Modulation of CNOT3 expression using antisense oligomers to treat retinitis pigmentosa 11

3541690

Janya Grainok[1,2,3], Ianthe Pitout[1,2], Fred Chen[4], Carla Jackson[1,4], Anja Stirnweiss[1], Kim Rice[1], Wissam Chiha[1], Suzy Juraja[1], Anna Mills[1], Laura Florez[1], Jessica Nichols[1], Danie Champain[1], Samuel McLenachan[4], Paula Cunningham[1], Sue Fletcher[1,2]

[1]PYC Therapeutics, Nedlands, WA, Australia. [2]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Murdoch, WA, Australia.

[3]Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. [4]Lions Eye Institute, The University of Western Australia, WA, Australia.

Retinitis Pigmentosa 11 is caused by mutations in pre-mRNA processing factor 31

Retinitis pigmentosa 11 (RP11) is caused by heterozygous mutations in pre-mRNA processing factor 31 (*PRPF31)*. Functional defects in PRPF31 lead to degeneration of photoreceptor and retinal pigment epithelial cells, which subsequently causes loss of peripheral vision and potential for complete blindness.

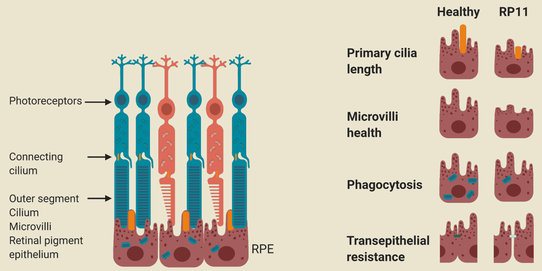

Figure 1. Key disease phenotypes in retinal pigment epithelium (RPE) derived from patients with RP11 including shortened cilia length and microvilli, reduced phagocytosis capability and loss of RPE tight junctions.
Image from biorender.com

CNOT3 is a negative regulator of *PRPF31* and is linked with incomplete disease penetrance of RP11

The CCR4-NOT transcription complex subunit 3 (CNOT3) is a major disease modifier that regulates *PRPF31* levels via transcription inhibition. RP11 cases show higher expression than nonpenetrant mutation carriers.

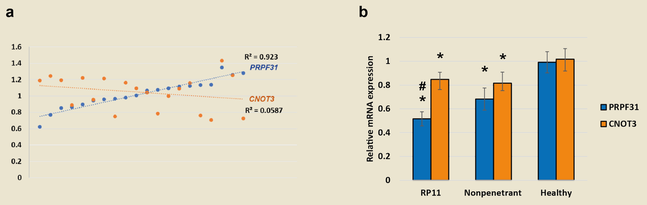

Figure 2. *CNOT3* expression is inversely correlated to *PRPF31* expression. **(a)** *PRPF31* and *CNOT3* mRNA expression levels in fibroblasts derived from healthy individuals (n=20). *CNOT3* is a negative regulator of *PRPF31*, exemplified by the inverse correlation between *CNOT3* and *PRPF31* mRNA levels in fibroblasts. *PRPF31* and *CNOT3* transcript levels were assessed by quantitative reverse transcription-PCR (qRT-PCR). **(b)** qRT-PCR analysis of *PRPF31* and *CNOT3* mRNA expression in induced pluripotent stem cell (iPSC)-derived RPE from RP11, nonpenetrant and healthy individuals (set to 1). **p*<0.05 compared to healthy control, #*p*<0.05 compared to a nonpenetrant carrier.

PYC's VP-001 demonstrates key functional improvement in RP11 patient iPSC-derived retinal pigment epithelium

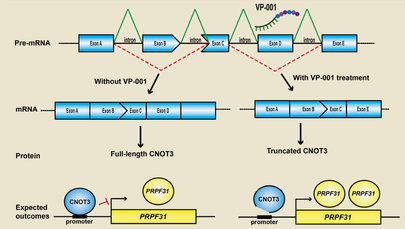

Figure 3. VP-001 comprises a cell penetrating peptide (CPP) conjugated to a PMO (phosphorothioate morpholino oligomer) designed to exclude an in-frame exon in the *CNOT3* pre-mRNA, resulting in a truncated form of CNOT3 with no, or reduced, function. CNOT3 is a negative regulator of *PRPF31* expression and regulates *PRPF31* transcription by directly binding the promoter. Loss of CNOT3 function leads to an increase in PRPF31 levels in a mutation agnostic manner.

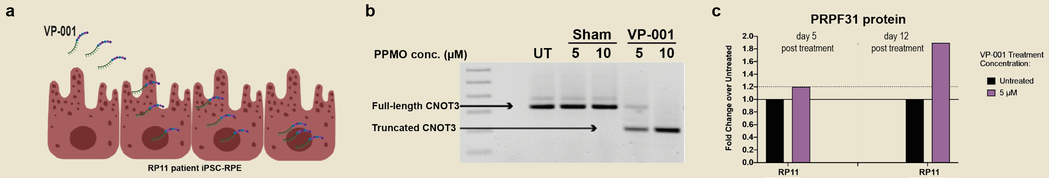

Figure 4. VP-001 mediated *CNOT3* exon skipping to induce PRPF31 expression. (a) RP11 iPSC-RPEs were incubated with VP-001 and cells were harvested for assesment of *CNOT3* exon skipping and PRPF31 protein expression. **(b)** Representative image of *CNOT3* exon skipping following VP-001 treatment. **(c)** PRPF31 protein upregulation was obsereved as a consequence of *CNOT3* exon skipping, determined using western blot assay.

