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ANALYSIS OF OCULAR CHANGES IN A CYP1B1<sup>−/−</sup> MOUSE

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Abstract

The goal of the research is to investigate the structural changes seen in Primary Congenital Glaucoma using a mouse model. Eye sections of a Cyp1b1<sup>−/−</sup> mouse were compared to eye sections of a healthy normal mouse (wild type, WT). Initial examination of the cornea, iris, and iridocorneal angle showed structural changes in Cyp1b1<sup>−/−</sup>. These changes were similar to those described in the literature using other animal models. This suggests that a mutation in Cyp1b1<sup>−/−</sup> in a mouse results in a phenotype similar to that seen in PCG human patients and this model could be used to investigate the pathophysiology of PCG.

Introduction

Primary Congenital Glaucoma (PCG) is the most prevalent form of pediatric glaucoma. This form of glaucoma is present at birth but its manifestations may not be recognized until infancy or early childhood. PCG affects approximately 0.04% of ophthalmic patients and causes 5% of childhood blindness. PCG affects all races, but male patients are found to have a higher incidence. This disease is caused by an inherited abnormality of anterior chamber angle and trabecular meshwork (TM), which results in impediment of aqueous outflow leading to increased intraocular pressure (IOP). The optic nerve can also be damaged due to the high IOP and if left untreated it can cause gradual visual loss and blindness (3). TM is located within the iridocorneal angle where the cornea and iris meet and the sclera transitions into the cornea (1). Abnormal aqueous outflow, corneal opacification, photophobia, epiphora, blepharospasm, and buphthalmia cause IOP (6).
Currently the treatment options for PCG include medical therapies and surgical procedures. Medical therapies such as, beta-blockers, carbonic anhydrase inhibitors, prostaglandin analogues, and other drugs are currently used for lowering the IOP. Cyclodestructive Procedures are another treatment used in pediatric glaucoma for patients with anatomic abnormalities that preclude traditional surgeries. This procedure is administered through ablation of the ciliary body and resultant reduction of aqueous production (2). Goniotomy and trabeculotomy are two of the more invasive surgical procedures that have been used to treat this disease. In 1938, Otto Barkan preformed the first successful goniotomy using a knife to incise the trabecular tissue; however, this treatment is nearly impossible in eyes with signification corneal edema (2). Trabeculotomy is a more recent surgical procedure being used to treat PCG; it involves inserting a trabeculotome into the schlemm’s canal, which tears through the TM into the anterior chamber (3). Both surgical procedures are associated with numerous postoperative complications that include hypotony, late bleb leakage, and endophthalmitis. These problems lead to additional surgeries and/or placement of drainage devices to lower the IOP and prolong the effects of the surgery.

PCG is caused by mutations in three genes: cytochrome p 450 b1 (CYP1B1), latent transforming binding protein 2 (LTBP2) and TEK. Mutations in CYP1B1 have been found to cause a severe phenotype in PCG patients. CYP1B1 encodes a 543-amino-acid dioxin inducible member of the cytochrome p450 gene superfamily (7). The enzyme is involved in the metabolism of a variety of substrates, including steroids and retinoids that act as potential morphogens during development (4). Its expression is increased in fetal eyes compared to adult eyes, which suggest it plays a role in the development and
maturation of ocular tissues.

In this qualitative study, we sought to investigate the effect of \textit{CYP1B1} mutation on the ocular structures of a mouse. The long-term goal of this study is to establish a PCG mouse model.

\textbf{Methods:}

\textit{Mouse Model:} Eyes from mice carrying a mutation in \textit{CYP1B1} (\textit{Cyp1b1}^{-/-}) were compared to eyes from age-matched healthy mice (WT).

\textit{Histology:} Immediately after enucleation, eyes were fixed in 10\% Buffered Neutral Formalin, processed and fixed in paraffin. Paraffin blocks were then cut into thin (6 \textmu m) tissue sections using a microtome.

\textit{Hematoxylin and Eosin (H&E) Staining:} Sections were stained using H&E staining. Eosin is an acidic dye that is negatively charged and stains basic structures red or pink. Hematoxylin is a basic dye used to stain acidic structures purplish blue. This made it possible for the cells to be visible under the microscope.

\textit{Microscopy:} H&E stained sections were then examined using and Olympus bright field microscope at 10x, 20x and 40x magnification. Images of the iridocorneal angle, cornea, and iris were qualitatively compared between \textit{Cyp1b1}^{-/-} and WT mice. Measurements of the epithelial layer of the cornea were taken under 20x magnification.

