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## REVIEW

# Human pluripotent stem cells for the modelling of retinal pigment epithelium homeostasis and disease: A review

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## Abstract

Human pluripotent stem cells (hPSCs), which include induced pluripotent stem cells and embryonic stem cells, are powerful tools for studying human development, physiology and disease, including those affecting the retina. Cells from selected individuals, or specific genetic backgrounds, can be differentiated into distinct cell types allowing the modelling of diseases in a dish for therapeutic development. hPSC-derived retinal cultures have already been used to successfully model retinal pigment epithelium (RPE) degeneration for various retinal diseases including monogenic conditions and complex disease such as age-related macular degeneration. Here, we will review the current knowledge gained in understanding the molecular events involved in retinal disease using hPSC-derived retinal models, in particular RPE models. We will provide examples of various conditions to illustrate the scope of applications associated with the use of hPSC-derived RPE models.

## KEYWORDS

human pluripotent stem cells, retinal disease, disease modelling, retinal pigment epithelium

## 1 | INTRODUCTION

In 2007, it was demonstrated that human adult somatic fibroblasts can be reprogrammed to a stem cell state through the overexpression of four pluripotent transcription factors, octamer-binding transcription factor (OCT3/4), sex-determining region Y-box 2 (SOX2), myc-proto-oncogene (C-MYC) and Kruppel-like factor 4 (KLF4).<sup>1</sup> The resulting induced pluripotent stem cells (iPSCs) exhibit the morphology and cell marker genes of human embryonic stem cells

(hESCs).<sup>1</sup> The advent of human pluripotent stem cells (hPSCs) has led to the development of a ‘disease-in-a-dish’ concept, allowing the study of molecular and cellular mechanisms using relevant human cells of interest in vitro. Combining hPSC models with genome editing technology, gene regulation analysis and phenotypic analysis now provides powerful tools to interrogate events of human development, physiology and disease. The eye and the retina are no exception to this expanding field of research, with hPSCs having already been used to recapitulate events of

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development, normal physiology and pathophysiology including of the retinal pigment epithelium (RPE). The role of the RPE is central to many retinal diseases, from inherited retinal diseases (IRD) to age-related macular degeneration (AMD). The use of hPSC-derived retinal pigment epithelium (RPE) models do not yet recapitulate the complex neuroanatomy of the retina, but the culture of this specific cell type allows the precise study of a single layer of the retina that is affected in various retinal conditions. This subsequently enables the development of novel methodologies for testing therapeutic efficacy of compounds in retinal diseases, an important consideration for drug trials, as preliminary screens of patient personalised RPE could provide greater insight into candidates for clinical trials.

## 2 | THE RETINAL PIGMENT EPITHELIUM

The RPE lines the back of the retina as a layer of pigmented, post-mitotic and polygonal cells.<sup>2</sup> The RPE has many fundamental functions that ensure the homeostasis and functioning of the retina, including: phagocytosis of shed photoreceptor outer segments, secretion of proteins for paracrine cellular communication, active transport of biomolecules, ion homeostasis, isomerization of all-trans-retinol to 11-cis retinal, and light absorption in melanosomes. Although not sensory cells themselves, the RPE cells are involved in intimate bioprocessing with photoreceptors, providing a critical role in their structural and functional viability.<sup>3</sup> The RPE is the most efficient phagocyte in the body, removing 10% of the length of the photoreceptor in outer segments daily.<sup>4</sup> These photoreceptor outer segments are phagocytosed, metabolically processed, and secreted through the Bruch's membrane into the choroidal circulation.<sup>5</sup> The morphologically polygonal RPE cells form tight junctions, creating a diffusion-impermeable barrier. Thus, the RPE must actively transport glucose and other nutrients between the basolateral choroid and the apically embedded photoreceptors, allowing visual processing to occur.<sup>6</sup> It is unsurprising that RPE dysfunction is connected to severe vision loss as its health and function is essential to photoreceptor survival and maintaining the balance of ions, water and pH within the subretinal space.

