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# Pluripotent stem cell-derived models of retinal disease: Elucidating pathogenesis, evaluating novel treatments, and estimating toxicity

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ABSTRACT

Blindness poses a growing global challenge, with approximately 26% of cases attributed to degenerative retinal diseases. While gene therapy, optogenetic tools, photosensitive switches, and retinal prostheses offer hope for vision restoration, these high-cost therapies will benefit few patients. Understanding retinal diseases is therefore key to advance effective treatments, requiring *in vitro* models replicating pathology and allowing quantitative assessments for drug discovery. Pluripotent stem cells (PSCs) provide a unique solution given their limitless supply and ability to differentiate into light-responsive retinal tissues encompassing all cell types. This review focuses on the history and current state of photoreceptor and retinal pigment epithelium (RPE) cell generation from PSCs. We explore the applications of this technology in disease modelling, experimental therapy testing, biomarker identification, and toxicity studies. We consider challenges in scalability, standardisation, and reproducibility, and stress the importance of incorporating vasculature and immune cells into retinal organoids. We advocate for high-throughput automation in data acquisition and analyses and underscore the value of advanced micro-physiological systems that fully capture the interactions between the neural retina, RPE, and choriocapillaris.

## 1. Introduction

Diseases of the outer retina present significant societal challenges due to the current paucity of effective treatments which, coupled with their frequency in ageing populations, makes them a matter of utmost concern. Unfortunately, these conditions pose substantial therapeutic challenges given their multifactorial contributions and heterogeneous cellular involvement. In particular, age-related macular degeneration (AMD) and inherited retinal dystrophies (IRDs) stand out as the first and third most prevalent causes of irreversible blindness worldwide yet there are few effective treatments (Quartilho et al., 2016). This review explores recent advances in using pluripotent stem cell (PSC)-derived retinal tissues to understand and treat these challenging conditions. AMD, prevalent among the elderly, constitutes half of all blindness cases in the UK. Its prevalence surges from 3.5% in those aged 55–59 to a staggering 17.6% at ages 85 and above, affecting approximately 196 million worldwide and projected to reach 288 million by 2040. Despite progress in managing 'wet', or exudative, AMD, its 'dry' form has historically lacked treatments until recently with two treatments, namely complement C3 and C5 inhibitors, approved by the US Federal Drug Agency (FDA) in February and August 2023 respectively. To address this, we must develop tools to probe the interplay of factors such as cellular metabolism disruptions, senescence, and oxidative stress on retinal tissue homeostasis, crucial in understanding the transition from normal aging to AMD (Copland et al., 2018).

IRDs affect one in 2000 individuals, exceeding two million globally (Berger et al., 2010). Occurring in early childhood, these diseases are especially concerning as, without curative therapy, they may progress to

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complete and irreversible sight loss, imposing enormous burdens on individuals, families, and communities. Over 20 IRD-linked ocular phenotypes have been described, including rod-dominated diseases, cone-dominated diseases, generalised retinal degenerations, and vitreoretinopathies. Despite exceptions, like Luxturna® (voretigene neparvovec-rzyl, *Novartis,* Basel, Switzerland) for *RPE65*-mediated retinitis pigmentosa (RP), most IRDs lack treatments due to their genetic complexity. Complicating matters, disease-causing variants extend beyond genes with clear retinal functions, often yielding divergent phenotypes within the same gene. The interdependence of cell types, particularly between the retinal pigment epithelium (RPE) and photoreceptors, further obscures disease sequences, especially in non-retinal specific processes.

In this review, we also explore prospects for treating retinoblastoma, a devastating childhood cancer affecting 8600–9000 children annually, predominantly before the age of five (Kivela, 2009; Stacey et al., 2021). While survival rates are high, focus has shifted to improving globe preservation and visual outcomes, which are variable (Warda et al., 2023). Despite the longstanding identification of causative mutations in the retinoblastoma (*RB1*) tumour suppressor gene, the early tumour cell biology and cell of origin remain disputed, critical for early diagnosis and treatment.

The key to increasing our understanding of outer retinal disease mechanisms is the establishment of *in vitro* experimental models of the human retina that replicate disease pathology and permit the quantitative assessment of parameters for drug discovery. Relying solely on experimental animals is no longer sufficient, as differences in physiology and disease mechanisms often exist between these and humans. PSCs offer great promise in this respect. The groundbreaking study by Sasai and colleagues in 2011 demonstrated the replication of retinal development in three-dimensional (3D) culture conditions using murine embryonic stem cells (ESCs) (Eiraku and Sasai, 2011; Eiraku et al., 2011). Subsequently, this achievement was replicated using human ESCs and induced pluripotent stem cells (iPSCs) and the resulting cellular constructs are now routinely referred to as retinal organoids - 3D laminated structures comprising all essential retinal cell types, faithfully recapitulating retinal development and function, including interconnected and light-sensitive photoreceptors (Hallam et al., 2018).

The capacity for self-assembly of a stratified replica of the retina is of enormous value as this allows us to interrogate cellular interconnectedness – an aspect impossible with isolated retinal cells. This proves particularly valuable for assessing the impact of candidate drugs on the overall retina, so there is considerable excitement about the potential utility of retinal organoids for this purpose. However, it is crucial to acknowledge the artificial nature of these constructs, marked by several limitations, notably the absence of vascular input and immune system influence. While limited types of immune cells can be introduced, currently restricted to microglia, incorporating an RPE layer and integrating replicas of Bruch's membrane (BrM) and the choroidal vasculature demand further study.

Nevertheless, retinal organoids are already making unprecedented contributions to our understanding of retinal disease. In this review, we discuss the progress achieved in the last two decades concerning the differentiation of human ESCs and iPSCs into RPE cells and retinal organoids. Our focus includes their applications in disease modelling for age-related and inherited retinal diseases, the evaluation of new therapies, as well as contributions to drug repurposing, biomarker discovery, and toxicity assessment. We will not delve into the use of iPSC- and ESC-derived retinal tissues for cell-based replacement therapies given extensive recent literature on this subject (Gasparini et al., 2019; Singh et al., 2020; Zarbin et al., 2019).

## 2. PSCs evolution

The use of PSCs in global efforts to model human retinal development and to generate cell types for potential treatments spans at most the last 25 years. PSCs, which encompass both ESCs and iPSCs, have the potential to divide indefinitely and differentiate into any cell type. The genesis of PSCs traces back to 1981 when Evans and Kaufmann, along with Martin, derived these from early mouse embryos (Evans and Kaufman, 1981; Martin, 1981). This milestone involved dissecting embryonic sections, cultivating them in suspension, and subsequently growing them on feeder layers composed of mitotically inactivated mouse fibroblasts. These feeder layers provided the necessary extracellular matrix components and growth factors needed for the attachment and proliferation of mouse ESCs (Evans and Kaufman, 1981).

Human ESCs were not derived until 1998 (Thomson et al., 1998), owing to limited availability of donated human embryos from *in vitro* fertilisation procedures and the need for more sophisticated culture media compositions to support their growth. Both mouse and human ESCs share a defining characteristic: pluripotency. This attribute denotes their ability to differentiate into most cell types found in developing embryos and adult organisms, while maintaining the capacity to undergo numerous population doublings, rendering them effectively immortal. Pluripotency distinguishes these cells from earlier embryonic stages, where cells (known as blastomeres) are considered totipotent, having the potential to differentiate into cells contributing to placental development and all other cell types. Notably, epiblast cells in the human blastocysts (unlike in the mouse) retain trophectoderm lineage plasticity cells, and human ESCs can assume this fate under appropriate culture conditions (Guo et al., 2021; Mischler et al., 2021).

Another type of PSCs, known as iPSCs, emerged through epigenetic reprogramming of somatic cells. This breakthrough, credited to Shinya Yamanaka in 2006, hails as a decade-defining achievement in stem cell research. iPSCs are generated by introducing a specific set of pluripotency transcription factors, known as the Yamanaka factors (Oct3/4, Sox2, c-Myc and Klf4), or the Thompson factors (Oct4, Sox2, Nanog, and Lin28) (Takahashi and Yamanaka, 2006; Yu et al., 2007). One of their remarkable attributes is that autologous iPSCs express the same histocompatibility antigens as the host, thus mitigating the risk of post-transplantation rejection of somatic cells differentiated from the iPSCs (Zimmermann et al., 2012). However, PSCs are susceptible to chromosomal abnormalities during culture, with trisomy of chromosomes 12 and 17 notably increasing proliferation rate and tumorigenicity in PSCs (Ben-David et al., 2014; Lamm et al., 2016). These abnormalities may disrupt DNA replication dynamics, perturb energy metabolism, or impair their differentiation (Lamm et al., 2016; Pucci et al., 2013). The presence of such chromosomal abnormalities raises safety concerns about PSC-based therapies, as undifferentiated PSCs with unstable genomes may expand more readily and lead to teratoma formation. Consequently, vigilant monitoring for genomic abnormalities at all stages of cell processing is vital to eliminate PSCs or somatic cell types produced during differentiation that may pose clinical risks. Despite these challenges, PSCs lend themselves to gene editing technologies, enabling the correction of the genome in autologous iPSCs before their transplantation (De Masi et al., 2020). This technology presents the opportunity to generate patient-specific cells, tissues, and organs for modelling human diseases, drug development and screening, evaluating the therapeutic effects of novel drugs and gene therapies, and personalised regenerative cell therapy, as discussed in Sections 4–7 of this review. Hence, substantial interest surrounds the use of these cells for retinal disease understanding and treatment.

## 3. PSCs differentiation to photoreceptors and RPE cells

To realise the possibility of retinal modelling, we require robust and reproducible methods to differentiate PSCs into component cells of the adult retina. This is a complex tissue (Fig. 1A) containing a network of sensory and neuronal cells supplied with oxygen and nutrients by the retinal and choroidal circulations. Specialised photoreceptor cells (rods and cones) convert visual stimuli into cellular polarisation events which modulate the release of neurotransmitters that stimulate the activity of

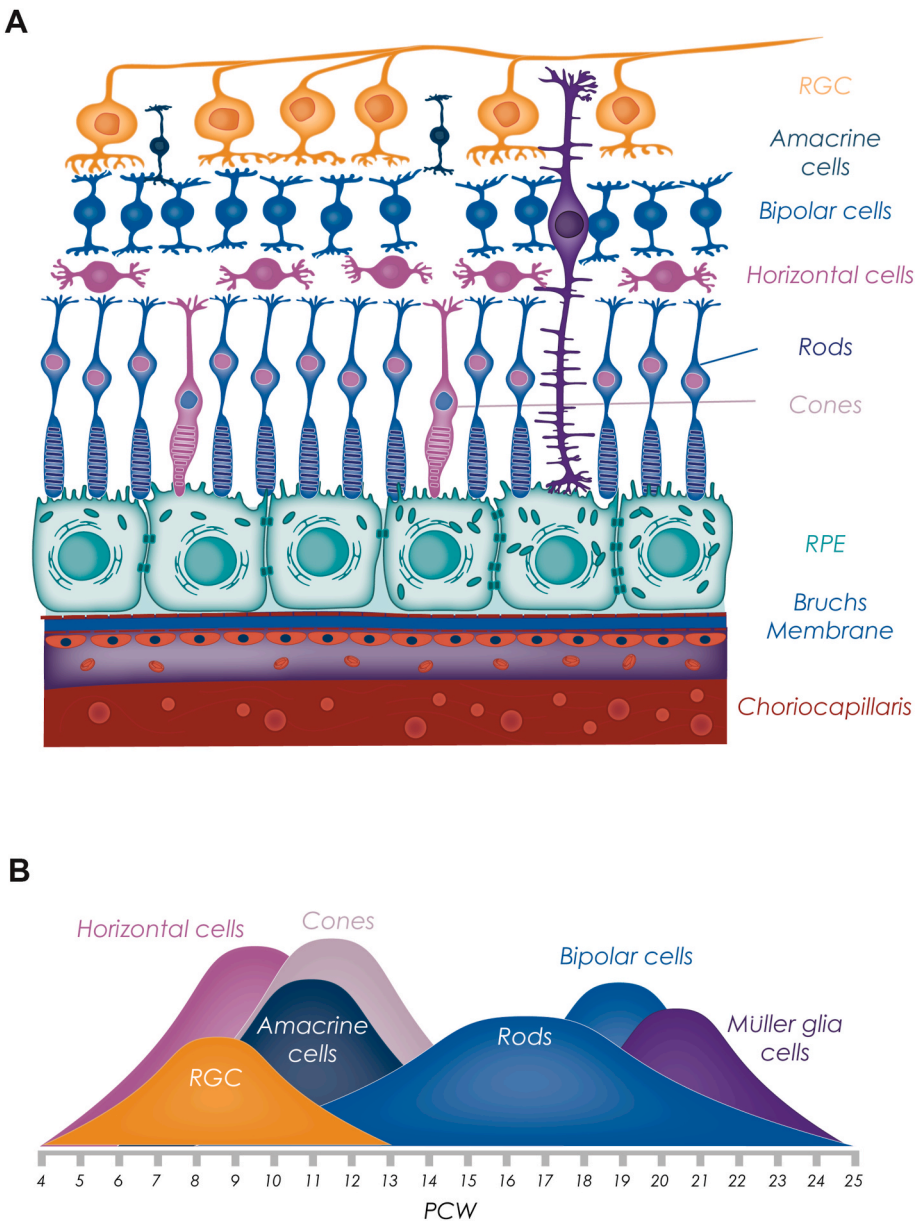


**Fig. 1. Schematic presentation of choriocapillaris, RPE and all retinal cells arranged in their appropriate layers (A) together with their sequential emergence during human retinal development (B).** The data for panel A has been imputed from Mellough et al. and Lu et al. publications (Lu et al., 2020; Mellough et al., 2019).

specialised interneurons called bipolar cells. These in turn cause action potentials in the retinal ganglion cells that are transmitted to the primary and secondary visual cortex of the brain to enable visual sensation. The phototransduction capability of the photoreceptors in humans utilizes three opsin photopigments whose active moiety is the chromophore 11-*cis*-retinal (Sato et al., 2017). Photo-stimulation of opsins is achieved by isomerization of 11-*cis* retinal to all-*trans*-retinal, but the photoreceptor cells lack the ability to reconvert the *trans*-back to the active *cis*-isomer. This function is provided by the RPE and Müller glia cells thereby completing the so-called visual cycle. Further types of cells present in the mature retina include amacrine cells which interact with bipolar interneurons and retinal ganglion cells and provide both paracrine functions and possible 'fine tuning' or modulation of photoreceptor responses, and horizontal cells which modulate the output of photoreceptors and play an important role in early visual processing important for colour opponency, contrast enhancement and generation of centre surround receptive fields (Marc et al., 2014). Müller glia contribute to retinal homeostasis, mechanical stability, and possibly to a limited degree of regenerative capacity under pathological conditions by differentiating to neural progenitor/stem cells which regenerate lost photoreceptors and neurons in zebrafish and to lesser extent in chickens, but not humans (Reichenbach and Bringmann, 2013).

Retinal function requires the presence and correct interaction of all these cells so the challenge for PSC differentiation is to recapitulate this structure as far as possible. This is a task of considerable complexity, so it is unsurprising that initial attempts to generate retinal cells from PSCs focussed on obtaining individual types of cells in isolation. The general approach to differentiation of PSCs *in vitro* is to replicate the processes that control the development of specific tissues and organs during embryogenesis. Development of the eye begins late in gastrulation when the eye field is established in the neural plate located between the telencephalon and the diencephalon of the nascent central nervous system (Miesfeld and Brown, 2019). Morphogen gradients comprising insulin-like growth factor 1 (IGF-1), Wnt proteins, fibroblast growth

factors (FGFs), and bone morphogenetic proteins (BMPs) are responsible for expression of the eye field transcription factors including Rax, Six3, Lhx2, and Pax6, which along with cell-cell signalling pathways, collectively initiate eye field formation in mice, frogs and other vertebrates (Grindley et al., 1995; Mathers et al., 1997; Porter et al., 1997; Zuber et al., 2003). The eye field then bifurcates to form the left and right optic vesicles which extend towards the embryonic surface ectoderm where they invaginate to form the optic cups and induce the lens placode to form the lens vesicle. During optic vesicle extension towards the surface ectoderm, the proximal portion of the vesicle forms the optic stalk that will develop into the optic nerve, and the distal portion invaginates to form the optic cup. The optic cup rim will become the iris and ciliary body while the remaining part gives rise to the retina with the thinner, outer layer forming the RPE and the inner layer forming the neural retina (Fuhrmann, 2010). Again, a combination of morphogen gradients including sonic hedgehog (Shh), Wnt proteins, transforming growth factor beta (TGFβ), and FGFs specify cells that will become the future RPE and neural retina (Mellough et al., 2014).

Cell lineage tracing performed in animal models and more recent transcriptomic studies indicate that retinogenesis follows a precise spatio-temporal pattern with cell differentiation proceeding in concentric waves that spread out from the inner layer of the optic cup (Lu et al., 2020; Mellough et al., 2019; Prada et al., 1991). Individual cell types appear according to a strict timescale (in a manner reminiscent of CNS development) (Fig. 1B) with retinal ganglion cells and horizontal cells appearing as the earliest differentiation events followed by cone photoreceptors, amacrine cells and rod photoreceptors, and the late born cell types such as bipolar and Müller glia cells (Prada et al., 1991; Young, 1985). Differentiation and maturation of specific cells retinal cells relies on both intrinsic and extrinsic factors, which have been thoroughly reviewed elsewhere so we will not discuss them further; however, it is notable that the combination of intrinsic self-organisation complemented with extrinsic growth factors, signalling pathway modulators and extracellular matrix (ECM) forms the basis for the generation of photoreceptors, retinal organoids and RPE cells which will be explored in Sections 3.1-3.3 below (Grigoryan, 2022; Hoon et al., 2014; Neumann and Nuesslein-Volhard, 2000; Stenkamp, 2015; Zhang and Yang, 2001).

### 3.1. Differentiation of PSCs to RPE cells

As discussed, AMD is a leading cause of blindness, especially in older adults, and RPE dysfunction contributes significantly to the loss of neural retinal cells. Therefore, differentiating PSCs into RPE holds great promise not only in retinal disease modelling and translational biomedical applications, but also in understanding of the fundamental biology of the tissue and its role in the visual system. The RPE shares features common to all epithelia: a polarised monolayer of cells in which neighbouring cells are joined via tight junctions, that mediate size-selective passive diffusion of solutes, create boundaries between apical and basolateral plasma membrane compartments, and contribute to the epithelial barrier function (Naylor et al., 2019). The RPE layer maintains the outer blood-retina barrier (oBRB) separating the neural retina from the underlying BrM and choriocapillaris and plays a central role in visual function, including transport of nutrients and growth factors to the neural retina and phagocytosis of outer segments shed from the photoreceptors. Other crucial roles of the RPE include the involvement in the visual cycle by re-isomerising of all-*trans*-retinal into 11-*cis*-retinal, and preventing light scattering back to photoreceptors in the outer nuclear layer due to its darkly pigmented colouration (Strauss, 2005). These functions exemplify the close functional interaction of RPE with the photoreceptors, abnormalities of which lead to retinal dysfunction and visual loss.

Early attempts at RPE differentiation from PSCs relied on spontaneous differentiation, facilitated by the ease of identifying pigmented RPE (Buchholz et al., 2009). In these methods, PSCs spontaneously differentiated into pigmented foci upon removal of basic fibroblast growth factor (bFGF) from the culture medium, which suppresses PSC differentiation (Ferguson et al., 2015). Mechanical selection and subculture between differentiation Days 60–90 led to the formation of polarised confluent monolayers of cells capable of phagocytosing bovine rod outer segments. These cells displayed similar morphology to primary and adult human RPE, and expressed genes involved in pigment synthesis as well as *ZO1*, *CRALBP,* and *RPE65* present in mature RPE cells. The gene products of *CRALBP* and *RPE65* are involved in the visual cycle and, therefore, expressed in the mature RPE. Although present in the PSC-RPE cells, their gene expression levels were lower compared to the foetal RPE. Additionally, the *Pax6* gene coding for a transcription factor known to be expressed during RPE development, was detected by Buchholz et al., which indicated that in some instances PSC-RPE cells may be similar to immature RPE. Notably, long-term culture (8 months) was shown to be beneficial for RPE65 protein expression, suggesting that terminal differentiation is influenced by the extended time in culture (Buchholz et al., 2009).

Nevertheless, spontaneous differentiation has variable success both within and between PSC lines (Kawasaki et al., 2002; Klimanskaya et al., 2004; Leach and Clegg, 2015; Rowland et al., 2012). It is also labour intensive, time-consuming, and more difficult to control due to the undefined signalling cues being exploited, potentially resulting in relatively low yield and high variability. In addition, such methods do not lend themselves to larger scale manufacturing needed to deliver the numbers of cells required for clinical applications while adhering to the principles and standards of good manufacturing practice. Subsequent experimental efforts therefore focussed on defined and shorter differentiation conditions (reviewed in detail in (Leach and Clegg, 2015)). Nicotinamide and activin A have been recognised as potent retinal fate inducers promoting mature and functional RPE differentiation under defined conditions (Idelson et al., 2009). While nicotinamide prevents neural cell apoptosis, activin A has been shown to have a role in RPE development *in vivo* by inducing expression of RPE specific markers, such as *Mitf*, *Wnt13* and *Mmp115*, and downregulating expression of the neural retina genes *Pax6*, *Chx10* and *Optx2* (Fuhrmann et al., 2000). Further developments of protocols utilised the addition of small molecules and growth factors at defined time points to recapitulate retinal development, increase the efficiency and reproducibility of RPE production from PSCs, and pursue the aim of faster and large-scale automated manufacture of cells for clinical use (Choudhary et al., 2017; Foltz and Clegg, 2017; Leach et al., 2015). Subsequently, Regent et al. demonstrated that the sequential use of nicotinamide, activin A and CHIR99021 in a defined protocol was sufficient to increase the yield and reduce the time required for RPE differentiation. This protocol was also shown to be amenable to robotic automation to bypass the manual pigmented RPE patch picking, and the collection (banking) of mature RPE cells obtained at differentiation Day 84 (Regent et al., 2019).

The application of ECM components also proved beneficial in promoting RPE differentiation (Rowland et al., 2013). Inclusion of ECM components is a reasonable strategy given that RPE cells adhere to BrM via integrins expressed at the cell surface (Aisenbrey et al., 2006; Zarbin, 2003). BrM contains several ECM proteins that bind integrins expressed by RPE cells. The integrin ligands include laminins (specifically laminin-111, -332, -511 and -521, collagens I and IV, fibronectin, and vitronectin). Mouse laminin-111 appears to specifically promote differentiation of PSCs to RPE cells and supports their ongoing maintenance in culture. Laminin-111 was also found to promote a high degree of pigmentation (Rowland et al., 2013). Notably, Matrigel™, was shown by Rowland et al. to be an excellent substrate for RPE differentiation and maintenance. Given that laminin is the major component of Matrigel™, it is likely that this component supports the high yields of enriched RPE cells. Human laminin-521 has been shown to be similarly effective with human PSCs, also, crucially, supporting the production of clinically compliant RPE (Plaza Reyes et al., 2016).

Additionally, cell surface makers were identified to prospectively enrich RPE cells from both embryoid body and monolayer cultures

(Choudhary and Whiting, 2016; Plaza Reyes et al., 2020). These markers include CD59, CD184, CD56, GD2 and CD140b, although CD59 has been shown to be expressed in undifferentiated and partially differentiated cells as well, indicating relatively low specificity. As extensively studied by Plaza Reyes et al., the identified markers could be used for positive (CD140b) and negative selection (GD2, CD184 and CD56) to ensure capture of homogenous populations of mature RPE, with no progenitor cells present. Although CD140b is not unique to RPE cells, it is not expressed in PSCs and therefore it could be considered a specific RPE marker in an *in vitro* pluripotent cell differentiation setting, assisting with the generation of pure RPE cells that could be used for clinical cell transplantation purposes (Plaza Reyes et al., 2020).

RPE cells obtained through the aforementioned methods displayed typical RPE functionality, including polarised expression of specific markers, tight junction formation (indicated by transepithelial electrical resistance), polarised secretion of vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF), and the phagocytosis of photoreceptor outer segments. Melanosomes within these cells localised towards the apical membrane, nuclei were positioned basally, and apical processes were present; features well-visualised on transmission and scanning electron microscopy and consistent with the ultrastructural characteristics of foetal RPE in culture (Farnoodian et al., 2022; Hallam et al., 2017; Maminishkis et al., 2006; Plaza Reyes et al., 2020). Importantly, these cells have been shown to integrate into the RPE of animal models, such as rabbits, and to rescue photoreceptor cells from induced damage, indicating their fidelity to their *in vivo* counterparts (Carr et al., 2009; Plaza Reyes et al., 2016).

Cultured RPE cells are indispensable in disease modelling, and this will be discussed in more detail in Section 4. For the purpose of this section, it is striking that the introduction of a culture system involving the growth of RPE cells on porous supports has significantly enhanced assessment of the functionality of these cells and the ability to observe features seen in human aged or diseased RPE, such as the extracellular drusen and lipid accumulation (Johnson et al., 2011; Maminishkis et al., 2006). This cell culture model system has been used widely in PSCs-derived RPE maintenance and proves beneficial in understanding RPE biology (e.g., secretion, functionality of the tight junctions and apical-basal polarity) and disease mechanisms (Bharti et al., 2022; Buskin et al., 2018; Cerniauskas et al., 2020). This principle of designing complex model systems that have biological relevance has recently been taken forward by Manian et al. to generate a 3D PSCs-RPE-choriocapillaris model (Manian et al., 2021). In this model, PSC-derived RPE, endothelial cells and mesenchymal stem cells were integrated into a hydrogel-based engineered extracellular matrix, facilitating the formation of organised cellular architecture mimicking the *in vivo* complex including a polarised RPE monolayer and underlying fenestrated vasculature. The modular nature of the culture model also allowed the investigation of specific intercellular interactions via trophic factors, demonstrating the importance of RPE secreted factors in the development of fenestrated choriocapillaris-like vasculature, as well as in initiating choriocapillaris atrophy in a pathological setting. Altogether, this novel RPE-choriocapillaris model system lays the foundation for elaborate model generation to investigate retinal physiology and, importantly, elucidate disease mechanisms for drug target identification and pharmacological testing.

