PDZD2 and primary open-angle glaucoma: Characterization of PDZD2-deficient mice and human PDZD2 SNPs

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INTRODUCTION

Glaucoma is the second **leading cause of blindness** worldwide and it is estimated that around 80 million of the world population would be affected by 2020[1]. Primary open-angle glaucoma (POAG), the most common type of glaucoma, is characterized by increased intraocular pressure (IOP), optic nerve head damage, and progressive retinal ganglion cells (RGCs) death[2,3]. As most glaucoma patients remain **asymptomatic** until late stages and **no curative treatment** is currently available[4,5], there is an urgent need to uncover its early diagnostic markers.

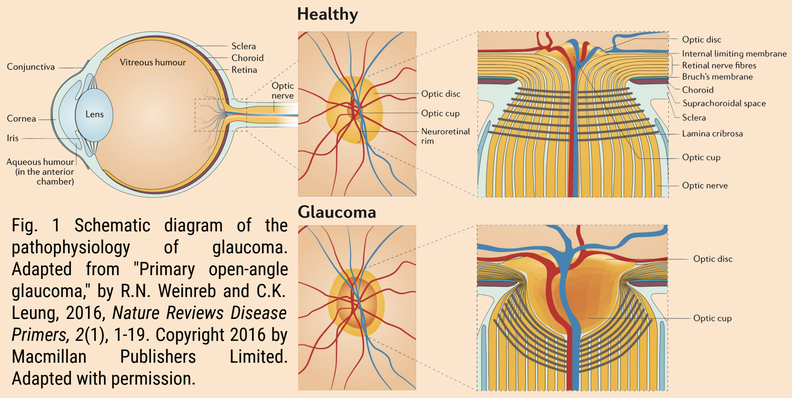


Fig. 1 Schematic diagram of the pathophysiology of glaucoma. Adapted from "Primary open-angle glaucoma," by R.N. Weinreb and C.K. Leung, 2016, *Nature Reviews Disease Primers, 2*(1), 1-19. Copyright 2016 by Macmillan Publishers Limited. Adapted with permission.

<u>PDZD2-deficient mice</u>

It is known that: 1) Sonic hedgehog (Shh), one of the hedgehog homologues, inhibits neurite growth in the retina[6], and a high concentration of Shh suppresses RGC differentiation[7]; and 2) both the full-length PDZD2 and the secreted form PDZD2 (sPDZD2), generated from proteolytic cleavage of endogenous PDZD2[8], are negative modulators of hedgehog signaling[9]. Hence, it is proposed that **PDZD2 plays a protective role** in the development and function of RGCs, thereby preventing POAG, through the inhibition of hedgehog signaling. Characterization of PDZD2-deficient mice would be performed to determine the phenotypes of POAG.

<u>PDZD2 SNPs</u>

It was identified that mutation in multiple genes, like myocilin and optineurin, induces POAG[10,11]. Recently, genome-wide association meta-analysis uncovered that POAG endophenotypes are strongly associated with a **single nucleotide polymorphism (SNP)** at rs72759609 located within a ~900-bp conserved region in the PDZD2 locus[12], implying a potential correlation between PDZD2 and POAG.

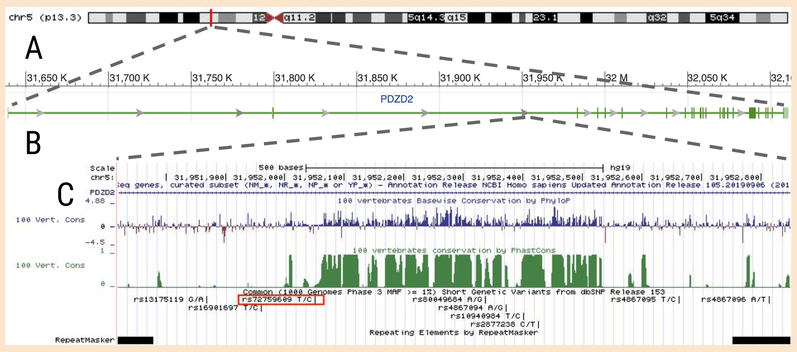


Fig. 2 PDZD2. (A) Schematic diagram showing the position of human PDZD2 gene (red line) on chromosome 5. (B) Schematic diagram showing the distribution of introns (thin line) and exons (bold line) on the PDZD2 gene (Adapted from NCBI). (C) Conservation analysis of human PDZD2 gene in comparison with 100 vertebrate genomes by phyloP (blue) phastCons (green) with the SNP associated with POAG indicated in red box (Adapted from UCSC Genome Browser).

