Retina Australia Research Report

Summary of Grant Activities: January to December 2015

Project Title: Correcting inherited retinal disease through gene editing.

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Breakthroughs in cellular technology have led to the ability to generate stem cells from adult tissue. This offers the unique ability to interrogate pathological processes in tissue, which cannot be easily obtained pre-mortem (e.g. retina). In this study we used recently developed molecular techniques for genome editing (CRISPR-Cas9 technology) to correct and assess three specific mutations, which cause three distinct blinding retinal diseases (Best Disease, Doyne Honeycomb Retinal Dystrophy and Sorsby Fundus Dystropy). Combining these technologies for ocular disease is novel and will lead to the next generation of gene therapy.

The following scientific progress has been made during the research period:

- Skin cells (fibroblasts) were extracted from two Best Disease patients, three Doyne Honeycomb Retinal Dystrophy patients, three Sorsby Fundus Dystrophy patients. The fibroblast cells are needed for the generation of patient-specific induced pluripotent stem cells (iPSC).
- iPSCs have been generated and characterised from the fibroblasts of one Best Disease, three Doyne Honeycomb Retinal Dystrophy and three Sorsby Fundus Dystrophy patients.
- We have established and optimised the CRISPR-Cas9 technique in our laboratory including the generation of CRISPR constructs, introducing the CRISPR constructs into the patient derived iPSCs and to screen for the gene-corrected iPSC clones.
- **Gene correction was successful** for the following patient-derived iPSC using CRISPR-Cas9 technology:
    - Best Disease (one corrected iPSC line)
    - Doyne Honeycomb Retinal Dystrophy (five corrected iPSC lines; Figure 1)

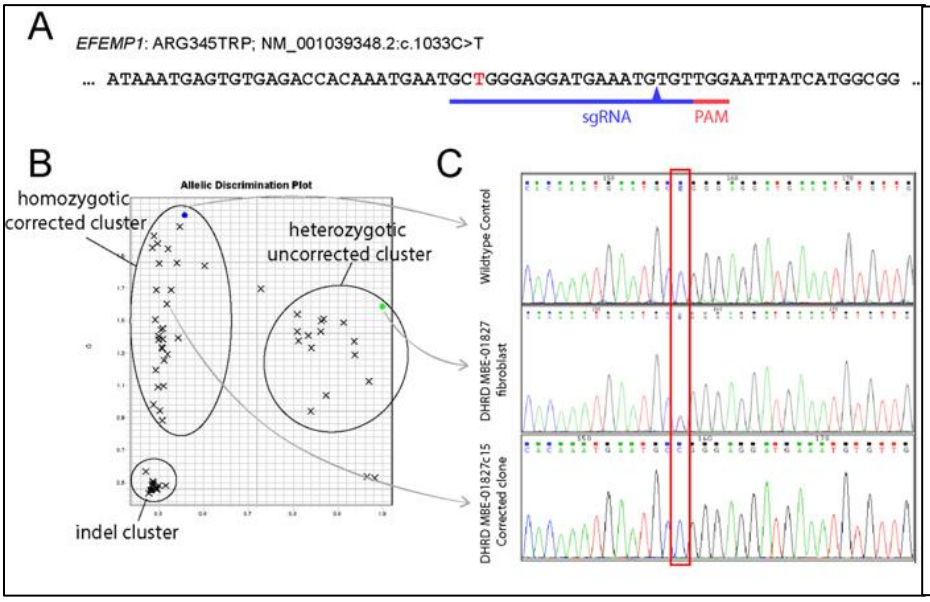

Figure 1. (**A**) Diagram of CRISPR guide RNA design targeting the gene mutated (EFEMP1) in Doyne Honeycomb Retinal Dystrophy patients. (**B**) An example of screening of the CRISPR-corrected iPSC clones. (**C**) Mutation correction was confirmed after the primary screening by Sanger sequencing.

- Correction of Sorsby Fundus Dystrophy patient-derived iPSC is currently underway.
- Phenotypic analysis is currently underway for Best Disease (Figure 2). Preliminary results suggest retinal pigmented epithelium (RPE) derived from our Best Disease patient iPSCs show a defect in their ability to engulf particles, which is one of the fundamental roles of RPE in the eye.

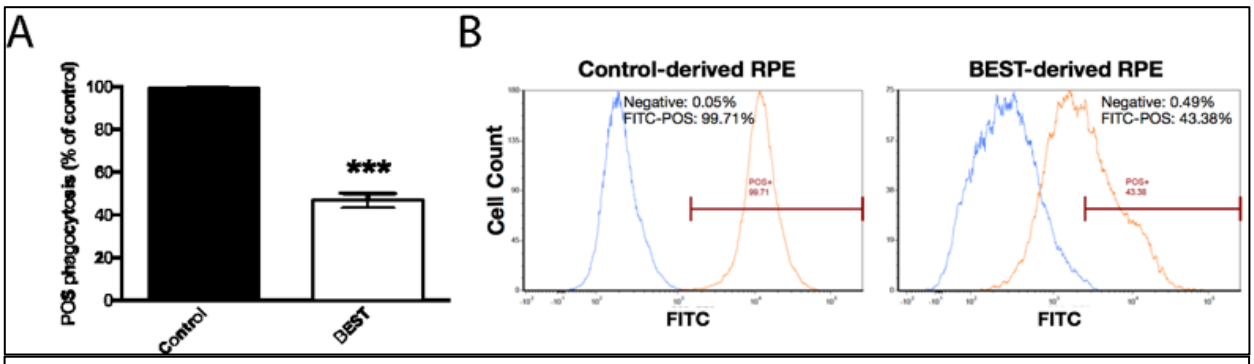

Figure 2. Functional analysis of Best Disease patient iPSC derived RPE. (**A**) Quantification of phagocytosis in control and Best disease patient iPSC-derived RPE showing internalisation of photoreceptor outer segments after 5 hour incubation ($p<0.001$). (**B**).The percentage of internalised POS (red) relative to the untreated (blue, negative control) was quantified using FACS.