### 3.2. Differentiation of PSCs to rod and cone photoreceptor precursors

Several key publications have shown that it is possible to drive the differentiation of human PSCs toward photoreceptors using developmental cues (Hirami et al., 2009; Lamba et al., 2006; Meyer et al., 2009; Osakada et al., 2008). For example, Lamba et al. generated putative photoreceptor like cells by directing PSCs to an anterior neural fate using Noggin, Dkk-1 and IGF in a brief suspension culture followed by attachment of retinal progenitor cells on Matrigel™ or poly-D-lysine coated plates, and their differentiation over a three-week differentiation in which significant numbers of cells expressing the photoreceptor precursor marker CRX were detected (Fig. 2A) (Lamba et al., 2006). Despite this encouraging finding, few of the CRX expressing cells progressed to express markers of more mature photoreceptors such as rhodopsin, and only co-culture with explants of mouse retinas enabled the generation of greater numbers of rhodopsin expressing cells. Maturation of human PSCs derived CRX $^{+}$ photoreceptor precursors was improved further by injection into the subretinal space of *Crx*$^{-/-}$ mice with limited restoration of light responsiveness in the transplanted animals; however, many of the transplanted cells failed to develop photoreceptor outer segments (Lamba et al., 2009, 2010). Across these studies, the differentiation efficiency was variable and rather low, reaching at best around 20%, when the Wnt and Nodal pathway were antagonised during the early stage of suspension culture and subsequently retinoic acid and taurine were added to aid photoreceptor specification under serum free conditions (Hirami et al., 2009; Osakada et al., 2008).

The efficiency of PSC differentiation was improved by Mellough et al. through systematic analysis of the culture conditions and growth factors required to generate rod and cone photoreceptors (Mellough et al., 2012). This protocol directed the stage-specific differentiation of PSCs toward an anterior neural fate, followed by retinal determination, retinal progenitor specification and the differentiation to photoreceptor lineage. The appearance of these phenotypes occurred in a sequential manner consistent with retinal development *in vivo,* leading to generation of rod (18%) and cone (67%) photoreceptors in a relatively short time window (45 days) compared to previous studies. Despite this much improved efficiency, the PSC-derived photoreceptors declined between Days 45 and 60 of differentiation, suggestive of lack of appropriate culture conditions and/or other cell types necessary for the survival of postmitotic photoreceptors.

To optimise culture conditions, Meyer and colleagues dissected optic vesicle like structures from the attached differentiating PSCs and continued to differentiate those in suspension, achieving a 56% efficiency in generation of photoreceptor precursors that were electrophysiologically similar to native photoreceptors (Meyer et al., 2011). Together, these findings highlight suspension (also referred to as 3D) culture conditions in combination with tailored made media as being beneficial for photoreceptor specification from PSC-derived retinal progenitor cells. By generating controlled size EBs and replacing the Wnt inhibitor Dkk1 with IWR1e, Markus and colleagues increased the efficiency of photoreceptor precursor generation to 87% (Markus et al., 2019; Schick et al., 2020). They were also able to demonstrate that the expression of voltage gated ion channels known to be expressed in mature photoreceptors increased up to Day 90 of differentiation. This was mirrored by the electrophysiological maturation of photoreceptors, which occurred in parallel to the increase in expression of CRX, a specific photoreceptor precursor marker (Schick et al., 2020). The high differentiation efficiency of this protocol enabled the generation of large-scale photoreceptor precursor cultures for transplantation and drug screening purposes. Nonetheless, the differentiation period was longer than *in vivo* development of human retina (Fig. 1B), where the first conception of photoreceptors and inner cells synapses with the outer plexiform layer are observed around 8 post conception weeks (Gupta et al., 2016). To shorten the differentiation window, a transcription factor driven differentiation protocol relying on the ectopic expression of the transcription factors *CRX* and *NEUROD1* was recently reported, offering a simplified protocol to generate photoreceptor precursors within 14 days, and electrophysiologically active photoreceptors by 30 days (Otsuka et al., 2022). Despite the considerable shortening of differentiation period, this direct conversion of PSCs to photoreceptors has a relatively low efficiency (34%): this could be increased to 44% by suspension culture conditions, but in both cases photoreceptor cells with typical ultrastructure including an outer segment were not generated, demonstrating that further improvements in the introduced transcription factor or culture conditions were needed to achieve the desired

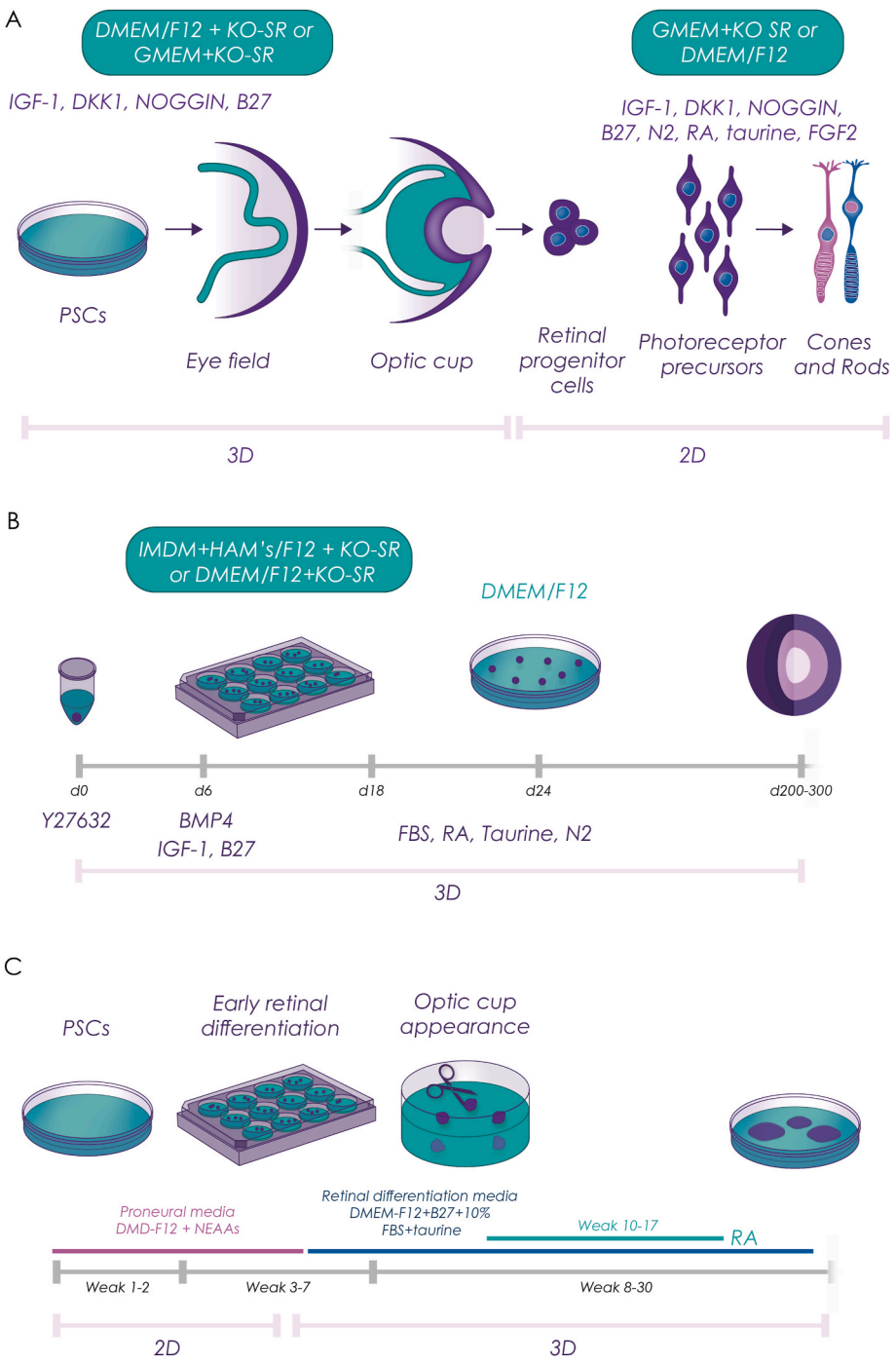


**Fig. 2.** Schematic presentation of differentiation protocols used for PSC differentiation to photoreceptors (A) and retinal organoids (B,C) using a combination of 2D and 3D culture conditions.

mature differentiation status.

### 3.3. PSCs differentiation to retinal organoids

Understanding and recapitulating retinal function requires the interactions among various cell types, which may not be adequately achieved by studying each cell type in isolation. To address these limitations, researchers have increasingly turned to 3D micro-physiological systems to better assess tissue or organ function. The formation of such complex 3D system requires that the various cell types aggregate and self-organise in a manner that recapitulates the spatial cellular organisation and functionality of the specific organ *in vivo*. In earlier studies, it was established that chick embryonic retinal cells were capable of self-aggregation, organisation and histogenesis *in vitro* (Layer and Willbold, 1989, 1993, 1994). This fundamental work laid the foundation for the first seminal paper published by Eiraku et al. demonstrating that mouse ESCs were able to self-organise into laminated retinal structures under serum free floating embryoid body (SFEB) culture conditions with the addition of extracellular matrix components (Matrigel™, laminin or nidogen) and exogenous components (TGFβ ligand nodal), which resulted in formation of hollow spheres with a polarised neuroepithelium (Eiraku et al., 2011). Subsequent evagination led to the formation of hemispherical vesicles expressing genes typical of the early embryonic neural retina on the internal surface, and those associated

with RPE development on the outer surface. These vesicles then underwent invagination to produce optic cup-like structures like those of E10.5 mouse embryos. These structures expressed genes of retinal progenitor cells such as *Chx10*, and over time, they formed a continuous, stratified epithelial structure resembling the spatial arrangement of cells found in early neonatal mouse eyes, with photoreceptors in the outermost layer, RGCs in the innermost layer, and other cells, such as bipolar cells, in between (Fig. 2B).

Through addition of Wnt inhibitor and Matrigel™ in the early stages, and foetal bovine serum and the hedgehog smoothened agonist (SAG) later during the differentiation process, the same group demonstrated that stratified retinal organoids expressing markers of all retinal cell types could be generated from human ESCs (Nakano et al., 2012). Transient treatment of retinal organoids in the very early stages of differentiation with BMP4 (Day 6), promoted the formation of neural retina, whilst the inhibition of WNT and FGF signalling pathway from Day 18–24 of differentiation (known as induction-reversal culture) enabled the generation of neural retina with patches of RPE cells, often located at the opposite end of the organoids (Kuwahara et al., 2015). Importantly, the retinal organoids generated with this method contained a putative ciliary margin zone, which contributed to retinal progenitors, allowing the expansion of organoids *in vitro* and mimicking retinogenesis *in vivo*. This method has become an established technique for generating retinal organoids from PSCs and has been successfully adopted to multi-well plate format, enabling studies of drug screening and toxicity that will be discussed in Sections 5 and 7 of this review (Hallam et al., 2018). Notwithstanding the ease of application, it is worth stating that not all human PSCs respond equally to this differentiation protocol.

To evaluate the functionality of retinal organoids for potential applications in the development of treatments for retinal diseases, it is crucial to demonstrate that they closely mimic adult retinal function *in vivo*. While the emergence of laminated retinal tissue is an encouraging sign, it does not guarantee the functionality of the resident photoreceptors and other retinal cell types within the organoids. As a technique to investigate the generation of action potentials and in particular the activities of specific ion channels that permit this function, patch clamping is highly valuable, but it cannot be used to record output from multiple cells of microphysiological systems. Multi-electrode arrays offer the possibility of recording from organoids/tissues in which many electrophysiological active cells connect with numerous electrodes. This approach was first applied to induction-reversal retinal organoids generated by Hallam et al. who, exposed RGCs by cutting organoids in half and placing them onto 4096 electrode arrays (Hallam et al., 2018). These RGCs responded to pulsed illumination by either increasing or decreasing their spike formation activity in artificial cerebrospinal fluid. Although the responses of individual RGCs were slow compared to those of mature retina, the change in activity was consistent with the response patterns of immature ON- and OFF-centre RGCs corresponding to the perinatal period. To confirm that these responses were driven by photoreceptors via bipolar cells, as opposed to photosensitive RGCs, the photoreceptor dark current was stimulated by adding membrane permeable 8-bromo-cGMP. This experiment identified RGCs with decreased spiking activity as being ON-centre photoreceptor driven, providing valuable insights into the functional connectivity within retinal organoids.

IGF-1 is a known enhancer of eye formation and retinal progenitor induction (Lamba et al., 2006; Richard-Parpaillon et al., 2002). To exploit these properties, Mellough and colleagues, differentiated PSCs in IGF-1 containing media, showing the formation of numerous optic vesicle-like structures that matured and formed the bilayered optic cup that gave rise to neural retina and RPE. The photoreceptors situated within the putative outer nuclear layer of the organoid displayed inner and outer-like segments, and phototransduction responses as early as 6.5 weeks of differentiation. Like the induction-reversal protocol discussed above, the IGF-1 protocol showed variable responses amongst several PSC lines, corroborating data provided by other groups working in this field (Chichagova et al., 2020; Cooke et al., 2023). Such variability has led to usage of a few amenable and well characterised PSC lines in many research groups; however, it is an unavoidable issue when working with newly derived patient specific iPSCs or gene-edited PSCs, which also show variable differentiation efficiency. To date, the underpinning mechanism for such variability has not been elucidated; however, DNA methylation and epigenetic landscape (chromatin looping, histone modifications) have been put forward as potential factors that may define the differentiation efficiency of PSCs (Wang et al., 2018). A potential solution to overcome such variability is to generate naïve pluripotent stem cells, but this would add more costs and duration to already long differentiation protocols and would not be feasible for large scale studies (Zhou et al., 2023). Thus, a set of markers has been put forward to allow researchers to predict retinal differentiation propensity of hESCs or different iPSC clones generated during the reprogramming process or CRISPR-Cas9 gene editing (Cooke et al., 2023).

While the above protocols have been focused on 3D generation of retinal organoids, a further method which combines both 3D and 2D culture conditions was developed and tested by several groups, who excised optic cup like structures from attached (2D) cultures, and further maintained those under floating conditions in media that supported the differentiation of all retinal cell types, arranged in their appropriate layers (Fig. 2C) (Meyer et al., 2011; Reichman et al., 2014; Zhong et al., 2014). This protocol has been used and modified by multiple groups, which has added to the complexity of comparing organoids generated in different laboratories with various PSC lines (Capowski et al., 2016, 2019; Gonzalez-Cordero et al., 2017; Phillips et al., 2018; Regent et al., 2020; Sanjurjo-Soriano et al., 2022). To this end, Capowski and colleagues designed a practical staging system, based on bright phase appearance, structural organisation, immunocytochemistry, metabolic imaging, and electron microscopy ultrastructural analyses, that should enable both experienced and inexperienced researchers to stage their organoids (Capowski et al., 2019). Stage 1 organoids are defined by the presence of proliferative retinal progenitors at the apical rim and abundance of retinal ganglion cells in the basal aspect of the organoids, while Stage 2 is characterised by specification of photoreceptor precursors and retinal interneurons. In Stage 3, metabolically active photoreceptors with specialised ribbon synapses, abundant Müller glia cells that contribute to the formation of outer limiting membrane and well-developed plexiform layers should be present. A key feature of Stage 3 is the development of photoreceptor outer segments, which in most protocols resemble foetal outer segment structures with unstacked discs attached to the mitochondria rich inner segment via the connecting cilia. Changing the composition of culture media by supplementation with lipids and antioxidants from Week 12 of differentiation significantly improves the formation of both cone and rod outer segments with discrete membranous structures and closely stack discs, and the proportion of photoreceptors that form outer segments, enabling robust models to investigate retinal disease and perform drug screening and toxicity studies (West et al., 2022).

In addition to photoreceptor maturity, the presence of functional synapses is critical for the transmission of light responses from photoreceptors to second or third order retinal neurones. Recent studies have shown the presence of ribbon synapses at photoreceptor axon terminals, often found in close contact with bipolar and horizontal cells, suggesting the presence of functional synapses between photoreceptors and second order neurones (Capowski et al., 2019; Cowan et al., 2020; Saha et al., 2022; Wahlin et al., 2017). Moreover, retinal organoid derived neurons can reform synaptic connections after cell dissociation, which provides evidence of their potential to re-establish connectivity upon transplantation *in vivo* (Ludwig et al., 2023). Notably, cone photoreceptors display robust intrinsic light evoked responses, akin to adult macaque foveal cones: these start at approximately Day 210 of differentiation when photoreceptors build their outer segments and amplify between Days 250 and 260 both in amplitude and proportion of responders (Saha

et al., 2022). Together, these data validate the potential of retinal organoids to generate functional human photoreceptors for cell replacement therapies and obtain unique insights into human retinal development.

However, as with every tool, retinal organoids should be used with careful consideration of their strengths and limitations. Current strengths include recapitulation of retinal development timeline, presence of mature photoreceptors and the synaptic connections that enable light transduction (Cowan et al., 2020). Limitations include lack of adjacent RPE, immune cells, and vasculature, which most likely limits the nutrient provision and waste disposal, leading to progressive disorganisation over time and decay of inner retinal neurons. Co-culture efforts have demonstrated that externally added microglia can transiently populate retinal organoids, without affecting their development, maturation or light responses (Chichagova et al., 2023). Within retinal organoids, microglia can be found in the outer and inner plexiform, as well as ganglion, cell layers akin to the location of retinal microglia cells *in vivo,* and are able to respond to endotoxin challenge (Usui-Ouchi et al., 2023). Microglia also arise spontaneously in mesenchymal-like cysts adjacent to retinal organoids generated via unguided protocols, but do not infiltrate the retinal neuroepithelium, demonstrating some incompatibility between the current static retinal culture conditions and the milieu needed for microglia incorporation and long-term survival within the retinal organoids (Usui-Ouchi et al., 2023). One possible solution may be to provide enhanced support for the neural retina by creating so-called assembloids which are composed of several tissue type organoids. An example of this are the assembloids of retinal and cortical or thalamic organoids, which enhance retinal ganglion cell survival, that extend axons deep into the assembloids, mimicking the projections of the visual system (Fligor et al., 2021).

Despite the ongoing advancements, another major challenge in generating retinal organoids is the scalability of cultures and maintaining the necessary conditions for an extended period (>200 days). To overcome this, innovative approaches such as microarray well seeding combined with checkerboard scraping and bioreactor-based protocols have been developed (DiStefano et al., 2018; Ovando-Roche et al., 2018). These methods facilitate the accelerated and reproducible generation of retinal organoids at scale while enabling continuous monitoring of physical and chemical conditions.

In summary, retinal organoids and the emerging assembloids represent significant advancements in the study of retinal development and function compared to traditional 2D differentiation methods. Their ability to generate complex, stratified retinal structures, with functional properties, holds great promise for advancing our understanding of retinal diseases and potential therapies. To fully recapitulate retinal microenvironments, more complex physiological systems, which incorporate the RPE and choriocapillaris need to be developed. This is an active area of research which we briefly discuss in Section 3.4.

### 3.4. Advanced co-culture models

The choriocapillaris, BrM, RPE, and neural retina form a closely integrated functional unit *in vivo* (Fig. 1A), so it is imperative that *in vitro* models mimic these anatomical interactions (Fields et al., 2020). The RPE is important for maintaining retinal homeostasis since it recycles photopigments as part of its visual cycle function and provides growth factors to support photoreceptor viability. Similarly, while oxygen and metabolites are delivered directly to the inner neural retina by the retinal vasculature (e.g., branches of the central retinal artery), it is the choriocapillaris, which meets the high metabolic demand of the RPE and outer neural retina including the outer nuclear layer (Levin et al., 2011). Given these contributions to retinal function, it is unsurprising that dysfunctions in the choriocapillaris and RPE either distinctly or in combination with problems arising in the neural retina, can give rise to outer retinal pathologies. AMD, for example, is thought to arise at least in part from complement system dysregulation within the choriocapillaris, which may contribute to pathology across the whole retina.

BrM plays a similarly important role. Sandwiched between the RPE and the choriocapillaris, this is a unique and complex membrane comprising the RPE basement membrane, three collagen and elastin-rich layers and the choriocapillaris basement membrane (Hammadi et al., 2023). BrM functions as a selective barrier between the choriocapillaris and the RPE but the formation of drusen and lipid deposition within the inner most layers of BrM is an early and highly prognostically important pathological event in the development of AMD. In the next section, we will discuss efforts to model the interactions between the choriocapillaris and RPE as well as retinal organoids and RPE.

#### 3.4.1. Co-culture of RPE and endothelial cells to mimic the choriocapillaris

An important feature of the endothelial cells forming the capillary vessels of the choriocapillaris is the presence of fenestrations which allow transport of larger molecules and seems to be essential for choriocapillaris function (Kim et al., 2020). This suggests that recapitulation of such fenestrae would be an important component of any model system. There is evidence to suggest that RPE regulates angiogenesis in the choroid as Hamilton et al. demonstrated that Human Umbilical Vein Endothelial Cells (HUVECs) could be induced to a fenestrated choriocapillaris endothelial cell phenotype with expression of VE-cadherin and ZO-1 when co-cultured with ARPE-19 cells on opposite sides of amniotic membrane (Hamilton et al., 2007; Tretiach et al., 2004). Further, Sakamoto et al. demonstrated that primary bovine choroidal endothelial cells could form choriocapillaris like tubes in 3D culture comprised of type 1 collagen-rich gel, but the co-culture with RPE modulated the formation of capillary-like structures by the endothelial cells more so than fibroblast and pericytes (Sakamoto et al., 1995).

Constructing models that mimic the oBRB is also of great interest since understanding the bi-directional relationship between RPE and the choriocapillaris is useful for developing therapies for outer retinal disorders (Spencer et al., 2017). Studies in this area suggest that combinations of RPE, a BrM mimic and endothelial cells with a choroidal phenotype are all necessary to model the oBRB. For example, the transfer of a 4 kDa dextran fluorescein tracer was almost totally inhibited by human umbilical vein endothelial cell (HUVEC)-amnion-RPE trilayer mimicking the barrier properties of the oBRB, but not a HUVEC-amnion-corneal epithelial cell tri-layer, or an amnion-RPE bilayer alone. The formation of RPE tight junctions appears to be dependent on the presence of stiff collagen anchor points in the basement membrane, the deposition of which is regulated by choroidal endothelium through yet unidentified paracrine factors. These findings demonstrate that RPE on its own does not fully allow the generation of a physiological oBRB and that co-culture with BrM and choriocapillaris is beneficial for achieving RPE maturation (Fig. 3). It has been suggested

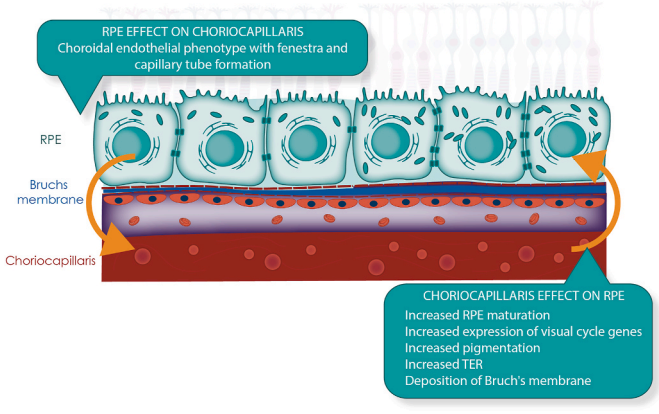


**Fig. 3.** The RPE and choriocapillaris are interdependent for full maturation in culture, with a number of phenotypic 'enhancements' observed by co-culture.

that the effect of the choriocapillaris may be partly mediated by basement membrane cues as the ARPE-19 phenotype is modulated by transwell co-culture with HUVECs, with enhanced basement membrane deposition, phagocytic activity, ZO-1 expression and expression of visual cycle genes including RPE-65 (Spencer et al., 2017). This is corroborated by transcriptomic analyses which demonstrate that the transcriptional programme of the developing choroid is focused on development and proliferation, while adult choroidal endothelium is focussed on extracellular matrix deposition and cellular adhesion (Benedicto et al., 2017). These findings are important for the development of *in vitro* models as they suggest that an engineered biomaterial with the correct physical properties may be able to maintain a mature RPE phenotype at least partly without the need for co-cultured choroidal endothelium.

Demonstrating the utility of these models for therapy development is important and it is encouraging that in their landmark publication, Song and colleagues engineered a native-like 3D-oBRB by bioprinting endothelial cells, pericytes, and fibroblasts on the basal side of a biodegradable scaffold and establishing an RPE monolayer on top (Song et al., 2023). In this 3D-oBRB model, a fully polarised RPE monolayer provided barrier resistance, induced choriocapillaris fenestration, and supported the formation of a BrM-like structure by inducing changes in gene expression in cells of the choroid. Complement activation in the 3D-oBRB triggered dry AMD phenotypes (including sub-RPE lipid-rich deposits similar to drusen, and choriocapillaris degeneration), and HIF-α stabilization or STAT3 overactivation induced choriocapillaris neovascularisation akin to a wet AMD phenotype. Together these findings demonstrate that inclusion of the choroid in *in vitro* models is beneficial for accurate modelling of the RPE in health and disease, and for developing regenerative therapies that need to target both RPE and choriocapillaris in diseases such as AMD.

