Retina Australia 2018 Project Grant Report

TITLE: Dual AAV retinal gene therapy approach for Usher 1F treatment

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PROJECT PROGRESS - RESULTS

1. Usher 1F patient samples

Samples from two separate Usher 1F patients were studied during this project, Patient A and Patient B. Patient A harbors a compound heterozygous pathogenic mutations in the *PCDH15* gene encoding a premature stop codon and a cryptic splice-site ([c.2986C>T];[c.3516+2T>C]). A skin sample was collected through the WARD study by Dr Fred Chen, and this project allowed the dermal fibroblasts to be maintained and expanded and also the generation of an iPSC line for this patient. This iPSC line has now been validated and can be used for future studies.

Patient B carries a homozygous R245X mutation ([c.733C>T];[c.733C>T]) in exon 8 of the *PCDH15* gene. Two induced pluripotent stem cell (iPSC) lines were already derived from this patient who previously donated dermal fibroblasts for gene editing and reprogramming by collaborators Dr Alice Pébay (University of Melbourne) and Dr Alex Hewitt (University of Tasmania). Our collaborators shared these iPSC lines with our LEI team. The two iPSC lines are a mutation-corrected isogenic control and the patient line with the homozygous mutation. Furthermore, our project also evaluated a second control iPSC line derived from an unaffected person.

2. Generation of Usher 1F iPSC-derived retinal organoids

Using the two iPSC lines derived from Patient B, we were able to generate retinal organoids for further study and testing of the dual AAV treatment approach. All iPSC lines exhibited healthy stem cell morphology and formed aggregates (embryoid bodies) which subsequently developed into retinal organoids (ROs). There were no apparent gross morphological differences between Patient B and control ROs during differentiation.

2.1.Characterisation of ROs

ROs were collected on days 30, 45 and 60 of differentiation and showed a progressive downregulation of pluripotency markers OCT4, SOX2 and NANOG, and progressive upregulation of germ layer markers TBXT, SOX7 and DCX, indicating that the ROs were differentiating correctly. Expression of retinal progenitor (PAX6, RAX) and photoreceptor (NRL, RHO) markers were present on days 30 and 60 of differentiation in both control and patient ROs, indicating they were progressing towards a retinal profile. On day 60 of differentiation, the cone-specific marker ARR3 was also expressed, indicating the presence of differentiated photoreceptors. The presence of the developing photoreceptor connecting cilium, where PCDH15 is located, was examined using a cilium marker, ARL13B. At day 60, there were no clearly observable differences in connecting cilium between patient and control lines.

3. AAV transduction

Our data showed that both AAV2/9 and AAV2/Anc80 are capable of transducing patient fibroblast and retinal organoids (ROs). Using a reporter fluorescent construct we showed that patient fibroblast had observable reporter gene expression starting from 5 and 9 days post-transduction with AAV2/Anc80 and AAV2/9, respectively. Fibroblast transduction levels was quantified at 14 days post-transduction and showed that AAV2/Anc80 was 22-fold more efficient than AAV2/9.

Transduction of ROs was also observed starting from 5 days post-transduction. When observed under fluorescent microscopy, AAV2/Anc80L65-transduced ROs had noticeably higher reporter expression

levels compared to AAV2/9-transduced ROs across all cell lines, biological replicates, and post-transduction time points.

