Chapter XX

Bestrophin1: A gene that causes many diseases

Joseph J. Smith[1], Britta Nommiste[1] and Amanda-Jayne F. Carr[1].

[1]UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK.

Corresponding Author: A-J. F. Carr. UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK. **e-mail:** a.carr@ucl.ac.uk

Abstract Bestrophinopathies are a group of clinically distinct inherited retinal dystrophies that lead to the gradual loss of vision in and around the macular area. There are no treatments for patients suffering from bestrophinopathies and no measures can be taken to prevent visual deterioration in those who have inherited disease-causing mutations. Bestrophinopathies are caused by mutations in the Bestrophin 1 gene (*BEST1*), a protein found exclusively in the retinal pigment epithelial (RPE) cells of the eye. Mutations in *BEST1* affect the function of the RPE leading to the death of overlying retinal cells and subsequent vision loss. The pathogenic mechanisms arising from *BEST1* mutations are still not fully understood, and it is not clear how mutations in *BEST1* lead to diseases with distinct clinical features. This chapter discusses BEST1, the use of model systems to investigate the effects of mutations and the potential to investigate individual bestrophinopathies using induced pluripotent stem cells.

X.X Introduction

Bestrophinopathies are a collection of inherited retinal diseases caused by mutations in Bestrophin1 (*BEST1*). These diseases, including Best disease, adult onset vitelliform macular degeneration (AVMD), autosomal recessive bestrophinopathy (ARB), autosomal dominant vitreoretinochoroidopathy (ADVIRC) and autosomal dominant retinitis pigmentosa (adRP), have varying times of onset and significantly different clinical manifestations (Table 1; For review see Leroy, 2012). Diagnosis of a bestrophinopathy is usually confirmed by an abnormal light-to-dark ratio in the electrooculogram (EOG), a test that assesses the standing potential at the back of the eye; an indirect measurement of retinal pigment epithelium (RPE) cell function. This pathognomonic test, together with protein localisation, hyperpigmentation, the accumulation of fluid and autofluorescent material within and around the macula, suggests that the origin of BEST1-related diseases resides within the RPE.

X.X RPE

The RPE is a tight barrier of cells that resides as a pigmented cobblestone-like monolayer on Bruch's membrane, between the neural retinal and the choriocapillaris, where it forms a crucial component of the blood retina barrier. The RPE is involved in a wide range of functions essential for the maintenance of the neural retina, including the daily phagocytosis of shed photoreceptor outer segments, recycling of retinol in the visual cycle, secretion of signalling molecules and growth factors, absorbance of stray light and the transport of water, ions, metabolites and nutrients, to and from the retina (For review see Strauss, 2005).

X.X Bestrophin1

Bestrophin1 was first identified as the gene responsible for Best disease in the 1990's. Genetic linkage analysis mapped the gene to chromosome 11q13 (Stone et al. 1992) and in 1998, the gene responsible, termed *vitelliform macular degeneration 2* (*VMD2*) or *Bestrophin1* (*BEST1*) was identified (Petrukhin et al. 1998). The *BEST1* gene spans 11 exons and produces a 585a.a. protein with a molecular weight of 68kDa. Approximately 300 mutations in *BEST1* have been identified, with allelic heterogeneity leading to the variety in phenotypes associated with bestrophinopathies.

Bacterial and chicken BEST1 proteins provide structural models suggesting a highly conserved N-terminus containing four transmembrane domains and a long, diverse cytosolic portion that contribute to the formation of an ion channel (Dickson et al. 2014; Yang et al. 2014). Five BEST1 protein subunits form a pentameric channel with a small extracellular region and larger intracellular region. The five subunits are symmetrically arranged round a central axis to produce a barrel shaped pore with a hydrophobic neck. BEST1 acts as a Ca^{2+}-activated Cl^- channel; the binding of Ca^{2+} to the intracellular Ca^{2+} clasps of the BEST1 subunits is associated with dilation of the gate and flux of Cl^- ions. The majority of disease-causing mutations reside

in hot spots of conserved residues within the N-terminal portion of BEST1, with many mutations, localised to the Ca^{2+} clasp and hydrophobic neck, thought to alter the permeability of the channel. Various studies have shown that BEST1 acts as a Ca^{2+}-activated Cl^- channel (Soria et al. 2009). It may also act as a volume-activated anion channel (Fischmeister and Hartzell, 2005), and be involved in the regulation of voltage-gated Ca^{2+} channels (Yu et al. 2006), the recruitment of Ca^{2+} from endoplasmic reticulum stores (Neussert et al. 2010), intracellular trafficking (Milenkovic et al. 2011) and the mediation of neurotransmitter release (Woo et al. 2012).