### 3.4.2. Organ-on-a-chip

The co-culture system described above are essentially static in that they do not allow controlled movement of culture media and addition/removal of nutrients, metabolites, or xenobiotic substances as *in vivo* systems. So called 'organ-on-chip' or microphysiological systems combine *in vitro* co-culture models with biomaterials and/or microfluidics to recapitulate one or more key functions of a tissue. They offer control over metabolite/drug delivery and waste removal, cellular compartmentalisation, simulation of flow and movement, and real-time physical measurements. A recent application of microphysiological systems in the retina, was provided by Paek et al. who designed a polydimethylsiloxane (PDMS) culture chamber, which they filled with primary choroidal endothelium and choroidal fibroblasts laden in a fibrin/type 1 collagen hydrogel (Paek et al., 2019). They observed self-assembly of endothelial vascular networks which anastomosed with an input-output circuit and were thereby perfused with culture media. They cultured PSC-derived RPE on this hydrogel and found greater basement membrane deposition, melanosome expression and RPE65 expression in their co-culture model versus mono-cultures (Fig. 4).

Co-cultured neural retina and RPE explants, arranged in an organotypic fashion, allow functional interaction between the photoreceptors and RPE cells, while allowing easy access to an artificial sub-retinal space for delivery of cell therapies (Yanai et al., 2015). To adequately model a physiological RPE-retinal interface and subretinal space, retinal organoids could be combined with RPE and/or choriocapillaris, as has been achieved using *ex vivo* explants. To this end, PSC-derived mature retinal organoids and polarised RPE sheets bound together with alginate have been generated and subretinally transplanted into immunodeficient Royal College of Surgeons rats at advanced stages of retinal degeneration (Thomas et al., 2021). The authors reported that with short term culture the RPE migrated into the organoids and caused structural changes suggesting that co-culture conditions weren't optimal. To preserve retinal organoid structure, Achberger and colleagues co-cultured 180-day-old human PSCs retinal organoids

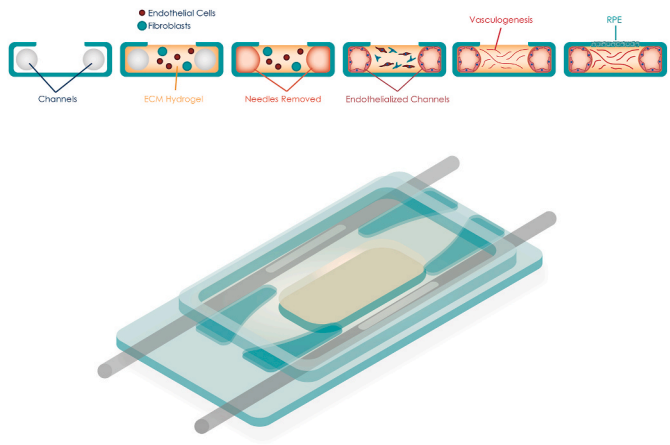


**Fig. 4. Schematic of a perfused culture chamber system as used by Paek et al (Paek et al., 2019).** Using needles inserted into the side channels the central chamber was filled with a fibrin/type 1 collagen hydrogel seeded with primary choroidal endothelium and fibroblasts. After withdrawal of the needles, the side channels were then also seeded with choroidal endothelial cells. After media perfusion, self-assembly of a 3D endothelial vascular network occurred, anastomosed with the input-output circuits and reminiscent of a microvascular plexus. RPE was then grown as a monolayer on the apical surface.

embedded in a hyaluronic acid-based hydrogel over a layer of human PSCs-RPE (Fig. 5) (Achberger et al., 2019). This co-culture perfused with a microfluidic system below a membrane made from Polyethylene Terephthalate with a pore diameter of 3 μm and a thickness of 10 μm, enabling more physiologically controllable conditions in terms of removing metabolic by-products and introducing nutrients and oxygen. Enhanced maturation of photoreceptor outer segments and importantly, RPE phagocytosis of photoreceptor outer segments were reported, despite the short co-culture period. Although the retinal organoids were far from adult retina in terms of maturation, and the choroid was not represented, this study marks a clear advance in our ability to co-culture RPE cells with retinal organoids without losing retinal lamination. That said, there was some disparity in the timing as compared to *in vivo*, at which co-culture of retinal organoids and RPE was initiated. Human neural retina and RPE develop in close contact from approximately Day 30 *in vivo* so it may be necessary to use retinal organoids at less than 180 days to recapitulate human retinogenesis (O'Rahilly, 1975). This could

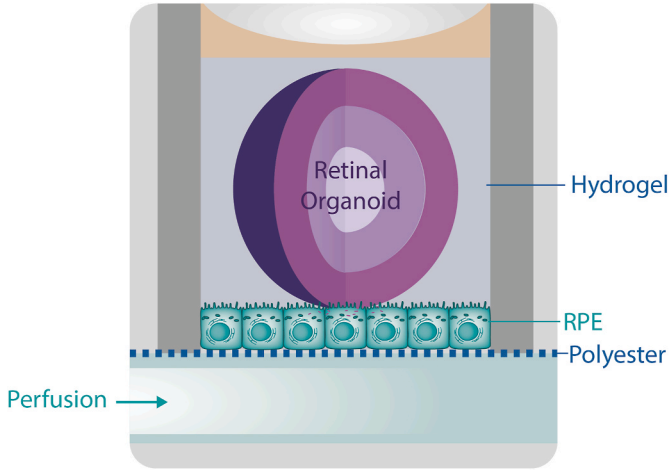


**Fig. 5. Schematic of co-culture system employed by Achberger et al (Achberger et al., 2019).** Retinal organoids were embedded in a hyaluronic acid-based hydrogel on a layer of human iPSC derived RPE. This was grown as a monolayer on a porous polyester transwell above a perfused microfluidic system.

help to increase the longevity of retinal organoids, avoid the presence of a necrotic core often observed at their centre, and improve their maturation, which is essential for disease modelling.

A recent study, where *USH2A* patient specific retinal organoids were co-cultured with *USH2A* RPE cells that were perfused of a microfluidic channel under the RPE, found that fewer photoreceptors underwent apoptosis on the perfused chip, compared to static conditions. Collagen IV and laminin significantly increased on the perfused chip with greater RPE pigmentation under the perfused conditions suggesting that the microfluidic system could facilitate both retinal organoid and RPE development. Similarly, microphysiological systems may also be used to investigate local effects of cell therapy e.g. on glial activation, and to optimise synaptic integration of transplanted neural retina (Dodson et al., 2015; Su et al., 2015). Su et al. observed synaptogenesis between two populations of developing mouse retinal precursors in 4 μm guiding channels (Su et al., 2015). Compartmentalised experimental setups such as this could be used to optimise synaptogenesis between PSCs-derived retina and host neurones.

In summary, it is likely that co-culture models will advance our understanding of outer retinal physiology and disease. Furthermore, they could help optimise and investigate the delivery, integration, maturation, and functionality of stem cell-derived retinal sheets for transplantation. Future work could build on the advanced culture systems presented and use patient-derived PSCs to model for example the effect of high- and low-risk AMD genotypes on co-cultures of RPE, photoreceptors and choriocapillaris. Such work may help to elucidate the early events which lead to AMD pathogenesis and help to understand the mechanisms underpinning diverse clinical presentations in the AMD patient population. In our search, we have not identified a co-culture system which incorporates neural retina, RPE and choriocapillaris. As we have shown, all the techniques to achieve such a system are available. Future work may examine the optimum way to integrate retinal organoids into co-culture with RPE and/or choriocapillaris in a model that mimics retinogenesis and the developmental interactions. Factors such as differentiation age and co-culture media components need to be optimised. Biomaterials can play an important role in recapitulating the retinal environment. Although much work has been done in identifying the building blocks of retinal ECM, more work needs to be done in identifying the 3D organisation of tethering domains and physical properties which emulate the healthy or diseased retinal ECM. However, it is also true that these *in vitro* models may need only to capture certain essential elements of the complex *in vivo* interactions to reproduce insight, depending on the question being asked. For example, *in vitro* models for optimisation of neural retina transplant may benefit from focusing on recapitulating the environment of the host sub-retinal space, the important elements of which may be the neural retina, RPE and interphotoreceptor matrix. Models for the optimisation of RPE transplant may focus on modelling diseased BrM and choriocapillaris.

## 4. Disease modelling with iPSCs

In this section we will describe progress in the use of iPSCs to model a range of retinal diseases. Given the impact of the disease, it is perhaps unsurprising that much effort has been devoted to making models of AMD, but we also cover work on models of IRDs, childhood retinal cancer (retinoblastoma) and mitochondrial diseases.

### 4.1. AMD

AMD is usually asymptomatic in its early stages, marked by the accumulation of lipid and protein rich deposits (63–125 μm) at the RPE-BrM interface, called drusen. In intermediate AMD, drusen progress to a larger size (>125 μm) and may be accompanied by pigmentary changes in the RPE. The advanced stages of AMD comprise geographic atrophy ('dry') and exudative ('wet') AMD, both of which, if left untreated, commonly lead to irreversible central vision loss – rapidly in the exudative type, owing to subretinal leakage of blood or serous fluid from unstable neovascular membranes, or insidiously in geographic atrophy as growing lesions of dying choriocapillaris, RPE, and photoreceptor engulf the macula (Holz et al., 2014; Mitchell et al., 2018). The primary cell type involved in AMD pathogenesis is debated; although the RPE is the first to be visibly affected, it is the accompanying photoreceptor dysfunction and atrophy that ultimately leads to vision loss (Curcio et al., 1996). Over the last decade, intravitreal anti-vascular endothelial growth factor (anti-VEGF) injections have emerged as an effective option to prevent and reverse acute vision loss in exudative AMD. However, the underlying disease process continues unabated in most, with up to a third of patients continuing to lose their vision over time (Parravano et al., 2021; Rofagha et al., 2013).

AMD's multifactorial nature has complicated attempts to model it experimentally. Other than age, smoking is the most consistent environmental risk factor (Lambert et al., 2016). However, AMD also has substantial contributions from common and rare genetic variants, with heritability estimated at approximately 50% (Fritsche et al., 2016; Tzoumas et al., 2022). Particularly strong associations have been observed for genes involved in complement activation and regulation, as well as a risk haplotype encompassing *ARMS2* and *HTRA1* (Fritsche et al., 2016; Tzoumas et al., 2021). Notably, a single nucleotide polymorphism (rs1061179) in the complement factor H (*CFH*) gene causing the protein change p.Y402H, and shared by a third of Europeans, confers a two-fold higher risk of late-AMD per allele copy (Sofat et al., 2012; Tzoumas et al., 2021). The elucidation of AMD's complex genetic architecture led to the first treatments for geographic atrophy, the complement C3 inhibitor pegecetacoplan (Syfovre®, *Apellis Pharmaceuticals*, MA, US) and the complement C5 inhibitor avacincaptad pegol (Izervay™, previously known as Zimura, Iveric Bio, NJ, US), were recently approved by the FDA in February and August 2023, respectively, following promising randomised trials showing reduced anatomical progression by 15–30% per year depending on patient characteristics and treatment regimen (Jaffe et al., 2021; Liao et al., 2020). Despite this breakthrough, the visual benefits of complement inhibitors remain inconclusive, and the optimal treatment strategy has yet to be established (Tzoumas et al., 2023).

To address these clinical uncertainties, numerous animal models of AMD have been developed. Although these display several important AMD features, they do not fully recapitulate the pathophysiology of the disease (Pennesi et al., 2012). Modelling of AMD in animals is challenging due to the heterogeneous nature of the disease, which is underscored by a plethora of molecular interactions involving altered cellular metabolism, senescence, inflammation, and oxidative stress (Lambert et al., 2016). Anatomical differences between animal and human retina are also considerable, such as the lack of the cone-rich macula in rodents, with unclear implications for translation (Pennesi et al., 2012). The pathology of RPE, thought by some to be the site of the primary lesion in AMD, has become a major focus of *in vitro* models, using RPE cell lines (primary and immortalised) and iPSC-derived RPE (Bharti et al., 2022; Young, 1987). The advancement of iPSC disease modelling has offered the opportunity to interrogate the pathobiology of AMD using patient specific RPE cells, allowing the production of genetic risk models from patients with high risk alleles linked to AMD.

The utility of iPSCs for basic research of AMD and for the development of novel therapeutics is underscored by the recent initiative of the New York Stem Cell Foundation (NYSCF) Research Institute and the NIH National Eye Institute (NEI) (nyscf.org). A resource of iPSCs from AMD patients, recruited as part of the Age-Related Eye Diseases Study (AREDS), with some of the patient cell lines also available with matching isogenic controls, has been created to springboard vision research and therapeutic innovations (Wright et al., 2020). The currently deposited cell lines have mutations in genes associated with a high risk of developing AMD, such as variants in *CFH*, *ARMS2/HTRA1*, *CFB* and *C3* genes. To facilitate genotype-phenotype analyses and to understand how genetic variants affect cell biology and contribute to the

disease process over time, the resource includes extensive clinical and medical information on the donors. The clinical and research community understands that early diagnosis, tracking disease progression (in particular identifying biomarkers of progression to the blinding stages of either geographic atrophy or neovascular AMD), and treating early-stage disease are among the top priorities in addressing the challenge of increasing burden of AMD (Wright et al., 2020). As early AMD usually spares visual function, iPSC-derived patient specific retinal cells provide a valuable opportunity to understand the early-stage disease at a cellular level, and potentially foster the development of biomarkers and drug targets.

The *CFH* and *ARMS2/HTRA1* loci explain most of the genetic predisposition to AMD (Fritsche et al., 2016). Accordingly, several basic research studies have focused on the derivation and characterisation of iPSCs from AMD patients with variants in those genes. For instance, Hallam et al. generated an iPSC-RPE model from patients with wet AMD and homozygous for the *CFH* p.Y402H polymorphism known to convey a high risk for the disease, and control lines carrying the low risk genotype (Hallam et al., 2017). iPSC-derived RPE presented features characteristic of native RPE, such as the formation of pigmented monolayers of hexagonal cells expressing markers of mature RPE and characterised by high transepithelial electrical resistance, an indicator of monolayer integrity (Sparrow et al., 2010; Strauss, 2005). A systematic characterisation of the patients RPE in comparison to RPE from age matched control individuals with the low risk *CFH* Y402 genotype, revealed disease-associated cellular phenotypes and functional deficiencies. Importantly, the cell model recapitulated the key features of AMD, such as the deposition of drusen-like deposits beneath the RPE cell layer, accumulation of intracellular lipid droplets and features of inflammation and cell stress at a molecular (altered gene expression) and ultrastructural level (e.g. cytoplasmic vacuoles and mitochondrial abnormalities). Further characterisation of the *CFH* p.Y402H RPE cells revealed a significant defect in autophagic degradative function under basal conditions, manifested by changes in abundance and activities of specific lysosomal markers (Cerniauskas et al., 2020). Notably, this work correlated RPE deficiencies in endo-lysosomal compartments with deposition of crucial lysosomal components in drusen-like deposits (Fig. 6). This functional association implies a pertinent role of dysfunctional lysosomes in the process of drusen formation, with drusen being a recognised feature of degenerating retina. Additionally, a direct link between complement system dysregulation and lysosomal damage has been shown: the Hallam et al. study demonstrated that *CFH* p.Y402H high risk cells are affected by an abnormally increased turnover of the complement C3 component, which was associated with increased deposition of the terminal complement C5b-9 complex within the lysosomal compartments. In conjunction with previous reports, these findings indicate a possible protective mechanism against the damage from complement overactivation, through enhanced endocytosis and destruction of complement components within lysosomes (Georgiannakis et al., 2015). Administration of a complement C3 inhibitor reduced disease-associated cellular lesions, providing further evidence for the involvement of complement dysfunction in RPE damage and corroborating outcomes of clinical trials of complement inhibitors in dry AMD (Cerniauskas et al., 2020).

Sharma et al. extended the analyses of complement-mediated mechanisms in AMD pathogenesis in a comprehensive study of multiple iPSC lines from homozygous or heterozygous participants for the *CFH* p.Y402H high risk allele (Sharma et al., 2021). It has been demonstrated that the exposure of iPSC-RPE, regardless of *CFH* p.Y402H carrier status, to activated complement molecules from human serum led to features indicative of AMD: the formation of lipid rich drusen-like deposits and RPE atrophy associated with cytoskeletal abnormalities, suggesting epithelial cell de-differentiation. These phenotypes were attributed to pathological complement activation within the RPE, induced by exposure to activated complement via stimulation of C3aR1 and C5aR1 complement receptors. Detailed and unbiased transcriptomic

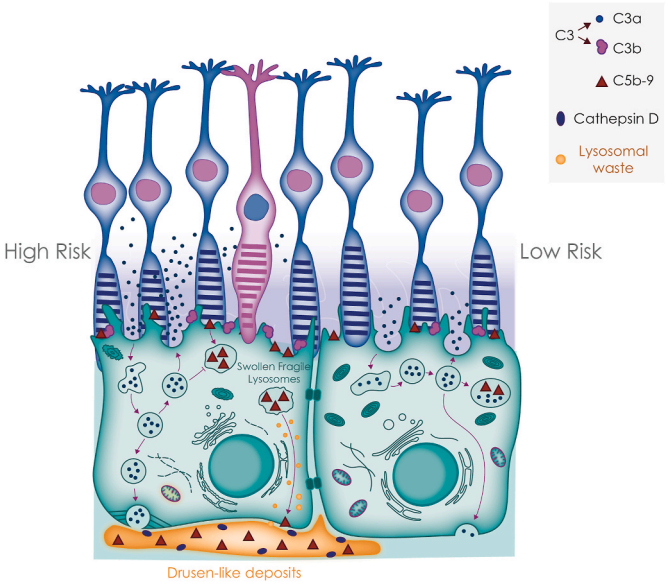


**Fig. 6. AMD modelling using Y402H iPSC-RPE.** This cell model recapitulated key features of AMD, including the deposition of drusen-like deposits beneath the RPE (Hallam et al., 2017), and cell stress and defects in lysosomal autophagic functions which directly correlated with complement system dysregulation and its abnormal intracellular localisation (Cerniauskas et al., 2020). The RPE intracellular defects had a manifestation in altered extracellular signalling via enhanced secretion of EVs with disease linked cargo (Kurzawa-Akanbi et al., 2022). Notably, the EV-mediated propagation of disease features to other retinal cells may be important for the progressive phenotype of AMD and provides insights into other retinal disorders.

analysis, validated by experimental approaches, revealed disrupted autophagy and activated NF-κB pathways, indicating their relevance to RPE health regardless of genetic background. Notably, these two functional cellular anomalies were shown to precede the formation of APOE-positive sub-RPE deposits, providing an independent validation to other studies linking drusen formation to RPE autophagic dysfunction (Cerniauskas et al., 2020; Golestaneh et al., 2017; Wang et al., 2009). While the harmful effects of activated complement were evident in all RPE cells, likely due to exposure to the anaphylatoxins C3a and C5a, the vulnerability of the *CFH* p.Y402H high risk variant carriers to retinal disease may be attributed to higher baseline deposition of these drusen-like deposits, suggesting underlying retinal vulnerability and lower survival threshold (Sharma et al., 2021).

Another study using iPSC-RPE generated from multiple donors with AMD and the high risk *CFH* p.Y402H genotype underscored the metabolic anomalies associated with complement genetic variation (Ebeling et al., 2021). Under basal conditions, RPE cells with the high risk genotype were characterised by significant defects in mitochondrial function, irrespective of the donor's disease status, which is an intriguing observation highlighting biological pathways that could be preconditioning retinal cells to the disease development. Overall, a cumulative body of evidence suggests that the role of *CFH* polymorphisms in disease susceptibility may extend beyond the regulation of the extracellular complement cascade. Changes in mitochondrial function, ultrastructure, and autophagy, together with accumulations of cytoplasmic lipid droplets, among other features, have been observed in primary RPE cultures from AMD donors (Golestaneh et al., 2017). The consistency of findings of the metabolic abnormalities in primary RPE and iPSC-derived RPE support the latter as a valid *in vitro* platform to elucidate disease mechanisms, particularly linked to a specific genetic background, and with the advantage of scalability for therapeutics testing (Ebeling et al., 2021).

The native RPE is a tight-junction polarised monolayer of cells with

asymmetric morphological and functional features that are essential for normal maintenance of the epithelium and interaction with the neural retina (apical side) and choroid/systemic circulation (basolateral side) (Strauss, 2005). Studies have shown that iPSC-derived RPE cells exhibit the same characteristics, including polarised secretion of a large portfolio of molecules, as well as the secretion of extracellular vesicles (EVs), which are involved in intercellular communication (Chen et al., 2022; Flores-Bellver et al., 2021; Hallam et al., 2017). As demonstrated for primary porcine RPE cell cultures, under homeostatic conditions, the protein contents of EVs secreted from the apical and basolateral sides were vastly different (Klingeborn et al., 2017). Similarly, human iPSC-RPE cells were shown to secrete various proteins and EVs in a polarised fashion, with the EVs cargo consisting of proteins involved in pathways linked to retinal disease and drusen formation, such as the oxidative stress, inflammation, immunological response and complement pathway (Chen et al., 2022; Flores-Bellver et al., 2021). Notably, some of the disease-related protein cargo of iPSC-RPE EVs increased in levels upon the cell exposure to smoke extract, which is believed to mimic the environmentally induced AMD phenotype (Khan et al., 2006). The chronic exposure of RPE to smoke extract was reported to lead to the appearance of sub-RPE basal deposits and ultrastructural changes indicative of cell stress, modelling the early AMD phenotype. Interestingly, the secretion of EVs appeared to be enhanced upon RPE exposure to stress conditions, preferentially to the apical side, with the increased abundance of disease related cargo (Flores-Bellver et al., 2021). Extended analyses have been performed of iPSC-RPE EV secretome on the *CFH* Y402H high risk variant carriers in comparison to iPSC-RPE with the low risk *CFH* genotype, to fully understand the potential role of EVs in a genetic model of predisposition to AMD (Kurzawa-Akanbi et al., 2022). These analyses found that under basal conditions the secretion of EVs is enhanced in the high risk iPSC-RPE compared to the low risk RPE, with the apical secretion being most affected in the high risk variant carriers (Fig. 6). These findings recapitulate those from Flores-Bellver et al. meaning that both environmental stressors and genetic predisposition factors have an early manifestation at a cellular level altering the RPE cell physiology. Accordingly, it was observed that the cargo of apical and basal-secreted EVs in high risk RPE cells is different to that of the low risk counterparts, and with clear links to AMD pathobiology (e.g. drusen components, induction of vascularisation and cell stress factors), which improves our understanding of the origin of drusen. Importantly, it was shown that the supplementation of control iPSC-RPE with high risk apical EVs is independently sufficient to induce AMD-like features in the healthy cells. These recent findings stand as a paradigm of early disease changes at a cellular level, and that iPSC-RPE cells are a suitable model to interrogate those mechanisms as they mimic the biology and function of native RPE.

iPSC-RPE cells provide ways to overcome the challenge of the restricted availability of *in vivo* RPE due to the invasive nature of the RPE harvest and reliance on post-mortem material. The unlimited possibility of generating patient specific RPE cells can fuel the molecular and genetic studies of RPE from large cohorts of AMD patients and unaffected individuals to understand the inherent features and mechanisms that could precondition the cells to disease development. Thanks to this approach, the genetic, transcriptomic, and proteomic signatures were unravelled for 127,659 RPE cells from 43 individuals with geographic atrophy and 36 controls, revealing the expression of adult RPE markers and validating the use of this model in exploration of disease mechanisms, although some populations of cells were identified to be less transcriptionally mature than others (Senabouth et al., 2022). The differential gene expression and proteomic analyses jointly identified key pathways changed in AMD and which are related to mitochondrial metabolic activity, ATP metabolic processes and oxidative stress, along with increased levels of extracellular matrix components, known drusen proteins and complement pathway components. Reassuringly, this work cross-validated earlier studies identifying the aforementioned pathways as important to AMD development. The retina is a highly metabolically active tissue, and the uncovered characteristics of model diseased cells are evidence of early metabolic perturbations in disease and cellular remodelling mechanisms (Senabouth et al., 2022).

The utility of iPSCs for disease modelling was further demonstrated by Saini et al., whereby a patient specific model of AMD RPE associated with the tightly linked homozygous risk genotypes in the age-related maculopathy susceptibility 2 (*ARMS2*) and the high-temperature requirement A serine peptidase 1 (*HTRA1*) was generated (Saini et al., 2017). The study showed that iPSC-RPE cells expressed markers associated with AMD and drusen formation, further validating the relevance of iPSC-based disease modelling to human disease. Notably, certain complement system and inflammatory factors were expressed at a higher level in all AMD patient-derived RPE (including those without the homozygous risk variants), and at an even higher level in the *ARMS2/HTRA1* polymorphisms carrying cells, underscoring the functional relevance of the genetic risk factors in AMD, as well as the presence of inherent molecular features in those cells predisposing them to disease processes.

