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Developing a Quantitative Method For Determining Lipofuscin Content in Mouse RPE: An Age Comparison of Wild Type and Immunoproteasome KO Mice

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ABSTRACT

Introduction. Aging of post-mitotic cells is associated with the accumulation of lipofuscin, which can lead to deleterious changes in the body and increased susceptibility to certain diseases. This study focused on measuring lipofuscin in the aging retina and determining whether the absence of immunoproteasome affected this age-related process. We hypothesize that lipofuscin increased with aging and the absence of immunoproteasomes.

Objective I. Develop a method to quantify lipofuscin content in retinal pigment epithelial (RPE) cells.

Objective II. Compare the accumulation of lipofuscin in mice of different ages and in wild type (WT) and immunoproteasome (L7M1) knockout (KO) mice.

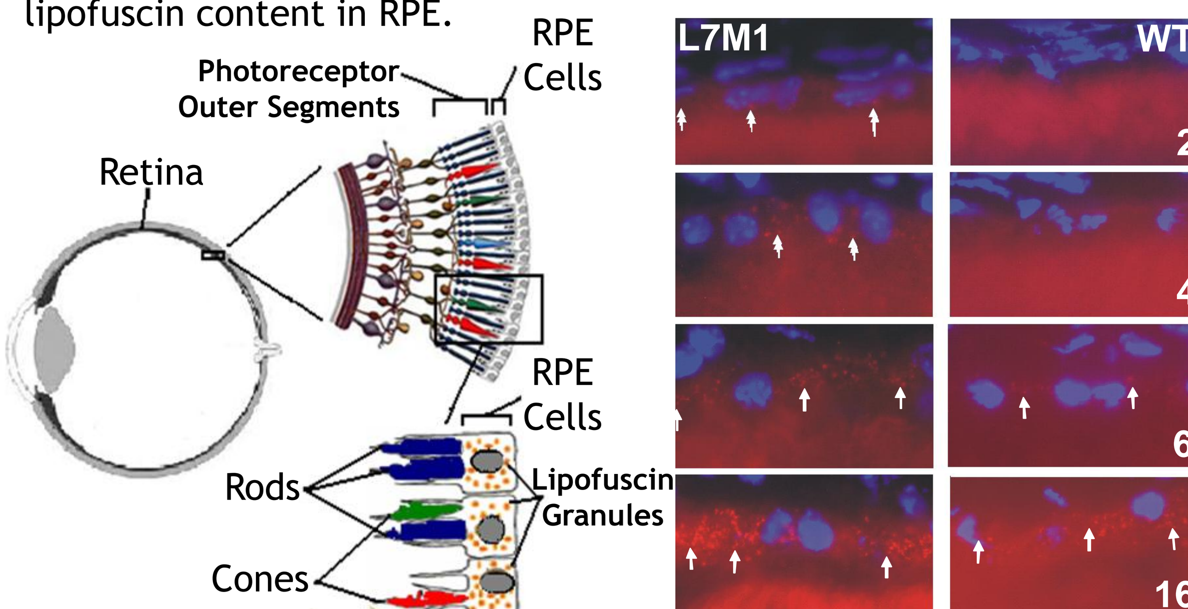
Materials and Methods. RPE cells from mice of different ages, including wild type and L7M1 KO strains, were used. The cells were processed for lipid extracts containing lipofuscin. The lipid extracts were then used to measure lipofuscin content using fluorescence spectroscopy.

Results. The optimal method for measuring lipofuscin was developed, including homogenization with PBS buffer, and extraction under dark condition. Fluorescence intensity increases with age in KO mice, but decreases with age in WT mice. The intensity was also observed to be higher in KO compared with WT. Intensity-average-emission-maximum (IAEM) values were found to vary within different age groups.

Conclusion. Our method of quantifying lipofuscin could detect differences in content between retinas from mice of different ages and between strains. The higher content of lipofuscin in KO mice supports our hypothesis. Varied IAEM suggests different fluorescent species developed with aging.