X.X Animal Models of Bestrophinopathies

In the eye, BEST1 is expressed in the RPE cells, where it is localised to the basolateral membrane (Marmorstein et al. 2000). Its role in the development of human RPE cell pathology has remained elusive as post-mortem tissues are often at end stage disease and reveal little of the molecular sequelae leading to vision loss. Animal models of bestrophinopathies have provided some insights into the disease. Mouse and rat models of Best disease, where mutated BEST1 proteins have been introduced to the eye using adenovirus, have subretinal deposits and altered light peak electroretinogram responses, indicative of defects in the Cl^--induced hyperpolarization of the RPE basal membrane (Marmorstein et al. 2009). However, these animals lack a macula so may not fully represent the full spectrum of human bestrophinopathies. One of the most informative animal models of bestrophinopathy is canine multifocal retinopathy (cmr), an autosomal recessive retinal disorder observed in multiple breeds, caused by naturally occurring c*BEST1* mutations. Although dogs do not have a macula, there is a fovea like region containing a high concentration of cones, known as the area centralis. The presence of multiple lesions in the majority of cmr cases is similar to that observed in the recessive human bestrophinopathy, ARB, where homozygous or compound

heterozygous mutations in c*BEST1* abolish channel activity resulting in a null phenotype (Guziewicz et al. 2011). Canine models of bestrophinopathy are helping to further our understanding of disease progression and deposit formation in bestrophinopathies, and suggest that abnormal apical microvilli in diseased retinae weaken the interaction between the RPE and overlying photoreceptor cells. Canine models are also aiding in the development of *BEST1* AAV gene therapy, which reverses disease induced retina-RPE microdetachments in the canine retina (Guziewicz et al. 2018).

X.X Induced Pluripotent Stem cells

Understanding the precise molecular sequelae of full range of bestrophinopathies is key to developing effective therapeutics, cellular therapies and personalised medicines for these diseases. Culture models, such as RPE and non-RPE epithelial cell lines, have been used to investigate RPE function, however issues with transdifferentiation of cells and incorrect polarisation of cells limit their use. Stem cell research is now revolutionising what we know about eye diseases, with induced pluripotent stem cells (iPSC) providing researchers with new means to investigate human blinding diseases in a dish. In 2007, a pivotal study demonstrated that human adult skin cells could be reprogrammed to a pluripotent state using a defined combination of embryonic transcription factors (Takahashi et al. 2007). As iPSCs can be generated from skin/blood/urine cells of patients and then differentiated into specific cell types, this technology offers the unique opportunity of studying genetic mutations in a patient's own cells. These cells provide a direct link between clinical disease and changes at the molecular and cellular level, and can be used as a platform to develop and test novel therapies (Figure. 1). iPSCs are particularly amenable to the study of ophthalmic diseases originating in the RPE due to the relative ease with which these cells can be differentiated and purified from iPSCs (Carr et al. 2009). iPSC-derived RPE can be cultured as a pigmented cobblestone monolayer

of cells, which express key markers of developing and mature RPE including and possess many of the key physiological aspects of native RPE e.g. transepithelial resistance, phagocytosis of retinal debris, secretion of growth factors, the presence of functioning voltage-gated ion transporters and the mobilisation of intracellular Ca^{2+} in response to ATP (Carr et al. 2009; Kokkinaki et al. 2011; Vaajasaari et al. 2011).

X.X Investigating bestrophinopathies using iPSC-RPE

Stem cell studies, using iPSC-RPE derived from bestrophinopathy patients, have revealed a number of pathways associated with RPE cell functions compromised by *BEST1* mutations. The phagocytosis of photoreceptor outer segment (POS) membrane discs is a vital process performed daily by the RPE to maintain the retina. This process is disrupted in best disease iPSC-RPE, with delays in both the internalisation and degradation of POS by the RPE (Marmorstein et al. 2018; Singh et al. 2013). Prolonged exposure to POS leads to the build-up of autofluorescent material and increased oxidative stress in cells (Singh et al. 2013). Ion flow is also affected in patient cells. In WT iPSC-RPE, stimulation with calcium elicits a decrease in intracellular Cl^-, this response is absent in cells from best disease patients (Moshfegh et al. 2016). Severely reduced Cl^- currents also suggest that BEST1 may function as a volume regulated ion channel regulating cell volume (Milenkovic et al. 2015). Epithelial cell water transport is often coupled to ion transport, failure of the Cl^- driven transport of water along with defects in phagocytosis could promote the build-up of extracellular fluid and material in the subretinal space, reducing retinal-RPE adhesion, ultimately contributing to pathological issues such as the vitelliform lesion and macular oedema.

