Retina Australia Research Report
Summary of Grant Activities: January to December 2017

Project Title: Large scale generation of retinal pigment epithelium cells from patient induced pluripotent stem cells

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Lay Description: The retina is a light sensing tissue that lines the back of the eye. Photoreceptors are the main cells of the retina that detect light. They convert light into electrical signals, which are sent to the brain via the optic nerve and are used to construct the images we see. The retinal pigment epithelium (RPE) is an essential tissue of the retina which supports the health and functions of photoreceptors and is essential to the overall homeostasis of the retina. Many blinding diseases manifest through RPE cell death, which cause the subsequent death of photoreceptors.

The extreme difficulty in obtaining tissue from the eyes of living people represents a major barrier to understanding the mechanisms behind retinal diseases and developing new treatments. Recent breakthroughs in stem cell technology have led to the ability to generate stem cells from skin samples, and these "induced pluripotent stem cells" (iPSCs) now represent a powerful disease modelling tool. iPSCs are cells that are generated from adult tissue and can be changed to any cell of interest. This potentially provides an endless supply of cells with disease specific properties.

We successfully derived patient-specific iPSCs and differentiated them into RPE cells, in particular from patients with BEST disease, Doyne Honeycomb Retinal Dystrophy (DHRD) and Sorsby's fundus macular dystrophy. **Here, we show how we established the control group needed to study retinal dystrophies using patients' stem cells. We created a large bank of RPE cells from control (no disease) stem cells using an automated platform unique in Australia to compare diseased to non-diseased cells, in order to model and understand fundamental disease processes in a culture dish. Obtaining a large control group is important to compare with diseased cells with the certainty that observed variation in cellular behaviour are due to the disease itself and not an artefact of the artificial manipulation of cells.**

Scientific Outcomes:

This project has been very successful, as demonstrated by the number and quality of peer review publications arising from this work.

1. We have successfully generated a bank of control iPSCs from >150 individuals. Most lines have now been fully characterised and passed quality control. Examples of our quality controls and automation steps are described in *Crombie et al 2017* (enclosed and Fig. 1). We have also optimised best method of iPSC maintenance of iPSCs (*Daniszewski et al,* submitted to iScience).

