

**A cellular disease model system of ARB: The creation of iPS-RPE from a patient with a premature stop mutation (p.R200X).**

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Abstract

**Purpose :** Autosomal-recessive bestrophinopathy (ARB) is a distinct inherited bestrophinopathy that is caused by mutations in bestrophin 1 (BEST1), a protein located in the retinal pigment epithelium (RPE). The p.R200X mutation is a premature stop mutation that causes alterations in the RPE and subretinal deposits in the macular area. Patients with this mutation have an absent EOG light rise and reduced ERGs, presenting with central vision loss early in life. Currently, patients with bestrophinopathies, such as ARB, do not have any available treatments and visual loss cannot be prevented. Resolving the exact function of the bestrophin 1 in RPE cells is an essential step in identifying viable therapeutics for these patient groups. Here we are using induced pluripotent stem cell (iPSC)-derived RPE created from a patient with the p.R200X mutation to investigate the role of BEST1 in the RPE.

**Methods :** We have created iPSCs from p.R200X patient fibroblasts by reprogramming with episomal vectors (c-myc, Klf4, Lin28, Oct4, Sox2). iPSC colonies were isolated, expanded and differentiated into RPE by spontaneous differentiation method. After approximately 6 weeks pigmented foci were purified by manual dissection and cells seeded to encourage monolayer formation. Patient iPSC and iPSC-derived RPE cells were assessed by standard molecular and cellular protocols, including immunocytochemistry (ICC) and PCR, in comparison to control cells, and whole cell patch clamp was used to assess RPE cell function.

**Results :** Sequencing of the starting material confirmed presence of pR200X mutation in the patient line. Whilst immunocytochemistry revealed absence of BEST1 protein in the pR200X patient iPS-RPE cells, confirming true null BEST1 mutation. Our patch clamp experiments confirmed involvement of BEST1 in the ion currents of the pR200X patient iPS-RPE cells.

Our results suggest that BEST1 may be involved in volume regulation within the RPE. Morphological assessment of the pR200X patient iPS-RPE revealed significant difference in size, in comparison to the control iPS-RPE cells.

**Conclusions :** Examination of the p.R200X mutation in patient derived iPSC-RPE cells has provided new insight into the role of BEST1 in the RPE cell. A greater understanding of the effects of BEST1 mutations on pathological mechanisms will assist the development of novel therapeutics for bestrophinopathy patients.

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