## 3 | CURRENT MODELS OF RPE, ADVANTAGE OF HPSC DERIVED MODELS

To adequately capture the underlying molecular features of retinal diseases and subsequently elucidate potential

therapies, it is essential to first establish appropriate models using human cells that recapitulate the disease phenotype. However, retinal diseases are challenging to model *in vitro* due to the paucity of access to human retinal tissue. As an alternative to a diseased human eye, given their genetic similarity to humans and parallel anatomical features, primate models have been developed, though are expensive to acquire and maintain. Comprehensive housing requirements and the need for highly experienced staff make them beyond reach of many research laboratories.<sup>7</sup> Rodents are a cost effective alternative, and commonly used to investigate diseases of the optic nerve and the retina.<sup>8</sup> Due to numerous orthogonal genes between rodent and human genomes, genetic studies in rodents are proving to be particularly enlightening with respect to disease-causing variants and pathological mechanisms. Furthermore, laboratory mice live for only a handful of years and often develop diseases that can take years to advance in humans.<sup>7</sup> Anatomically, rodent retinas differ from humans', for example, rodent optic nerves are superior colliculus making their eyes easily surgically accessible.<sup>7</sup> Rodents do not have a macula or a fovea, the site of trauma in many human macular diseases in humans, and up to 90% of rodent optic nerve axons decussate to the opposite side of the brain as compared to humans.<sup>9</sup> Approximately, 1% of human genes have no identifiable mouse homologues,<sup>10</sup> thus primary cultures from human cadavers are a popular method for studying ocular disease *in vitro*. However, primary tissues begin to degrade following death of the donor.<sup>11</sup> Therefore, swiftly obtaining and culturing primary tissue is imperative. Since it is logistically difficult to predict death and process a sample before degeneration, this medium of study is also limiting. While immortalised cell lines overcome this issue, a number of established lines fail to exhibit the morphological and functional characteristics of the native tissue or have an abnormal karyotype.<sup>12</sup> The culture of hPSC-derived RPE cells enables unrestricted access to the cells that comprise the RPE layer as it exists *in vivo*. Furthermore, genome editing methods such as CRISPR/Cas systems, can be used to generate isogenic hPSC lines that differ only at specific loci, thus the effects of chosen genetic variants can be precisely interrogated,<sup>13</sup> for instance to uncover downstream changes in gene expression caused by a disease-associated mutation,<sup>14,15</sup> or reveal the influence of a specific variant on a disease phenotype.<sup>16</sup>

## 4 | DIFFERENTIATING HIPSCS TO RPE

The simplest way of producing a somatic cell from a pluripotent stem cell is routinely referred to as spontaneous differentiation. Pluripotent stem cells move out of pluripotency

and down the developmental path of one of the three germ layers: ectoderm, endoderm or mesoderm.<sup>17</sup> Spontaneous differentiation uses adherent or suspension culturing methods and relies on the inherent cell fate specification mechanisms of the cells to reach somatic maturity. This route is inefficient, lengthy and highly variable between cell lines for generation of a single specific cell type for research purposes, and relies on excision of pigmented regions from heterogeneous cultures. However, spontaneous differentiation methods are commonly used for confirmation of pluripotency of a hPSC line for quality assurance.<sup>18</sup> Knowledge gained through embryology studies of developmental signalling pathways have allowed researchers to develop more focused, sequential, directed differentiation protocols. Directed, or guided, differentiation uses conditioned media containing growth factors or small molecules such as such as Basic Fibroblast Growth Factor (bFGF), the Bone Morphogenetic Protein (BMP) antagonist Noggin, the Wnt antagonist Dickkopf-1 (DKK1), nicotinamide, casein kinase I inhibitor 7, the ALK4 inhibitor SB-431542 and the Rho-associated kinase inhibitor Y-27632 to modulate specific signalling pathways, mimicking endogenous communication and driving the lineage decision and cell fate of the hPSC.<sup>19–23</sup> Many protocols have been established for the in vitro directed differentiation of RPE from hESCs<sup>19,21,24</sup> and iPSCs.<sup>25–27</sup> hPSC derivatives—including RPE cells—are relatively immature, demonstrating a closer similarity to foetal-like cells than to adult counterparts.<sup>28</sup> However, they demonstrate characteristics that validate them as valuable models of human adult pathophysiology. In particular, hPSC-derived RPE cells have consistently been shown to display expression of RPE markers,<sup>29,30</sup> and key functions observed in native mature RPE cells.<sup>31–33</sup> Importantly, iPSC-derived RPE retain the complex genetic signatures and variants associated with pathogenesis, making them a valuable tool in disease modelling.