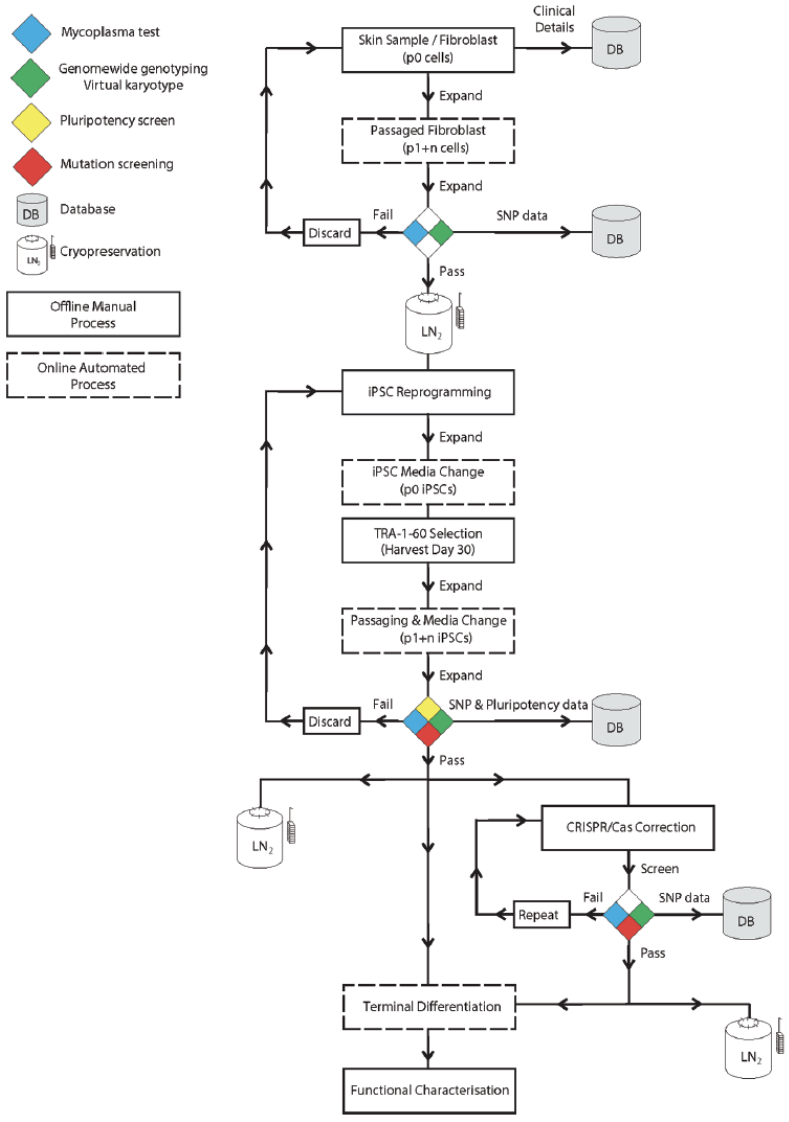


Figure 1: Workflow for the automation of stem cell culture. Quality controls and potential incorporation of gene editing are also indicated. From Crombie *et al* 2017.

2. We have set up and optimised procedures for generation of RPE on the automated platform. This automated aspect of our work has now been published in *Crombie et al 2017* (enclosed, Figure 2).

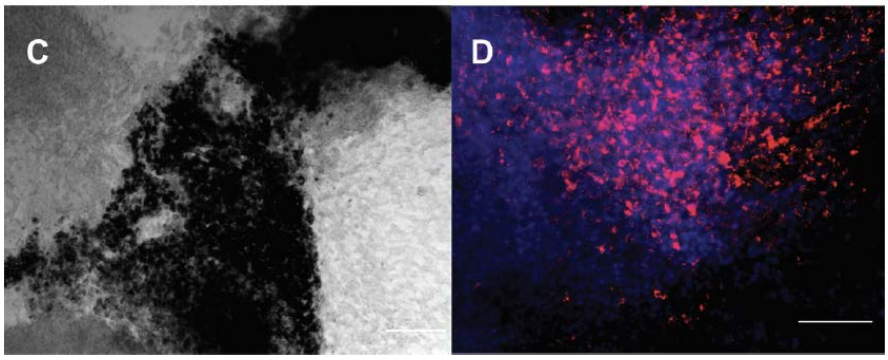


Figure 2: RPE differentiation of human pluripotent stem cells on the automated platform. Representative Bright field (Left) and fluorescent (Right) images of human pluripotent stem cell-derived RPE cells at day 35, showing pigmentation, cobblestone morphology (Left) and expression of the RPE marker PMEL (Right, red) with DAPI counterstained (blue). From Crombie *et al* 2017.

3. We have also set up procedures for sequencing of single cells from our cultures. This is exemplified in our recent publication from *Daniszewski et al 2017* using iPSC-derived retinal ganglion cells (Figure 3). In light of our results, we have now decided that all RPE samples to be used for disease modelling will be sequenced at the single cell level, which will bring further valuable information to the "bulk" sequencing of samples. This aspect of the work will continue thanks to the award of a MDFA research grant for 2018-19.