\textit{Statistical Analysis:} A t-test was used to compare the width of the epithelial layer of the cornea for the two mouse models.
Results:

Wild-type mouse  \[ \text{Cyp1b1}^{-/-} \] mouse

\[ \text{Figure 1.} \] Images of the cornea from (A) wild-type mouse and (B) \( \text{Cyp1b1}^{-/-} \) mouse. Images taken at 20x magnification. Note the irregular organization of the collagen fibers in the PCG corneal stroma (blue arrow) and a thicker epithelium cell layer (red arrow) compared to the normal cornea. S: stroma. Scale bar: 1\( \mu \text{m} \).

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>CYP1B1^{-/-}</th>
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<tbody>
<tr>
<td>Average ( \mu \text{m} ) ± SEM</td>
<td>0.675 ± 0.093</td>
<td>1.14 ± 0.371</td>
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\[ \text{Table 1.} \] Averages for the width of epithelial layer for the two mouse models. The WT mouse had an average width of 0.675 \( \mu \text{m} \) and the \( \text{Cyp1b1}^{-/-} \) mouse had an average width of 1.14 \( \mu \text{m} \). These averages show that there was a difference in the width of the cornea.
There is a statistical difference in the width of the epithelial layer between the wild-type mouse and the Cyp1b1-/- mouse. The average width of WT mouse was 0.675 μm and the average width for the Cyp1b1-/- mouse was 1.14 μm (n=10; p<0.05).

Analysis of corneal sections:

Figure 1 shows a comparison between the cornea of a Cyp1b1-/- and a healthy mouse eye. The collagen fibers within the corneal stroma of the Cyp1b1-/- mouse are no longer organized and have become more condensed (B). This leads to loss of the corneal transparency and therefore the cloudiness seen in PCG patients. Figure 2 shows the statistical analysis of the width of the epithelial layer between the two mouse lines. A t-test was used to compare the two mouse models. It was concluded that there was a statistical difference in the width of the epithelial layer of the cornea between the WT mouse and the Cyp1b1-/- mouse (n=10; p<0.05). The Cyp1b1-/- mouse has undergone structural corneal changes seen in PCG.
Figure 3A. Images of the iris from (A) wild-type mouse and (B) $Cyp1b1^{-/-}$ mouse. Images taken at 10x magnification. Scale bar: 1µm

Figure 3B. Images of the iris from (A) wild-type mouse and (B) $Cyp1b1^{-/-}$ positive mouse. Images taken at 40x magnification. Note the vascularization in the $Cyp1b1^{-/-}$ iris (arrows) compared to normal iris. Scale bar: 1µm

Analysis of iris sections:

Figures 3A and Figure 3B show the difference between cells within the iris of the WT mouse and $Cyp1b1^{-/-}$ mouse. We can see in both figures that the $Cyp1b1^{-/-}$ mouse (B) has condensed and more disorganized structures. Increased vascularization is also
present in both the iris and the optic nerve, as a result of angiogenesis. Angiogenesis is the formation of new blood vessel, which occurs as a result of cells start to proliferate and differentiate.

![Wild-type mouse](image1.png) ![Cyp1b1−/− mouse](image2.png)

**Figure 4.** Representative photos of normal TM, SC, and normal iridocorneal angle histology of (A) wild-type mouse and *Cyp1b1*−/− mouse (B). Images taken at 20x magnification. Note the altered structure of the ciliary body (CB) and iris (I) and the cornea (C) and compressed structures in the *Cyp1b1*−/− mouse compared to the WT mouse. Scale bar: 1µm

**Analysis of the iridocorneal angle:**

**Figure 4** shows structural changes in the iridocorneal angle structure in the *Cyp1b1*−/− mouse compared to WT mouse. These included compressed structures of the angle, altered ciliary body, iris and cornea. These changes may be the cause for angle closure in PCG, which is caused by the sudden increase in IOP. In PCG, the angle is thick and immature. These structural changes are evident when you compare (B) to the control (A).
Discussion:

PCG is caused by an abnormality in the anterior chamber angle and trabecular meshwork. This abnormality prevents aqueous outflow from draining properly, which results in increased IOP. This increase in pressure results in many ocular changes, such as structural changes in the cornea and iris. The increased vascularization seen in $Cyp1b1^{-/-}$ gives reason to believe that this mutation could be linked to p53 expression, and further research will test this theory. The structural changes seen in the $Cyp1b1^{-/-}$ mouse are consistent with changes seen in PCG.

This study demonstrated that there were numerous ocular changes seen in mice with a $CYP1B1$ mutation, which is one of the most causative genes of PCG. These changes are the result of failure of the tissues to develop properly, as well as elevated IOP levels. This analysis confirmed that a mutation in $CYP1B1$ has the ability to cause PCG. Future plans are to use this information to establish a PCG mouse model, and to test the effectiveness of alternative treatment methods.
References