Patient iPSC-RPE are also helping to reveal details of BEST1 channel activators. The Best disease p.I201T mutation, which resides within a proposed ATP binding motif, decreases the binding of ATP and activation of BEST1 in iPSC-RPE, suggesting the presence of an ATP

dependent mechanism regulating BEST1 channel gating (Zhang et al. 2018). Intriguingly, ATP released by photoreceptor cells has been postulated to be the light peak substance observed in the electrooculogram, a test normally used to diagnose bestrophinopathies. Defects in ATP responses could be the determinant behind EOG changes in patients with *BEST1* mutations.

Given the varying clinical pathology of bestrophinopathies, iPSC-RPE may be important in defining the mechanisms behind discrete molecular pathologies underlying these disparate diseases. The pathology behind these diseases could be accounted for by the effects of the mutation on channel formation, protein function or protein localisation. For example, Best disease and ADVIRC both result from dominant *BEST1* mutations, however, while Best disease affects the macula, ADVIRC has widespread effects in the peripheral retina and affects eye development. ADVIRC was predicted to result from aberrant splicing of *BEST1*, however, normal splicing occurs in ADVIRC iPSC-RPE with the V235A mutation, instead protein was observed at both the apical and basal membrane of cells (Carter et al. 2016), which suggests that mislocalisation across the membrane may play a role in this disease. The six known ADVIRC mutations are clustered in the cytosolic regions of the second and third transmembrane domains of BEST1, which suggests they may affect a critical localisation and/or functional domain.

X.X Conclusions

Induced pluripotent stem cells offer a powerful alternative model to investigate inherited diseases such as bestrophinopathies by creating diseased human RPE cells in a dish that recapitulate the spectrum of BEST1-related diseases observed in human patients. Studies using patient derived iPSC-RPE will compliment on-going studies in alternative models to fully understand the role of BEST1 in RPE cells and provide vital insight into the contribution of

BEST1 mutations to individual bestrophinopathies, assisting in the development of disease specific therapies.

References

Carr A-J, Vugler A , Hikita ST, Lawrence JM et al (2009) Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. PLoS One 4:e8152

Carter DA, Smart MJK, Letton WVG et al (2016) Mislocalisation of BEST1 in iPSC-derived retinal pigment epithelial cells from a family with autosomal dominant vitreoretinochoroidopathy (ADVIRC). Sci Rep 6.

Fischmeister R, Hartzell HC (2005) Volume sensitivity of the bestrophin family of chloride channels. J Physiol 562:477–491.

Guziewicz KE, Cideciyan A V., Beltran WA et al (2018) *BEST1* gene therapy corrects a diffuse retina-wide microdetachment modulated by light exposure. Proc Natl Acad Sci.

Guziewicz KE, Slavik J, Lindauer SJP et al (2011) Molecular consequences of BEST1 gene mutations in canine multifocal retinopathy predict functional implications for human bestrophinopathies. Investig Ophthalmol Vis Sci 52:4497–4505.

Kane Dickson V, Pedi L, Long SB (2014) Structure and insights into the function of a Ca2+-activated Cl− channel. Nature 516:213–218

Kokkinaki M, Sahibzada N, Golestaneh N (2011) Human iPS-Derived Retinal Pigment Epithelium (RPE) Cells Exhibit Ion Transport, Membrane Potential, Polarized VEGF Secretion and Gene Expression Pattern Similar to Native RPE. Stem Cells.

Leroy BP (2012) Bestrophinopathies. In: Genetic Diseases of the Eye, Second Edition, pp 426–436. Oxford University Press.

Marmorstein AD, Marmorstein LY, Rayborn M et al (2000) Bestrophin, the product of the

Best vitelliform macular dystrophy gene (VMD2), localizes to the basolateral plasma membrane of the retinal pigment epithelium. Proc Natl Acad Sci U S A 97:12758–12763.