The *ARMS2/HTRA1* risk haplotype associated with AMD contains several variants in strong linkage disequilibrium with each other, which makes their contributions to disease susceptibility indistinguishable in the cells from variant carriers. Although the existence of specific vulnerability to oxidative stress, and an association with an increased production of complement and inflammatory factors due to the *ARMS2/HTRA1* risk haplotype have been proposed, disentangling the effects of individual gene variants are difficult and the specific functions of each of the genes remain unclear (Saini et al., 2017; Yang et al., 2014a). In a recent report, Chang et al. demonstrated a state-of-the-art approach to model specific genetic risk factors by creating gene-edited iPSC-RPE cells, with a particular aim to discern the specific gene functions in this locus (Chang et al., 2023). The individual risk variants were isolated by genetic engineering in an isogenic parental low risk homozygous background, to dissect the specific vulnerabilities arising from the particular gene polymorphisms. Given the considerations that iPSC-RPE may resemble foetal RPE more than adult cells, the cells were cultured long-term and subjected to pharmacological ageing using $H_2O_2$, A2E and blue light - mimicking the oxidative environment exposure and processes naturally occurring in RPE due to the cells' roles in phagocytosis of shed photoreceptor outer segments. The findings indicated that a single polymorphism in *ARMS2* may be responsible for the oxidative stress associated damage of RPE and the disease susceptibility in the *ARMS2/HTRA1* risk haplotype carriers, which was alleviated with an anti-inflammatory antioxidant compound, sodium phenylbutyrate (Chang et al., 2023). With the advent of bioengineering approaches, this study illustrates the unprecedented opportunities of iPSC-based modelling in the development of risk models, dissecting disease mechanisms and supporting translational AMD research.

Although the majority of AMD modelling approaches have focused on the RPE, the gradual loss of choriocapillaris underlying the RPE layer from the earliest stages of disease development is also a recognised feature (Biesemeier et al., 2014). The dysregulated complement activation that has been linked to AMD and the aberrant function of high risk CFH p.Y402H protein is believed to lead to complement activation and deposition on the choroidal endothelial cells, causing endothelial cells lysis, as confirmed in human donor eye studies (Clark et al., 2010b; Mullins et al., 2011). The functional significance of this variant is unclear, but may involve reduced binding to polyanionic patterns in BrM (e.g. heparan sulfate), malondialdehyde, and monomeric C-reactive protein. Therefore, CFH p.Y402H may not effectively direct its regulatory activity in limiting complement activation and membrane attack complex (MAC)-induced cell lysis (Clark et al., 2006, 2010a, 2010b; Herbert et al., 2007; Molins et al., 2016; Weismann et al., 2011). iPSC-derived choroidal endothelial cells (iPSC-CECs) have been used to verify the hypothesis that choroidal complement and particularly CFH anomalies are important factors in initiating AMD at the choriocapillaris level (Mulfaul et al., 2022). iPSC-CECs derived from a patient with

severe dry AMD, who was homozygous for the *CFH* p.Y402H variant and also carried a separate early Stop mutation in *CFH*, showed pronounced cell surface MAC deposition when treated with normal human serum containing all complement components, in contrast to those same iPSC-CECs transduced with functional CFH protein using a lentiviral vector. These findings suggested a novel therapeutic option through the restoration of CFH levels in the choroid; nonetheless, this approach would probably be most beneficial at the earliest stages of disease progression (Mulfaul et al., 2022).

Manian et al. increased the complexity of analysis of the functional niche formed by the RPE and choriocapillaris by the generation of a 3D model of patient iPSC-derived RPE and choriocapillaris (Manian et al., 2021). In-depth characterisation demonstrated that the co-culture model recapitulated features of the *in vivo* tissue, with the ability of manipulation of the individual tissue components to interrogate the cellular crosstalk. The authors elegantly showed a strong effect of paracrine signalling from the RPE layer that is independently sufficient to initiate choriocapillaris atrophy, as well as choroidal neovascularisation. Moreover, the results suggested that the choriocapillaris loss is a direct effect of altered RPE signalling, rather than a consequence of drusen accumulation. This proposal is particularly interesting in the view of the recent studies of RPE EV-mediated signalling and experimental evidence showing the detrimental impact of EVs, derived from RPE cells of patients with AMD, on RPE cells from healthy volunteers, and their pro-angiogenic effect towards endothelial colony forming cells (Kurzawa-Akanbi et al., 2022). It would be tempting to speculate that the EV-mediated signalling may be responsible for the paracrine effect observed in the RPE-choriocapillaris model, however this remains to be elucidated.

In summary, the development of iPSC technology and complex tissue modelling approaches constitute a powerful and vitally important platform to improve our understanding of retinal biology in health and disease. This knowledge is imperative for expediting the identification of drug targets and the creation of novel treatment avenues, particularly in anticipation of the rising prevalence of AMD with the ageing global population.

### 4.2. IRDs

IRDs are a heterogeneous group of diseases of the retina caused by pathogenic variants in genes that lead to photoreceptor cell death and vision loss. The prevalence of IRDs in the population is around 1 in 2,000 individuals, with a pattern of inheritance that can be autosomal recessive, autosomal dominant, or X-linked. Today, up to 281 genes have been identified in total for IRDs (RetNet, https://web.sph.uth.edu/RetNet/, August 2023).

Retinitis pigmentosa (RP, OMIM #268000) is the most common form of IRD (1 in 3,000–5,000), with over one million people affected worldwide. RP is characterised by initial dysfunction and death of rod photoreceptors, followed by cone degeneration. Key features of RP include progressive degeneration of the mid-peripheral retina, visual field constriction, night blindness, and eventual loss of visual acuity. RP is presented as autosomal recessive (50–60% of patients), autosomal dominant (30–40% of patients), or X-linked (5–15% of patients) (Hartong et al., 2006). 15–20% of autosomal dominant RP cases are caused by mutations in six pre-mRNA processing factors (PRPFs): *PRPF8*, *PRPF31*, *PRPF3*, *PRPF4, PRPF6* and *SNRNP200* and are known as spliceosomopathies.

Cone-rod dystrophies are less common than RP (1 in 20,000–100,000) and are characterised by primary dysfunction or degeneration of cone photoreceptors followed by rod photoreceptors (Roosing et al., 2014). Leber Congenital Amaurosis (LCA, OMIM #204000) is one of the most severe forms of IRDs, with an early-onset in the childhood and a prevalence of 1 in 30,000–80,000. This devastating condition leads to severe visual impairment or blindness in the adolescence. LCA is characterised by vision loss associated with nystagmus, an initially benign fundus appearance, but with reduced to non-detectable ERG responses (Cideciyan and Jacobson, 2019).

IRDs and AMD can appear very similar, since AMD also has a genetic basis, as described in the previous Section 4.1. While AMD is age-related, early-onset inherited macular dystrophy is called Stargardt's disease (STGD1) (1 in 8,000–10,000 individuals). STGD1 is associated to variants in the visual cycle-associated gene *ABCA4*, the most commonly mutated gene in IRDs (Allikmets et al., 1997), which will be addressed in Section 4.2.3.1.

#### 4.2.1. Spliceosomopathies

*4.2.1.1. Insights into spliceosome and spliceosomopathies.* Precursor messenger RNA (pre-mRNA) splicing is a fundamental process that results in removal of the introns and generation of the mature messenger (mRNA), which is used as a template for the synthesis of protein during translation. Several conserved intronic sequences (namely the 5′ and 3′ splice site, and the branch point typically located 18–40 bp upstream of the 3′ splice site) facilitate splicing out of the intron from the pre-mRNA. Definition of intronic borders also requires the involvement of RNA binding proteins, which interact with specific sequences located near splice sites. The intron splicing is carried out in two transesterification reactions with the first cleaving the 5′ splice site and joining the 5′ end of the intron to the branch point, and the second cleaving the 3′ splice site and the subsequent ligation of the 5′ and 3' exons (Will and Luhrmann, 2011). In addition to constitutive splicing, pre-mRNAs can undergo alternative splicing in a tissue, cellular, or developmental time-regulated pattern, resulting in selection of specific exons/introns and expression of multiple protein isoforms from a single gene (Ule and Blencowe, 2019). Both constitutive and alternative splicing processes are catalysed by a complex cellular machinery, the major spliceosome, which is composed of five small nuclear ribonucleoproteins (snRNPs) [U1, U2, U4, U5 and U6] and >200 polypeptides that assemble on pre-mRNAs to catalyse intron excision (Will and Luhrmann, 2011). A second spliceosome, known as the minor spliceosome, is present in all eukaryotes. However, it is involved in recognising a very small subset of introns (0.35% of all human introns), and its activity relies on the U12 snRNA rather than U2 snRNA subunit (Yang et al., 2022). Herein we will focus on the major spliceosome and its specific functions in retinal cells.

During the splicing process, the spliceosome complex exists in seven

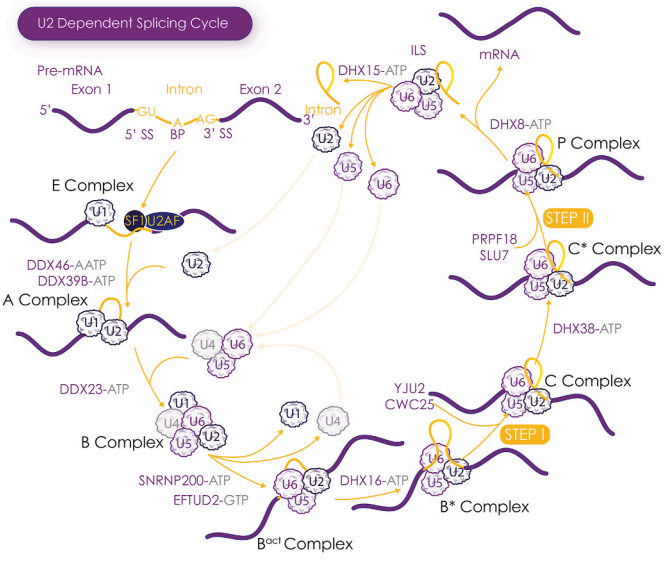


**Fig. 7. Major spliceosome splicing cycle showing the formation of various complexes.** 5′SS-5′ splice site, 3′SS-3′ splice site, BP-branch point. Figure adapted from Will and Luhrmann (Will and Luhrmann, 2011).

different configurations (Fig. 7), directed by the ordered interactions of the spliceosomal snRNPs with other accessory splicing factors (Yang et al., 2022). In the earliest intron-spliceosome interaction, named the E complex, the U1 snRNP is recruited to the 5′ splice site enabling interaction of non-snRNP factors SF1 and U2AF with the branch point and 3′ splice site respectively. In the next step, the U2 snRNP associates with the branch point displacing SF1 and forming the pre-spliceosome also known as the A complex. The U4/U6.U5 tri-snRNP is then recruited to form the pre-catalytic B complex. Significant rearrangements in RNA-RNA and RNA-protein interactions result in destabilization of the U1 and U4 snRNPs, which together with pairing of the 5′ end of U6 snRNA with the 5' splice site and U2 duplexing with the branch point, lead to the formation of the $B^{act}$ complex. Catalytic activation of the $B^{act}$ complex by DEAH-box ATP-dependent RNA helicase DHX16 results in formation of the B* complex which catalyses the first transesterification reaction. The C complex is generated after the first splicing step: this is activated by the DEAH-box ATPase DHX38, resulting in formation of the C* complex which catalyses the second transesterification reaction. At the end of second step, the P complex contain the excised intron and the spliced mRNA. Another DEAH-box ATPase dependent protein named DHX8 releases the spliced mRNA from the P complex, which forms the intron lariat spliceosome (ILS). The latter dissociates allowing the released snRNPs to engage in additional rounds of splicing.

Mutations of spliceosome components, known as spliceosomopathies, would be expected to disrupt splicing function in all cells due to their ubiquitous expression and requirement for splicing. Yet, these conditions typically affect specific cell types resulting in distinct disease phenotypes such as skeletal dysplasia, myelodysplastic syndromes, spinal cord atrophies, and retinitis pigmentosa (Fig. 8). The suggested underlying pathomechanisms of spliceosomopathies stem from the notion that mutations in splicing factors interfere with spliceosome assembly, kinetics and function. This disruption leads to accumulation of tissue-specific mis-spliced transcripts. Notably, mutations in the splicing factors are mostly heterozygous mutations, thus the spliceosome as a whole is expected to be at least partially functional. This suggests that the affected tissues and organs have a lower threshold for tolerating the shift in the ratio between normal and mis-spliced transcripts. Equally, it is plausible that the aberrantly spliced transcripts confer a detrimental function and/or toxic phenotype leading to cell death and degeneration in a tissue-specific fashion (Yuan et al., 2005). We will explore these hypotheses in the context of RP spliceosomopathies and the current patient specific iPSC retinal models in the next section.

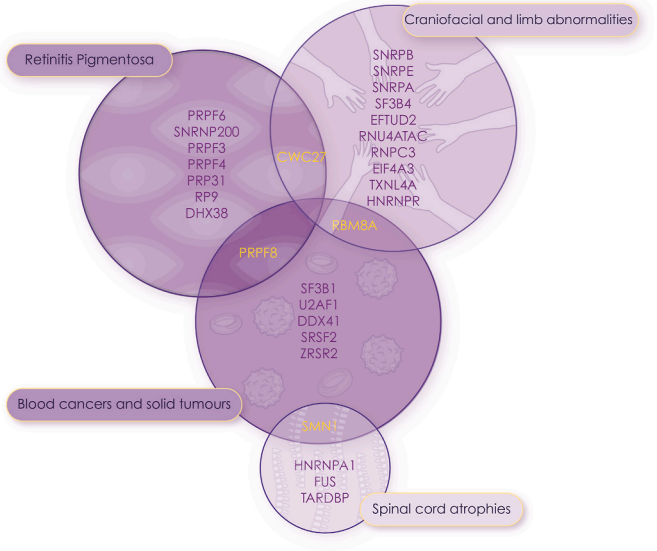


**Fig. 8.** Mutations of spliceosome components, known as spliceosomopathies, affect specific cell-types and produce distinct disease phenotypes such as skeletal dysplasia, myelodysplastic syndromes, spinal cord atrophies and retinitis pigmentosa.

*4.2.1.2. PRPF-RP.* Autosomal dominant inheritance in RP accounts for 30–40% of RP, with an estimated 15–20% of these caused by mutations in six pre-mRNA processing factors (PRPFs): *PRPF8*, *PRPF31*, *PRPF3*, *PRPF4, PRPF6* and *SNRNP200* (Fig. 8). All RP-related PRPFs are part of the U4/U6.U5 tri-snRNP (Table 1). They play crucial roles in maintaining the tri-snRNP and facilitating its integration into the spliceosome. Additionally, these PRPFs contribute to the formation of the catalytically active spliceosome, thereby exerting a significant influence on the regulation and precision of alternative splicing (Tanackovic et al., 2011b; Tanackovic and Rivolta, 2009; Wickramasinghe et al., 2015).

PRPFs are ubiquitously expressed, but mutations predominantly cause retinal-specific degeneration, raising the fundamental question of why retinal cells are more susceptible to splicing deficiencies. It has been shown that retina expresses the highest amount of spliceosomal snRNAs and processes the largest volume of pre-mRNAs (Tanackovic et al., 2011b). This may suggest that retina could have a lower threshold for tolerating deficiencies in spliceosomal components and function than other human tissues. Such a hypothesis is corroborated by the phenotype of PRPF-RP patients, who despite having splicing deficiencies in other cell types (e.g., lymphoblasts), manifest disease only in the retina. It is possible that other tissues may accumulate splicing defects at a sub-pathological level, which may not be obvious during a human lifetime, but because of the high pre-mRNA splicing activity, retinal cells could undergo degeneration and cell death due to progressive accumulation of aberrantly spliced transcripts or deregulation of circular RNAs as shown recently in the degenerating cerebellum of homozygous mice with RP-*Prpf8* variants (Krausova et al., 2023).

A related view, which may work in parallel with the low retina sensitivity threshold to spliceosome dysfunction, would rely on pathomechanisms involving selective mis-splicing of key genes that are essential for photoreceptor and RPE cell function, which is partly corroborated by the mis-splicing of a key phototransduction gene (Rhodopsin) in *Prpf31*-deficient retinal cells (Yuan et al., 2005). Notably, retinal photoreceptors are characterised by a specific splicing programme initiated before the development of outer segments which is driven by the Musashi protein (Murphy et al., 2016). This specific splicing programme affects transcripts encoding components of photoreceptor primary cilia and outer segments, both of which are essential for phototransduction. Strikingly, the splicing programme uses retinal-specific miniexons and mutually exclusive exons that are included in photoreceptor transcripts but are excluded in non-retinal cell-types (Ciampi et al., 2022; Murphy et al., 2016). Our own integrated transcriptional analysis of the developing human retina has shown that transition from foetal to adult retina is characterised by a large increase in the percentage of mutually exclusive exons that code for proteins involved in photoreceptor cilia, pre-mRNA splicing and epigenetic modifications (Mellough et al., 2019). Together these data suggest that PRPF-driven insufficiencies in spliceosome function are very likely to affect splicing of a subset of genes encoding proteins involved in cilia and outer segments, both of which are essential for photoreceptor and RPE function and survival. It is also possible that some introns are more sensitive to deficiencies in tri-snRNPs and spliceosomal complexes than others. Consistent with this hypothesis, it has been shown that repression of *Prpf8* affects the splicing of introns with weak 5' splice sites (Wickramasinghe et al., 2015). While it is not known if retinal specific genes are enriched in these suboptimal introns, it can be envisaged that if these were found in key transcripts, such as those regulating phototransduction, ciliary protein transport or outer segment renewal, they could contribute to disease onset only in the retina, but not in other tissues that do not express these proteins or depend on their function.

An alternative view is that RP manifests due to the accumulation of

**Table 1**
Summary of PRPFs involved in RP, their inferred function in animal models, patient cells and/or mammalian cell lines.

| PRPF/ associated snRNP | Function | Animal model findings | RP patients samples and mammalian cell lines findings |
|---|---|---|---|
| Prpf31/U4 | Assembly and stability of U4/U6.U5 tri-snRNP (Makarova et al., 2002; Schaffert et al., 2004) | ***Prpf31***$^{+/-}$ ***mice***: decreased RPE phagocytosis; morphological changes in ageing RPE and loss of RPE adhesion (Graziotto et al., 2011); ***Prpf31***$^{A216P/+}$ ***mice:*** RPE degeneration with drusen-like deposits, Prpf31 accumulation in insoluble cytoplasmic aggregates, impaired alternative splicing; ***prpf31 haploinsufficient zebrafish***: reduced visual function, loss of photoreceptor outer segments, changes in expression of retinal genes (Linder et al., 2011); ***prpf31 haploinsufficient fruitflies***: photoreceptor degeneration under light stress (Ray et al., 2010). | Reduction in expression of the wild type protein (Rio Frio et al., 2008); accumulation of essential spliceosome components in Cajal bodies (Schaffert et al., 2004), compromised tri-snRNP and spliceosome assembly and pre-mRNA splicing (Makarova et al., 2002), reduction in rhodopsin expression and apoptosis of rhodopsin positive retinal cells (Yuan et al., 2005). |
| Prpf3/U4, U6 | Binds the U4/U6 and stabilises the U4/U6.U5 tri-snRNP (Liu et al., 2006, 2015; Nottrott et al., 2002) | ***Prpf3***$^{T494M/T494M}$ ***mice***: decreased RPE phagocytosis; morphological changes in ageing RPE; decreased rod function (Graziotto et al., 2011) ***prpf3***$^{+/-}$ zebrafish, no retinal degeneration ( Graziotto et al., 2008) | Tri-snRNPs assembly is not affected, but these contain reduced amounts of hSnu114 and SNRNP200 ( Tanackovic et al., 2011b); delayed spliceosome formation; impaired alternative splicing ( Tanackovic et al., 2011b) |
| Prpf4/U4, U6 | Part of the U4/U6 and U4/U6.U5 tri-snRNP, important for U4/U6.U5 tri-snRNP stability ( Liu et al., 2006; Makarov et al., 2000; Makarova et al., 2002) | ***Prpf4 haploinsufficient zebrafish***: reduced visual function, loss of photoreceptor outer segments; changes in expression of retinal genes (Chen et al., 2014; Linder et al., 2011) | Altered levels of splicing factors; changes in the morphology of nuclear speckles ( Chen et al., 2014) |
| Prpf6/U5 | Essential for interaction between U5 and U4/U6 and formation of the U4/U6.U5 tri-snRNP (Liu et al., 2006) | | Accumulation of mutant Prpf6 in Cajal bodies; inhibition of snRNP assembly, impaired splicing of pre-mRNAs ( Tanackovic et al., 2011a) |
| Prpf8/U5 | Necessary for formation of U5 snRNP, regulation of SNRNP200 activity and pre-mRNA splicing | ***Prpf8***$^{H2309P/H2309P}$ **mice**: decreased RPE phagocytosis; morphological changes in ageing RPE (Graziotto et al., 2011) ***Prpf8***$^{\Delta 17/\Delta 17}$ **mice**: reduction in the | Altered alternative splicing (Papasaikas et al., 2015; Tanackovic et al., 2011b; Wickramasinghe et al., 2015); the tri-snRNP assembly is not affected, however |

**Table 1** (*continued*)

| PRPF/ associated snRNP | Function | Animal model findings | RP patients samples and mammalian cell lines findings |
|---|---|---|---|
| | | retinal thickness; delayed b wave in ERG | these contain reduced amounts of hSnu114 and SNRNP200; inefficient spliceosome assembly and reduced pre-mRNA splicing ( Tanackovic et al., 2011b) |

insoluble and cytotoxic mutant protein aggregates in cells that can affect the tissue by loss-of-function (Comitato et al., 2007; Valdes-Sanchez et al., 2019). To be able to distinguish between these views, it is necessary to catalogue the tissue specific splice variants and their interactions with splicing machinery, assess their how they affect constitutive and alternative splicing and protein isoform generation, as well as post-translational modifications and localisation, in both affected and unaffected tissues. While some tissues are easily accessible in patient and unaffected cohorts (e.g., peripheral blood), others (such as retina and brain) are not, thus more complex approaches are needed to functionally assess the impact of tissue specific mis-splicing on disease pathogenesis. The advent of iPSCs combined with CRISPR-Cas9 gene editing enables an unprecedented approach to generate many patient specific tissues and isogeneic controls, which can be used for cross-comparative large scale omics studies, as well as platforms for validating the function of pathogenic splice variants. Their use in discovering the retinal specific PRPF-RP pathomechanisms is explored in the following section.

*4.2.1.3. What have we learnt so far from PRPF-iPSC models?.* The Prpf animal models have predominantly revealed RPE deficiencies, such as loss of RPE adhesion and decreased phagocytic activity; however, photoreceptor defects were rare or not observed (Table 1), demonstrating that these models are insufficient for mimicking the RP disease phenotype. As discussed in sections 2 and 3, patient specific iPSCs and their isogeneic controls (generated via CRISPR-Cas9 *in situ* gene editing), can be differentiated to both photoreceptors and RPE cells, and thus provide a unique opportunity to understand the cellular and molecular pathomechanisms of the disease as well as testing new therapies in a range of patients derived from diverse genetic backgrounds. Given that PRPF31 is the third most common contributor, behind mutations in *RHO* and *PRPH2* genes, accounting for approximately 5.5% of dominant RP, it is not surprising that the first patient iPSC-derived retinal models focused on patients with *PRPF31* mutations (Sullivan et al., 2006). Buskin et al. derived iPSC lines from three subjects with *PRPF31* c.1115_1125del11 heterozygous mutation and one with severe RP harbouring the *PRPF31* c.522_527 + 10del heterozygous mutation, reporting reduced expression of PRPF31 in both patient iPSC-derived RPE cells and retinal organoids (Buskin et al., 2018). This systematic study revealed a large range of RPE phenotypic defects including loss of apico-basal polarity, reduced phagocytic ability, impaired trans-epithelial barrier, and ultrastructural abnormalities (shorter microvilli, basal deposits), disrupted tight junctions and defects in ciliogenesis, as well as the presence of stressed and dying photoreceptors within the apical layer of retinal organoids (Georgiou et al., 2022). Importantly, this study demonstrated a more severe decrease in PRPF31 expression in RPE cells compared to other cell types assessed (iPSCs, fibroblasts and retinal organoids), which was accompanied by the highest level of transcripts with retained introns and alternative 3' splice sites. Since PRPF31 expression in the murine RPE is much higher than neural retina, a drastic decrease in its expression is likely to render the RPE more sensitive to *Prpf31* mutations (Valdes-Sanchez et al., 2019). In

support of this, global proteomic analyses demonstrated that the impact of altered pre-mRNA splicing was also much more noticeable in the RPE cells, which led to the suggestion that the RPE is the most affected cell type in PRPF31-RP. The PRPF31-RPE cellular and functional phenotypes were replicated in PRPF6-RPE cells generated from an iPSC cells of a patient harbouring the c.G2699A:p.R900H mutation, indicating a crucial role for PRPFs in RPE cell structure and function (Liang et al., 2022).

A follow up study by Rodrigues et al. derived iPSC lines from two patients with either duplication in exon 8 (c.709_734dup) or deletion in exon 4 (c.269_273del) and corroborated in full the RPE defects uncovered by Buskin et al. previously (Rodrigues et al., 2022). RPE loss of adhesion and disorganised cell to cell contact was also observed in line with altered expression of genes related to cell adhesion, adherens junctional complexes and ECM (Rodrigues et al., 2022). This group documented a significant decrease of rod photoreceptors from Day 130 of retinal organoid differentiation and a severe alteration in rod morphology particularly at the outer segments, followed by cone reduction after Day 175 of differentiation, recapitulating the RP disease phenotype. This was also seen at the molecular level, with patient retinal organoids demonstrating changes in expression of genes involved in outer segment formation, maintenance of extracellular matrix and phototransduction.

In both studies, restoration of PRPF31 expression either via CRISPR-Cas9 in iPSCs or AAV-mediated gene transfer in retinal organoids reversed the RPE and photoreceptor endophenotypes, highlighting haploinsufficiency as disease mechanism and gene augmentation therapy as a promising therapy for PRPF31 patients. While the function of individual cells was restored at a global level, AAV-mediated PRPF31 augmentation only partially restored normal phagocytosis and cilia function, hence further studies which increase the RPE targeting efficiency at earlier stages of the disease are needed to assess if restoration of RPE function will be sufficient to preserve vision in PRPF31-RP patients (Brydon et al., 2019).

