Spotlight Summary: Quantitative Fundus Autofluorescence and A2E Increase With Aging in Wild-Type and “Stargardt” Mice

Robert Zawadzki¹ and Edward N. Pugh Jr²

¹University of California-Davis Eye Center, Department of Ophthalmology, Sacramento, California

²University of California-Davis, Department of Physiology and Membrane Biology, Davis, California; enpugh@ucdavis.edu

Stargardt disease is a recessively transmitted retinal degeneration arising from mutations in *ABCA4*, the gene encoding an ATP-binding cassette “flippase” transporter expressed in rod and cone outer segment disc membranes. Stargardt disease typically results in severe vision loss in afflicted subjects by age 20. A diagnostic hallmark of Stargardt disease is an abnormally rapid increase of fundus autofluorescence with age. Previous work by several laboratories have shown that *Abca4*^{-/-} mice recapitulate some aspects of Stargardt disease. In this issue of *Investigative Ophthalmology & Visual Science*, Sparrow, Delori and colleagues¹ report quantitative fundus autofluorescence (qAF) measurements with a confocal scanning laser ophthalmoscope, and parallel measurements of the bisretinoid lipofuscin component A2E in mice defective for the *Abca4* transporter. The care in quantification of the qAF signals by use of an internal fluorescence reference, and meticulous control of mouse eye condition stand out in this investigation.

Fundus autofluorescence arising from RPE cells increases steadily throughout life at a rate of about 7% per year,² but much more rapidly in patients with Stargardt disease. In the midspectral emission region, most fundus autofluorescence arises from lipofuscin,³ an oily composite of inclusion bodies comprising proteins, neutral lipids, phospholipids, and retinoid derivatives. RPE cell lipofuscin is generated principally from photoreceptor material ingested during the daily cycle of rod and cone outer segment phagocytosis, but imperfectly digested by lysosomes. Lipofuscin droplets are thought to arise from partially or completely defunct lysosomes.

A specific fluorescent component of lipofuscin is the bisretinoid A2E.⁴ The precursor of A2E is generated in photoreceptor outer segments, as all-*trans* retinal and possibly 11-*cis* retinal in the visual cycle escape from normally protective reactions and bind covalently to phosphatidylethanolamine, forming initially N-retinylidene phosphatidylethanolamine (NR-PE; one vitamin A moiety), and then more rarely A2-PE (two vitamin A moieties).⁴ Although NR-PE can be removed from the inner leaflet of disc membranes by the *Abca4* transporter and subsequently be subjected to hydrolysis on the cytoplasmic face of the disc, A2-PE cannot, with the result that A2-PE is eliminated from photoreceptors only when the discs are phagocytosed by RPE cells. In the RPE cell, the lipid is removed from A2-PE during lysosomal degradation, but the residuum, A2E, appears not to be biodegradable and likely remains in the lysosomal membrane. A2E absorbs blue light ($\lambda_{\text{max}} \sim 435$ nm), and acts as a photo-oxidizing agent, contributing to the malfunction and demise of lysosomes.⁴ In Stargardt disease, as recapitulated in the *Abca4*^{-/-} mouse, inadequate *ABCA4* function leads to a more rapid buildup of lipofuscin and RPE cell demise. The buildup of lipofuscin is also hypothesized to be a causal factor in the development of AMD.

In their current article, Sparrow et al.¹ measure fundus qAF, total A2E, and outer nuclear layer (ONL) thickness in *Abca4*^{-/-}, *Abca4*^{+/-}, and *Abca4*^{+/+} albino mice from age 2 to 12 months. During the first 8 months of life, qAF increases at an approximately 2-fold higher rate, and A2E almost 5-fold more rapidly, in *Abca4*^{-/-} than in wild-type mice, despite modest ONL thinning in the *Abca4*^{-/-} mice. These changes reveal that autofluorescence increases, although correlated with A2E buildup, underrepresent the magnitude of the A2E buildup.

The results of Sparrow et al.¹ lay a strong foundation for additional work characterizing the time course and cellular mechanisms by which lipofuscin and A2E buildup lead to RPE and photoreceptor cell damage and death. They also open the door to noninvasive monitoring of disease progression and testing of therapies in a more efficient and controlled way in the mouse model of Stargardt disease. Moreover, the present and future work with the qAF method should enhance the translation of information obtained from Stargardt and aging wild-type mice into the clinical setting.

References

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