Conserved sequences usually have important functional roles[13]. Latest work in the lab also found that the deletion of the ~900-bp region up-regulated the expression of PDZD2. It was, therefore, suggested that the ~900-bp region is a **silencer element**. To serve as a control, this SRF scheme aimed to study the regulatory effects of the two ~900-bp regions flanking the silencer on the expression of PDZD2. It was hence expected that no regulatory effects on the expression of PDZD2 would be observed.

METHODOLOGY

Remove sequences flanking the silencer by **CRISPR-Cas9** and;

Measure PDZD2 expression by **dual-luciferase reporter assay**

1 Western blotting to identify PDZD2 expression in 293 cell line

Harvest 293 cells → Gel electrophoresis → Gel transfer → Tag 1° antibody (PDZD2: α-PDZD2; β-actin: α-β-actin) → Tag 2° antibody (PDZD2: α-rabbit; β-actin: α-goat) → Develop x-ray film

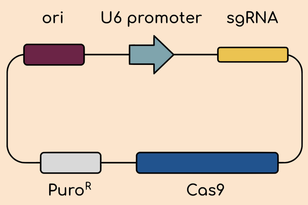


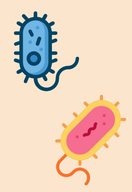
Fig. 3 Schematic diagram of the Cas9 plasmid (PX459).

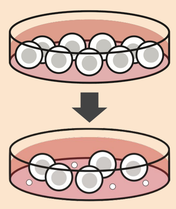
2 Subclone sgRNA into Cas9 plasmid

Design and order sgRNA → Digest plasmid → DNA electrophoresis on EB gel to confirm only one band → Extract and purify plasmid from gel → Measure plasmid conc. using NanoDrop → Ligate sgRNA into plasmid

3 Plasmid amplification

Transform plasmid into DH5α E.coli → Set culture → Miniprep for plasmid extraction → Digest some of the plasmids → DNA electrophoresis on EB gel to confirm the correct insert size





4 Transfect plasmid into 293 cell line

Follow protocol of FuGENE® HD Transfection Reagent by Promega → Screening → Run sequencing on cells

5 Dual-luciferase reporter assay

Transfect vectors into 293 cells → Follow protocol of Dual-Luciferase® Reporter Assay System by Promega (Harvest 293 cells → Add LAR II for luc2 → Measure luminescence → Add Stop & Glo® Reagent for hRluc → Measure luminescence)

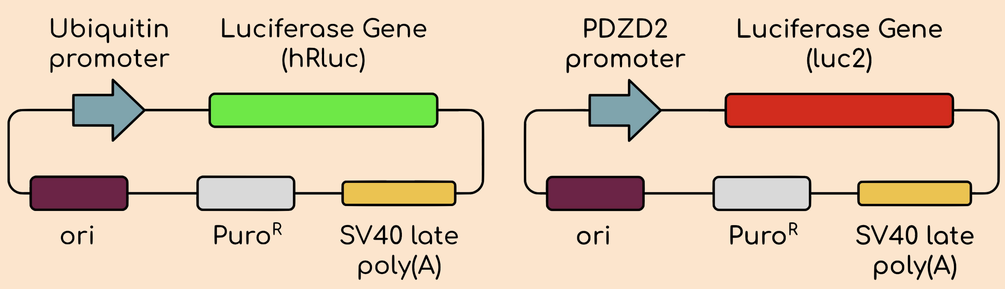


Fig. 4 Schematic diagram of the pGL4 luciferase reporter vectors.

RESULTS

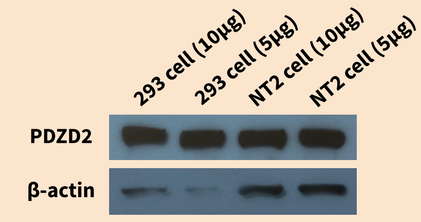


Fig. 5 Western blot image showed the expression of PDZD2 and β-actin in 293 and NT2 cell lines.