INTRODUCTION

Lipofuscin is an autofluorescent granule found mostly in post-mitotic cells, including neurons, myocardial cells, and RPE. Its composition consists of oxidized lipids and proteins and various fluorescent species, including A2E and its isomers, which fluoresce at different excitation wavelengths. As we age, lipofuscin accumulates in lysosomes and may contribute to oxidative stress and many degenerative diseases, such as age-related macular degeneration. The accumulation is a result of phagocytosis of photoreceptor outer segments adjacent to RPE cells. Our first **objective** in this study is developing a method to quantify lipofuscin in RPE using fluorescent spectroscopy. Using a previous method (counting lipofuscin granules), increased lipofuscin was observed with aging and was greater in immunoproteasome knockout mice (figure). However, this method is not quantitative. Therefore, new assay with optimal conditions needs to be developed. The second objective of this study is to measure lipofuscin content in mice of different ages. We hypothesize that lipofuscin autofluorescent granules increase with aging. In addition, we are also comparing the wild type and immunoproteasome (L7M1) KO mice. Immunoproteasome is upregulated in the central nervous system and degrades oxidized proteins in response to injury and stress. We **hypothesize** that the absence of immunoproteasome may result in increased oxidative damage and consequently, lipofuscin content in RPE.



MATERIALS AND METHODS

Quantitative Method Development

Materials. Mouse RPE cells with a C57BL/6 genetic background.

Tissue/Cell Homogenization. RPE cells from two pairs of eyes were homogenized using glass homogenizer with teflon pestle with PBS.

Lipid Extraction. The homogenates were mixed with chloroform and methanol at 1:2 ration and chloroform and PBS at 1:1 ratio. The solution was centrifuged with 3727 m/s^2 G force for 15 minutes. The organic phase (lipids) was isolated and dried with argon (Bligh and Dyer, 1959). The dried extract was dissolved in methanol. The procedure was performed in dark condition.

Fluorescence Spectroscopy. The dissolved extract was scanned inside a fluorometer cell using FluoroMax-2 and excited at wavelengths of 290 and 350 nm to generate emission spectra with 1 s/1 nm integration time.

Lipofuscin Content Comparison

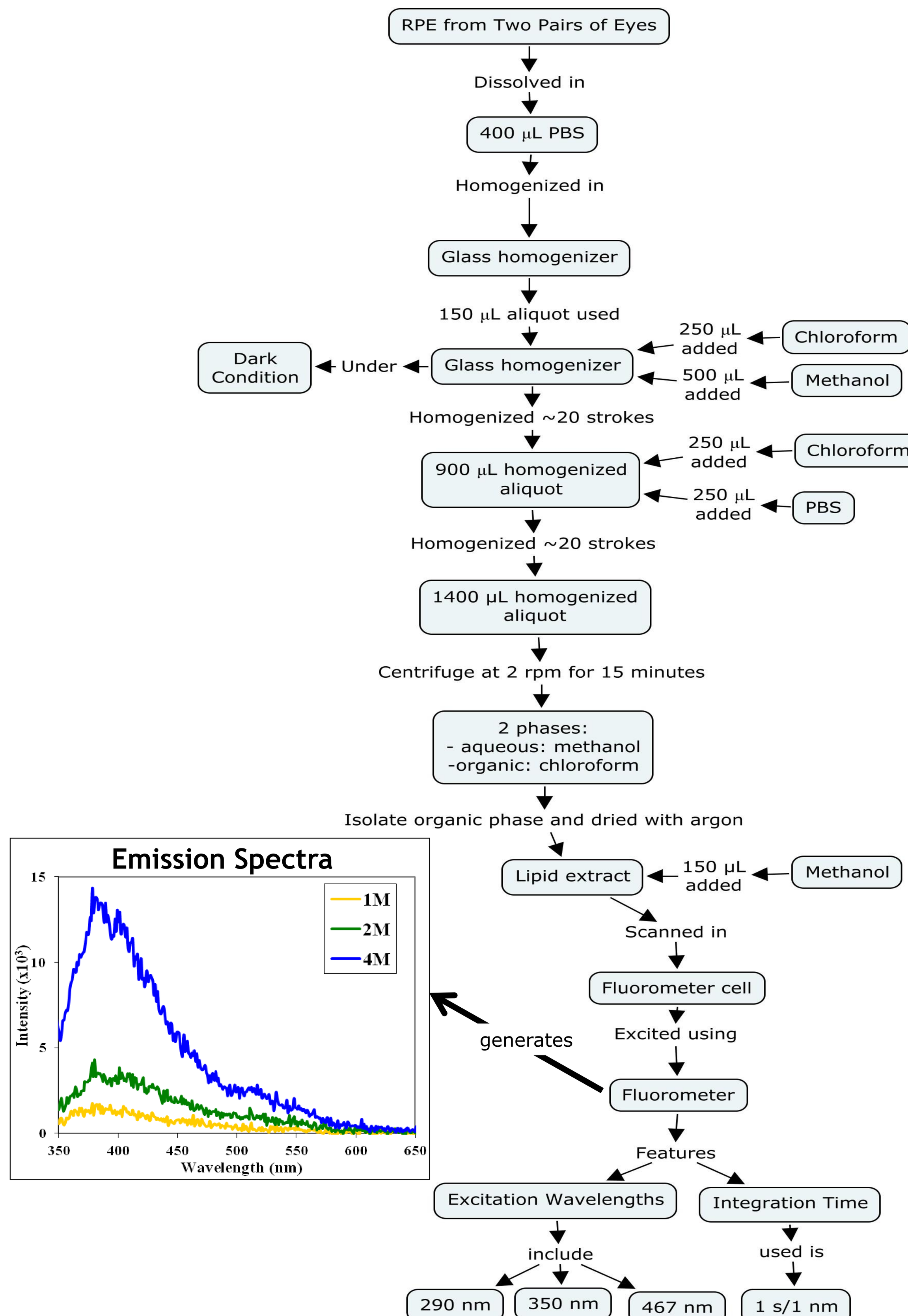
Materials. RPE cells from mice of different ages (2-3 months, 8-9 months, and 12-14.5 months) and strains (WT and L7M1 KO).