Marmorstein AD, Cross HE, Peachey NS (2009) Functional roles of bestrophins in ocular epithelia. Prog Retin Eye Res 28:206–226

Marmorstein AD, Johnson AA, Bachman LA et al (2018) Mutant Best1 Expression and Impaired Phagocytosis in an iPSC Model of Autosomal Recessive Bestrophinopathy. Sci Rep 8:1–14

Milenkovic A, Brandl C, Milenkovic VM et al (2015) Bestrophin 1 is indispensable for volume regulation in human retinal pigment epithelium cells. Proc Natl Acad Sci USA 112:E2630–E2639

Milenkovic VM, Rohrl E, Weber BHF et al (2011) Disease-associated missense mutations in bestrophin-1 affect cellular trafficking and anion conductance. J Cell Sci 124:2988–2996

Moshfegh Y, Velez G, Li Y et al (2016) BESTROPHIN1 mutations cause defective chloride conductance in patient stem cell-derived RPE. Hum Mol Genet 25:2672–2680.

Neussert R, Müller C, Milenkovic et al (2010) The presence of bestrophin-1 modulates the Ca2+ recruitment from Ca2+ stores in the ER. Pflügers Arch - Eur J Physiol 460:163–175

Petrukhin K, Koisti MJ, Bakall B et al (1998) Identification of the gene responsible for Best macular dystrophy. Nat Genet 19:241–247.

Singh R, Shen W, Kuai D et al (2013) iPS cell modeling of best disease: Insights into the pathophysiology of an inherited macular degeneration. Hum Mol Genet 22:593–607.

Soria RB, Spitzner M, Schreiber R et al (2009) Bestrophin-1 enables Ca2+-activated Cl- conductance in Epithelia. J Biol Chem 284:29405–29412.

Stone EM, Nicols BE, Strebet al (1992) Genetic linkage of vitelliform macular degeneration

(Best’s disease) to chromosome 11q13. Nat Genet 1:246–250.

Strauss O (2005) The Retinal Pigment Epithelium in Visual Function. Physiol Rev:845–881.

Takahashi K, Tanabe K, Ohnuki M (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. 131:861–872

Vaajasaari H, Ilmarinen T, Juuti-Uusitalo et al (2011) Toward the defined and xeno-free differentiation of functional human pluripotent stem cell-derived retinal pigment epithelial cells. Mol Vis 17:558–575.

Woo DH, Han K-S, Shim JW et al (2012) TREK-1 and Best1 channels mediate fast and slow glutamate release in astrocytes upon GPCR activation. Cell 151:25–40

Yang T, Liu Q, Kloss B et al (2014) Structure and selectivity in bestrophin ion channels. Science (80-) 346:355–359

Yu K, Cui Y, Hartzell HC (2006) The Bestrophin Mutation A243V, Linked to Adult-Onset Vitelliform Macular Dystrophy, Impairs Its Chloride Channel Function. Investig Opthalmology Vis Sci 47:4956

Zhang Y, Kittredge A, Ward N et al (2018) ATP activates bestrophin ion channels through direct. Nat Commun 9:3126

Disease	Symptoms	Inheritance - Prevalence
Best disease	Egg yolk-like vitelliform lesion to scrambled egg-like cyst stage, layering of subretinal material. RPE atrophy and loss of central vision loss. Abnormal EOG, CNV hyperautofluorescence. Early onset, some affected asymptomatic.	Autosomal dominant - 1-9/100,000
ARB	Diffuse RPE alterations, punctate subretinal deposits, retinal oedema, hyperautofluorescence, RPE atrophy, CNV. Abnormal EOG, central vision loss, early onset.	Autosomal recessive - 1/1,000,000
ADVIRC	Peripheral circumferential hyperpigmentation, white punctate opacities, RPE atrophy, CNV. Developmental abnormalities: microcornea, nanophthalmos, vitreous fibrillar condensation, angle-closure glaucoma, cataract optic nerve dysplasia. Visual field loss. Early onset.	Autosomal dominant - 1/1,000,000
AVMD	Similar to Best disease with late adult onset and slower progression. Subnormal EOG, mild central vision loss. Can develop CNV and RPE atrophy.	Autosomal dominant(?) - Unknown
adRP	Peripheral retinopathy, hyperpigmentation in peripheral retina, macula oedema. Abnormal EOG and constricted visual field loss. adRP (RP50) could be misdiagnosed	Autosomal Dominant - Unknown

Table 1. Symptoms associated with clinically distinct bestrophinopathies.