGC degeneration in glaucoma---however---can be influenced by pre-degenerative mechanisms.





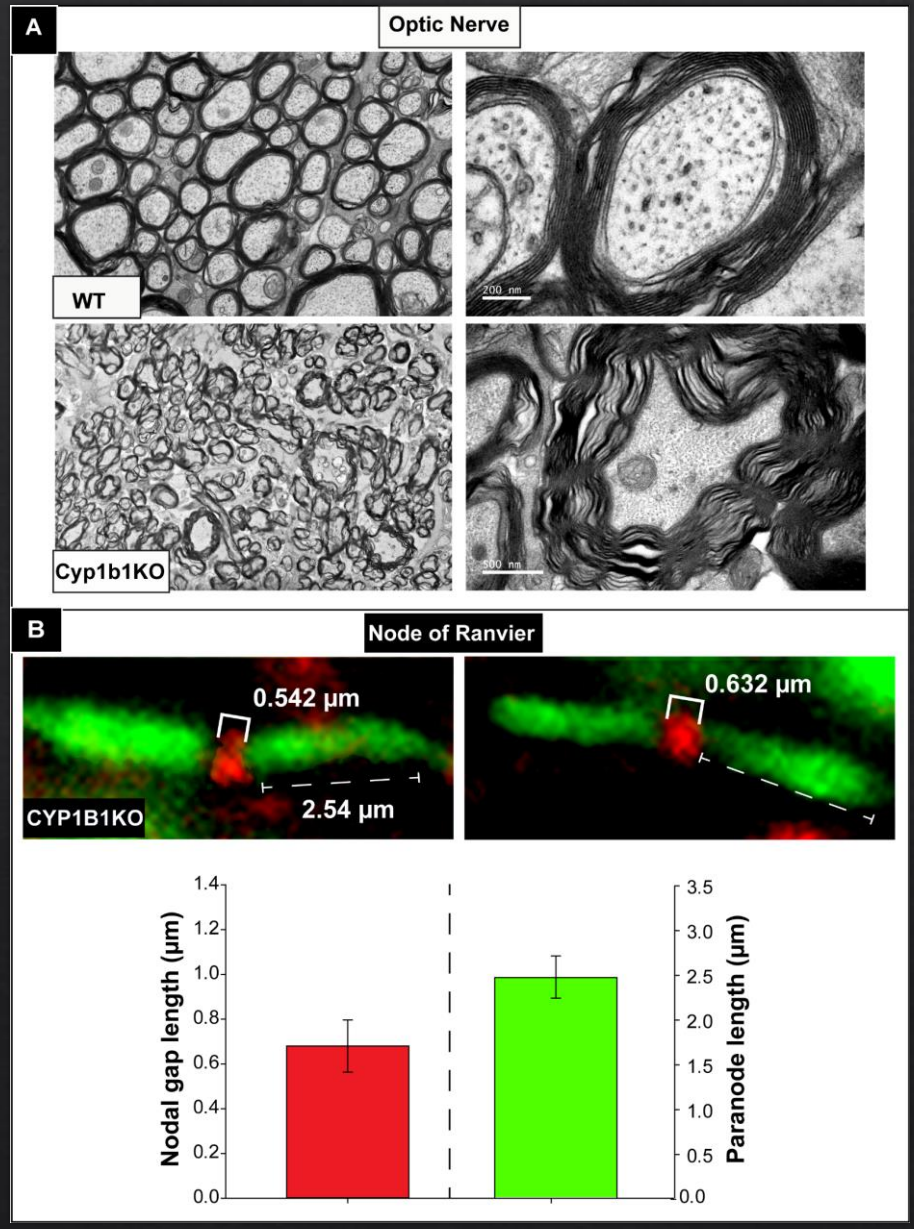
Results: Absence of RGC degeneration and IOP elevation in Cyp1b1-/-

- Cyp1b1-/- ocular pressure readings across the lifespan (P16 to 12-mo) maintain within normal physiological range in both eyes (panel A left).
- Cyp1b1-/-(red) retinal ganglion cell densities do not differ from WT retina (panel A right and B).
- PERG abnormalities cannot be traced to RGC somal loss in the retina BUT need to look at the whole system.