Many retinal dystrophies affect multiple cell types, therefore understanding intracellular networks is fundamental to pathogenesis, which homogeneous two-dimensional iPSC-derived culture cannot mimic. Newer methodologies are being developed and optimised to make three-dimensional in vitro models of the retina called retinal organoids. The laminar retinal organoid allows a further level of complexity to cell culture models.<sup>34,35</sup> These self-organising, neural retinas in a dish can be used to interrogate interactions between various cell layers in the retina, and some have even been shown to respond to light.<sup>28,29</sup> The advantage of this approach is the ability to develop a patient-derived neural retina in vitro to study the interactions between retinal cell types. Specifically, in cases of retinal disease wherein RPE degeneration results in photoreceptor death, the spatial organisation and temporal signalling between the

two cell types could be of importance to the mechanism of disease. Existing retinal organoid protocols produce tissue containing RPE and photoreceptors with rudimentary outer segments which are being developed as a source for potential cell transplantation.<sup>30</sup> Organoids are a powerful tool in mimicking the physiological structures in affected patients and allow for co-culture or explant culture of RPE for disease modelling.

## 5 | MODELLING INHERITED RETINAL DISEASE

Many blinding diseases manifest by the inherited degeneration of the RPE, and despite the identification of specific mutations causing many of the inherited retinal dystrophies, most of these conditions are currently untreatable. A few examples of inherited retinal diseases are given below, to illustrate how hPSCs can be used for modelling and studying these conditions.

### 5.1 | Sorsby fundus dystrophy

Sorsby fundus dystrophy (SFD) is an autosomal dominant IRD caused by variants in the tissue inhibitor of metalloproteinases-3 (*TIMP3*) gene. *TIMP3* is constitutively expressed in the RPE and its monomers and dimers have been found in drusen deposits of SFD eyes. The leading hypothesis to explain the presence of *TIMP3* accumulation in SFD eyes was related to a gain or loss of cysteine residues in the *TIMP3* variant associated with the disease, resulting in the formation of disulfide bonds and subsequent *TIMP3* dimers.<sup>31</sup> These intramolecular bonds are challenging to break down and since SFD eyes have a diminished ECM turnover rate, it is thought to be a consequence of the dimerisation and an explanation for the accumulation of *TIMP3* drusen deposits in the Bruch's Membrane.<sup>31</sup> Various studies have shown the value of hPSC-derived RPE cells for the modelling of SFD, with elucidation of key differences in molecular pathways<sup>32</sup> and retrieval of disease phenotypes observed in vivo, such as drusen-like deposits.<sup>33</sup> In comparing iPSC-derived RPE that have the SFD-causing variant *TIMP3* p.(Ser204Cys) and age-matched controls lacking the disease-causing variant, *TIMP3* dimers were observed in both SFD and control iPSC-derived RPE.<sup>32</sup> These surprising results rejected the commonly held notion that *TIMP3* dimerisation drives SFD pathology, instead suggesting excess accumulation of *TIMP3*, not a functional deficiency in *TIMP3*, is implicated in ECM changes in SFD.

iPSC studies of SFD have further shown that SFD-iPSC-derived RPE cells exhibit diminished trans epithelial

electrical resistance, and, as seen in vivo, increased basolateral accumulation of TIMP3.<sup>32</sup> TIMP3 regulates the thickness of the Bruch's membrane by inhibiting metalloproteinase (MMP) activity,<sup>36</sup> which catalyse breakdown of the ECM.<sup>31</sup> Interestingly, no change in TIMP3 expression could be observed across cohorts, and the diseased lines maintained MMP activity.<sup>32</sup> Proteomic analysis showed enriched expression of cytoskeletal remodelling proteins, and angiogenesis-related pathways in SFD-iPSC-derived RPE.<sup>32</sup> These results suggest that since MMP activity is retained, the accumulation of active TIMP3 within RPE promotes continuous MMP inhibition, enabling successive ECM synthesis with diminished turnover, resulting in the dysregulated thickening of the BM.

Drusen-like deposits have also been observed underneath iPSC-derived RPE cell cultures from both healthy and SFD individuals, yet these were more abundant in the SFD lines and of different composition than in the control lines.<sup>33</sup> This established the concept that genesis of drusen is RPE-autonomous, as accumulation of the deposits occurred in the disease model independent of the apically situated photoreceptors or the basal interactions of the innermost capillary structure of the choroid, the choriocapillaris (CC).<sup>33</sup> It also highlights a quantifiable disease phenotype in vitro that can be used for further understanding of disease pathogenesis. For instance—and in an attempt to understand the role the choriocapillaris plays in macular disease aetiology and in choroidal neovascularization—Manian et al. developed a multicellular model comprised of iPSC-derived RPE cells and mesenchymal stem cells, a necessity for developing the fenestrated CC-endothelium, crosslinked with MMP-degradable peptides to mimic the RPE-choriocapillaris interface.<sup>37</sup> This construct allowed the study of locally secreted factors in the progression of choriocapillaris atrophy, a pathology observed in degenerative macular diseases, and described that choroidal neovascularization and choriocapillaris atrophy can be initiated by RPE-secreted factors exclusively.<sup>37</sup> Using this model with SFD lines carrying the *TIMP3* disease-causing variant, the authors showed that choriocapillaris atrophy and choroidal neovascularization at the RPE-choriocapillaris interface preceded drusen formation and ECM thickening,<sup>37</sup> which thus brings confirmation on the chronology of SFD progression.