To uncover the pathomechanism of disease, multi-omics and biochemical studies were conducted by our group. These revealed three key findings that may explain the retinal tissue phenotypes in PRPF-RPs:

***Retinal specific global splicing dysregulation:*** Both PRPF31-patient RPE cells and retinal organoids, but not iPSCs or fibroblasts, demonstrated impaired *in vivo* splicing assessed through transduction of a minigene reporter, mimicking the retinal specific phenotype of PRPF31-RP (Buskin et al., 2018). The patient RPE and retinal organoids displayed the highest number of alternative splice events when compared to fibroblasts and iPSCs. These appeared to be predominantly confined to the splicing programmes that affect RNA processing itself, generating a vicious circle that possibly exacerbates the impact of PRPF31 on the spliceosome function (Fig. 9). In support of this, there were no differences in tri-snRNP stability between patient iPSCs and isogenic controls, but PRPF31 photoreceptors displayed decreased expression of U4 and U6 snRNAs, and a significant accumulation of U5 snRNA in Cajal bodies (where the assembly and maturation of tri-snRNPs occurs), demonstrating a retinal specific defect of tri-sn-RNP formation. This in turn resulted in a prominent reduction of active spliceosomes in PRPF31-photoreceptors and RPE cells, and reduced nuclear speckles, the site where the spliceosomal complexes are stored (Georgiou et al., 2022). The question is whether this splicing dysregulation is specific to PRPF31- retinal cells? Published findings indicate that this is not the case, for PRPF8-iPSC-RPE cells derived from one patient with p.Val2325_Glu2331del also demonstrate global splicing defect with the most prominent mis-splicing observed for spliceosome components (e.g. *PRPF4, PRPF31*), suggesting that PRPFs malfunction may propagate to key spliceosome components to generate the disease phenotype (Arzalluz-Luque et al., 2021).

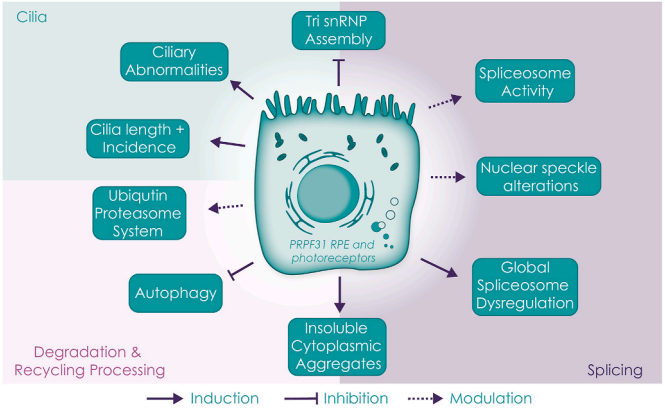


**Fig. 9.** *PRPF31* mutations cause global splicing dysregulation, ciliary abnormalities and impairment of waste disposal resulting in formation of toxic cytoplasmic aggregates in RPE cells and photoreceptors.

***Ciliary abnormalities***: As splicing factors, PRPFs are localised within the nuclear speckles of both RPE cells and photoreceptors (Buskin et al., 2018). Unexpectedly, a seminal study published by Wheway and colleagues in 2015, showed that several PRPFs involved in RP, are localised to the base of cilia and are required for normal cilial growth and function (Wheway et al., 2015). This has led to speculation that PRPFs may have specific roles in RNA metabolism and transport, independent of their role in splicing (Johnson and Malicki, 2019). Photoreceptors contain a highly specialised non-motile cilium namely the outer segment, where phototransduction takes place (Fig. 10). The connecting cilium in the photoreceptors is the intracellular link between the inner and outer segments, (equivalent to the transition zone of cilia in other cells) and is essential for transporting cargo between these two compartments (Khanna, 2015). Dysfunction of photoreceptor cilia due to mutations in photoreceptor-specific and common cilia genes lead to specific IRDs (Bujakowska et al., 2017; Chen et al., 2021; Sanchez-Bellver et al., 2021). In view of this, it has been debated whether PRPFs misfunction in splicing or ciliary function is the route case for PRPF-RPs. Herein we review PRPF cilia localisation and phenotypic defects in PRPF-iPSC retinal cell models to reach an understanding of whether this can be the case. As observed for other cell types, PRPF31 was found in the base of the cilia of both iPSC-derived RPE cells and photoreceptors. Notably, the cilia incidence and cilia length were significantly reduced in PRPF31-RPE cells and photoreceptors, with multiple cilia showing an abnormal, bulbous shape with misaligned microtubules were observed (Buskin et al., 2018). The localisation of several proteins along the ciliary axoneme and transition zone (IFT88, CC2D2A, RPGRIP1L) was altered, suggesting defects within intra-flagellar transport. Interestingly, gene expression profiling revealed that transcripts encoding proteins involved in cilia assembly and organisation, centriole, and microtubule organisation were the most prominent across the differential exon usage transcripts identified in PRPF31-retinal organoids and fibroblasts (Buskin et al., 2018; Nazlamova et al., 2022; Rodrigues et al., 2022). By extracting the sequences of all splice sites in controls and the exons skipped in PRPF mutant samples (including PRPF31-retinal organoids), and calculating the splice site strength, Nazlamova and colleagues showed that spliceosome defects due to *PRPF31* and *PRPF6* mutations, predominantly affected the splicing of exons with weak splice sites (Nazlamova et al., 2022). Importantly, consistent enrichment of genes with the ontology terms “microtubule”, “centrosome”, “centriole”, and “cilium” were observed within the affected exons with weak splice sites, suggesting that transcripts encoding microtubule, centrosomes, centriole and cilia proteins may be more susceptible to mis-splicing in cells with *PRPF* mutations. Would this on its own lead to a retinal phenotype? Fibroblasts and cell lines with heterozygous *PRPFs* knock-in mutations or PRPF knockdowns, also show ciliary abnormalities, yet they do not display overt endophenotypes (Nazlamova et al., 2022; Wheway et al., 2015). However, these cells may not depend on their cilia function as much as photoreceptors, leaving open the possibility that

ciliary dysfunction may play an important role in PRPF-RP pathogenesis.

***Progressive accumulation of toxic cytoplasmic aggregates***: Mislocalisation and accumulation of misfolded proteins into aggregates is a pathological feature of many neurological diseases including IRDs (Kosmaoglou et al., 2008; Smith et al., 2015). Several causes are thought to induce protein aggregation, and these include mutations, errors in protein synthesis, environmental factors and importantly splicing defects, which often lead to formation of misfolded proteins. The latter are ubiquitinated and targeted for degradation by the proteolytic degradation machinery; however, if this is defective, these aggregates accumulate over time, starving the affected cells of key proteins needed for their function. Earlier findings reporting aggregation of mutant Prpfs into insoluble cytoplasmic deposits in photoreceptor and RPE cells, but not epithelial or fibroblasts cell lines in mouse mutants, led to the speculation that retinal cell degeneration in PRPF-RP may be due to progressive accumulation of cytotoxic aggregates, which affect cell survival (Comitato et al., 2007; Valdes-Sanchez et al., 2019). There is evidence this also occurs in human retinal cells. Using a PRPF31-iPSC-RPE cell model, we demonstrated a significant reduction of wild type PRPF31 in the nucleus and accumulation of mutant PRPF31 in insoluble cytoplasmic aggregates, which also trapped therein misfolded proteins, belonging to visual cycle, mRNA splicing and stress response (Georgiou et al., 2022). These events led to upregulation of multiple proteins acting on the unfolded protein response and autophagy. These would normally remove damaged organelles and protein aggregates; however, key proteins acting on these pathways were also recruited into the aggregates, resulting in the dysfunction of waste disposal mechanism in PRPF31-RPE cells and progressive accumulation of these cytotoxic aggregates, which affected RPE cell survival. Furthermore, the activity of both proteasome and autophagy mediated waste clearance was downregulated, most likely as the system was overburdened with accumulated and unprocessed cellular aggregates. To relieve the burden of cellular aggregates, the PRPF31-RPE cells were treated with several compounds shown to activate autophagy or the unfolded protein response pathway. Of those, only rapamycin was able to significantly reduce aggregation and bring about an improvement in cell survival, mirroring similar clearance of cytoplasmic mutant proteins (e.g., α-synuclein, huntingtin, or tau mutant proteins) from the brains of transgenic mouse models (Rodriguez-Navarro and Cuervo, 2010; Sarkar and Rubinsztein, 2008; Webb et al., 2003).

Together the new findings derived from the iPSC-RPE models have an important significance for highlighting pathomechanisms. They demonstrate that it is not one single scenario at play in PRPF31-RP, but an integrated set of mechanisms (Fig. 9), which start with retinal specific spliceosome dysfunction that leads to a global splicing deregulation. In turn this generates misfolded proteins, that accumulate progressively into insoluble cytoplasmic aggregates, and burden the waste disposal system over time, mimicking the RPE disease phenotype and starving the retinal cells of key resources needed for normal function and survival. Superimposed on these, is the sensitivity of ciliary transcripts to spliceosome misfunction. Mis-splicing of these transcripts, results in mislocalisation of ciliary proteins in the cilia, abnormal ultrastructure, and intra-flagellar transport, that most likely affect the function of "already stressed" photoreceptors and "over-burdened" RPE cells.

Whether this integrated set of mechanisms holds true for other PRPF-RPs remains to be investigated. Early findings on the PRPF8-RPE suggest no changes in localisation of PRPF8 between patient and control cells, despite the global splicing dysregulation (Arzalluz-Luque et al., 2021).

As more PRPF-iPSC models become available, we will be able to perform integrative and cross-comparative data analyses and to ask key questions such as:

- How do different PRPFs interact with snRNPs and the 5′ and 3′ splice sites?
- What are the PRPF targets in RPE and photoreceptor cells?
- Are these shared between different PRPFs, or unique to each of them?
- Are these targets specific to retinal tissues?
- Do they have unique exon/intron features to make them more susceptible to PRPF-induced splicing deficiencies?
- Is aggregate accumulation and waste disposal dysfunction a feature of all PRPF-RPs?
- Are the RPE or photoreceptor cells the most affected?
- Is haploinsufficiency, dominant negative, or a combination of both at play in different PRPF-RPs?

Recent findings that show reduced expression of wild type PRPF31 protein in RPE cells and photoreceptors together with the inability of the mutant PRPF31 protein to be incorporated into the spliceosome, further support AAV.PRPF31 gene augmentation as an optimal approach for gene therapy treatment in PRPF31-RP patients. This approach is very likely to improve RPE and photoreceptor cell function in the early stages of the disease, where protein aggregation is not a major issue. However, for RP patients with advanced disease, both PRPF31 gene augmentation and aggregate clearance need to be combined to maintain RPE survival and function. A recent study has identified two FDA-approved compounds, which activate autophagy in RPE cells, without affecting their overall health and function (Zhang et al., 2021). It is important to assess these combined approaches in PRPF-RPE models for efficacy before moving to Phase 1 clinical trials.

#### 4.2.2. *Retinal ciliopathies*

Primary cilia are microtubule-based organelles that function as a sensor of the extracellular environment. The outer segments of photoreceptor cells are modified cilia arranged as a stack of membrane discs (Fig. 10) packed with light sensitive opsin molecules that serve as light-sensing organelle (May-Simera et al., 2017; Mill et al., 2023; Wheway et al., 2014). A short ciliary bridge or connecting cilium links the outer segment to the photoreceptor inner segment, where proteins are synthesised. Daily, about 10% of all the outer segment material is renewed and phagocytosed by the adjacent RPE, creating a high metabolic demand for the proper maintenance of this structure (Young, 1967).

Pathogenic variants in cilia genes cause a range of diseases called ciliopathies, that affect different organs, including retinal degeneration as a common feature. Importantly, around 20–25% of IRDs are associated with cilia genes (Bujakowska et al., 2017). Retinal ciliopathies are phenotypically variable depending on the cilia gene affected, presenting as non-syndromic (RP or LCA), or syndromic forms. Therefore, understanding of the molecular mechanisms underlying retinal cilia function and ciliopathies is important for the development of therapies for IRDs.

iPSCs-derived retinal organoids provide an excellent tool to investigate retinal ciliopathies, since photoreceptors contain a cilium that is observable at the apical edge of the retinal organoid (Corral-Serrano et al., 2020). In addition, cilia can be observed in other retinal organoid cell types, e.g., amacrine and retinal ganglion cells, but these have not been studied in detail yet (Ning et al., 2023). In this section, we will review recent literature on stem cell models for retinal ciliopathies, specifically for autosomal recessive RP, X-linked RP, LCA, Usher syndrome and syndromic ciliopathies. *CEP290* is the most commonly affected gene in non-syndromic LCA, accounting for ~20% of the cases (den Hollander et al., 2006). *CEP290* variants can also cause a series of syndromic ciliopathies including Joubert Syndrome (JBS), Senior–Løken syndrome, Meckel-Grüber Syndrome and Bardet-Biedl syndrome (Baala et al., 2007; Coppieters et al., 2010; Helou et al., 2007; Leitch et al., 2008; Sayer et al., 2006; Valente et al., 2006). The most common LCA variant is the deep intronic change c.2991+1655A>G, which results in alternative splicing and inclusion of a cryptic exon containing an immediate premature stop codon, p.Cys998Ter. CEP290 is essential for the initiation of ciliary transition zone assembly (Wu et al., 2020). Disruption of CEP290 in mice causes absence of photoreceptor cilia and a phenotype similar to Joubert syndrome (Rachel et al., 2015). The

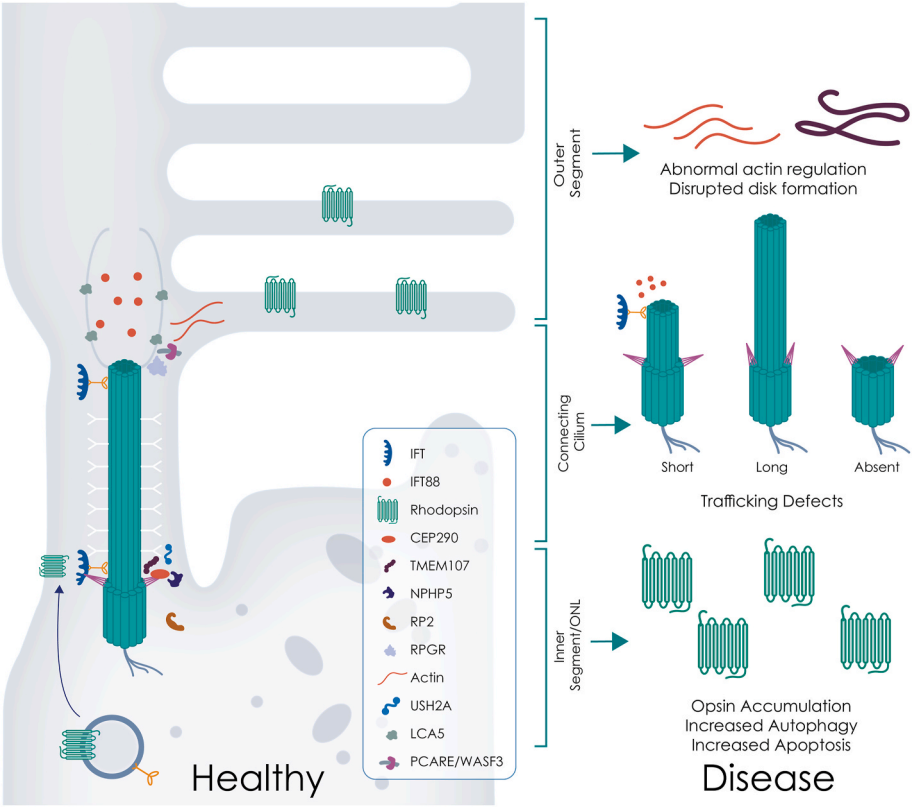


**Fig. 10. Schematic of retinal ciliopathy proteins that have been investigated using retinal organoids, and the effects observed in the disease phenotype.** Left: schematic of a photoreceptor cell focusing on the inner segments/connecting cilium/outer segments (IS/CC/OS) interface. Rhodopsin is transported to the OS via the ciliary membrane, driven by microtubular motors (kinesin-II/IFT/myosin VIIa). The transition zone proteins NPHP5, CEP290, TMEM107 and RPGR play an important role as ciliary gate keepers, controlling the access to the outer segment of membrane and soluble proteins. RP2 functions as a GTPase regulating the traffic of membrane proteins into cilia. USH2A would assist protein transport at the periciliary region. LCA5 helps to maintain integrity of the bulge region at the OS base, where PCARE and WASF3 localise to promote actin-driven OS disc formation aided by the Arp2/3 complex. Right: schematics of the disease phenotypes observed. Defects in these proteins lead to a variety of ciliary phenotypes in the CC/OS region: either short, long, or absent cilia; trafficking defects; abnormal actin regulation, and/or disrupted OS disc formation. In the IS/ONL region, defects in these proteins may lead to accumulation of opsins, increased autophagy, and increased apoptosis.

transition zone is a ciliary domain distal of the basal body that functions as a gatekeeper of ciliary protein import and export. Super-resolution microscopy revealed that CEP290 localizes throughout the length of the photoreceptor cilium, in close proximity to the microtubule doublets (Potter et al., 2021).

Modelling of CEP290 disease using human PSCs has been important to enhance our understanding of disease mechanisms, especially for the common c.2991+1655A>G variant, since CEP290 mice do not fully replicate the human *CEP290* mRNA processing (Garanto et al., 2013). CEP290-deficient cells typically show short or absent cilia. This phenotype was first modelled and rescued in CEP290-LCA patient iPSCs-derived photoreceptor precursor cells (Burnight et al., 2014). CEP290 was later modelled in retinal organoids and RPE (May-Simera et al., 2018; Parfitt et al., 2016). iPSCs-RPE cells derived from CEP290-JBS patients showed no differences in ciliation, but shorter cilia, defective tight junctions and impaired phagocytosis, while *CEP290*-LCA c.2991+1655A>G iPSCs-RPE are relatively unaffected (May-Simera et al., 2018; Parfitt et al., 2016). By contrast, the correctly spliced *CEP290* transcript expressed in LCA c.2991+1655A>G retinal organoids was only 10%–20% of total *CEP290*, whereas in fibroblasts and iPSCs-RPE it was of 40% and 50%, respectively, highlighting specific differences in splicing in the retina, potentially explaining the non-syndromic manifestation of disease with this variant (Parfitt et al., 2016). CEP290-LCA retinal organoids show decreased ciliation and short cilia, under-developed mother centrioles, membrane-less cilia and incompletely formed ciliary vesicles (Fig. 10) (Parfitt et al., 2016; Shimada et al., 2017). Another characteristic feature of CEP290-LCA patient retinal organoids is the accumulation of rhodopsin in the ONL, indicating defective rhodopsin traffic to the outer segments (Corral-Serrano et al., 2023).

Patients with biallelic variants in *NPHP5* suffer from LCA with a similar phenotype as those with *CEP290* variants (Cideciyan et al., 2011; Estrada-Cuzcano et al., 2011; Stone et al., 2011). Like CEP290, NPHP5 is required in the transition zone for ciliogenesis. NPHP5-LCA patient iPSCs-derived RPE cells and retinal organoids show reduced CEP290 protein levels and abnormally long ciliary axonemes, and accumulation of the intraflagellar transport protein IFT88 (Kruczek et al., 2022). This phenotype contrasts with earlier reports showing that inhibition of NPHP5 impeded ciliation (Barbelanne et al., 2013). The elongated ciliary axoneme phenotype could be due to the activity of residual mutant NPHP5, but this needs to be further evaluated. Rhodopsin was localised to the ONL, as observed with CEP290, suggesting it may be a hallmark of the disease (Corral-Serrano et al., 2023; Kruczek et al., 2022).

A third transition zone protein, TMEM107, has recently been investigated using retinal organoids. *TMEM107* variants are found in patients with Meckel–Gruber syndrome, Oral-facial-digital syndrome, and Joubert syndrome (Iglesias et al., 2014; Lambacher et al., 2016; Shaheen et al., 2015; Shylo et al., 2016). TMEM107 functions with NPHP4 to regulate cilium integrity, transition zone docking and assembly of membrane to microtubule Y-link connectors (Lambacher et al., 2016). The TMEM107 phenotype in retinal organoids is very

severe, with lack of primary cilia development and loss of expression of differentiation genes (Dubaic et al., 2023).

Variants in the gene *LCA5* have been linked to LCA and RP, but not to syndromic disease (den Hollander et al., 2007). Deletion of *Lca5* in mice results in delayed development of photoreceptor outer segments and disordered outer segment structure, which eventually leads to rapid degeneration of the outer and inner segments of photoreceptor cells (Boldt et al., 2011). LCA5 is part of the bulge region of photoreceptors, and it is essential for the integrity of this structure in mice (Faber et al., 2023). The bulge region of photoreceptors is an important structure of the photoreceptor cell. At this site, new outer segment discs are formed, through an actin-driven process mediated by photoreceptor cilium actin regulator PCARE, the Wiskott-Aldrich syndrome protein family member 3 (WASF3), and the Arp2/3 complex (Corral-Serrano et al., 2020). Modelling of LCA5 in patient-derived retinal organoids showed opsin accumulation in the ONL, which was rescued in isogenic gene-edited controls (Afanasyeva et al., 2023).

Retinitis Pigmentosa GTPase Regulator (*RPGR*) variants account for 70–80% of all X-linked RP cases and 10–20% of all RP cases (Chivers et al., 2021). RPGR disease modelling in iPSC-derived photoreceptor cultures displayed abnormal actin polymerisation (Megaw et al., 2017). This led to perturbation of outer segment formation and mislocalisation of rhodopsin to the inner segment, which could be rescued by over-expression of the actin capping protein gelsolin (Megaw et al., 2017). In another study, *RPGR* mutations produced shorter outer segments and deficient rhodopsin trafficking in retinal organoids (Deng et al., 2018).

iPSCs-RPE from RP patients carrying a premature stop mutation in the *RP2* gene, which is associated with up to 20% of X-linked RP, displayed defects in Golgi cohesion, and Gβ1 trafficking defects, whereas RP2 retinal organoids had kinesin trafficking defects (Schwarz et al., 2015, 2017). Furthermore, RP2 patient and RP2 knock-out retinal organoids had reduced number of rhodopsin positive photoreceptors during organoid development, which was associated with increased photoreceptor apoptosis at day 150, and reduced ONL thickness at later stages (Lane et al., 2020).

Usher syndrome is characterised by early-onset deafness, followed by photoreceptor degeneration and vision loss later in life. *USH2A* is the most commonly mutated gene in non-syndromic RP and was first modelled in iPSCs-derived photoreceptor precursor cells by Tucker and colleagues (Tucker et al., 2013). They reported that the patient variant c.7595-2144A>G caused aberrant splicing and a new exon in intron 40 of *USH2A*, while the *USH2A* Arg4192His variant caused disease through protein misfolding and ER stress (Tucker et al., 2013).

Modelling of a USH2A non-syndromic RP patient with the *USH2A* c.8559-2A>G mutation by the Jiansu Chen lab, suggested that there was abnormal organoid production and increased apoptosis, with no photoreceptor formation (Guo et al., 2019). However, using a microfluidic chip-based approach, the same authors were able to improve the differentiation of the USH2A patient retinal organoids up to 180 days, highlighting discrepancies between differentiation protocols (Su et al., 2022). In a more recent publication, USH2A non-syndromic RP patient retinal organoids showed compromised photoreceptor differentiation, with rods primarily affected and reduced rhodopsin, PDE6B and PRHP2 staining, while CRISPR correction partially improved the phenotype (Sanjurjo-Soriano et al., 2023). USH1B retinal organoids in contrast did not display any characteristic marker of degeneration, apoptosis, oxidative stress or reactive gliosis (Leong et al., 2022).

### 4.2.3. Defect in visual cycle and phototransduction

Vision is initiated by the conversion of light into electrical pulses that go from the retina to the brain. The cascade of biochemical events in the photoreceptors and RPE cells that enable this are known as visual cycle and phototransduction (Travis et al., 2007). One of the main challenges that occurs in studying both processes *in vitro* is the tissue specificity of the majority of the genes involved. The development of patient-derived retinal organoids and RPE cells makes now possible to investigate tissue specific genetic, transcriptional and post-transcriptional changes in the retina, using a human model *in vitro*.