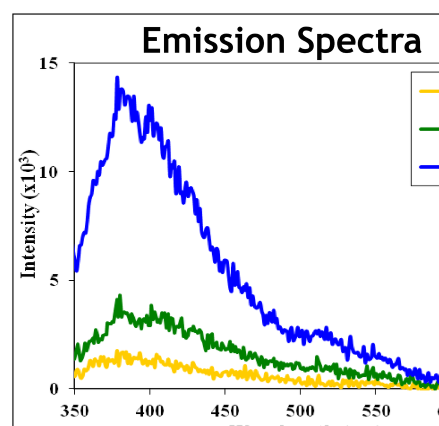
Testing RPE Cells with Optimized Conditions of Fluorescence Measurement. RPE cells were homogenized and extracted with the optimized procedure described above. Lipofuscin in the extracts were quantitated using fluorescence spectroscopy.

Data & Statistical Analysis. The total intensity of the emission spectra and the Intensity-average-emission-maximum (IAEM) for each sample were calculated. One-way ANOVA (Tukey post-hoc test) and t-test were used to test for significance between mice of different ages and strains, respectively.

RESULTS

Quantitative Method Development



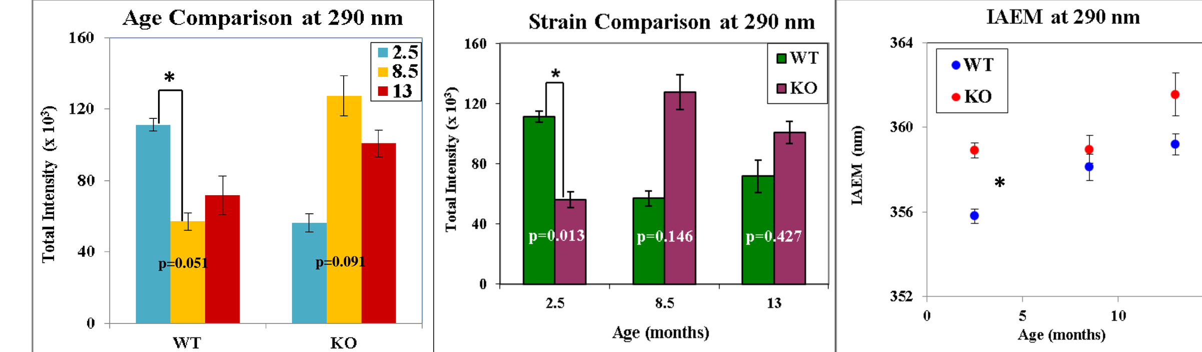


RESULTS (CONT'D)