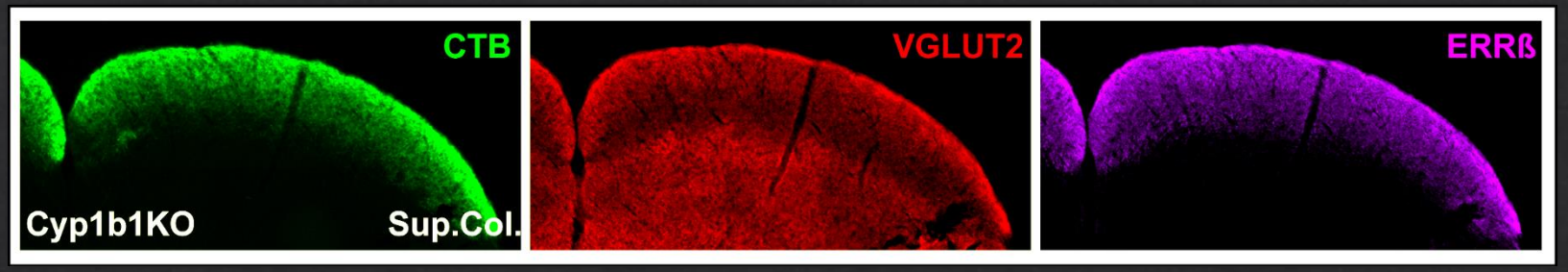


Results: Abnormal axon morphology in Cyp1b1-/-

- Optic nerve cross sections reveal discontinuous focal myelin separations and increased cytoskeletal density /compaction in Cyp1b1-/- axons.
- Cyp1b1-/- nodes of Ranvier appear absent of major morphometric changes in the node (Nav1.6, red) and paranode (Caspr, green).



Results: Intact axonal transport and gross synaptic connectivity in Cyp1b1KO



- SC lack signs of axonal transport, synapse or axon loss that typically hallmarks glaucomatous pathology

- Intraocular injection of cholera toxin-B conjugated -alexafluor488 (CTB488, green)

VGlut2: red, RGC presynaptic terminals
Estrogen related receptor-B: magenta, RGC axon + presynaptic axon terminals

Work left to be done

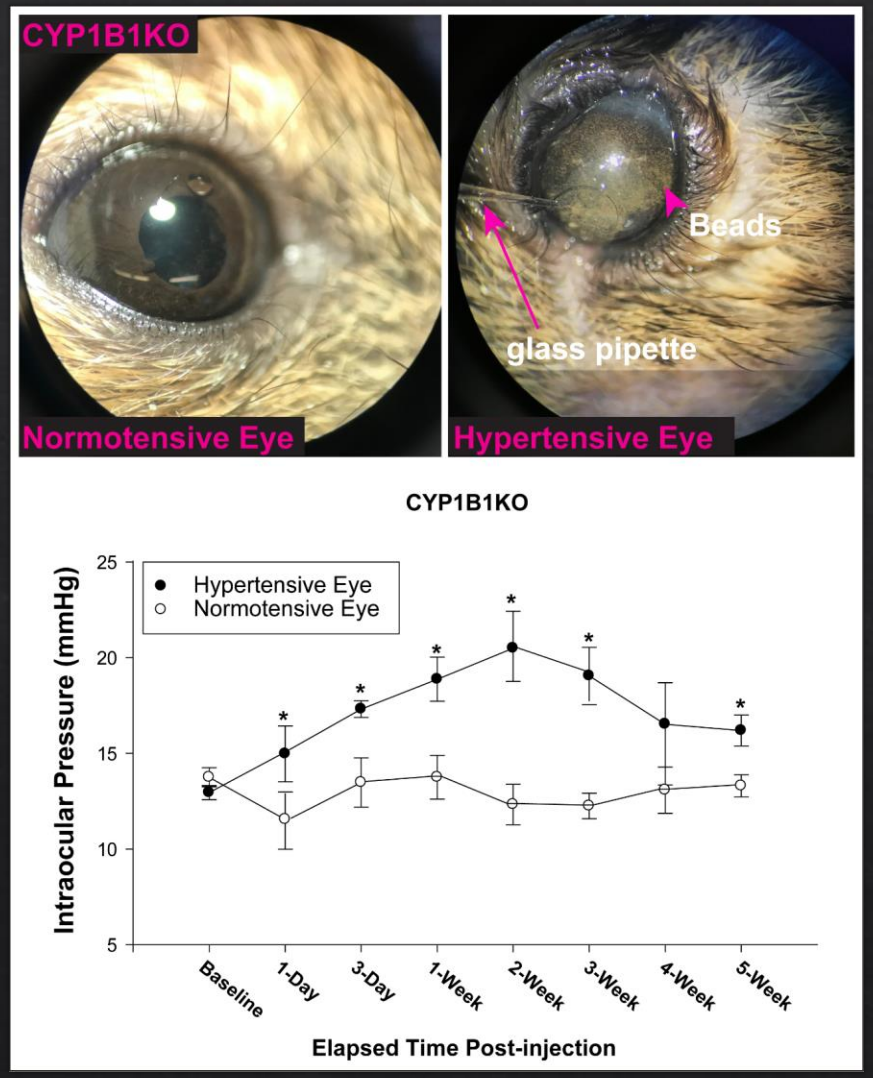
- Cyp1b1-/- mice present with a defect in RGC activity. Remains uncertain what is responsible.
- Defect appears for the most part not a result of major anatomical changes to the visual projection.
- Still need to assess retinal synapses (bipolar to RGC). Given CYP1B1 expression in both bipolar and RGCs under normal conditions its absence could alter the connectively of these cells.
- BUT what about pressure…….

The microbead occlusion model (MOM)

- Given that these animals do not spontaneously develop abnormal elevation in IOP, it is necessary to add pressure back into the equation to see how they respond.