#### 4.2.3.1. Modelling the visual cycle.

Rhodopsin is an archetypal G-protein couple receptor (GPCR) that localizes in the rod photoreceptor outer segment discs and covalently binds the chromophore 11-*cis*-retinal. The Schiff base linkage between opsin and the inverse agonist 11-*cis*-retinal helps to keep rhodopsin in an inactivate state. The first event that characterises vision is the isomerization of 11-*cis*-retinal into all-*trans*-retinal in response to absorption of a photon of light. This *cis-trans* isomerization changes the state of rhodopsin into photo-rhodopsin and then meta-rhodopsin I and II. Meta-rhodopsin II is the active form of the protein and, when it decays, it turns into apo-opsin and releases free all-*trans*-retinal. The ATP-binding cassette transporter type 4 (ABCA4) facilitates the transport of all-*trans*-retinal from the outer segment disc membrane to the photoreceptor cytoplasm, where it is reduced into all-*trans*-retinol by all-*trans*-retinol dehydrogenases, such as RDH12 (Fig. 11). The all-*trans*-retinol is then released by rods and cones, taken

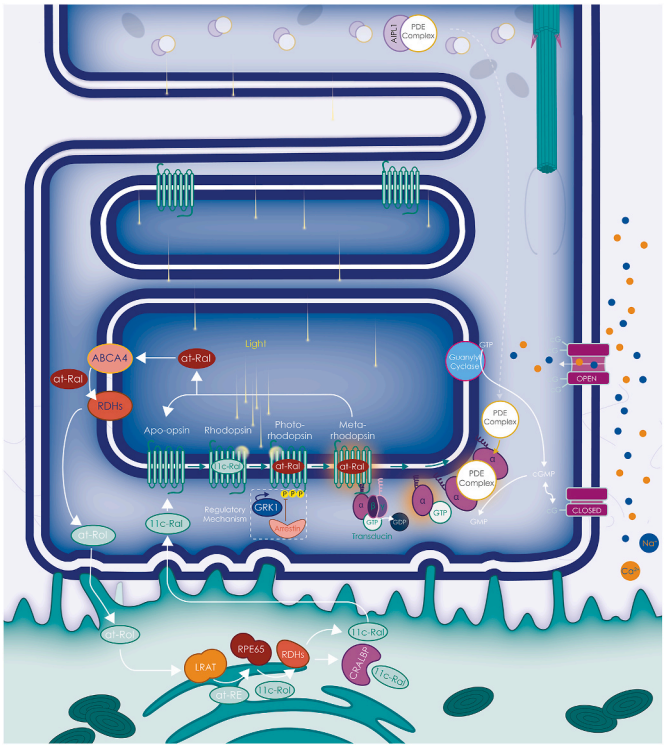


**Fig. 11. Schematic of the visual cycle and the phototransduction cascade.** In the photoreceptor outer segments, the absorption of a photon of light induces the photoisomerization of 11-*cis*-retinal (11c-Ral) into all-*trans*-retinal (at-Ral) and the conversion of rhodopsin into its active states: photo-rhodopsin first and then meta-rhodopsin. When meta-rhodopsin decays, it released at-Ral in the intradiscal space, while turning into apo-opsin. At-Ral is transported by ABCA4 into the photoreceptor cytoplasm where it is reduced into all-*trans*-retinol (at-Rol) by the RDHs. At-Rol from the photoreceptors diffused into the RPE and LRAT esterify it to al-*trans*-retinyl ester (at-RE). RPE65 isomerises al-RE into 11-*cis*-retinol (11c-Rol), while RDHs oxidise 11c-Rol into 11c-Ral. 11c-Ral can bind CRALBP in the RPE cytosol or be transported back to the photoreceptor outer segment, where it binds apo-opsin to form rhodopsin. In addition, after the *cis-trans* photoisomerization, in the photoreceptor outer segment, meta-rhodopsin activates transducin by exchange of GDP for GTP. Transducin in turn activates the PDE complex that hydrolyses cGMP and induces the closure of cGMP-gated channels. The influx of $Na^+$ and $Ca^{2+}$ leads to the hyperpolarization of the photoreceptor. The correct folding of the PDE complex subunits is driven by AIPL1. Importantly, a regulatory mechanism controls rhodopsin state of activation. GRK1 phosphorylates rhodopsin reducing its activity, while arrestin bound to phosphorylated rhodopsin leads to a full inactivation.

up by the RPE cells and esterified by the lecithin:retinol acyl transferase (LRAT). The all-*trans*-retinyl ester is a substrate for RPE65, an isomerase that catalyses a reaction to obtain 11-*cis*-retinol plus a free fatty acid. An accumulation of 11-*cis*-retinol can inhibit the isomerization reaction itself. The role of the cellular retinaldehyde-binding protein (CRALBP) is to prevent this phenomenon by binding 11-*cis*-retinol. LRAT can also esterify 11-*cis*-retinol back into 11-*cis*-retinyl ester to facilitate retinoid storage. Moreover, the 11-*cis*-retinol can be oxidised into 11-*cis*-retinal by 11-*cis*-retinol dehydrogenases, such as RDH5 and RDH11, to be released by the RPE. Thereby, the free 11-*cis*-retinal can bind the apo-opsins on the photoreceptor outer segment, form rhodopsin and start a new cycle (Choi et al., 2021; Travis et al., 2007).

Pathogenic variants in the genes involved in the retinoid cycle are associated with STGD1, RP and LCA (Travis et al., 2007). Biallelic pathogenic variants in *ABCA4* are associated with autosomal recessive STGD1. The generation of patient STGD1 and *ABCA4* gene edited derived-2D photoreceptor precursor cells (PPCs), RPE and retinal organoids encoding pathogenic variants of *ABCA4* has facilitated the investigation of disease mechanisms and potential therapeutic interventions. For example, patient and gene edited retinal organoids have been used to show how deep-intronic and common near-exonic variants in *ABCA4* lead to aberrant *ABCA4* transcript splicing. Moreover, these ABCA4 model retinal organoids have supported the development of new therapeutic approaches for STGD1 designed to block the insertion of pseudo-exons or stimulate the inclusion of skipped exons using specific antisense oligo nucleotides (AONs) (see Section 5.2.3) (Kaltak et al., 2023; Khan et al., 2020).

In 2018, Radu and colleagues demonstrated that ABCA4 expression could also be detected in RPE cells from PSC and murine models, as well as photoreceptors (Lenis et al., 2018). Recent studies have described the localisation of ABCA4 in iPSCs-derived RPE and confirmed that the loss of function of ABCA4 in the RPE could play a role in the pathogenesis of STGD1. In patient-derived RPE, the activity of ABCA4 was impaired, and both STGD1 patient-derived RPE and ABCA4 knock out RPE cells showed an intracellular accumulation of lipids and ceramides, when fed with wild type OS, together with an increase in the complement C3 activity (Farnoodian et al., 2022; Ng et al., 2022).

Loss-of-function pathogenic variants of the isomerase RPE65 are also associated with LCA and autosomal recessive RP (Travis et al., 2007). iPSCs-derived RPE cells were used to test an *RPE65* gene augmentation therapeutic approach, showing a non-linear correlation between the increase in the *RPE65* transcript and protein level (Udry et al., 2020). Moreover, patient-derived RPE cells encoding a common *RPE65* c.11+5G>A variant showed a reduction in the level of *RPE65* transcript in a tissue-specific manner (Vázquez-Domínguez et al., 2022).

*4.2.3.2. Modelling phototransduction.* Prior to decay, meta-rhodopsin II, the active state of rhodopsin, interacts with transducin $G_t$ and initiates a cascade of signalling within the photoreceptors known as phototransduction. Activated transducin exchanges GDP for GTP and consequently activates the phosphodiesterase (PDE) complex to hydrolyse cGMP (Fig. 11). In this way, the concentration of cGMP in the outer segment decreases leading to the closure of the cGMP-gated channels which blocks of the influx of $Na^+$ and $Ca^{2+}$. Therefore, the activation of rhodopsin by light triggers a hyperpolarization in membrane potential (Lenahan et al., 2020). Rhodopsin activation needs to be tightly regulated in order to allow the recovery of the protein by dark adaptation. Phosphorylation is a key mechanism in rhodopsin regulation. G-protein coupled receptor kinase (GRK1) phosphorylates rhodopsin on its C-terminus, facilitating arrestin binding and decreasing the rate of transducin activation until the inhibition of the phototransduction cascade (Pulagam and Palczewski, 2010).

Similar to the visual cycle (Section 4.2.3.1), a perturbation in phototransduction proteins leads to pathological conditions. Variants of *RHO*, GRK1, arrestin, transducin and PDE6 are all associated with forms of IRDs (Retnet, https://web.sph.uth.edu/RetNet/disease.htm). Patient-derived PSCs encoding these variants have been used to investigate the role of phototransduction proteins *in vitro* and recapitulate the progression of the "disease-in-a-dish".

The first reported study was a photoreceptor 2D model, which suggested an ER stress upregulation in rudimentary rod photoreceptors generated from RP patients encoding $RHO^{G188R}$ (Jin et al., 2012), but these did not morphologically resemble photoreceptors, akin to those seen in retinal organoids. More recently, it was suggested that there was also ER stress in early stages retinal organoids generated from ESCs carrying $RHO^{P171L}$ (Yang et al., 2023); however, again these early stage retinal organoids did not develop morphologically distinct photoreceptors. Moreover, mature retinal organoids were used to characterised late-onset RP associated with a multi-copy variant in *RHO* identified as a novel cause of autosomal dominant RP. Higher levels of rhodopsin were observed in the patient-derived retinal organoids carrying the copy-number variation, leading to a defect in outer segment formation and to the mislocalisation of rhodopsin in 200–300 day old retinal organoids. The phenotype was rescued by a pharmacological treatment targeting NR2E3, a protein involved in rod gene expression (Kandoi et al., 2023). Photoregulin3 (PR3) treatment, by reducing NR2E3 expression, can decrease the level of rhodopsin transcript and rescue rod photoreceptor maturation.

*PDE6B* variants were investigated in late-stage retinal organoids generated from *PDE6B* c.694G > A homozygous patients and recapitulated the increase in cGMP levels observed in mouse models. A mislocalisation of rod cells, defect in synapse formation and photoreceptor maturation were also reported (Gao et al., 2020). Severe forms of IRDs can be recapitulated in retinal organoids. The Aryl hydrocarbon receptor-interacting protein-like 1 (AILP1) is a retina-specific co-chaperone involved in the correct folding and assembly of the PDE6 complex (Liu et al., 2004; Ramamurthy et al., 2004). Variants in *AIPL1* are associated with LCA4, an early-onset IRD. LCA4 patient-derived iPSCs, gene corrected and AIPL1 KO iPSCs were used to produce retinal organoids that showed a decrease in the levels of AIPL1, PDE6A and B, and an increase in cGMP without affecting photoreceptor maturation (Leung et al., 2022; Lukovic et al., 2020; Perdigao et al., 2023). Interestingly, the expression of full length AIPL1 could be partially rescued in patient-derived retinal organoids with premature termination codon (PTC) variants by a translational readthrough treatment with PTC124 (Leung et al., 2022).

*4.2.3.3. Limitations of iPSCs-derived retinal organoids and RPE cells to model visual cycle.* Patient-derived retinal organoids and RPE cells have been used extensively to study disease mechanisms and to test potential therapies targeting genes involved in the visual cycle and phototransduction. However, functional assessment of light responses in these disease models has proved challenging due to several factors; including, the endogenous amount of 11-*cis*-retinal present in culture and the spatial distribution of RPE cells and photoreceptors in retinal organoids. While in the human retina RPE cells and photoreceptors interact physically, in retinal organoids the two cell-types develop in opposition, and co-culture of retinal organoids with RPE cells has not yet been shown to be stable, as discussed in **Section 3.4**. Nevertheless, several studies have reported light driven responses in retinal organoids using different kind of assays such as patch clamp, microelectrode array (MEA) or calcium signalling (Cowan et al., 2020; Hallam et al., 2018; Zhong et al., 2014). In addition, robust cone driven light responses have been shown to resemble those of the primate fovea (Saha et al., 2022). Nevertheless, it remains to be shown how disruption of the essential genes in the visual cycle and phototransduction pathways affect these light responses.

### 4.3. Childhood tumours: retinoblastoma

#### 4.3.1. Clinical and pathological features of human retinoblastoma

Retinoblastoma (RB) is a malignant neoplasm arising during retinal development from biallelic inactivation of the tumour suppressor gene *RB1* or *MYCN* amplification, with an estimated frequency ranging from 1 in 15,000 to 1 in 18,000 live births (Dyer, 2016; Pandey, 2014). It accounts for about 4–6% of all cancers in children younger than 15 years (Kivela and Hadjistilianou, 2017; Rabinowicz et al., 2013; Yang et al., 2005). Globally 8600–9000 children are affected each year with most cases diagnosed below the age of four years. The disease can manifest as unilateral in about two thirds of the patients, with the remaining one third presenting with bilateral RB. A key clinical presentation of RB is the presence of leukocoria, a white pupillary reflex due to the presence of tumours at the posterior pole of the fundus, (Fig. 12A); however, other presentations may include decreased vision, retinal detachment, glaucoma, ocular pain or inflammation (O'Brien, 2001). Treatment of RB depends on the number, position and size of the tumours in the eye, with good survival rates (e.g., 85–97% for unilateral cases and 88–100% for bilateral cases) in high-income countries, but can result in significant visual deficits and adversely affects quality of life, especially for patients that undergo eye removal (enucleation) (MacCarthy et al., 2009). Although thankfully rare in high-income countries, metastatic RB accounts for up to 50% of cases in low-income countries, where inefficient

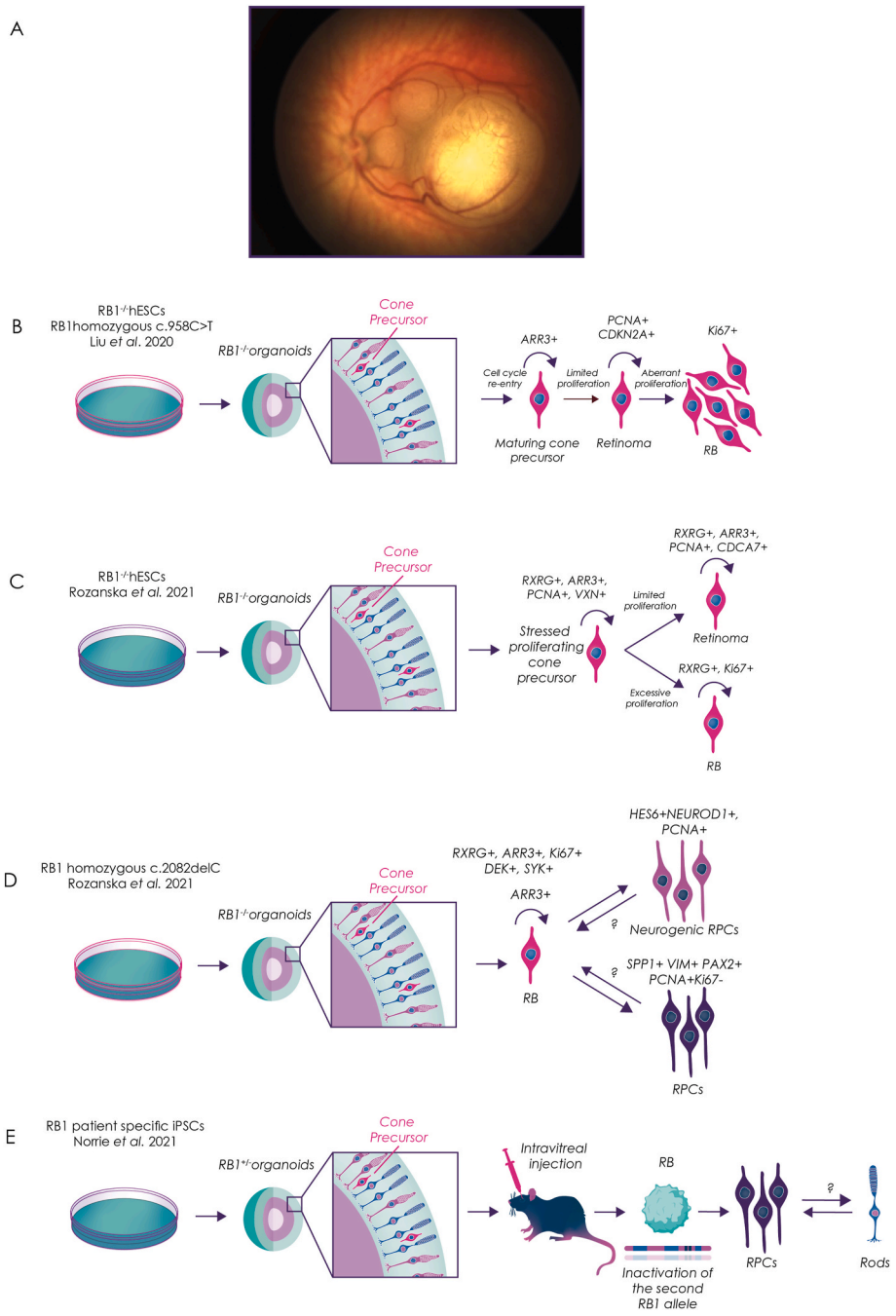


**Fig. 12. Clinical presentation of Retinoblastoma and summary of iPSC disease modelling studies. A)** Wide field colour fundal image showing a multicentric retinoblastoma at the posterior pole of the left eye in a 3-year-old child. The right eye was also affected; **B)** Retinoblastoma iPSC disease modelling studies have pinpointed proliferating cone precursors as cell or origin (Liu et al., 2020; Rozanska et al., 2022). An accumulation of retinal progenitor cells was also reported in both retinoblastoma organoids *in vitro* and tumours *in vivo* (Norrie et al., 2021; Rozanska et al., 2022).

healthcare systems result in delayed diagnosis and suboptimal care (Fabian et al., 2020; Rodriguez-Galindo et al., 2008). If the disease has spread from the eye to extraocular tissues, optic nerve, or central nervous system even with intensive treatment involving high dose chemotherapy, stem cell transplant and external beam therapy, the outcome is often fatal.

RB1 functions as a negative cell cycle regulator through its ability to bind the transcription factor E2F, leading to repression of genes needed for S phase. RB1 is also involved in chromatin organisation and regulation of cell migration, invasion, cell death and dedifferentiation, and malignant transformation events (Dyson, 2016). A two step, biallelic inactivation of the *RB1* tumour suppressor gene is required for tumour initiation in approximately 98% of Rb patients (Knudson, 1971). However, it has been suggested that biallelic *RB1* inactivation alone leads to a non-proliferative retinoma, the benign precursor to RB, and progression to malignancy requires additional genetic aberrations (Dimaras et al., 2008). Reported alterations in the *RB1* gene are well characterised and heterogeneous, including single nucleotide variants, deletions, rearrangements of the whole gene as well as chromosomal deletions (Dommering et al., 2014). However, the spectrum of cooperating gene mutations remains largely unknown, and most studies have been limited to identification of recurrent chromosomal copy number alterations including gains at 1q32, 2p24, 6p22 and losses at 16q22, most likely leading to activation of oncogenes or inactivation of tumour suppressors genes at these regions (Bowles et al., 2007; Kooi et al., 2016a, 2016b). Recent targeted next generation sequencing studies have identified a high frequency (46%) of additional somatic and likely pathogenic alterations beyond *RB1* biallelic inactivation, which correlate with aggressive histopathological features. These include focal high-level amplification of oncogenes such as *MYCN* and *MDM4* and truncating mutations in tumour suppressor genes including *ARID1A, MGA* and *BCOR* (Afshar et al., 2020). In many tissues, the loss of *RB1* is not sufficient to drive malignant transformations, thus raising questions as to why retinal cells are more prone to malignant transformation.

Treatments for RB includes local therapy with laser, cryotherapy or plaque, chemotherapy (systemic, intra-arterial, and intravitreal), external beam radiotherapy or enucleation (Yanik et al., 2015). To treat smaller tumours local therapy may be sufficient, but for eyes with more advanced tumours a combination of treatments may be required (Chawla and Singh, 2017). In the last decade there has been a shift towards conservative treatment with the aim of preserving both the globe and vision while minimising toxicity. Intra-arterial and intravitreal chemotherapy offer an alternative route of delivery, avoiding the complications of systemic chemotherapy including bone marrow suppression, alopecia and nutritional compromise in the short term and nephrotoxicity and ototoxicity in the longer term. However, these approaches are also associated with ocular and systemic morbidity such as vascular retinopathy, cystoid macular oedema, anterior segment toxicity and orbital fat atrophy, which limit their effectiveness as salvage options (Bohm et al., 2018; Dalvin et al., 2018; Munier et al., 2011; Panthagani et al., 2020; Stathopoulos et al., 2021). There is a clear need for new drugs with lower toxicity, good penetration across lipid barriers and a wide therapeutic window, as well as lab models to test candidate drug safety and efficacy. Earlier work in mice has shown that inactivation of one copy of *Rb1* does not lead to the development of RB at a detectable frequency; furthermore, no eye abnormalities were observed upon inactivation of both copies of *Rb1*, thus a mouse model for RB does not exist (Clarke et al., 1992; Jacks et al., 1992; Lee et al., 1992). Induced RB tumours in mice display enrichment of amacrine and horizontal cell interneurons (Ajioka et al., 2007). Together these data indicate that different mechanism underly the RB pathology between humans and mice and necessitate the usage of human models for revealing the molecular basis of RB.

Using RNA interference to knock down the *RB1* gene in human foetal retinal cells followed by assessment of malignant conversion *in vivo* through a xenograft model, it was shown that human RB tumours may initiate from *RB1*-depleted human maturing cone precursors (ARR3+), which enter the cell cycle, proliferate and form retinoma- and RB-like lesions. The cone photoreceptors first differentiate from the outer part of the outer neuroblastic zone during the 8th post-conceptional week of human development; however their sensitivity to RB loss occurs at the onset of ARR3 expression, around weeks 15 and 22 in the central and peripheral retina, respectively (Singh et al., 2018). This developmental window is inaccessible for research for ethical reasons, but the developments in generation of patient-specific iPSCs and differentiation to laminated 3D retinal organoids that contain all the key retinal cell types have opened new avenues for understanding the impact of *RB1* in normal retinal development and human disease. In the next section, we review how RB1-depleted PSC-derived retinal organoids have enhanced our understanding of human RB.

#### 4.3.2. Retinoblastoma PSC-derived organoid models

One of the first PSC-derived organoid models, was generated by Liu et al. who introduced a bi-allelic *RB1* common mutation (p.R320X; c.958C > T) or ablated *RB1* gene ($RB1^{-/-}$) in hESCs (Liu et al., 2020). Notably, inactivation or ablation of *RB1* in hESCs had no impact on their cell cycle, genome stability, self-renewal or pluripotency, demonstrating the cell-type specific effect of RB1 depletion and corroborating two other concurrent studies (Deng et al., 2020). Retinal organoids derived from the RB1-depleted human ESCs recapitulated key features of RB tumours during their differentiation process, showing the presence of tumour foci, inner cavities resembling Flexner–Wintersteiner rosettes that are characteristic of RB, and fleurette-like patterns which are typical of retinomas.

Altered cell cycle regulation, gene expression and DNA methylation, especially at key *RB1*-related genes such as *RXRG, SYK, MKI67* were identified (Liu et al., 2020). Moreover, at the transcriptional level the RB1-depleted organoids were more closely correlated to human primary RB tumours than the RB-derived cell lines, demonstrating that the organoids recapitulate key features of human RB as far as gene expression, DNA methylation and protein markers are concerned. *In vivo*, injection of RB1-depleted organoids cells into the subretinal or vitreal space resulted in formation of RB-like tumours with high efficiency. Those again demonstrated key features of tumours including the presence of tumour foci, expressing at high levels proliferation (Ki67) and oncogenic markers (SYK, DEK) as well as packed clusters of cells with a high nucleocytoplasmic ratio, and mitotic index. The authors used single cell RNA-Seq to analyse the cellular composition of RB1-depleted organoids, revealing the presence of four unique clusters representing RB-like cells, retinoma-like cells, stressed cells characterised by the expression of UPR-related genes, and an excessive clusters of cone precursors which expressed at high level both cone (*RXRG, ARR3*) and proliferation markers (*MKi67*). The early photoreceptor precursors ($CRX^{+}ARR3^{-}$) are postmitotic and thus do not express proliferation markers. In contrast, the abundant $ARR3^{+}$ identified in the RB1-depleted retinal organoids, showed high expression of Ki67 by immunohistochemistry, indicating their re-entry into the cell cycle. Pseudo-time analyses which help to infer the cell order based on gene expression data, demonstrated the presence of these maturing $ARR3^{+}MKi67^{+}$ cone precursors at a branch point, that was followed sequentially by the retinoma-like and the RB cell clusters, demonstrating that these could be the cell of origin for RB (Fig. 12B). Partially these data were corroborated by a follow-up study published by Rozanska et al., who differentiated $RB1^{-/-}$ human ESCs to 3D retinal organoids, showing cell growth in an anchorage-independent manner typical of tumour cells, the presence of mitotic chromosomes, and rosette-like structures, resembling pathologic features of RB (Rozanska et al., 2022). By single cell transcriptomic phenotyping of $RB1^{-/-}$ organoids, both an RB- and a retinoma-like cluster were identified. Those were thought to have risen by uncontrolled proliferation of stressed cone precursors, with high expression of UPR-related genes, which went on to generate either the retinoma or the RB-like cluster (Fig. 12C).

Both of the previous models were generated by biallelic knock-in in of a common *RB1* mutation or inactivating both copies of the *RB1* gene in hESC; thus the patient specific genetic background was not considered. To address this, Rozanska and colleagues, derived iPSCs from an RB patient with a heterozygous *RB1* mutation (c.2082delC), which were subjected to one round of CRISPR-Cas9 gene editing to generate homozygous RB1-depleted iPSCs or a fully corrected isogeneic control (Rozanska et al., 2022). Similar to $RB1^{-/-}$ organoids, the patient specific RB1 organoids with a homozygous c.2082delC mutation, showed ultrastructural features (presence of rosettes, decrease in mitochondria cristae number etc) typical of RB tumours and anchorage-independent growth in soft agar assays. Notably these features were absent in organoids derived from the patient iPSC line with the heterozygous c.2082delC mutation, or heterozygous c.623deltT mutation generated by Li et al., demonstrating that inactivation of both copies of the *RB1* gene is essential for tumorigenesis (Li et al., 2022). This corroborates the findings reported by Norrie et al., who showed that retinal organoid cells derived from RB patient-specific iPSC lines a heterozygous germline mutation gave rise to RB tumours with indistinguishable features of human RB, when injected into the vitreous cavity of immunocompromised mice (Norrie et al., 2021). These tumours developed stochastically and showed inactivation of the second *RB1* allele and no other mutations in other known cancer genes. Importantly, the authors found copy number gains in *MDM4* and *MYCN*, which are common events in RB (Norrie et al., 2021).