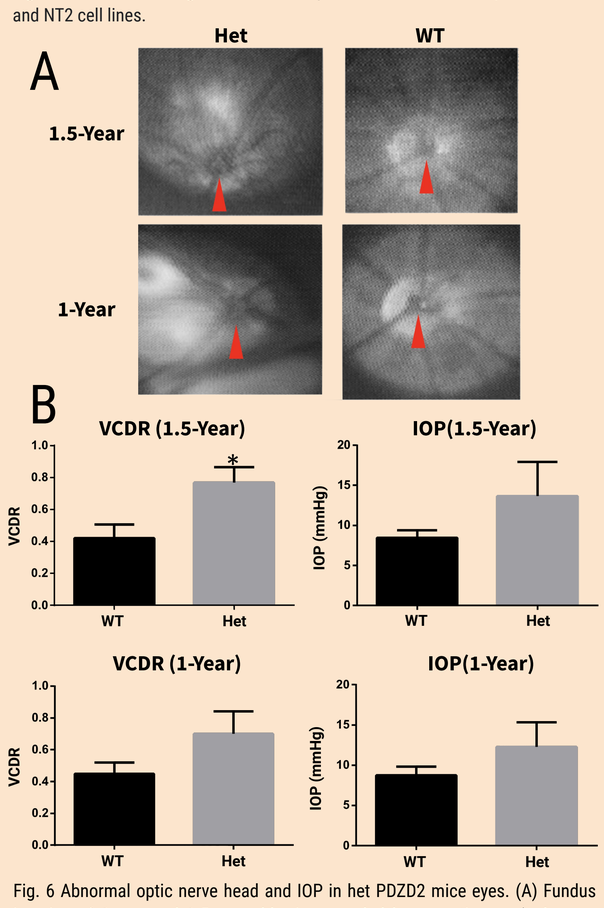


Fig. 6 Abnormal optic nerve head and IOP in het PDZD2 mice eyes. (A) Fundus imaging of 1-year and 1.5-year old mice revealed larger optic cup (red arrow heads) in het PDZD2 mice than the WT littermates. (B) Average vertical cup disc ratio (VCDR) and IOP were higher in both 1-year and 1.5-year old het mice than the WT littermates. (Results produced by Dr. Yao).

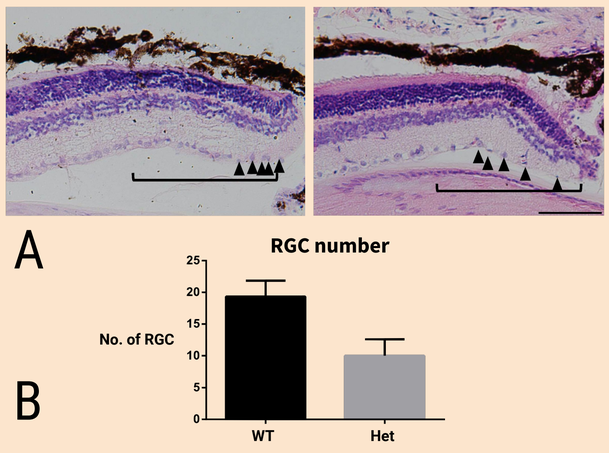


Fig. 7 Reduced number of RGCs in 2-month old het PDZD2 mice. (A) H&E staining revealed fewer RGCs (black arrowheads) at the peripheral retina in het PDZD2 mice when compared to WT littermates. Scale bar, 100mm. (B) Cell counting of RGCs at the peripheral retina (within 200mm starting from the ora serrata) in 6 sections from 2 pairs of het and WT mice. (Results produced by Dr. Yao).

DISCUSSION

Latest work in the lab found that the PDZD2+/- heterozygous (het) mice displayed POAG phenotypes, including decreased number of RGCs in the ganglion cell layer, increased vertical cup-disc ratio, and increased IOP. Hence, it is suggested that the deficiency in PDZD2 is associated with the development of POAG. As sPDZD2 is derived from PDZD2 and functions as an extracellular signaling molecule[8], the plasma level of sPDZD2 might be a **diagnostic or screening marker for POAG**.

This SRF scheme provided control evidence to support the silencing role of the ~900-bp region. SNPs within the region would thus up-regulate PDZD2 expression, **protecting the RGCs**. With the strong association between POAG and the SNP, it is therefore proposed that the SNP might reduce endophenotypes of POAG in patients. With tools for tissue-specific site-directed mutagenesis now available[14,15], the SNP at rs72759609 might be a **one-time treatment or even a cure for POAG patients deficient in PDZD2**, however, further studies are required.

Moreover, it is known that the up-regulation of PDZD2 is strongly associated with the early development of prostate cancer[16]. This research hence revealed the possibility of combining the SNPs within the silencer with the existing **genetic markers** to increase the predictive value for **prostate cancer** by DNA genotyping.

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