## 5.2 | Stargardt disease

Stargardt disease (STGD1) is a progressive macular dystrophy that is genetically heterogeneous with over 90% of cases attributed to a biallelic pathogenic mutation in the *ABCA4* gene that encodes the ATP-binding cassette, subfamily A, member 4 transporter protein.<sup>38</sup> In general,

ATP-binding cassette transporters (ABC transporters) are fueled by ATP hydrolysis and translocate substrates across cellular membranes.<sup>39</sup> Within the normally functioning retina the role of *ABCA4* is to work as a retinaldehyde flippase, which facilitates vitamin A shuttling in the photoreceptor outer segments. Specifically, *ABCA4* shuttles all-trans-retinal from the outer segment disk to the outer segment cytoplasm in the form of retinylidene-phosphatidylethanolamine (*N*-retinylidene-PE). By transporting *N*-retinylidene-PE to the cytoplasm side of the disk membrane, it can be dissociated, allowing the released all-trans-retinal to enter the visual cycle.<sup>38</sup> In patient eyes with STGD1, the *ABCA4* flippase activity is delayed and the *N*-retinylidene-PE lingers long enough in the outer segment to be covalently bound to another retinyl moiety irreversibly forming the cytotoxic, insoluble, bis-retinoid known as phosphatidylpyridinium bisretinoid (A2PE) and its derivative *N*-retinyl-*N*-retinylidene ethanolamine (A2E). These are then shed in photoreceptor outer segments and phagocytosed by the RPE. A2PE and A2E subsequently accumulate and engorge the RPE causing vermillion fundus. Extensive accumulation appears as yellowish deposits known as pisciform flecks, and the inevitable result is death of the RPE which leads to loss of vision.<sup>38</sup>

*ABCA4* displays extensive allelic heterogeneity with over 1000 pathogenic variants to date.<sup>40</sup> Various iPSC lines have been generated to represent the myriad of *ABCA4* mutations seen in STGD1.<sup>41,42</sup> Using iPSC-derived tissue to model a disease such as STGD1 is useful in attempts to link causality and severity with expression levels, since patient lines retain the complex genetic signatures created via natural splicing and editing that occurs in vivo. In one study, eight iPSC lines from patients harbouring a single pathogenic *ABCA4* variant and normal controls were differentiated into RPE and subjected to RNA sequencing and protein analysis.<sup>43</sup> The resulting principal component analysis of the iPSC-derived RPE transcriptomes showed the controls clustering distinctly from the patient samples, indicating the differentially expressed genes are driven by *ABCA4* mutations.<sup>43</sup> In a patient line containing two *ABCA4* splice variants, aberrantly produced polypeptides were short-lived in the endoplasmic reticulum.<sup>43</sup> The allele-specific expression results showed normal *ABCA4* transcripts were present across splice variants, however, the corresponding protein was not detected.<sup>43</sup> In this study, all patient lines showed upregulation in the unfolded protein response pathway implying membrane mislocalization and protein misfolding, indiscriminate between missense and splice variants.<sup>43</sup> In comparing patient phenotypes, the higher the missense variant expression, the more deleterious outcomes for the patient, and iPSC



studies confirmed this genotype–phenotype correlation in the severity of *ABCA4* variants.<sup>38,43</sup> Consistent with STGD1 disease phenotypes, the transcriptomic analysis of the patient iPSC-derived RPE cells, links oxidative and endoplasmic reticulum stress, likely symptoms of reactive responses rather than primary protein alterations due to *ABCA4* variants.<sup>38,43</sup> This study highlights the benefits of using iPSC-derived RPE to assess casual variants in difficult to detect genomic regions.