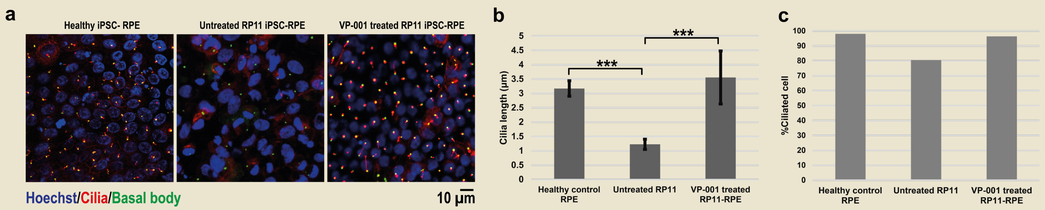

Figure 5. VP-001 rescues primary cilia defects in RP11 patient iPSC-RPE. (a) Immunostaining of RPE with cilia marker ARL13B (red) and basal body marker Pericentrin (green) in healthy and RP11 RPE following treatment with VP-001 (10 μM) for 5 days. **(b)** Cilia length was significantly increased. Bar chart represents mean±SEM from ~ 300 ciliated cells. **(c)** The percentage of ciliated RPE cells, counted from >1,000 cells/sample. Biological replicate = 1.

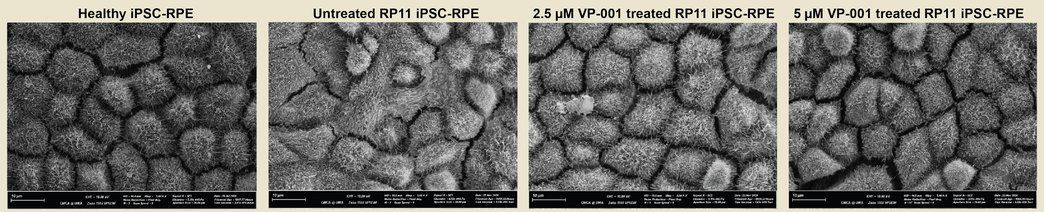

Figure 6. VP-001 rescues microvilli defects in RP11 patient iPSC-RPE. RPE morphology and microvilli were evaluated in healthy iPSC-RPE and RP11 iPSC-RPE monolayers by scanning electron microscopy (SEM) 28 days post-treatment with VP-001 (2.5 μM, 5 μM). Scale bars: 10 μm.

Enhanced phagocytosis capability of RP11 iPSC-RPE following VP-001 treatment

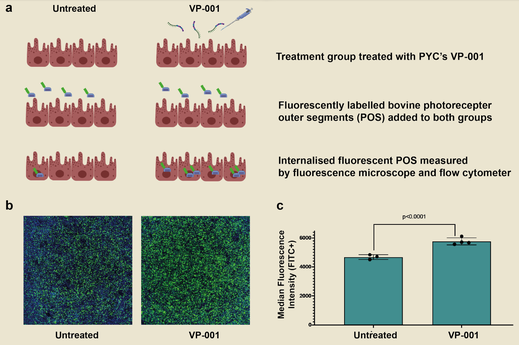

Figure 7. VP-001 rescues phagocytic function in RP11 patient iPSC-RPE. **(a)** Phagocytosis was evaluated in RP11 patient iPSC- derived RPE by incubating cells with fluorescently labelled bovine photoreceptor outer segments (POS) and assessing internalised POS, measured by **(b)** confocal microscopy or **(c)** flow cytometry. Results showing increased engulfment of FITC-labelled POS by RP11 RPE following treatment with VP-001. A single treatment with VP-001 (5 μM) led to a significant increase in mean fluorescence intensity 28 days post-treatment compared to untreated cells ($p<0.05$, one way ANOVA; untreated n=3, VP-001 treated n=4)

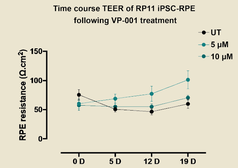

Figure 8. VP-001 rescues tight junction defects in RP11 patient iPSC-RPE. Trans-epithelial resistance (TER) assays demonstrate a marked increase in resistance following treatment of RP11 patient RPE (5 μM) compared to untreated after 19 days.

Conclusions

Subtle changes in *CNOT3* and *PRPF31* levels in retinal cells determine disease penetrance in *PRPF31* mutation carriers within an affected family. Modulating expression levels of these two proteins can halt the cellular disease phenotype in RP11. Antisense oligomers are effective modulators of CNOT3 expression and function with the ability to increase *PRPF31* transcription from the unaffected allele to an expected therapeutic level.

Disclosures

JG, IP, CJ, AS, KR, WC, SJ, AM, LF, JN, DC, PC and SF are the employees of PYC Therapeutics. JG, IP, FC, SM and SF are named as co-inventors on International application number PCT/AU2020/050516, a treatment for RP11.

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