In contrast to hESC RB1-depleted models, a retinoma like cluster was not found in homozygous patient specific RB1-organoids. Instead, an RB-like cluster characterised by high expression of cone precursors markers (*RXRG, ARR3*), proliferation markers (*MKi67, PCNA*) and RB typical markers (*DEK, SYK*) and exclusive to homozygous patient organoids was found in addition to several clusters of retinal and neurogenic progenitor cells (Fig. 12D). The latter were enriched in RB1-depleted organoids but were also present in the heterozygous and isogenic control derived organoids, which the authors interpreted as result of a differentiation block rather than evidence of a progenitor signature. A bias towards retinal progenitors and rods was instead found in RBs generated by intravitreal injection of patient specific RB1 organoids in immunocompromised mice (Fig. 12E) (Norrie et al., 2021). In those, there was evidence of both progenitor transition to photoreceptors as well as the opposite pattern. Both studies also demonstrated that tumour cells have a mixed gene expression signature of multiple cell types. For example, Rozanska et al. showed that proliferating cone precursors which were highly enriched in RB1-depleted organoids, expressed markers of RGCs and horizontal cells, while Norrie et al. demonstrated expression of cone specific markers (e.g. PDE6H) across of a range of various retinal cell types in RB. The transient presence of cone precursor cells with gene expression signatures of several cell types has also been documented in human foetal retina, hence it is possible that cone precursors cells that re-enter the cell cycle resume a gene expression profile akin to transient neurogenic progenitors, recently shown to give rise to all the retinal neurones in a stepwise fashion (Lako et al., 2023; Sridhar et al., 2020). Equally, it possible that retinal progenitor cells give rise to more differentiated tumour cells, but this needs to be further addressed by combining clonal analysis with single cell phenotyping to determine the relationship between the various cell populations in RB as well as the dynamic changes in gene expression over time.

After reviewing all current published models, the merits of each RB-PSC organoid model depend on the specific question(s) being asked. For those interested in determining the events that result in the second inactivating *RB1* hit and development of RB, the model developed by Norrie and colleagues is particularly valuable. This allows the spontaneous *in vivo* development of RB in a similar fashion to human patients. The disadvantages of this model are the low efficiency (<5%) and time-consuming process (12–18 months per tumour) as well as the more permissive microenvironment of immunocompromised mice, which is different to human RB patients. However, if drug discovery/repurposing is the question being addressed, the RB1-depleted models developed by Liu et al. and Rozanska and colleagues are ideal, as they represent the end phenotype of RB development (Liu et al., 2020; Rozanska et al., 2022).

The models developed by these two groups have been used for testing of known RB therapeutics (e.g. melphalan, topotecan, TW-37, vincristine, etoposide, carboplatin), showing a significant reduction in the number of proliferating cone precursors or proliferating cells in a dose dependent manner. These models are easily scaled up in 96 well plates, enabling high throughput of drug testing and determination of most suitable dose that does not affect the viability of healthy adjacent retinal tissue. Their usefulness is further enhanced by the potential to discover new roles for RB1. To this end, Rozanska and colleagues discovered a decrease in RGCs, horizontal and amacrine cells alongside an accumulation of retinal progenitor cells, suggesting a differentiation block and an important role for RB1 in retinal cell differentiation. Noteworthy were their observation of retinal progenitor cell accumulation concomitant with amacrine cell reduction even in heterozygous RB1-derived organoids, which indicates that even 50% reduction in RB1 expression can affect the differentiation of these two retinal cell types. Together these findings iterate the enormous utility of PSC-derived organoids to recapitulate key events of RB development, to provide a scalable, easy to use platform for therapeutic drug development and enhance our understanding of human retinal development under normal physiological conditions and malignancy.

### 4.4. Mitochondrial disease

As the organelles responsible for generation of ATP via oxidative phosphorylation (OXPHOS), mitochondria are indispensable for cell survival and function, but they also fulfil several other roles such as calcium homeostasis, cell signalling and the regulation of apoptosis. Mitochondria have a small genome, distinct from the much larger nuclear genome and this is encoded by a 16.5 kb double stranded DNA circle (Fig. 13). This encodes only 37 genes of which 13 are involved in the OXPHOS system, two are ribosomal RNAs, and 22 are transfer RNAs, which are used for mitochondrial RNA translation (Wallace and Chalkia, 2013). In addition to these, the structural components of mitochondria and their multiple roles require more than 1100 proteins which are encoded by genes in the nuclear genome. Unlike the nuclear genome,

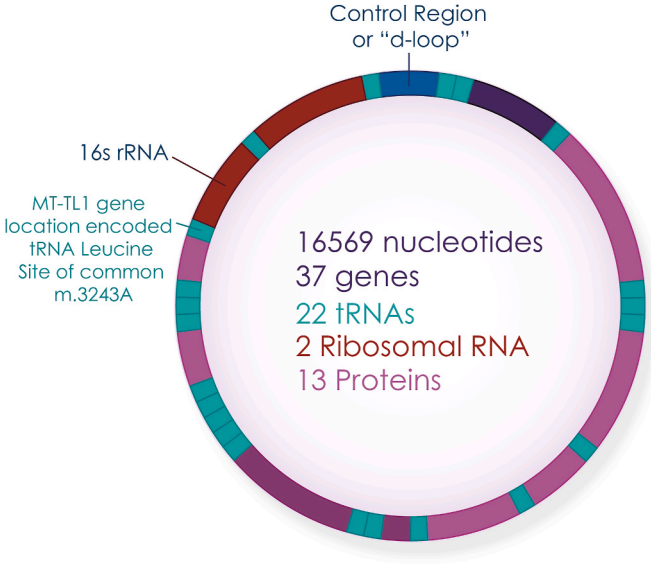


**Fig. 13. Schematic to illustrate the structural organisation of mitochondrial genome.**

mitochondrial DNA is entirely maternally inherited and each cell contains hundreds to thousands of copies (Rius et al., 2019; Shmookler Reis and Goldstein, 1983). Mitochondrial diseases arise from mutations primarily in the genes encoding oxidative phosphorylation proteins resulting in deficient ATP production.

The retina can be affected by both mitochondrial DNA variants and by acquired mitochondrial dysfunction. The outer retina has a high metabolic activity and is exposed to near constant oxidative and endoplasmic reticulum stress due to the high metabolic requirements of phototransduction, with the constant synthesis of visual pigments and outer segment discs (Shen et al., 2005). Photoreceptor cells are also relatively hypoxic, due to the absence of retinal vasculature in the outer nuclear layer. Cone photoreceptors are particularly sensitive to oxidative stress as they contain 10 times as many mitochondria as rods in primates, packed into the inner segments (Hoang et al., 2002). There is known accumulation of mitochondrial dysfunction with age linked to various age-related eye diseases including AMD, and glaucoma. Chronic oxidative damage in the RPE leads to the accumulation of damaged cellular organelles including mitochondria, and impaired mitochondrial autophagy (mitophagy) which leads to reduced clearance of damaged mitochondria contributing in turn to RPE degeneration (Sridevi Gurubaran et al., 2020). A notable difference between the mitochondrial and nuclear genomes is that the former has less effective DNA repair systems. This, coupled to the high concentrations of reactive oxygen species generated during oxidative phosphorylation, predisposes mitochondria to accumulate DNA mutations which can contribute to age related mitochondrial dysfunction. Classical mitochondria eye disease however, mostly occurs by inheritance of existing mitochondrial DNA mutations that have arisen historically and the unique potential for the use of iPSC in these diseases is now discussed.

Inherited mitochondria DNA mutations can be associated with macular disease, as well as retinal ganglion cell loss in inherited optic neuropathies (DiMauro and Davidzon, 2005; Schrier and Falk, 2011). The m.3243A>G mutation in the mitochondrial gene *MT-TL1* encoding tRNA Leucine is one of the most common mitochondrial DNA mutations with a prevalence of at least 3.5 per 100,000 in the UK (Gorman et al., 2015). It has a broad phenotypic spectrum ranging from the classical syndrome of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), to varying combinations of neurological and ophthalmological manifestations, including maternally inherited diabetes and deafness (MIDD) (Majamaa et al., 1998; Moraes et al., 1993). The mitochondrial dysfunction caused by m.3243A>G is probably explained by the decreased translation of mitochondrial RNA transcripts, especially Leu-rich proteins, such as NADH: ubiquinone oxidoreductase (complex I in the respiratory chain) core subunit 6 (ND6) (Schaffer et al., 2014). Ocular abnormalities are common with over half of all patients developing at least one ophthalmological manifestation, commonly progressive external ophthalmoplegia and ptosis, but also visual loss with a pigmentary maculopathy, or more rarely optic atrophy (Grönlund et al., 2010; Yu-Wai-Man et al., 2016; Zhu et al., 2017). Indeed, macular pigmentary abnormalities and RPE atrophy like those in AMD have been identified in about one in five of all m.3243A>G mutation carriers. The changes can often be subtle with more widespread abnormalities detected perifoveally on autofluorescence imaging (Fig. 14). Pale subretinal deposits have also been reported around the areas of RPE atrophy, which are morphologically distinct from the typical central round drusen found in AMD. Although many patients with pigmentary maculopathies can be asymptomatic, central visual loss can occur in the advanced stages (Rath et al., 2008).

The pathogenesis of mitochondrial disease has been difficult to study, largely because available patient cell lines such as fibroblasts do not represent the disease phenotypes of affected cells and accessing

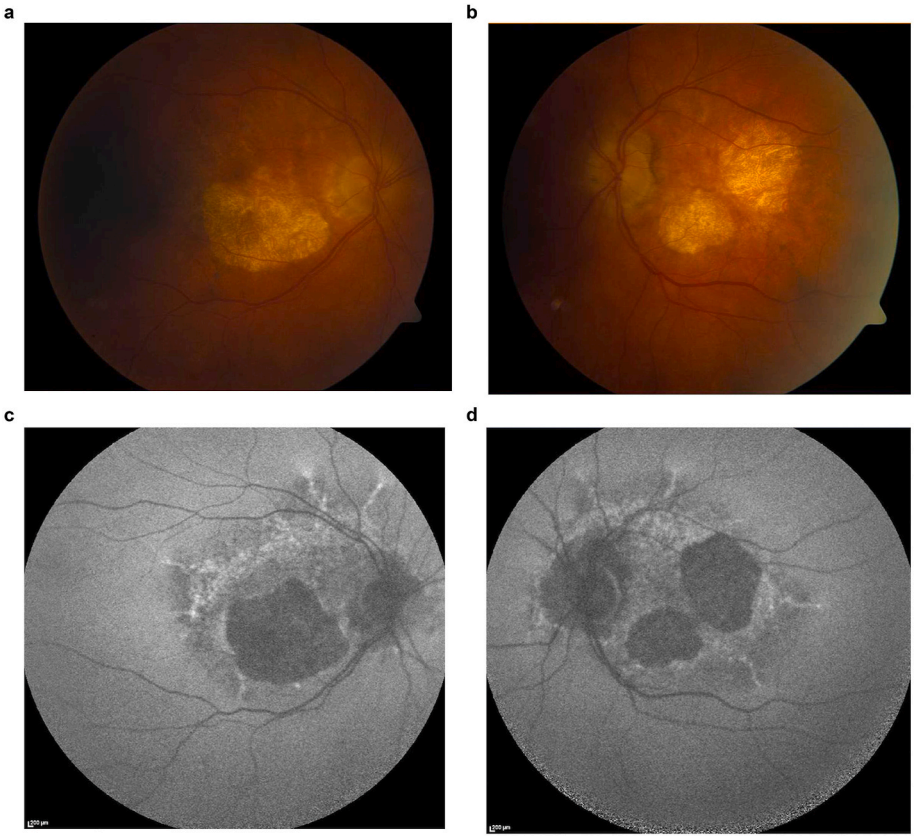


**Fig. 14. Colour and autofluorescence fundal images of a patient with a mitochondrial DNA variant associated maculopathy (A3243G).** Patches of bilateral outer retinal atrophy with RPE and choriocapillaris loss is evident, with relative foveal centre sparing. The changes are more extensive and evident on the blue wavelength autofluorescence imaging.

tissues effected by disease especially in the eye is typically not possible. An added complication is that mitochondrial diseases are frequently heteroplasmic, meaning that cells contain both healthy and mutated copies of the mitochondrial DNA. Healthy somatic cells are typically homoplasmic (i.e., all copies of the mitochondrial DNA are identical). However, cells can contain both mutated and healthy mitochondria in varying proportions, termed heteroplasmy (Payne et al., 2013). The level of the heteroplasmy is responsible for both the clinical manifestations of mitochondrial disease and the degree of metabolic dysfunction. High levels of heteroplasmy are found not only in patients with mitochondrial disease but are also seen with normal aging and increase with AMD severity (Lin et al., 2011). Heteroplasmy can be affected by several factors, including a genetic bottleneck during embryogenesis and genetic drift over generations (Floros et al., 2018; Wonnapinij et al., 2008). iPSCs have many advantages for modelling mitochondrial disease. iPSCs being highly glycolytic and not reliant on OXPHOS for energy generation, means that mitochondrial mutant iPSCs should be stable and grow normally although problems may emerge when the iPSC differentiate into somatic cell types with greater OXPHOS requirements (Lees et al., 2017). The problem of heteroplasmy still applies in iPSC-derived models. In general, heteroplasmy appears to bias toward a decreased mutant load following either reprogramming or extended cell culture (Fig. 15) (Perales-Clemente et al., 2016; Sercel et al., 2021). In contrast, directed differentiations appears to maintain the heteroplasmy level of the starting PSCs (Kosanke et al., 2021; Zambelli et al., 2018).

The use of isogenic controls is a key part of iPSC modelling. CRISPR-Cas9 gene editing can be done to correct nuclear DNA patient mutations (Fig. 16A). For mitochondrial DNA disorders, cybrids can be used to make wild type controls (Vithayathil et al., 2012). Additionally, the reprogramming process shapes the spectrum of mtDNA heteroplasmic variants, with loss of variants from the fibroblasts and appearance of the specific iPSC variants (Carelli et al., 2022; Wei et al., 2021). This can result in clones with varying levels of heteroplasmy (Fig. 16B), including some with wild type homoplasmy, which can be used as isogenic controls for mitochondrial DNA-based stem cell studies (Hamalainen et al., 2013; Prigione et al., 2011). Gene editing of the mitochondrial genome is less straightforward than nuclear DNA due to the lack of

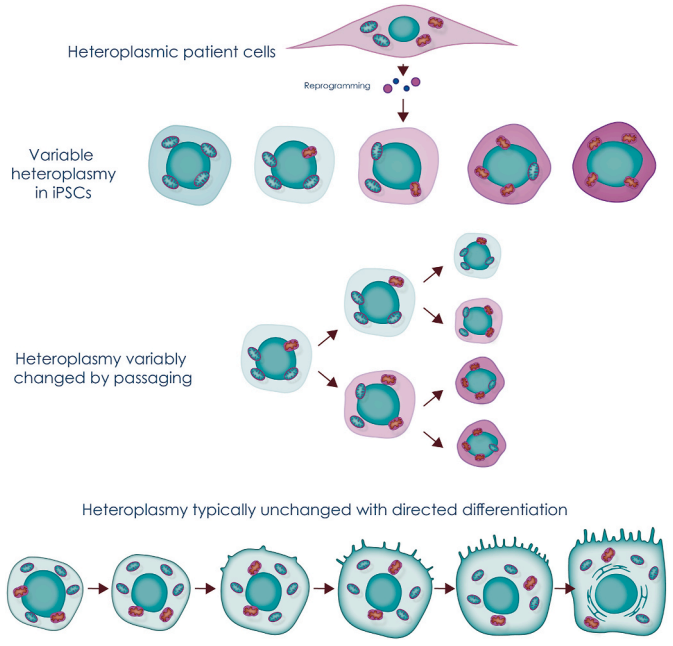


**Fig. 15. Variable levels of heteroplasmy in IPSC clones after reprogramming**. These levels can change after cell passaging, sometimes resulting in generation of iPSC clones that lack mutant mitochondria. During directed differentiation heteroplasmy is typically stable.

mitochondrial RNA import machinery meaning that CRISPR-Cas9 is not effective for editing mitochondrial DNA (Gammage et al., 2018). However, other options include mitochondrial targeted transcription activator-like effector nucleases (mitoTALENs), which target specific point mutations to decrease cellular heteroplasmy levels or generate isogenic controls with homoplasmic wild type mitochondrial DNA (Hashimoto et al., 2015; Yang et al., 2018) and high-fidelity base editors (Lee et al., 2023b).

Ultimately, the long-term goal of iPSC models is to identify a cellular phenotype that can be used to evaluate potential treatments. There are now various cellular assays, including functional ones that have been validated in differentiated cell types, particularly RPE to investigate underlying disease mechanisms and identify pathways that can be targeted for treatment. Transcriptomic and proteomic analyses can also be used to elucidate cellular compensation mechanisms and inform treatment studies, but it is important that heteroplasmy is carefully monitored and cell lines are not kept in extended culture without consistent validation of heteroplasmy.

Several groups, including our own, have generated iPSCs from patients carrying the m.3243A>G mutation and differentiated them into RPE cells with varying levels of heteroplasmy (Fig. 17) (Chichagova et al., 2017). Two high heteroplasmy clones were derived and one isogenic control with homoplastic wild type mitochondrial DNA. The isogenic control failed to differentiate to RPE so one control ESC and one control iPSC line were used. Attempts to derive iPSCs from another patient failed despite multiple attempts, with no metabolic reason discovered. We were able to show that IPSC-derived RPE cells derived from patients with MELAS and pigmentary maculopathies, contained morphologically abnormal mitochondria and melanosomes, and exhibited marked functional defects in phagocytosis of photoreceptor outer segments (Fig. 17). These had clear similarities to the pathological abnormalities reported in RPE cells studied from post-mortem tissues of affected m.3243A>G mutation carriers. Homma et al. derived iPSCs from one patient with MELAS and the m.3243A>G mutation and derived three clones with moderate heteroplasmy, demonstrating attenuated proliferation and survival of MELAS patient derived IPSC with the m.3243A>G mutation (Homma et al., 2021). Notably metabolomic analyses showed that oxidative stress markers were upregulated, but all these effects were rescued by taurine administration. iPSC-derived RPE cells showed epithelial mesenchymal transition (EMT), again suppressed by taurine. Bhattacharya et al. derived a 'high' and 'low' heteroplasmy iPSCs and RPE from one donor with MELAS and compared them to a control IPSC line and a primary RPE line. They observed heteroplasmy-dependent impairment of mitochondrial bioenergetics and reliance on glycolysis for generating energy in MELAS iPSC-derived RPE cells. The degree of heteroplasmy was directly associated with increased activation of signal transducer and activator of transcription 3, reduced adenosine monophosphate-activated protein kinase α activation, and decreased autophagic activity (Bhattacharya et al., 2022). In addition, impaired autophagy was associated with aberrant lysosomal function, and failure of mitochondrial recycling replicating changes seen in mitochondrial depleted RPE cells. Based on these findings, the authors suggested that therapies aimed at activating RPE mitophagy may have applications in the prevention or treatment of AMD.

As well as RPE, retinal organoids have high potential for use in the investigation of mitochondrial disease. Lee et al. analysed retinal organoids derived from patients with achromatopsia (a hereditary cone degeneration syndrome) with a bi-allelic loss of function mutation in activating transcription factor 6 (Lee et al., 2023a). They analysed bulk RNA-sequencing transcriptomes and relevantly showed marked dysregulation in mitochondrial respiratory complex gene expression and disrupted mitochondrial morphology. Gene expression from control and disease retinal organoids were compared with transcriptome profiles of seven major retinal cell types generated from recent single-cell transcriptomic maps of non-diseased human retina. This indicated

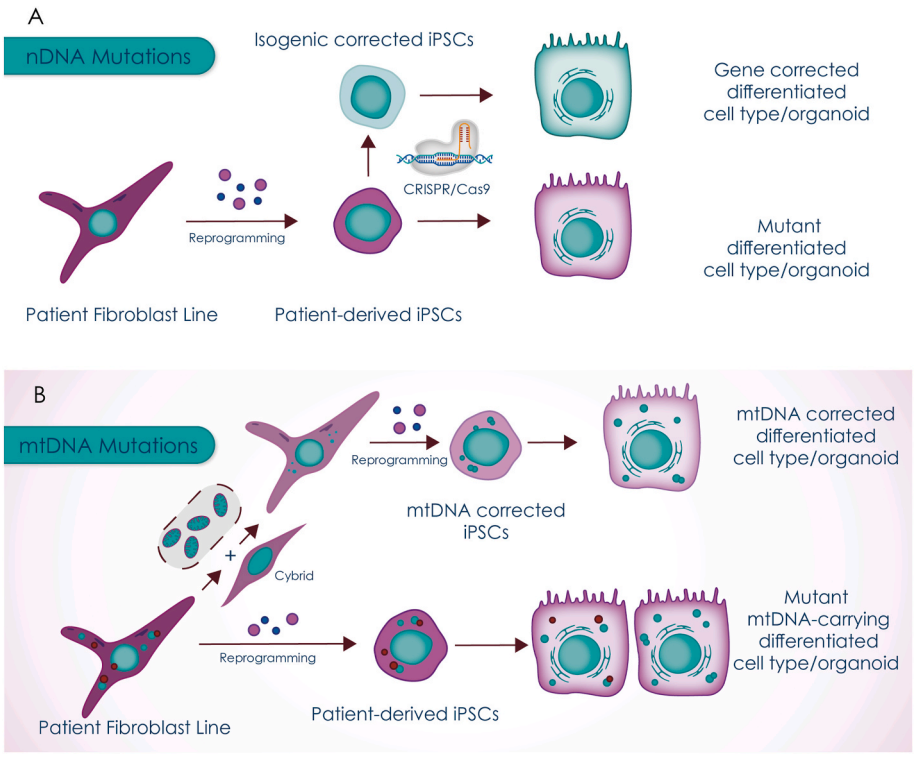


**Fig. 16. iPSC modelling of mitochondrial diseases.** Cells with nuclear DNA variants obtained from disease carrying patients are expanded. Gene editing techniques can be used either before, but more commonly after reprogramming to correct disease carrying lines and create isogenic controls. For mitochondrial DNA variants, generation of isogenic controls can be achieved prior to reprogramming by cybrid formation, as well as after reprograming in heteroplasmic controls using mitochondrial targeted transcription activator-like effector nucleases (mitoTALENs). Homoplasmic controls clones can also occur by chance during reprogramming.

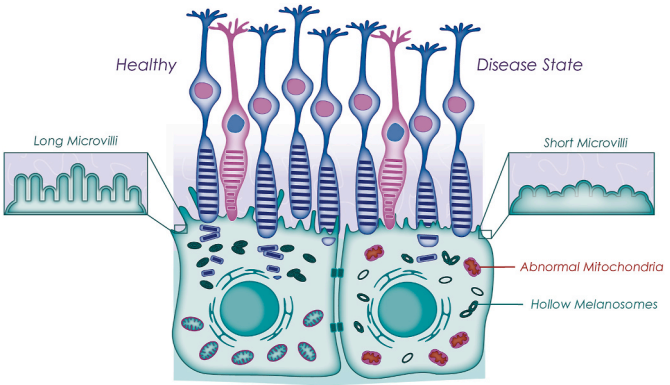


**Fig. 17. iPSC-derived RPE cells from patients with the mitochondrial DNA 3243A>G variant and pigmentary maculopathies display several key morphological and functional abnormalities.** Normal RPE cell typically display numerous mitochondria in a perinuclear distribution, with solid black melanosomes, predominantly positioned apically with long microvilli extending from their apical surfaces. (Left) The disease carrying cells displayed loss of the normal baso-apical distribution of organelles with smaller numbers of less mature and fragmented mitochondria and hollow melanosomes randomly distributed around the nuclei. There was approximately one third less cells within the population of patient-derived RPE cells that were able to phagocytose photoreceptor outer segment discs (Chichagova et al., 2017).

pronounced down-regulation of cone genes and up-regulation in Müller glia genes, with no significant effects on other retinal cells.

Although this review does not attempt to cover primary optic nerve disease it is worth mentioning the potential use of iPSC-derived cells and organoids for these disease modelling studies. Inherited optic neuropathies (IONs) encompass several genetically diverse disorders characterised by RGC loss with progressive irreversible visual loss in children and young adults. Autosomal dominant optic atrophy (ADOA) and Leber hereditary optic neuropathy (LHON) are the 2 most common IONs. They share overlapping clinical characteristics despite being genetically distinct conditions. About 70% of patients with ADOA carry variants in the nuclear-encoded *OPA1* gene encoding an inner mitochondrial membrane protein. OPA1-ADOA has been modelled in iPSC and iPSC-RGCs, differentiated in 2D. These studies showed that CRISPR correction of the nuclear *OPA1* gene can rescue the deficits in bioenergetics, mitochondrial networks, enhanced cell vulnerability and mtDNA changes associated with dominant variants in *OPA1* (Sladen et al., 2021, 2022).

Conversely, LHON is a primary mitochondrial DNA disorder, typically with point variants in genes encoding key subunits of the mitochondrial respiratory chain complex I. They remain incompletely understood diseases. Maternal inheritance is clear but the mechanism behind the marked male predominance in LHON remains unclear, and other genetic or environmental factor are assumed to play a role. Furthermore, the pathways that result in largely isolated RGC loss remains indistinct, and this lack of mechanistic insight partly accounts for the currently limited treatment options. The application of iPSCs technology has great potential to advance the understanding of IONs, and to determine the factors that cause some affected RGC to degenerate, while others compensate for the mitochondrial defects (Chen et al., 2016; Harvey et al., 2022; Nie et al., 2022). Although studies have begun to investigate the effects of disease-causing variants on RGC biology using 3D retinal organoids, there is typically RGC loss within the inner layers during organoid maturation, likely due to the lack of a terminal synaptic connections and/or nutrient deprivation. 2D RGC specific differentiation protocols have thus largely been used to date (Sladen et al., 2022; Wong et al., 2017; Wu et al., 2018; Yang et al., 2020). Studies using IPSCs have also explored the modifying effect of nuclear encoded mitochondrial genes in LHON while others have shown that

pathological autophagy and mitophagy affects the predisposition to LHON while being counterbalanced by compensatory mitobiogenesis (Chen et al., 2023b). Treatment with autophagy regulators and redox modulators, as well as genetically activating mitochondrial biogenesis have led to rescue of the cellular phenotype, proving potential paths towards clinical translation (Danese et al., 2022).