To date, there have been four attempts at cellular intervention in STGD1 patients.<sup>44–47</sup> The first clinical phase I/II trials delivered hESC-derived RPE cell suspension into the region between healthy and atrophic RPE in nine STGD1 patients, who had undergone low-dose immunosuppression the week prior.<sup>44</sup> In the patients assessed after 1 year, three showed improvement, no improvement but no decline in three patients, and one patient had vision decline.<sup>44</sup>

### 5.3 | Best vitelliform macular dystrophy

Best vitelliform macular dystrophy (Best disease, BD) is an autosomal dominant, early-onset macular dystrophy frequently caused by mutations in the bestrophin (*BEST1*) gene, with over 200 missense mutations in *BEST1* already identified.<sup>48</sup> BD is clinically characterised by bilateral vitelliform lesions in the subretinal space in the early stages. In many individuals, these lesions eventually rupture, giving a ‘scrambled egg’ appearance, leading to subfoveal fluid deposition, atrophy of the underlying RPE and progressive irreversible central vision loss. *BEST1* encodes a calcium-activated chloride channel found in the RPE, with disease-associated mutations clustering within regions encoding calcium or chloride-ion binding sites.<sup>49</sup> BD-iPSC-derived RPE cells with various *BEST1* mutations possess various functional defects, including a delayed degradation of POS, increased oxidative stress following chronic POS feeding,<sup>50,51</sup> defective chloride conductance,<sup>52</sup> or decreased fluid transport.<sup>53</sup> Work with BD-iPSC-derived RPE has also permitted exploring the potential of gene augmentation to treat BD. Dominantly inherited disorders are commonly excluded as candidates for gene replacement therapies, as it is challenging to silence the dominant allele without altering wild-type gene expression. Gene augmentation with optional genome editing approaches that allow precision in silencing or repairing pathogenic variants is a powerful solution. In a BD-iPSC-derived RPE disease model, gene augmentation restored *BEST1* calcium-activated chloride channel activity in two of the pathogenic model systems, representing two different variants both encoding ion-binding domains.<sup>48</sup> A third BD iPSC-derived RPE model did not respond to the lentiviral

constructed gene augmentation, however, the *BEST1* channel activity normalised after subsequent CRISPR-Cas9 editing of the pathogenic variant.<sup>48</sup> Each of the three rescued models then underwent gene editing to produce premature stops within the pathogenic *BEST1* alleles. Single cell profiling confirmed the RPE transcriptome remained undisturbed.<sup>48</sup> These results suggest the multi-pronged approach of personalising gene therapy wherein gene augmentation is used as a first pass and non-responders become eligible for alternative gene-editing approaches is a potential strategy for treating BD. iPSC-RPE model systems like these can be leveraged to maximise safety and efficacy while minimising cost for providing proof of concept gene therapies for impacted individuals. This could be useful and important in ensuring gene therapy development is efficacious and tested on human cells with the genotypic and phenotypic disease profile of interest.

### 5.4 | Retinitis pigmentosa

Retinitis pigmentosa (RP) is an irreversible degenerative disease that has been associated with over 40 genes expressed in photoreceptors or the RPE.<sup>54</sup> The tremendous heterogeneity makes the genetics of RP complex, however, the pathogenesis has been linked to disease-causing variants in Mer tyrosine kinase (*MERTK*), a gene that encodes a receptor of the Tyro3/Axl/Mer family of tyrosine kinases; and retinoid isomerohydrolase (*RPE65*), a gene that encodes a protein involved in visual transduction post-light exposure.<sup>55</sup> Mer tyrosine kinase (*MERTK*) is expressed by the RPE and critical for phagocytosis of the photoreceptor outer segments.<sup>56</sup> iPSC studies have compared iPSC-derived RPE with homozygous or compound heterozygous variants in *MERTK*, and healthy controls. The outcomes showed the cellular morphology remained similar across diseased and control cohorts, however, the engulfment of photoreceptor outer segments was significantly lower in the diseased RPE cells than in controls.<sup>56</sup>

Recessive homozygous disease-causing variants of *RPE65* occur ~2% of RP cases.<sup>57</sup> There is a regulatory-approved *RPE65* retinal gene replacement therapy available for patients suffering from severe RP. However, to be eligible for the therapy, patients must be biallelic for the risk variants, and maintain a specified minimum of uncompromised, functioning retinal cells.<sup>57</sup> Risk variants in *RPE65* include missense, frameshift, premature stop, in-frame deletion, and numerous splicing variants.<sup>57</sup> The onset or severity of RP due to harbouring of the pathogenic *RPE65* allele offers no correlation between genotype and resultant phenotype, providing no link to the