Funding by Retina Australia has enabled us to take this project to the next stage- to use the CRISPR-Cas9 technology in *in vivo* mouse model studies (Figure 3), providing an important step towards the development of next generation gene therapy to correct for hereditary mutation directly in the eye.

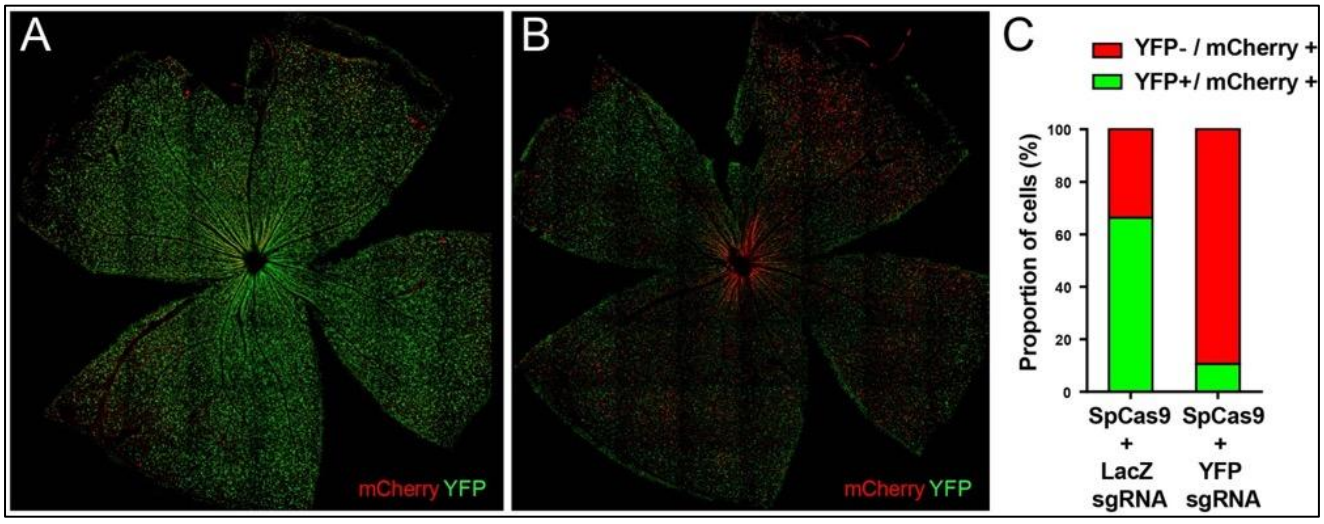

Figure 3. CRISPR/Cas mediated gene-editing in mouse retina. Representative fluorescence microscopy images of retinas from Thy1-YFP (green) mice exposed *in vivo* to an AAV2 CRISPR plasmid system carrying either control (LacZ) sgRNA (**A**) or sgRNAs targeting YFP (**B**). sgRNA plasmids expressed mCherry (red). Overall, the proportion of mCherry expressing cells (mCherry+), which lacked YFP (YFP-) was higher in CRISPR/YFP-sgRNA treated eyes (**C**).

With the support of the funding from Retina Australia we are currently in the process of publishing the following manuscripts:

1. Sandy SC Hung, Vicki Chrysostomou, Amy Fan Li, Jeremiah KH Lim, Jiang-Hui Wang, Leilei Tu, Maciej Daniszewski, Camden Lo, Raymond CB Wong, Jonathan G Crowston, Alice Pébay, Anna E King, Bang V Bui, Guei-Sheung Liu*, Alex Hewitt*. **AAV-mediated CRISPR/Cas gene editing of retinal cells in vivo.** Research paper under review with IOVS. (pre-printed: http://biorxiv.org/content/early/2016/02/09/039156) * equal contribution.

2. Sandy SC Hung*, Tristan McCaughey*, Olivia Swann, Raymond CB Wong, Alex Hewitt. **Genome Engineering in Ophthalmology: Application of CRISPR/Cas to the treatment of Eye Disease**. Review paper under review with Progress in Retinal and Eye Research. * equal contribution.

3. Katherine P Gill*, Sandy SC Hung*, Alexei Sharov, Camden Y Lo, Karina Needham, Grace E Lidgerwood, Stacey Jackson, Duncan E Crombie, Bryony Nayagam, Anthony L Cook, Alex W Hewitt, Alice Pébay, Raymond CB Wong. **Enriched retinal ganglion cells derived from human embryonic stem cells.** Research paper under review with Scientific Reports. * equal contribution.

4. Sandy SC Hung, Nicole J Van Bergen, Stacey Jackson, Helena Liang, David A Mackey, Damian Hernandez, Shiang Y Lim, Alex W Hewitt, Ian Trounce, Alice Pébay, Raymond CB Wong**. Study of mitochondrial respiratory defects on reprogramming to human induced pluripotent stem cells.** Research paper under review with Aging.

5. Sandy SC Hung, Alice Pébay, Raymond CB Wong. **Generation of Integration-free Human induced pluripotent stem cells using hair-derived keratinocytes.** (2015) Research paper published with JOVE. (publication link: http://www.jove.com/video/53174/generation-integration-free-human-induced-pluripotent-stem-cells?status=a55180k)