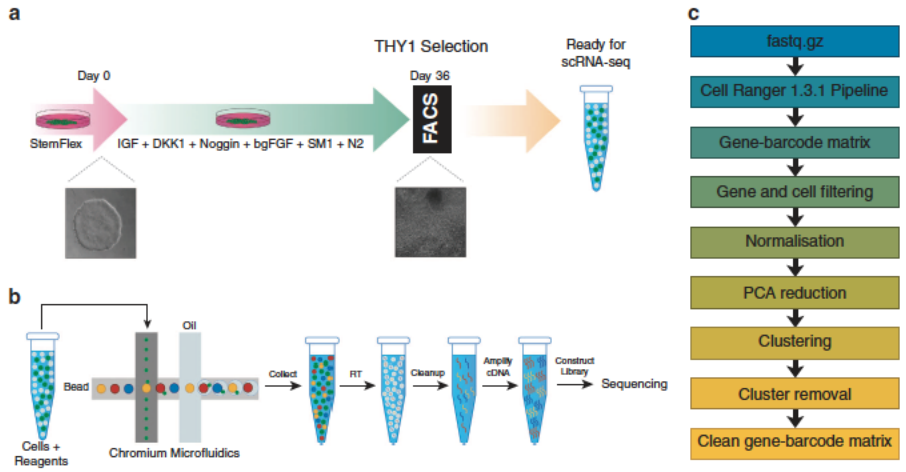
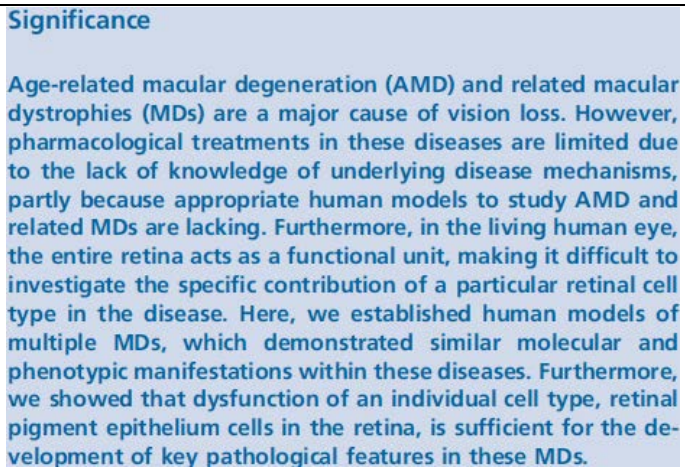


Figure 3: Schematic representation of a single cell sequencing workflow, using human pluripotent stem cell-derived retinal ganglion cells. (a) Guided differentiation of stem cells into ganglion cells; (b) method of harvest of cells to allow (c) sequencing and analysis at the single cell level. This method will now be used for RPE samples. From Daniszewski *et al* 2017.

4. In parallel, we have continued our gene editing correction of iPSCs obtained from patient with retinal dystrophies. In collaboration with Ruchira Singh, we corrected DHRD iPSC lines, a result published in PNAS (*Galloway et al 2017*, enclosed). This work allowed the identification of drusen in a dish, and of phenotypes being reduced in control and CRISPR corrected lines (Figure 4). Using the methodology described in the PNAS manuscript and in collaboration with Ruchira Singh, RPE cells from AMD patients and controls are now being investigated for the presence of Drusen following long term culture. This work is still ongoing.



Significance

Age-related macular degeneration (AMD) and related macular dystrophies (MDs) are a major cause of vision loss. However, pharmacological treatments in these diseases are limited due to the lack of knowledge of underlying disease mechanisms, partly because appropriate human models to study AMD and related MDs are lacking. Furthermore, in the living human eye, the entire retina acts as a functional unit, making it difficult to investigate the specific contribution of a particular retinal cell type in the disease. Here, we established human models of multiple MDs, which demonstrated similar molecular and phenotypic manifestations within these diseases. Furthermore, we showed that dysfunction of an individual cell type, retinal pigment epithelium cells in the retina, is sufficient for the development of key pathological features in these MDs.

Figure 4: Drusen in a dish using iPSCs: significance. Significance insert from Galloway et al, for which we generated iPSCs and CRISPR-corrected iPSCs. From Galloway *et al* 2017.

5. In term of ageing our cells, we originally planned to artificially age our RPE cells with Bisretinoid N-retinylidine-N-ethanolamine (A2E), the main fluorophore of lipofuscin. During optimization of this work, we have noted that A2E is not an optimal form of aging our cells. Indeed, this compound is highly autofluorescent, which does not allow visualization of cells under fluorescent microscopy. We have assessed impact of other potential stressors on RPE cells as way to mature/age the cells. We have generated novel data on the effect of the bioactive lipid Lysophosphatidic Acid (LPA) on the biology of the RPE. This work, accepted in *BBA Molecular and Cellular Biology of Lipids* (Lidgerwood *et al.*), indicates that LPA plays an important role in the integrity and functionality of the RPE (Figure 5). We could potentially use this molecule on RPE as a way to modulate their biology.