location of the causative variants associated. In some patients, the pathogenic *RPE65* mutation, is categorised as a variant of uncertain significance (VUS), regardless of if the phenotype mimics the disease. This disqualifies the patients from *RPE65* gene therapy. *RPE65* is highly expressed in the RPE, and very minimally in all other tissue types. Due to this fact, iPSC-derived RPE were generated from those carrying VUS. This allowed bioinformatic interrogation of *RPE65* mRNA in potentially impacted patients to uncover the consequence of this variant and determine the repercussions on retinal disease pathology. Functional studies of RNA expression using the iPSC-derived RPE confirmed this VUS to be pathogenic thus granting the patient's eligibility for the *RPE65* gene replacement therapy.<sup>57</sup>

Another severe form of RP is called X-linked retinitis pigmentosa (XLRP), and ~15% of all cases are due to variants in retinitis pigmentosa 2 (RP2) gene.<sup>58</sup> Retinal organoids obtained from patient-derived RP2 iPSCs and isogenic knockout controls showed that after 150 days in culture, RP2 patient organoids showed a peak in rod photoreceptor death, and by day 180 there was thinning of the outer nuclear layer. Adeno-associated virus-mediated gene amplification with RP2 rescued the outer layer degeneration phenotype in the isogenic controls.<sup>59</sup> This study provides evidence that retinal organoids can be used as a disease model of retinal degeneration and are a suitable platform for testing potential therapies.

### 5.5 | Bietti crystalline dystrophy

Bietti crystalline dystrophy (BCD) is an autosomal recessive inherited retinal disease characterised by crystalline deposits mainly in the macular RPE, resulting in progressive atrophy of the RPE and subsequent deterioration of the connected vasculature the choriocapillaris, and the apically embedded photoreceptors.<sup>60</sup> A pathogenic variant of *CYP4V2*, a gene belonging to the cytochrome P450 superfamily which encodes a fatty acid hydroxylase, has been identified as causative for BCD.<sup>60</sup> *CYP4V2* is localised most commonly to the endoplasmic reticulum within the RPE and participates in lipid recycling between the RPE and photoreceptors, allowing visual transduction to occur. iPSC-derived RPE cells from patients carrying the *CYP4V2* mutation exhibit lysosomal dysfunction and impaired autophagy functioning, leading to cellular death.<sup>61</sup> Mimicking the phenotype seen in postmortem samples from patients with BCD, iPSC-derived RPE showed degenerative changes such as vacuolated cytoplasm, and accumulation of free cholesterol.<sup>61</sup> The BCD-iPSC-derived RPE disease model associated the accumulation of free cholesterol with lysosomal

alkalinization and impairment resulting in dysfunctional RPE autophagy. Introducing cyclodextrins or  $\delta$ -tocopherol which reduced free cholesterol, rescued the pathogenic phenotype.<sup>61</sup> This BD-iPSC-derived RPE disease model provides evidence for a therapeutic pathway forward in reducing intracellular cholesterol as a target for BD. Recently, iPSC-derived RPE cells were also used to assess impact of targeted gene supplementation, with delivery of *CYP4V2* using AAV2 constructs providing functional *CYP4V2* to the BD-iPSC-derived RPE cells,<sup>62</sup> and further demonstrating the important and versatile roles hPSC-derived RPE cells have in preclinical applications.

## 6 | MODELLING COMPLEX DISEASE: AGE-RELATED MACULAR DEGENERATION

Modelling complex diseases such as AMD, require a population-scale of disease-specific iPSCs to achieve sufficient statistical power to identify pathogenic variants.<sup>63,64</sup> Worldwide, evidence of this necessity has been established by several large-scale initiatives to generate population-scale, disease specific-iPSC repositories. Over the past decade, GWAS and whole genome sequencing studies have identified various disease associated variants.<sup>65</sup> One genetic risk factor, a common variant in the *CFH* gene (*CFH* Y402H, rs1061170 C/C), is estimated to account for nearly half of all AMD risk.<sup>66,67</sup> Furthermore, variants at the *LOC387715/ARMS2/HTRA1* (rs10490924 T/T) locus have been identified as major contributors to AMD development.<sup>68,69</sup> To date, genome-wide association studies (GWAS) have identified over 30 independent loci where a common risk allele is associated with an increased risk of AMD.<sup>70-72</sup> These loci influence distinct biological pathways, such as the complement system, lipid transport, extracellular matrix remodelling, angiogenesis and cell survival.<sup>73</sup> In an attempt to provide a population-scale approach to iPSC-disease modelling for complex disease, we recently described both transcriptomic and proteomic profiles of iPSC-derived RPE cells from a large cohort of people with or without the advanced form of AMD geographic atrophy.<sup>74</sup> The comparison of single cell RNA sequencing from iPSC-derived RPE cells of 43 patients at the phenotypic end of AMD and of 36 unaffected controls implicated genes at loci definitively associated with disease such as the *CFH* and *ARMS2/HTRA1* loci, and also uncovered new pathogenic loci and pathways.<sup>74</sup> In particular, mitochondrial pathways were identified as dysregulated in geographic atrophy RPE cells, data confirmed by mass spectrometry and by functional analysis of mitochondrial respiration.<sup>74</sup>