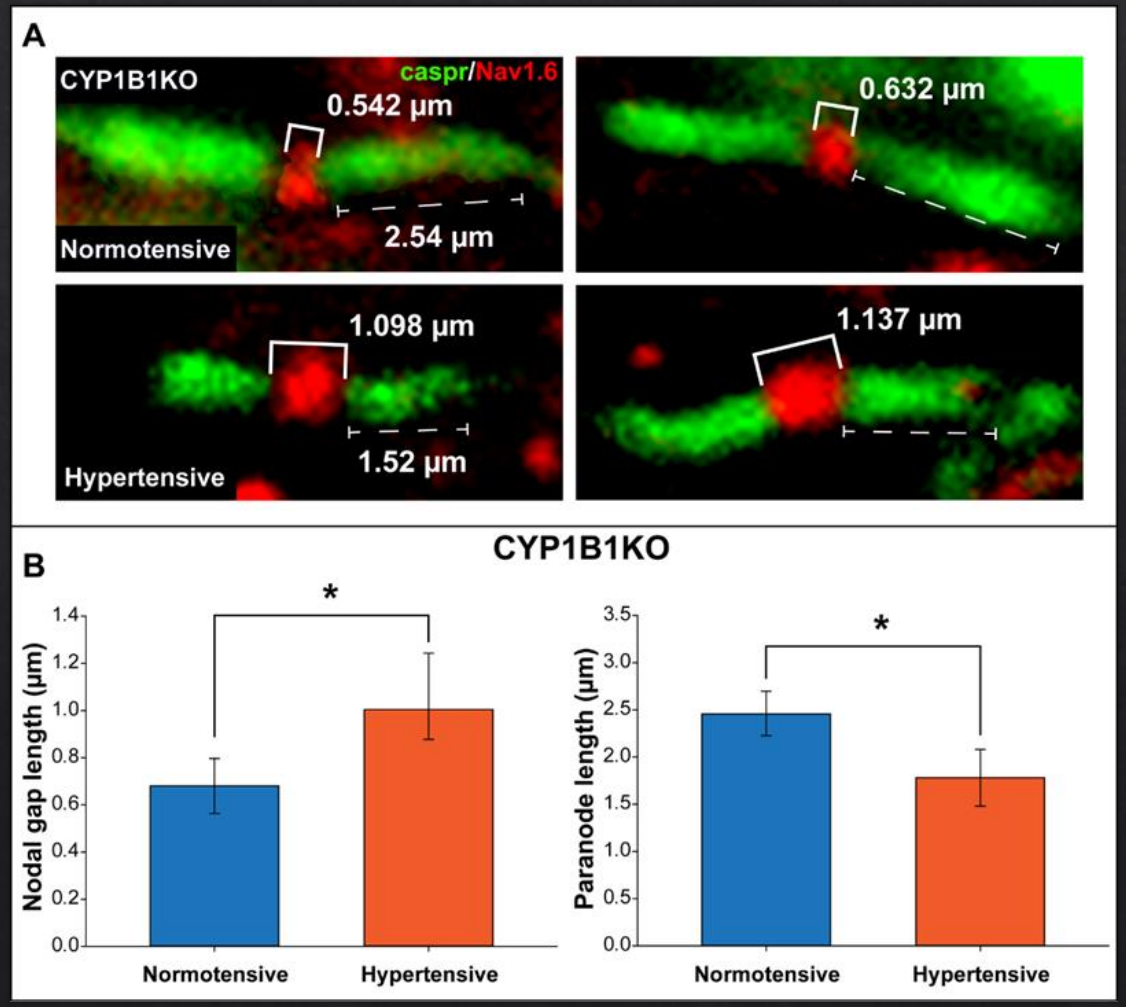
- Using the microbead occlusion model we increased intraocular pressure in the CYP1B1KO mice.

Microbead occlusion in CYP1B1-/-



Results: Node of Ranvier alterations present in CYP1B1-/- post ocular microbead occlusion

- Node of Ranvier alterations in CYP1b1-/- post ocular microbead occlusion.
- Increase in node length + reduction in paranode length. Sodium channels remain normally distributed.
- Node of Ranvier changes NOT normally seen as a result of IOP elevation post-microbead occlusion.



Major Conclusions

- A full assessment of the retinal projection in these animals was previously unreported.
- Absence of CYP1B1 alters visual function, but remains unclear by what mechanism.
- Absence of CYP1B1 alone is not sufficient to drive glaucomatous pathology.
- Mutations in CYP1B1 may subject RGCs to be more vulnerable to stressors such as changes in ocular pressure.

Acknowledgments

Prevent Blindness®
Our Vision Is Vision®

Smith Lab

PI: Matthew Smith, PhD

Brian Foresi, B.S. (U.Akron)

Collaborators

Rachida Bouhenni, PhD
(Akron Children Hospital)

Other

Neurodegenerative Disease & Aging Focus Group

Akron Children's Hospital