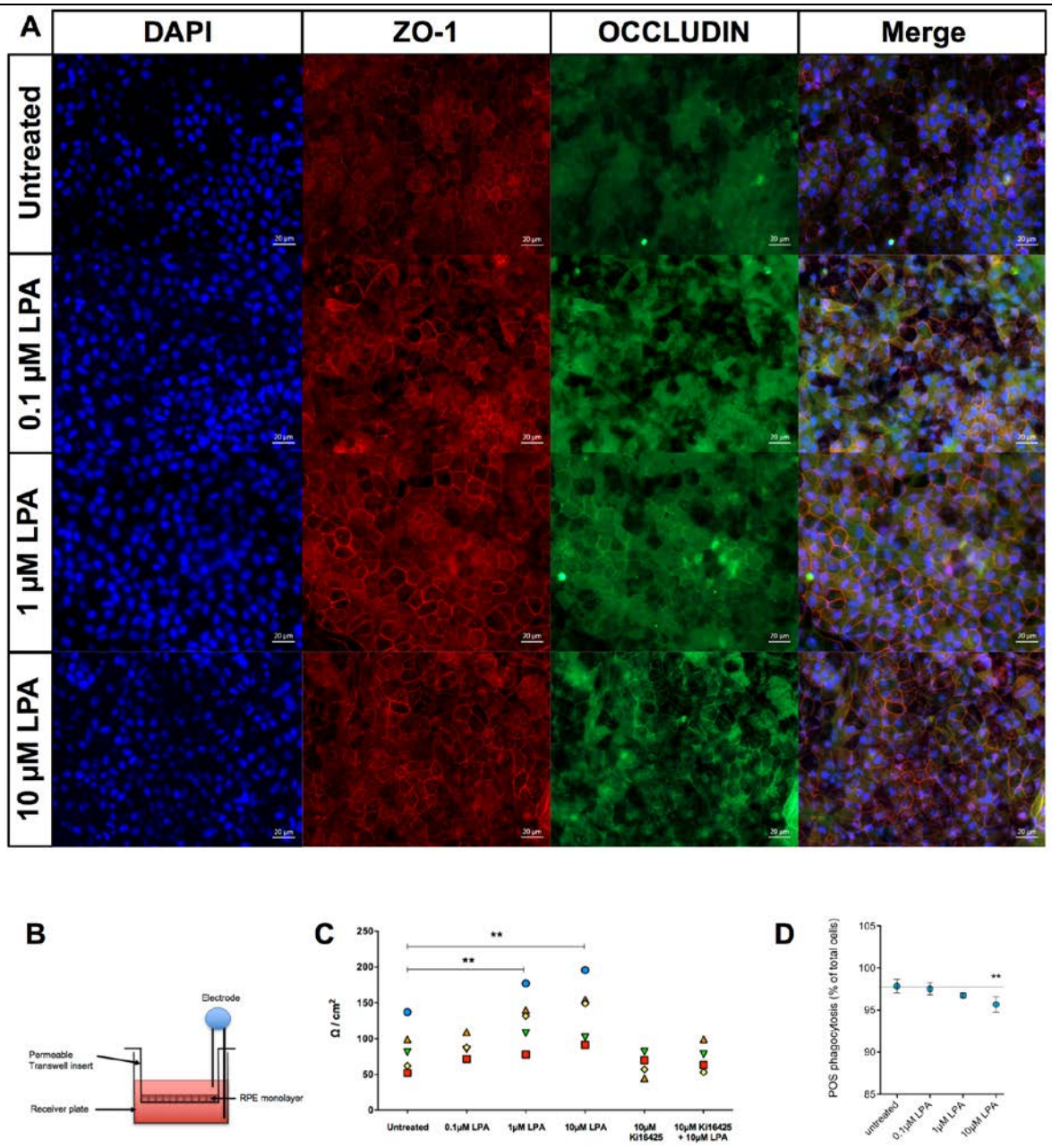


Figure 5. (**A**) **LPA modifies ZO-1 and OCCLUDIN expression in hPSC-derived RPE cells.** Representative images of mature hPSC-derived RPE cells untreated or treated with various doses of LPA (0.1, 1 and 10 µM) for 7 days and immunostained for ZO-1 (red) and OCCLUDIN (green), with DAPI counterstained (blue), and merged. Data presented are representative of 4 independent experiments. Scale bars: 20 µM. (**B-D**) **LPA modifies key RPE functions.** (**B**) Schematic representation of TEER analysis of polarised RPE cell cultures. (**C**) TEER measurements ($\Omega \cdot cm^2$) in mature hPSC-derived RPE cells untreated or treated with various doses of LPA (0.1, 1 and 10 µM), or Ki16425 (10 µM) for 7 days. Data were measured 3 hours after the last treatments, n=4-5, **p<0.01 paired t-test. Each individual experiment is colour and symbol coded. (**D**) Flow cytometry analysis of phagocytosis by control- (untreated) and LPA treated- (0.1, 1 and 10 µM, 7 days) hPSC-derived RPE cells showing phagocytosis after incubation with FITC- photoreceptor outer segments. Data are mean ± SEM, n=3 independent experiments. Statistical significance ** p<0.05 by paired t-tests of individual treatment versus control. From Lidgerwood *et al*, currently submitted.

Published (* indicate co-senior author)

1. Lidgerwood GE, Morris A, Conquest A, Daniszewski M, Rooney LA, Lim SY, Hernández D, Liang HH, Allen P, Connell PP, Guymer RH, Hewitt AW and Pébay A. Role of lysophosphatidic acid in the retinal pigment epithelium and photoreceptors. *BBA Molecular and Cellular Biology of Lipids*. Accepted 11.04.18