Other studies using small subsets of hPSCs have also identified phenotypes associated with AMD. iPSC-RPE cells derived from patients with AMD compared to healthy controls display a reduced activity of the mitochondrial antioxidant enzyme superoxide dismutase 2 (SOD2) and have a higher susceptibility to oxidative stress.<sup>75–78</sup> Additionally, AMD iPSC-derived RPE cells exhibit a decreased autophagy and complement dysregulation.<sup>75–77</sup> A hallmark of AMD is the accumulation of sub-RPE drusen-like deposits of lipids and proteins, and RPE degeneration follows soon after. Evidence of an adequate AMD iPSC-derived RPE disease model is presence of drusen. Drusen components such as apolipoprotein E (APOE),<sup>79</sup> vitronectin,<sup>80</sup> and TIMP3,<sup>81</sup> have been shown to accumulate in AMD disease models using iPSC-derived RPE from affected patients.<sup>33,81,82</sup> These in vitro studies have demonstrated that drusen-like deposits can accumulate with a homogeneous population of RPE. Drusen-like material has been observed by 3 months in culture of iPSC-derived RPE, and was more frequent and dense when RPE was derived from AMD patients.<sup>33</sup> It has been shown that by co-culturing with retinal progenitor cells (RPCs), large mounds of drusen-like deposits appear that functionally impact permeability,<sup>81</sup> thus mimicking the damaged Bruch's Membrane seen in AMD.

Drug discovery and screening using iPSCs provides the possibility of scalable, personalised therapies for patients suffering from any genetic disorder,<sup>65</sup> including retinal disease. One study used a panel of AMD biomarkers for a candidate drug screen, and in combination with transcriptome analysis, uncovered that the vitamin B3 derivative nicotinamide is a promising avenue for interrogation. After showing the iPSC-derived RPE produced drusen-related proteins, with the *ARMS2/HTRA1* AMD donor lines showing the most pronounced accumulation, the study found that nicotinamide ameliorated AMD-related phenotypes. It is known that *ARMS2/HTRA1* lines have a compromised superoxide dismutase 2 response, implying increased vulnerability to oxidative mitochondrial stress.<sup>75</sup> Nicotinamide has antioxidant and anti-inflammatory properties<sup>83</sup> that protect mitochondria against reactive oxygen species (ROS) and excessive fragmentation by decreasing mitochondrial protein acetylation<sup>84</sup> via upregulation of SIRT-1 a known chromatin modifying gene<sup>85</sup>. Nicotinamide inhibits drusen proteins and complement factors while upregulating nucleosome, ribosome, and chromatin-modifying genes. This study provides evidence towards developing therapeutics that target nicotinamide-regulated pathways.<sup>77</sup> More recently, a study used iPSC-derived RPE from AMD patients to test the efficacy of three mitochondrial maintenance drugs: AICAR (5-Aminoimidazole-4-carboxamide ribonucleotide), Metformin, and Trehalose. After acute and extended

drug exposure, the mitochondria of the RPE were analysed and the results showed differential responses to the drugs across patient lines.<sup>86</sup> This study shows the need for a personalised medicinal approach, and supports the feasibility of using iPSC-derived RPE to develop a personalised drug treatment plan for patients with AMD.

