Investigating the Effect of the Loss of Complement Factor D on Complement Activity in the Stargardt Disease Mouse Model

Kaitlin Griffith, Bridget Ryan, and Robert Chow

Department of Biology, University of Victoria

The Role of Complement in the Eye

- The complement system eliminates pathogens and damaged host cells through a proteolytic cleavage cascade
- Neuroprotection in the retina

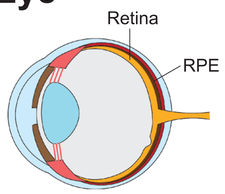
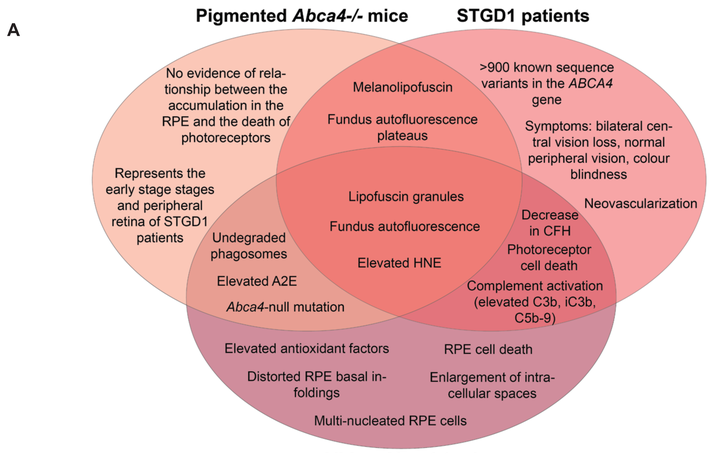


Figure 1. Cross-section of the human eye

Stargardt Macular Degeneration

Stargardt macular deneration is the most common form of macular degeneration affecting children and young adults. This disease is the result of mutations in the *ABCA4* gene, which normally encodes a critical transporter that is found in the outer segments of photoreceptors and in the RPE. As the result of this mutation, lipofuscin accumulates in the retinal pigmented epithelium (RPE) and causes local dysregulation of the complement system. As a consequence of this inflammation, the critical functions of the RPE are hindered. This leads to progressive photoreceptor degeneration and the loss of central vision.



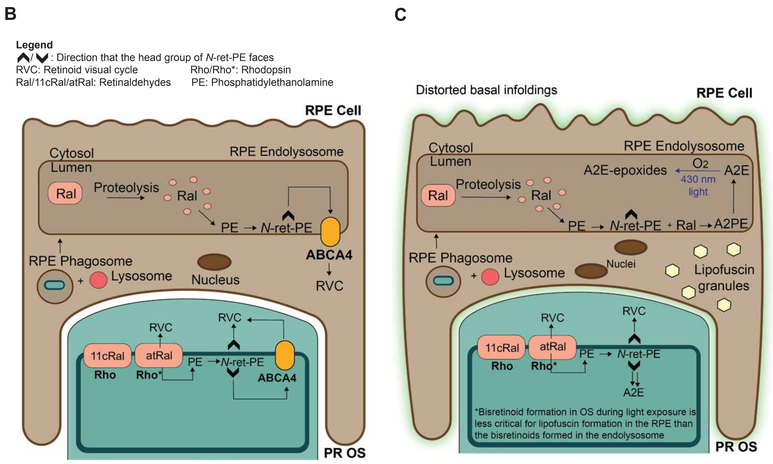


Figure 2. A) *Abca4-/-* mouse models recapitulate the phenotype demonstrated by Stargardt (STGD1) patients. **B)** ABCA4 prevents the formation of bisretinoids in RPE phagolysosomes in wildtype albino mice. **C)** Bisretinoids form in the RPE endolysosomes of albino *Abca4-/-* mice. Subsequent photo-oxidation of the bisretinoids generates damaging products and activates the complement system.

Genetic Rescue Approach

A Complement Factor D (*Cfd*) knockout mutation has been introduced into pigmented *Abca4-/-* mice to evaluate the potential for *Cfd* as a target for gene therapy to treat Stargardt disease.

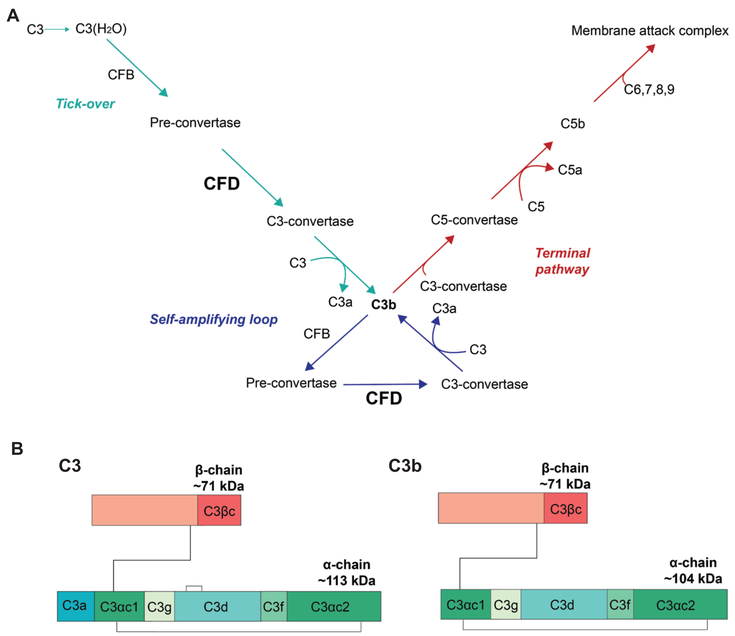


Figure 3. A) CFD catalyses the formation of active C3-convertases in the alternative complement pathway, which cleave C3 into C3a and C3b **B)** C3 and C3b are composed of α and β chains. C3b is progressively degraded to iC3b, C3c, and C3d through a series of enzymatic reactions.