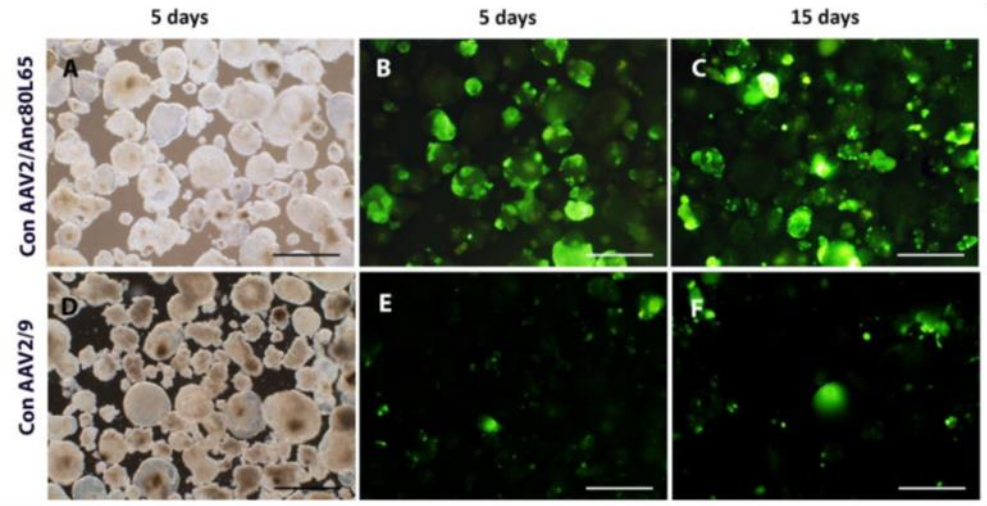

Fig 1. Transduction of ROs with reporter gene. (A, D) Brightfield images; (B,C,E,F) Fluorescent images. (G) Quantification of fibroblast AAV transduction at 14 days.

4. Assessment of PCDH15 transcript expression restoration by dual AAV therapy

Retinal organoids were treated with the two AAV constructs containing the separate halves of the *PCDH15* gene at either day 30 or 45. Early hybrid dual AAV treatment (day 30) of both AAV2/Anc80L65 and AAV2/9 resulted in an increase in PCDH15 on days 45 and 60 relative to untreated day 30 (baseline) expression. Hybrid dual AAV2/Anc80L65 resulted in a higher initial increase of PCDH15 expression in the first 15 days post-transduction relative to baseline (AAV2/Anc80L65 =2.1, AAV2/9=1.7) while AAV2/9 induced a greater increase (2.2-fold) between days 45 and 60 than AAV2/Anc80L65 (1.3-fold). At 30 days post-transduction, hybrid AAV2/9-transduced ROs had a greater overall increase in PCDH15 expression compared to AAV2/Anc80L65-transduced ROs (fold increase relative to baseline: 3.6 and 2.8, respectively). Similarly, ROs treated at a later time point (day 45) had higher induced PCDH15 expression at day 60 when transduced with hybrid AAV2/9 than hybrid AAV2/Anc80L65. The fold increase in *PCDH15* expression from untreated day 60 was 3.6 for AAV2/9-transduced ROs and 2.8 for AAV2/Anc80L65-transduced ROs.

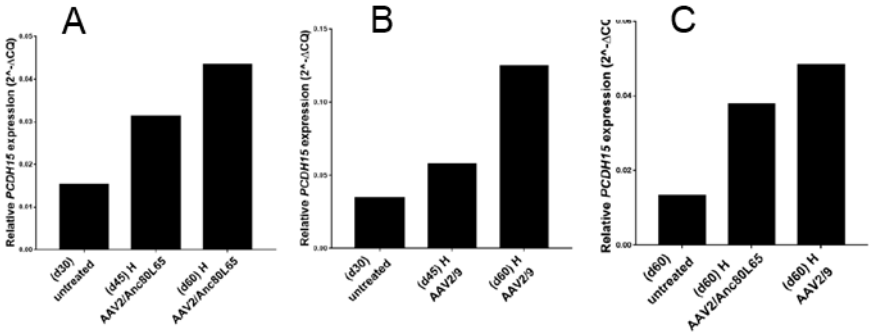

Fig 2. Assessment of PCDH15 expression in patient and control ROs after dual AAV delivery. Untreated ROs are compared with (A,B) single dose early-treated (day 30) ROs and (C) single dose late-treated (day 45) ROs.

CONCLUSIONS

This Retina Australia grant has allowed us to develop an effective *in vitro* model for Usher 1F and demonstrate proof-of-principle expression restoration of *PCDH15* using dual AAV vectors. Low-level dosage of hybrid AAV2/9 vectors was the most effective strategy tested to induce *PCDH15* expression. Future work can aim to assess restoration of full-length protocadherin-15 and validate our data in a larger number of replicates. Overall, our results are a starting point for follow-up studies, which can aim to investigate the viability of an optimised dual AAV approach for the therapeutic delivery of PCDH15 to Usher 1F patients.