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Bestrophinopathy: An RPE-photoreceptor interface disease

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ABSTRACT

Bestrophinopathies, one of the most common forms of inherited macular degenerations, are caused by mutations in the *BEST1* gene expressed in the retinal pigment epithelium (RPE). Both human and canine *BEST1*-linked maculopathies are characterized by abnormal accumulation of autofluorescent material within RPE cells and bilateral macular or multifocal lesions; however, the specific mechanism leading to the formation of these lesions remains unclear. We now provide an overview of the current state of knowledge on the molecular pathology of bestrophinopathies, and explore factors promoting formation of RPE-neuroretinal separations, using the first spontaneous animal model of *BEST1*-associated retinopathies, canine Best (cBest). Here, we characterize the nature of the autofluorescent RPE cell inclusions and report matching spectral signatures of RPE-associated fluorophores between human and canine retinae, indicating an analogous composition of endogenous RPE deposits in Best Vitelliform Macular Dystrophy (BVMD) patients and its canine disease model. This study also exposes a range of biochemical and structural abnormalities at the RPE-photoreceptor interface related to the impaired cone-associated microvillar ensheathment and compromised insoluble interphotoreceptor matrix (IPM), the major pathological culprits responsible for weakening of the RPE-neuroretina interactions, and consequently, formation of vitelliform lesions. These salient alterations detected at the RPE apical domain in cBest as well as in BVMD- and ARB-hiPSC-RPE model systems provide novel insights into the pathological mechanism of *BEST1*-linked disorders that will allow for development of critical outcome measures guiding therapeutic strategies for bestrophinopathies.

Contents

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1. Introduction

The retinal pigment epithelium (RPE) is an organized monolayer of highly specialized cells responsible for a sustained interaction with photoreceptors, a mission pivotal for normal visual function and retinal preservation (Hageman and Johnson, 1991; Bok, 1993; Strauss, 2005). These diverse functions are orchestrated by a broad array of molecules designed to access specific cascades of intra- and intercellular interactions in the retina (*reviewed in:* Steinberg, 1985; Strauss, 2005; Kevany and Palczewski, 2010; Bonilha, 2014). One of such RPE-specific molecules is BESTROPHIN 1 (OMIM #607854), a transmembrane channel protein encoded by *BEST1* gene and associated with the RPE basolateral membrane (Marmorstein et al., 2000; Sun et al., 2002; Gomez et al., 2013). Although BESTROPHIN 1 has been extensively studied and described as a multifunctional protein implicated in mediating anion transport, regulation of calcium signaling and cell volume (Rosenthal et al., 2005; Hartzell et al., 2008; Strauss et al., 2014; Kane Dickson et al., 2014; Yang et al., 2014; Milenkovic et al., 2015), its multifaceted nature and complex interactions with photoreceptors in health and disease still remain elusive.

Mutations in *BEST1* have been causally associated with several clinically heterogeneous retinal disorders, collectively termed bestrophinopathies (Petrukhin et al., 1998; Seddon et al., 2001; Yardley et al., 2004; Schatz et al., 2006; Burgess et al., 2008; Davidson et al., 2009; Boon et al., 2009a). The *BEST1* mutational spectrum underlying these retinopathies varies greatly, and involves over 200 distinct mutations (Boon et al., 2009a; Pasquay et al., 2015; Yang et al., 2015). Additionally, a considerable variability in age at onset, rate of disease progression and phenotypic expression has been reported, not only between unrelated patients harboring the same mutation, but also among affected individuals within a single family (Kramer et al., 2000; Kinnick et al., 2011; Bitner et al., 2012; MacDonald et al., 2012; Boon et al., 2013). This phenomenon of phenotypic and allelic heterogeneity highlights a significant phenotypic overlap among *BEST1*-linked disorders, posing a challenge in both determining the diagnostic specificity as well as predicting the outcomes of visual impairment. In recent years, the complexity of genotype-phenotype correlation in bestrophinopathies has proven difficult to explain with traditional models of disease pathogenesis (Allikmets et al., 1999; Boon et al., 2007; Yu et al., 2007; Querques et al., 2009; Booij et al., 2010; Liu et al., 2016), indicating a presence of potential genetic modifiers or still undetermined Best1 protein interactors, involvement of environmental components or a combination of both. This likely would point to a complex interplay of genetic susceptibility factors and modifiable environmental stimuli utilizing novel signaling pathways in the retina.

The broad spectrum of clinical presentations in bestrophinopathies ranges from the widespread symptoms affecting peripheral retina and vitreous in a rare condition of vitreoretinochoroidopathy (ADVIRC; OMIM#193220) to the well-defined clinical abnormalities restricted to the macula and central retina in Best Vitelliform Macular Dystrophy (BVMD) and autosomal recessive bestrophinopathy (ARB). BVMD (a.k.a. VMD2, OMIM#153700), inherited as an autosomal dominant trait with incomplete penetrance, and the recessive form (ARB; OMIM#611809) are the most common and best explored juvenile macular dystrophies among bestrophinopathies, characterized by a markedly abnormal electrooculogram (EOG) accompanied by an excessive accumulation of lipofuscin material within RPE cells, formation of focal and multifocal subretinal lesions, and consequently, loss of central vision (Pianta et al., 2003; Boon et al., 2009b; Pasquay et al., 2015; Fung et al., 2015).

An abnormal accumulation of lipofuscin is a major risk factor implicated in different forms of macular degeneration (Delori et al., 1995a; Marmorstein et al., 2002; Gerth et al., 2007; Biarnes et al., 2015), and also the most notable and consistent pathological finding in *BEST1*-linked maculopathies, serving as an indirect biomarker of metabolic activity between the photoreceptor outer segment (POS) turnover and RPE phagocytosis (Bakall et al., 2007; Piñeiro-Gallego et al., 2011; Lei et al., 2013; Singh et al., 2013a). Recent advances with noninvasive retinal imaging modalities have enabled detailed mapping and quantification of fundus autofluorescence (FAF) *in vivo*, and its correlation with increased levels of lipofuscin components in the aged and diseased retinae (Delori et al., 1995a, 1995b; Brunk and Terman, 2002; Boon et al., 2008; Duncker et al., 2014); however, the polymorphous nature of lipofuscin material and consequences of its buildup in the retina are still controversial.

To begin to address these questions, we used the spontaneous canine *BEST1* disease model (cBest a.k.a. canine multifocal retinopathy, *cmr*) (Guziewicz et al., 2007, 2011; Zangerl et al., 2010; Beltran et al., 2014; Singh et al., 2015) to characterize lipofuscin fluorophores in the cBest1 mutant RPE, and explore factors leading to the formation of subretinal lesions in *BEST1*-associated maculopathies. This study highlights matching spectral profiles of the native lipofuscin autofluorescence between human and dog bestrophinopathies, as well as robust biochemical and structural alterations at the RPE-photoreceptor interface that trigger formation of vitelliform lesions.

2. Canine models of human *BEST1*-related dystrophies

Over the recent decades, hundreds of spontaneous genetic conditions have been described in dogs, and most of them with clinically and genetically close counterparts to the human disorders (OMIA: http://omia.angis.org.au). The naturally occurring canine models of inherited retinal disorders in man have proved crucial in the investigation of disease mechanisms and development of new therapeutic strategies (Acland et al., 2001; Komáromy et al., 2010;

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