Results

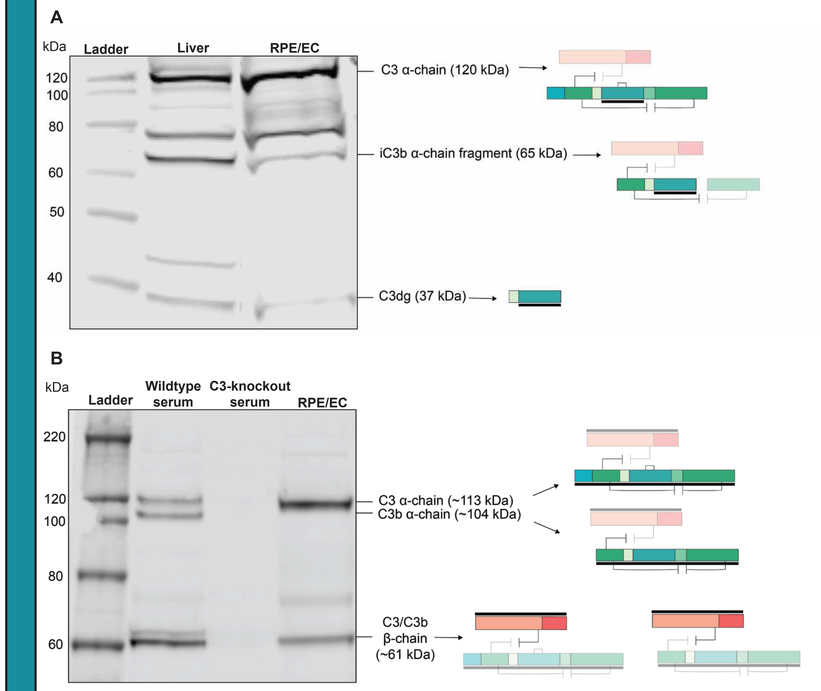


Figure 4. Protein fragments that are not included in the band are transluscent. Black bars on the schematics indicate the region recognized by the primary antibody. **A)** The α-chain of C3, C3b, iC3b, and C3dg was detected. Mouse liver lysate was used as a positive control. **B)** C3 was detected in the RPE/EC. Mouse serum lysates were used as positive and negative controls.

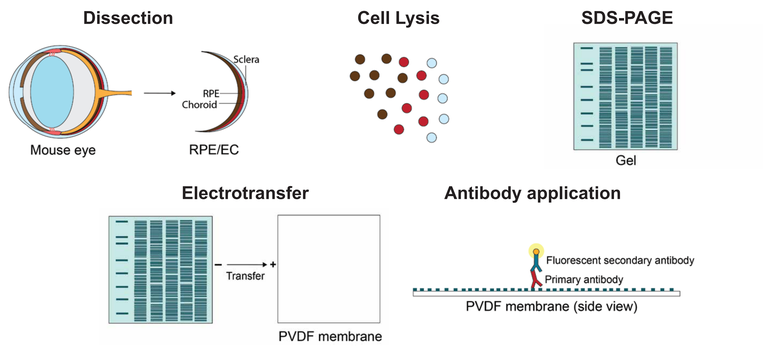
Objectives

- Develop a quantitative western blot assay to detect C3 and C3b in the mouse retinal pigmented epithelium and eyecup (RPE/EC)
- Quantify C3 and C3b in the RPE/EC of wildtype, *Cfd-/-*, *Abca4-/-*, and *Abca4;Cfd-/-* mice to evaluate complement activity

Future directions

- Explore other antibodies that are specific for C3b
- Test current protocols on older *Abca4-/-* or *Abca4;Rdh8-/-* mice that are expected to demonstrate a severe phenotype

Methods



References

Barratt, J. & Weitz, I. (2021) Complement Factor D as a Strategic Target for Regulating the Alternative Complement Pathway. Frontiers in immunology. 12, 12712572–712572.

Charbel Issa, P. et al. (2013) Fundus autofluorescence in the Abca4(-/-) mouse model of Stargardt disease--correlation with accumulation of A2E, retinal function, and histology. Investigative ophthalmology & visual science. 54 (8), 5602–5612.

Hu, J. et al. (2020) Evidence of complement dysregulation in outer retina of Stargardt disease donor eyes. Redox biology. 45, 37101787.

Klettner, A. K. & Dithmar, S. (2020) Retinal Pigment Epithelium in Health and Disease. 1st ed. 2020. Alexa Karina. Klettner & Stefan. Dithmar (eds.). Springer International Publishing.

Lenis, T. L. et al. (2018) Expression of ABCA4 in the retinal pigment epithelium and its implications for Stargardt macular degeneration. Proceedings of the National Academy of Sciences - PNAS. 115 (47), E11120–E11127.

Ng, E. S. Y. et al. (2022) Membrane Attack Complex Mediates Retinal Pigment Epithelium Cell Death in Stargardt Macular Degeneration. Cells (Basel, Switzerland). 11 (21), 3462.

Radu, R. A. et al. (2011) Complement System Dysregulation and Inflammation in the Retinal Pigment Epithelium of a Mouse Model for Stargardt Macular Degeneration. The Journal of biological chemistry. 286 (21), 18593–18601.

Taubitz, T. et al. (2018) Ultrastructural alterations in the retinal pigment epithelium and photoreceptors of a Stargardt patient and three Stargardt mouse models: indication for the central role of RPE melanin in oxidative stress. PeerJ (San Francisco, CA). 6, e5215–e5215.

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