2. Daniszewski M, Senabouth A, Nguyen QH, Crombie DE, Lukowski SW, Kulkarni T, Sluch VM, Jabbari JS, Chamling X, Zack DJ, Pébay A*, Powell JE* and Hewitt AW* (2018). Single Cell RNA Sequencing of stem cell-derived retinal ganglion cells. *Scientific Data.* 5:180013
3. Galloway C, Dalvi S, Hung SSC, MacDonald L, Latchney L, Wong RCB, Guymer RH, Mackey DA, Williams DS, Chung M, Gamm D, Pébay A, Hewitt AW and Singh R (2017). Drusen in a dish: Novel patient-derived hiPSC model(s) of macular dystrophies. *Proceedings of the National Academy of Sciences*: 114(39): E8214-E8223.
4. Crombie DE, Daniszewski M, Liang HH, Kulkarni T, Li F, Lidgerwood GE, Conquest A, Hernandez D, Hung SS, Gill KP, De Smit E, Kearns L, Clarke L, Sluch VM, Chamling X, Zack DJ, Wong RCB, Hewitt AW* and Pébay A*(2017). Development of a modular automated system for maintenance and differentiation of adherent human pluripotent stem cells. *SLAS Discovery*: 22(8): 1016-1025.
5. Daniszewski M, Crombie DE, Henderson R, Liang HH, Wong RCB, Hewitt AW and Pébay A*. Automated cell culture systems and their applications to human pluripotent stem cell studies. *SLAS Technology.* Accepted 06.05.17.

Submitted

1. Daniszewski M, Nguyen Q, Chy HS, Singh V, Crombie DE, Kulkarni T, Liang HH, Lidgerwood GE, Hernández D, Conquest A, Rooney LA, Chevalier S, Andersen SB, Senabouth A, Vickers JC, Mackey DA, Craig JE, Laslett AL, Hewitt AW*, Powell JE* and Pébay A*. Single cell profiling identifies key pathways expressed by iPSCs cultured in different commercial media. *iScience*.