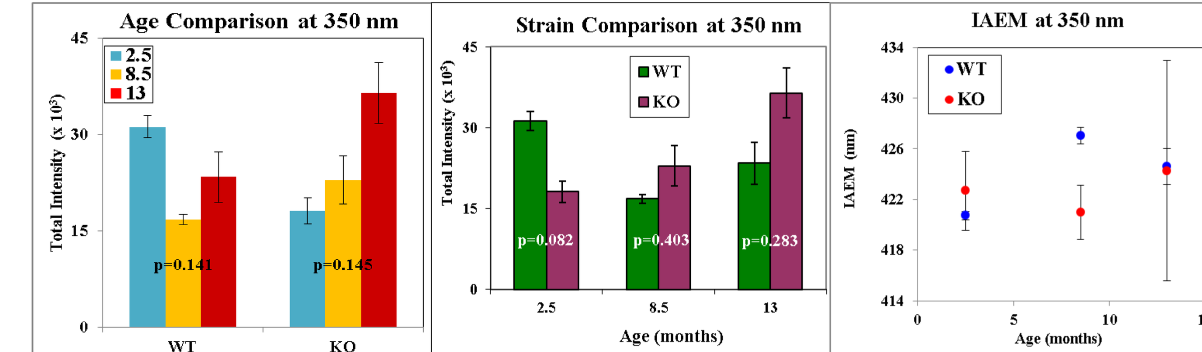
Lipofuscin Content Comparison

Total Intensity and IAEM at:

• 290 nm

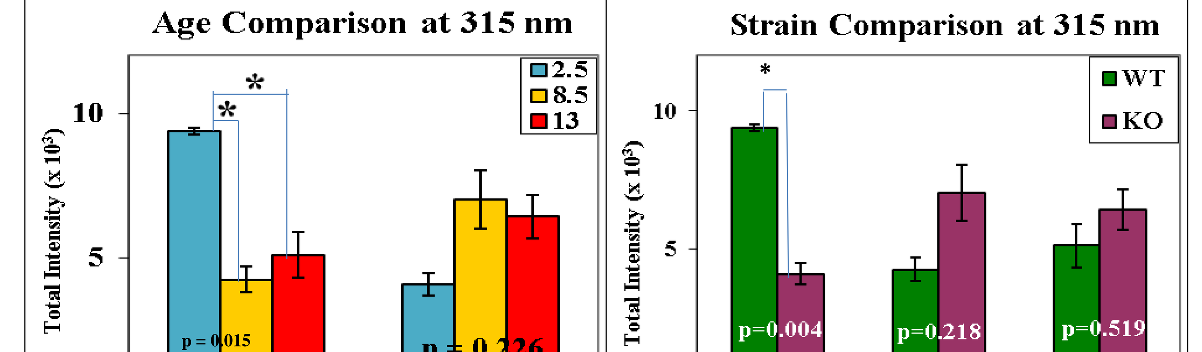


• 350 nm

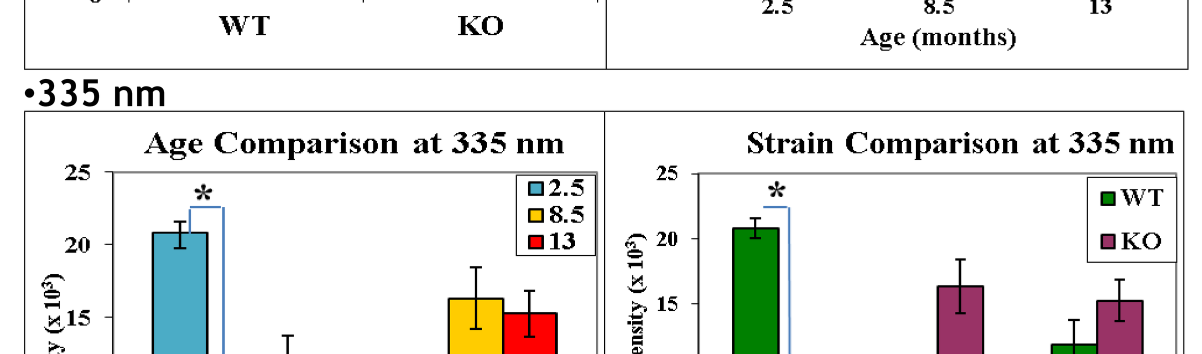


Emission Peaks at:

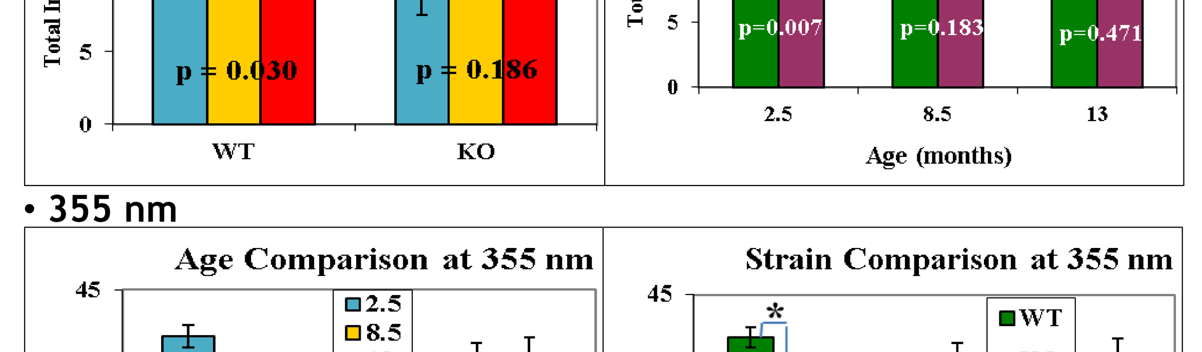
• 315 nm



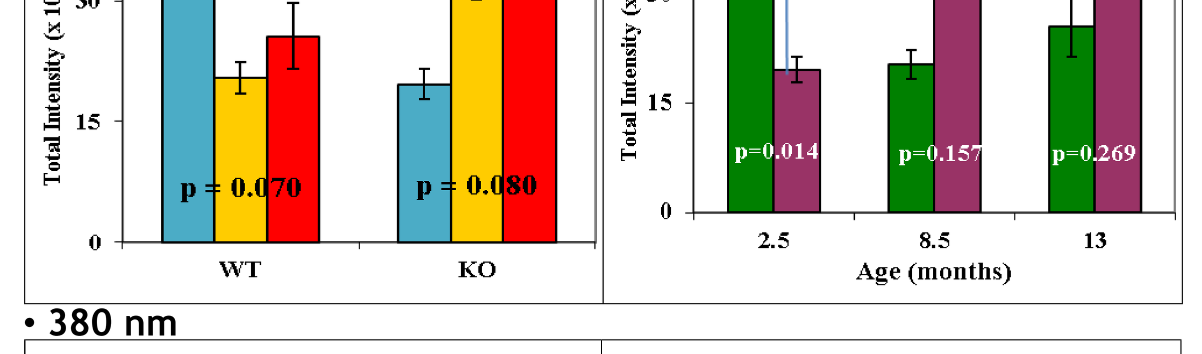
•335 nm



• 355 nm

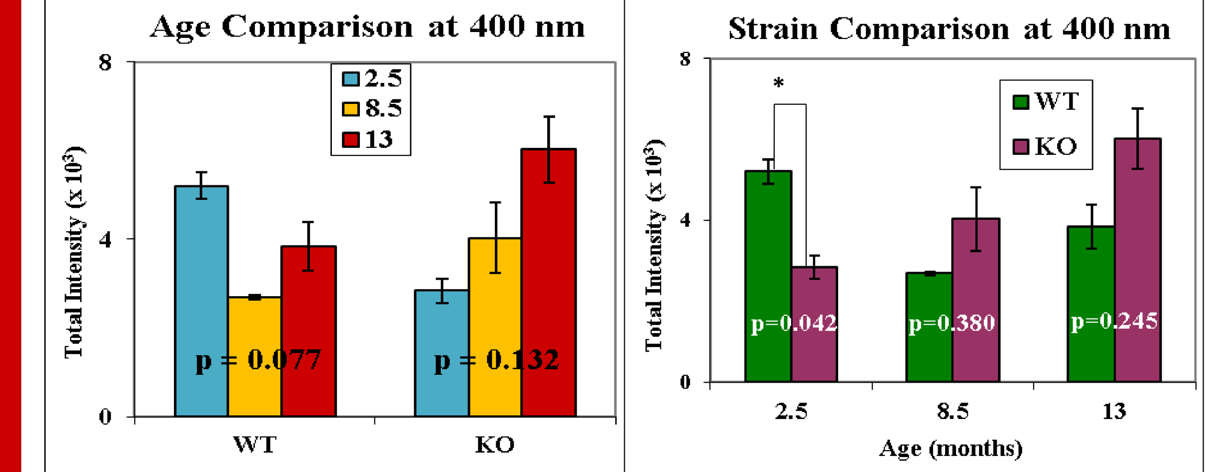


• 380 nm

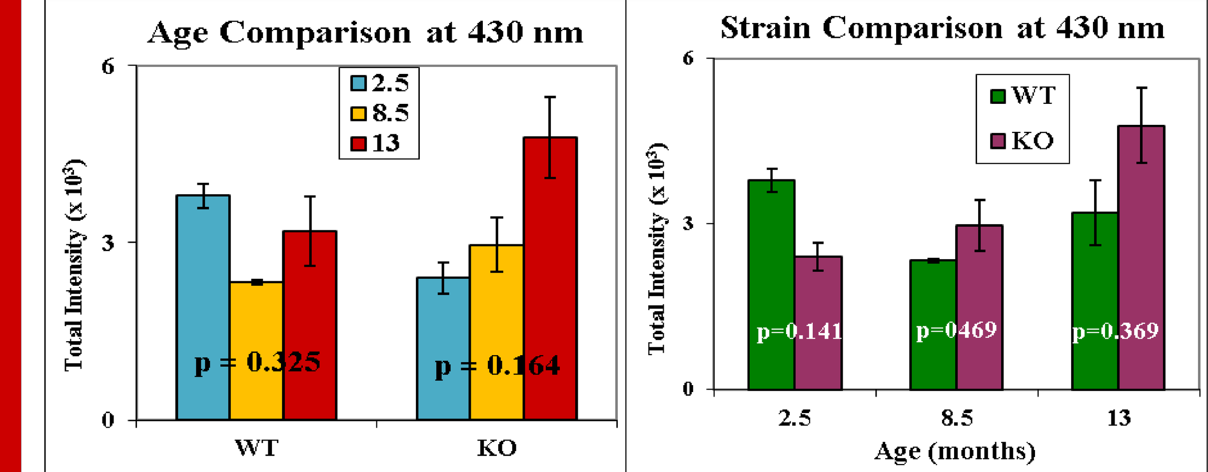


RESULTS (CONT'D)

•400 nm



• 430 nm



CONCLUSIONS

Quantitative Method Development

- Homogenization using PBS gives higher yield than using Tris/Urea/DNAse.
- Extraction procedure was performed in dark condition to reduce light exposure, preventing unwanted reactions.
- Fluorescence spectroscopy was more sensitive for measuring lipofuscin content in RPE compared to spotting TLC plates with lipid extracts and performing densitometry.

Lipofuscin Content Comparison

- In the WT mice, the lipofuscin content decreases with age, while in the KO strain, the lipofuscin content increases with age.
- Most of the KO mice have higher lipofuscin content compared to age-matched WT mice.
- The IAEM values of mice of the age 2-3 months were different significantly, which implies that different species of fluorescent molecules were formed in different ages.
-A minimum sample size of 5 is required for each group to have sufficient statistic power to detect differences of lipofuscin content between groups.

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