Finally, iPSCs have made the concept of autologous cellular replacement therapy a reality. Autologous cell replacement therapy minimises the risk of immune rejection in patients because the cells are derived from the patient themselves rather than a donor.<sup>87</sup> The eye is an ideal organ for stem cell based therapies due to its accessibility and the advent of high resolution in vivo retinal imaging such as optical tomography which allows for unambiguous and continuous monitoring.<sup>88</sup> Clinical trials have commenced wherein patient-derived iPSCs are differentiated into RPE and surgically implanted into the damaged retina of the patients.<sup>89–93</sup> Current stem cell therapies cater to outer retinal degeneration of the RPE and photoreceptors to treat AMD and RP. hPSC-derived RPE as cell suspension or monolayer sheets (with or without scaffolds) have been used to attempt to halt the disease progression of AMD by cellular transplantation and restore patient vision.<sup>89–93</sup> In comparison with RPE cell suspension, RPE sheet transplantation is more surgically invasive due to the need to create a larger incision and on occasion requires removal of choroidal neovascularization prior to insertion of the sheet. Retinal haemorrhage, edema and other adverse effects have been reported as outcomes of the RPE sheet surgery.<sup>89–91</sup> Outcomes of RPE sheet transplantation are promising and show either unchanged or improvement in visual acuity with no evidence of worsening vision.<sup>89–93</sup> There are predictions that RPE sheet transplantation could have implications long-term and severity could parallel scaffold composition, as two clinical trials used non-biodegradable scaffolds.<sup>88</sup> However, studies have confirmed that a subretinally transplanted autologous iPSC-derived RPE sheet can survive below the retina for at least 4 years with no adverse events.<sup>92</sup> Transplantation using RPE cell suspension is less invasive than sheets and there are no adverse consequences associated with surgical outcomes. However, adverse events reported with RPE cell suspension transplantation were associated with side effects of immunosuppressants such as mycophenolate mofetil and tacrolimus,<sup>44,45,94</sup> although recent reports suggest that long term immunosuppression may not be required.<sup>95</sup> There were reports of a thin layer of tissue forming on the retina referred to as an epiretinal membrane, which was attributed to cellular leakage from the site of transplant.<sup>44,45,94,96</sup> Unfortunately to date this therapy shows meagre outcomes with minimal improvement in retinal sensitivity but no statistically significant increase in visual acuity or patient reported quality of life.<sup>44,45,94,96</sup>

## 7 | CONCLUSION

There is a pressing and continuous need for human models of disease to improve our understanding of retinal degenerative diseases. hPSC-derived RPE, and retinal organoid models containing RPE are powerful tools for elucidating underlying disease genotypes and pathological phenotypes, developing clinically suitable therapeutics for treatment of IRDs and more complex conditions such as AMD. The genome editing technology which takes advantage of a bacterial immune system, clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein (Cas) and an RNA that guides the Cas protein to a specific location in the genome has emerged as a precise tool for correcting monogenic IRDs.<sup>97</sup> CRISPR-based genome editing allows for permanent replacement or repair of causal genetic variants, and shows promise in treating complex retinal degenerative diseases such as AMD. An in vivo editing vector has been developed to induce permanent inhibition of VEGF via CRISPR-mediated knockout in the RPE, which could prevent further neovascularization in AMD patients.<sup>98</sup> Furthermore, in a human embryonic kidney cell line a CRISPR/Cas-mediated strategy has been shown to correct an allele conferring AMD risk in the complement factor H gene.<sup>99</sup> Although in vivo CRISPR-based agents have been used to gene correct adults with congenital blindness,<sup>100</sup> the attractiveness of the therapeutic potential of precise genome modifications is often overshadowed by the safety aspects associated with the CRISPR/Cas technology. Marrying CRISPR/Cas and iPSC technology is an important tool for interrogating safety and efficacy of cellular therapeutics by improving delivery methods, promoting detection of rare variants, and discovering off-target effects. This technology is paving the way forward for personalised therapeutics, drug discovery, and cellular therapy at scale. It is however important to acknowledge the limitations associated with hPSC disease models and to highlight where these models are most valuable. For instance, a concern for using iPSCs to study complex conditions such as AMD, where genetics, age and environment play roles, is that iPSC-derived RPE have a transcriptional profile that resembles foetal cells<sup>101</sup> and do not live a lifetime of molecular events as their native counterpart would, which must still be carefully considered when interpreting data. Furthermore, in vivo, cells do not live in autonomy, but form parts of tissues and organs with intercellular exchanges and communications, a complexity that is not yet reproduced in current in vitro models. The development of better models could incorporate 'on demand' levels of maturity of cell populations, layering of various cell populations in set numbers, from the same embryonic lineage or other

lineages (such as endothelial cells and immune cells), and in environmental conditions closer to their native counterpart. Much effort is already dedicated to developing these complex models<sup>102</sup> that will undoubtedly provide powerful new tools in the fight against vision loss and blindness.

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## CONFLICT OF INTEREST


The authors declare no conflict of interest.

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