## 5. Using iPSC disease models to devise new treatments

### 5.1. Drug screening/repurposing

iPSC technology has revolutionised the drug discovery sector by facilitating the generation of patient and disease relevant models in a dish at an industrial scale. The key advantages of iPSCs over other model systems, such as the ability to proliferate almost indefinitely *in vitro,* capacity to differentiate into any cell type in a patient specific manner (retaining their genetics), scope for genome editing strategies and a large-scale production, allow high-throughput screening approaches to evaluate drug efficacy. With the advent of iPSC resources and a better understanding of the genetic and environmental determinants of retinal degeneration, disease modelling using iPSC-derived cells and organoids and complementary technologies has the potential to open new avenues for novel compound testing for safety and efficacy to inform patient stratification for clinical trials. These "clinical trials in a dish" offer an unprecedented opportunity not only to identify individuals who are more likely to respond to treatment (e.g., due to a specific genetic component) or experience drug-induced toxicity, but also to gain surrogate end points of trials by establishing specific biomarker signatures of the "responder" cells. The value of these potential applications have been recognised by the FDA, who are making efforts to bring those biotechnological advances to reality (Strauss and Blinova, 2017).

#### 5.1.1. The use of PSC-RPE in developing and testing therapies for AMD

Research studies continue to provide the critical foundation for the adoption of iPSC-based approaches for disease modelling and drug development and supporting advances in precision medicine. The ability to derive patient specific cells and test them in a lab dish provides feasibility to tailor treatments to patients' individual characteristics, such as the genetic makeup, and test drugs for efficacy and safety, before administering them to the patients. This personalised approach shows a particular promise in the discovery of drugs for AMD, owing to the complexity of its risk factors, and aligns with the elucidation of disease mechanisms. Correspondingly, as outlined in the previous section, patient derived models of AMD associated with the strongest genetic risk factors, polymorphisms in *CFH* and *ARMS2/HTRA1*, have been used to discern the effects of genetic susceptibility towards AMD on retinal cell functioning and to test potential therapeutic agents. For example, the evidence of inflammatory and complement component of AMD signature was supported by the efficacy in reducing disease associated cellular lesions using a compstatin analogue Cp40 which attenuates uncontrolled complement activation in RPE cells (Cerniauskas et al., 2020). The results of *in vitro* studies were confirmed in outcomes of clinical trials, bringing first two newly approved drugs for late-stage dry AMD to the clinics (Jaffe et al., 2021; Liao et al., 2020).

In another study, an *ARMS2/HTRA1* iPSC-derived model was used to evaluate repurposing of a drug, nicotinamide, which is in clinical trials for Alzheimer's disease (NCT00580931) and glaucoma (NCT05275738) (Hui et al., 2020; Saini et al., 2017). Nicotinamide is a vitamin B3 derivative, which has been shown to exert antioxidant and anti-inflammatory properties. Its beneficial effect on RPE overall health and survival was demonstrated to be mediated by modulating expression of genes and proteins consistently associated with AMD pathobiology, including inflammatory and complement factors, providing foundation for more in depth studies of nicotinamide's clinical utility (Saini et al., 2017). Chang et al. demonstrated feasibility for repurposing sodium phenylbutyrate (NaPB), an FDA-approved drug for urea-cycle disorders, with anti-inflammatory and antioxidant activity, in the *ARMS2/HTRA1* iPSC-RPE model (Chang et al., 2023). NaPB treatment was beneficial in reducing the effects of oxidative stress and ameliorating cell death in the *ARMS2* high risk variant carriers. Similarly, curcumin, a natural plant extract, was shown to counteract the effects of oxidative stress in patient-derived iPSC-RPE cells likely through the enhancement of cytoprotective antioxidant gene expression (Chang et al., 2014). Additionally, ciclopirox olamine was identified in a high throughput screen of 5065 compounds from the commercially available FDA-approved and bioactive drug libraries, as a potent inhibitor of oxidative damage in RPE (Cai et al., 2019). The mode of action was demonstrated to be through improving mitochondrial function, including energy production and maximal respiration, and increasing cell viability after exposure to oxidative stressors in patient-derived iPSC-RPE cells. Although still rather unclear, the protective effect towards mitochondria may be through the regulation of pro-apoptotic gene expression, as shown for human immortalised RPE line ARPE-19. Notably, the efficacy of ciclopirox olamine was not evident in iPSC-RPE cells from one donor, which suggests an inherent biological variability and the need for drug screening on large sample sizes (Cai et al., 2019). Overall, the above research data underscore the important role of oxidative stress in predisposition towards AMD, and strongly imply that antioxidant administration may be one approach to treating or preventing this disorder. These findings also provide support for the beneficial effects of AREDS supplements with antioxidant activity in AMD prevention (Age-Related Eye Disease Study Research Group, 2001).

Numerous studies have reported mitochondrial deficiency features in primary RPE cell cultures from AMD donors as well as patient-derived iPSC-RPE (Ebeling et al., 2021; Golestaneh et al., 2017; Hallam et al., 2017). While not a disease specific pathology, as mitochondrial dysfunction is a common finding in neurodegenerative disorders, ameliorating the effects of compromised mitochondrial function may be beneficial in preventing the disease or slowing down its progression. The disruption in mitochondrial function does not only result in reduced pools of cellular energy, but also correlates with increased oxidative stress, autophagy dysfunction and reduced cell viability, owing to the multitude of functions exerted by the mitochondria (Nunnari and Suomalainen, 2012). Ebeling et al. demonstrated the use of iPSC-derived patient specific RPE as a platform to test drugs targeting mitochondrial function (Ebeling et al., 2022): 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), metformin, and trehalose with reported roles in inducing mitochondrial biogenesis and energy metabolism, and autophagy (trehalose), were tested for efficacy in improving mitochondrial function parameters. The results demonstrated a significant cell line variability in drug-induced effects (from improvement, through no effect, to toxicity) for all drugs tested, suggesting patient specific responses, and demonstrating the utility of iPSC-RPE testing for developing personalised medicine approaches (Ebeling et al., 2022). The efforts to make individualised drug testing a reality would require high throughput approaches in iPSC-RPE derivation and drug testing. In response to this apparent need, there are advances being made towards automation of manual procedures with the aim to improve precision and reproducibility and allow for industrial scale compound testing. Accordingly, Truong et al. demonstrated the use of commercial Fluent® workstations (Tecan Group Ltd., Männedorf, Switzerland) for the purpose of automation of iPSC culture, RPE differentiation and high throughput drug testing and end-point screening for AMD (Truong et al., 2021). This proof-of-principle study tested 18 mitochondrial targeting compounds with automated end-point screening of mitochondrial bioenergetics using the Seahorse Flux Analyzer (Agilent). As shown in other studies, the screening revealed high variation in drug response of the individual cell lines tested (Ebeling et al., 2022; Truong et al., 2021). Overall, there is a strong indication that large-scale population studies are required to evaluate the effects of putative drugs and to generate clinically relevant data.

To this end, Sharma et al. provided a proof of concept that human iPSC-derived RPE cells generated to model AMD can be used as a high throughput platform for drug screening and discovery of disease suppressing compounds (Sharma et al., 2021). Based on the molecular, morphological, and physiological characterisation of the cells, leading to the hypothesis that calcium homeostasis is fundamental to AMD mechanisms, a drug screen was performed using a commercially available Library of Pharmaceutically Active Compounds (LOPAC) with 1280 bioactive small molecules. Drug treatment along with a chemical stressor known to cause RPE cell death through calcium signalling, led to the identification of two drugs, L745,870 and aminocaproic acid. These two compounds consistently demonstrated cytoprotective effects against complement-induced stress pathways activation, pathological intracellular lipid accumulation and RPE damage, recognising them as potential disease modifying therapeutics for AMD. While L745,870 is a dopamine D4 receptor agonist, aminocaproic acid is an inhibitor for proteolytic enzymes; and the hypothesised mode of action of the drugs may be through blocking downstream pathways relevant to AMD and inhibition of proteolytic activity leading to complement activation and anaphylatoxins generation, respectively (Sharma et al., 2021). These disease specific mechanisms of action make them suitable candidates for pharmacological evaluation and testing in clinical trials.

#### 5.1.2. The use of retinal organoids and RPE in drug screening and development in IRDs and RB

Altogether, the highlighted here studies re-enforce the utility of iPSC-derived RPE models to study genetic susceptibility and idiopathic disease at a cellular level, and as a high sensitivity platform facilitating drug discovery and screening. In contrast drug screening in retinal organoids has been hampered by the lack of quantitative assays that are suitable for 3D models, which are composed of multiple cell types often arranged in a tissue specific manner. This complicates diffusion of small molecules and makes the measurement of experimental variables more challenging due to the need for spatial resolution and the difficulty of assigning the outcomes to a particular cell type. To overcome some of these challenges, Vergara and colleagues developed a 3D automated reporter screening (3D-ARQ) platform, that allows accurate quantification of fluorescent reporters via an incorporated microplate reader with *xyz*-dimensional detection. The authors found that the retinal organoid size was the major source of variability; hence they double stained the retinal organoids with Bodipy TR and Calcein AM and used the fluorescence ratio of these two dyes to normalise for size difference. The system was tested with membrane-permeant dye JC-1, which is widely used to monitor mitochondrial membrane potential. Since the mitochondria are predominantly located in the inner segment of photoreceptors, the majority of JC-1 staining should be in the putative outer nuclear layer of retinal organoids. JC-1 stained retinal organoids were then treated with a mitochondrial uncoupler (CCCP), while the control group was exposed to the vehicle (DMSO). Longitudinal quantitative analyses in live retinal organoids, demonstrated a significant decrease in JC-1 fluorescence in the CCCP-treated group, providing proof-of principle that retinal organoids can be used for drug screening in high throughput assays (Vergara et al., 2017). In this context, Srimongkol and colleagues used RB organoids to screen 133 FDA-approved drugs, identifying Sunitinib as a highly cytotoxic agent against both *RB1*-deficient and novel *MYCN*-amplified RB organoids, with ability to significantly reduce the proliferation tumour cones, which re thought to be the cell of origin for RB tumours (Srimongkol et al., 2023).

To date, applications of retinal organoids for drug discovery remain mostly at infancy and driven by insights obtained from iPSC disease models. In a model of X-linked RP caused by a premature termination codon (PTC) in the *RP2* gene, defects in Golgi cohesion, Gbeta trafficking were found in RPE cells, as well as ciliary trafficking of Kif7 in retinal organoids (Schwarz et al., 2015, 2017). Readthrough drugs, namely Ataluren and G148 could restore the full length RP2, reversing the cellular phenotypes in RP2 patient retinal organoids and RPE cells, illustrating the ability of iPSC-derived retinal models for discovering potential therapeutics. Readthrough therapy was also shown to be effective against a PTC in the *MERTK* gene using patient derived iPSC-RPE (Ramsden et al., 2017). MERTK is essential for photoreceptor outer segment phagocytosis by the RPE and PTC124 was able to reinstate phagocytosis of labelled photoreceptor outer segments at a reduced, but significant level. Furthermore, Ataluren was shown in increase the level of detectable AIPL1 protein in a retinal organoid model of LCA4, but this was not able to rescue defects in PDE biosynthesis or cGMP (Leung et al., 2022). In addition, treatment of iPSC-RPE derived from choroidermia patients with PTC124 did not lead to any increase in REP1 or any downstream effects on Rab proteins, showing that the efficacy of readthrough of PTCs can depend on the type of PTC, the context of each PTC, gene, cell type and protein as well as the level of functional protein restoration required (Torriano et al., 2018).

A potential alternative to using human retinal organoids in drug discovery is to use mouse retinal organoids that differentiate much faster. Using retinal organoids from *rd16* mutant mice, Chen and colleagues performed a high-throughput screen of over 6,000 molecules to test their ability to improve development and survival of photoreceptors with dysfunctional CEP290 (Chen et al., 2023a). Reserpine, a compound approved by the FDA for the treatment of hypertension and schizophrenia, was found as the lead compound, as it increased rhodopsin and S-opsin positive staining in the treated mouse organoids. The effect of reserpine was confirmed in CEP290-LCA patient retinal organoids and *in vivo*. At the transcriptional level, reserpine modulates the expression of phototransduction genes, mainly by reducing their expression (Chen et al., 2023a). In CEP290 organoids, accumulation of autophagosomes indicated increased autophagy, which was reduced after reserpine treatment (Chen et al., 2023a). The flavonoid eupatilin has been demonstrated to improve ciliogenesis and cilium length in different *in vitro* models of CEP290 ciliopathies, including retinal organoids (Corral-Serrano et al., 2023; Kim et al., 2018). Eupatilin modulates ciliary and synaptic genes in control organoids, mainly by downregulation of these genes, as seen with reserpine (Corral-Serrano et al., 2023). The downregulation of phototransduction and cilia genes could be beneficial in retinal ciliopathies, through reducing the burden of protein transport to the outer segments.

While there are still many challenges for adoption of iPSC-derived retinal models (discussed in section 7), the genomic and cellular specific features of these models represents a unique opportunity for revolutionising drug development and personalised medicine. With the current technological advances in iPSC patient specific disease modelling and deploying robotics for automation and scalability, the “clinical trials in a dish” become the realistic future of personalised medicine more than ever before.

### 5.2. Gene therapy

The retina’s accessibility and immune-privileged characteristics, coupled with the predominantly monogenic nature of many retinal disorders, make it an ideal candidate for precision gene editing strategies. Using customised endonucleases such as CRISPR, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs), as well as adeno-associated viral vector (AAV)-based gene augmentation or antisense oligonucleotide (ASO)-mediated RNA modification, in conjunction with iPSC-derived retinal cells and organoids, can accelerate the development of disease models and targeted therapies for ophthalmic conditions.

#### 5.2.1. Gene vectors

A well-established method for retinal gene therapy involves gene augmentation with nucleic acid cargo, such as DNA, mRNA, or small interfering RNA (siRNA), delivered via viral or non-viral vectors. Adeno-associated viral (AAV) vectors have been the preferred choice for IRDs due to their sustained gene expression capabilities (Russell et al., 2017).

Historically limited by a small cargo capacity (around 5 kb), AAV vectors now use multiple vectors (dual or triple), each carrying distinct segments of a large transgene expression cassette, that reconstitute upon co-infection (Dyka et al., 2014; Lopes et al., 2013; Maddalena et al., 2018; Trapani et al., 2014). Intein-mediated protein trans-splicing has also enabled reconstitution of large proteins in animal models and human iPSC-derived retinal organoids (Tornabene et al., 2019).

iPSC-derived retinal tissues are gaining traction for assessing AAV serotype transduction efficiency across various cell types. Several AAV capsid serotypes have demonstrated successful transduction in mouse and human iPSC-derived retinal organoids and RPE cells (Gonzalez-Cordero et al., 2018; Völkner et al., 2021). Notably, the ShH10 and 7m8 capsids (Klimczak et al., 2009), exhibit high transduction efficacy across all stem cell-derived tissues (Gonzalez-Cordero et al., 2018). Additionally, AAV2 and AAV5 are efficient vectors for transducing mouse and human iPSC-derived RPE, photoreceptors, and Müller cells (Khabou et al., 2018; Mookherjee et al., 2018; Quinn et al., 2019), though distinctions likely exist among specific variants within these serotypes (Gonzalez-Cordero et al., 2018; McClements et al., 2022). Transduction efficiency can also be influenced by organoid maturity and differentiation protocols. Early-stage retinal organoids (e.g., before Week 13) may resist transduction due to underdeveloped photoreceptor outer segments (Gonzalez-Cordero et al., 2018; Petit et al., 2017), or the presence of the corresponding cell surface receptor, such as undetectable N-linked galactose (the primary AAV9 receptor) in organoids until at least 10 weeks of maturation (Garita-Hernandez et al., 2020).

iPSC-derived disease models have facilitated the assessment of targeted AAV-mediated gene correction therapies in various conditions including, as previously mentioned (Section 4.2.1.3), RP (Brydon et al., 2019; Lane et al., 2020; Xi et al., 2022; Yoshida et al., 2014), as well as STGD1 disease (Tornabene et al., 2019), LCA (Kruczek et al., 2021, 2022; Tornabene et al., 2019), CRB1 (Boon et al., 2023), choroideraemia (Cereso et al., 2014; Duong et al., 2018), RPGR (West et al., 2022) and Bietti crystalline corneoretinal dystrophy (Jia et al., 2023). These technologies can also be combined, as demonstrated by Lane and colleagues, who developed retinal organoid models of X-linked RP due to *RP2* mutations from patient-derived iPSCs and CRISPR-edited knockouts, highlighting the effectiveness of AAV-mediated RP2 gene therapy and identifying novel photoreceptor cell death patterns (Lane et al., 2020).

Additionally, iPSC-derived tissues and organoids have shown efficient transduction with lentiviral vectors derived from the human immunodeficiency virus (Cora et al., 2019; Kallman et al., 2020; Udry et al., 2020), benefiting from low immunogenicity (Griesenbach et al., 2012; Sinn et al., 2008). Moreover, retinal organoids offer opportunities to compare retinal administration routes for viral vector-mediated therapies, considering anatomical barriers to transduction (Hammadi et al., 2023), and assess emerging non-viral delivery systems (Banskota et al., 2022; Yin et al., 2014).

#### 5.2.2. Gene editing

CRISPR technology relies on bacterial nuclease proteins, such as Cas9 derived from *Streptococcus pyogenes*, and synthetic guide RNA (gRNA) to enable precise cleavage of complementary genomic sequences (Doudna and Charpentier, 2014). The recognition of the DNA target sequence by the Cas9/gRNA ribonucleoprotein hinges on the presence of an immediately upstream protospacer adjacent motif (PAM), comprising a 5′-NGG-3′ motif, where "N" represents any of the four nucleobases (adenine, thymine, cytosine, or guanine), followed by two guanine ("G") bases (Kleinstiver et al., 2015). PAM selection, usually done computationally, minimises off-target activity (Hsu et al., 2013). Subsequent processing of these DNA breaks by cellular repair mechanisms, like non-homologous end joining (NHEJ) and homology-directed recombination (HDR), allows for precise genetic modifications, such as gene knock-ins or knockouts (Xue and Greene, 2021). CRISPR's quick design and generation, compared to protein-based DNA targeting motifs like ZFNs and TALENs (Miller et al., 2011; Urnov et al., 2010), along with its greater precision and superior multiplex gene editing capabilities, make it invaluable for investigating polygenic disorders and identifying therapeutic targets (Xue and Greene, 2021).

As discussed earlier (**Section 5.1**), there is a growing trend in modifying retinal organoid and RPE cell protocols to facilitate high-throughput screening of therapeutic compounds (Hallam et al., 2018; Vergara et al., 2017). However, this application is constrained by the developmental variability and the inherent diversity of retinal organoids and RPE cells (Cahan and Daley, 2013; Germain and Testa, 2017). Even when the cellular phenotype of a specific mutation appears evident, it can be obscured by genetic and epigenetic differences (Gore et al., 2011; Mekhoubad et al., 2012; Sandoe and Eggan, 2013; Sterneckert et al., 2014). To overcome this challenge, gene editing tools like CRISPR-Cas9 can be leveraged to establish isogenic iPSCs with identical genetic backgrounds, differing only in disease-causing variants. This can involve the correction of variants in patient-derived iPSCs or introducing pathogenic variants into iPSCs from healthy controls, facilitating valid therapeutic comparisons (Afanasyeva et al., 2023; Buskin et al., 2018; Chang et al., 2023; de Jong et al., 2022; Deng et al., 2018; Farnoodian et al., 2022; Huang et al., 2019; Karjosukarso et al., 2023; Lane et al., 2020; Rodrigues et al., 2022).

Isogenic iPSC models edited with CRISPR technology have effectively demonstrated its potential for treating RP and X-linked retinoschisis (Bassuk et al., 2016; Buskin et al., 2018; Deng et al., 2018; Diakatou et al., 2021; Huang et al., 2019; Rodrigues et al., 2022). As discussed in Section 4.2.1, our group recapitulated the morphological and functional changes in PRPF31-driven RP using iPSC-derived RPE cells and retinal organoids (Buskin et al., 2018). We demonstrated that CRISPR-Cas9-mediated correction of *PRPF31* in affected retinal cells rescued the molecular and cellular phenotypes. Rodrigues et al. extended these findings, showcasing RP-like deficiencies due to *PRPF31* haploinsufficiency and highlighting CRISPR-Cas9's potential for preventing these deficiencies by inserting a functional *PRPF31* copy into isogenic iPSC lines (Rodrigues et al., 2022). Another example of CRISPR-Cas9 gene correction comes from retinal organoids derived from a patient with the homozygous nonsense variant in *LCA5* (c.835C>T; p.Q279*) (Afanasyeva et al., 2023). The authors used RNP as delivery strategy in iPSC, that improved efficiency and precision of HDR editing while decreasing cytotoxicity. Absence of LCA5 caused opsin retention in the ONL, which was rescued after gene editing (Afanasyeva et al., 2023).

However, CRISPR-Cas9's promise in treating retinal disorders (Hung et al., 2016; Ruan et al., 2017; Yiu et al., 2016), is accompanied by significant challenges, including off-target effects and variable gene editing efficiency, particularly in specific cell types or tissues. Moreover, off-target detection is crucial (Burnight et al., 2018) due to the potential for unintended genome damage (Fu et al., 2013), even in iPSCs (Yang et al., 2014b). On-target effects like local DNA changes and chromosomal rearrangements remain poorly understood (Leibowitz et al., 2021). Nevertheless, Afanasyeva et al. performed whole genome sequencing of LCA5 gene corrected iPSC clones and confirmed only the desired changes were introduced, any single nucleotide or structural variants detected were also present in the parental line (Afanasyeva et al., 2023). However, this might not be practical in all cell line construction and will not be possible *in vivo*. Addressing these issues is vital for improving CRISPR-Cas9's efficiency and safety. Co-transfection with AAV vectors, specifically AAV6, delivering DNA donor templates, offers a means to promote HDR efficiency without the need for selection markers (Martin et al., 2019). This approach has demonstrated notable efficacy in primary cell types such as undifferentiated iPSCs, but it is unlikely to be effective in the retina where other approaches, such as prime editing, MMHJ, or HiTi, are more effective (Martin et al., 2019; Tornabene et al., 2022).

#### 5.2.3. Antisense oligonucleotides

In addition to the above-mentioned approaches, alternative gene directed therapies are under development using iPSC-derived models. One such approach involves the use of antisense oligonucleotides (ASOs), which are short RNA molecules chemically modified to modify splicing, mRNA stability or translation. This approach was used to restore full length mRNA and protein function, as well as ciliogenesis, in retinal organoids derived from iPSCs of a patient with LCA due to the common deep intronic *CEP290* c.2991+1655A>G variant (Dulla et al., 2018; Parfitt et al., 2016), ultimately leading to promising clinical trials (Russell et al., 2022). Similarly, retinal organoids derived from patients with STGD1 disease and *USH2A*-related RP were used to show recovered protein expression and normal photoreceptor development following treatment with targeted ASOs, through either the suppression of pseudo exons caused by deep intronic variants in *ABCA4*, skipping of an in-frame exon that is a pathogenic variant hotspot in *USH2A*, or to enhance exon inclusion for a common severe near intronic variant in *ABCA4* (Dulla et al., 2021; Kaltak et al., 2023; Khan et al., 2020; Tomkiewicz et al., 2022).

#### 5.2.4. Prime editing

There has been growing recent interest in gene editing techniques that eliminate the need for error-prone DNA breaks. One such approach, prime editing, comprises just two components – a Cas9 nickase fused to a modified reverse transcriptase, and a prime editing guide RNA (pegRNA) (Anzalone et al., 2019). This complex binds to the target region, inducing a nick 3 bp upstream of the PAM site. This action releases a 3′ flap that interacts with the pegRNA's primer binding site, acting as a primer for new DNA synthesis. The edited 3′ flap displaces the unedited 5′ flap, removed by cellular endonucleases. This method builds on previous base editing strategies by expanding the potential nucleotide modifications available (Scholefield and Harrison, 2021). Enhanced precision may be achieved through a structured RNA motif in engineered pegRNAs, preventing degradation and improving efficiency across various cell lines (Nelson et al., 2022). There is now considerable interest in validating these techniques in PSC-derived structures, with AAV-mediated prime editing already used to reverse the causal *RPE65* mutation in mouse models of the congenital retinal dystrophy, Leber's congenital amaurosis (Jang et al., 2022; Park et al., 2023).

In summary, combining gene therapy with iPSCs and retinal organoids has the potential to accelerate the development of precision medicine for retinal disorders, reducing reliance on animal testing and enabling innovative combination therapies.

## 6. Biomarker development

A biomarker, according to the "Biomarkers, EndpointS, and other Tools" (BEST) resource developed by the FDA and the National Institutes of Health, is "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions" (Califf, 2018; FDA-NIH Biomarker Working Group, 2016). Biomarkers are used in a clinical setting to screen for diseases, monitor disease progression and monitor responses to therapies. Biomarkers are also particularly important for the pharmaceutical and biotechnological industry, where they can be used as screening tools in drug discovery and development to stratify patients for clinical trials, monitor effectiveness of drugs and adverse effects, to name a few uses.

The "accessibility" of the retina allows a detailed analysis of its anatomy and physiology, and unsurprisingly multimodal retinal imaging techniques, such as the digital retinal photography, optical coherence tomography (OCT) and fluorescein angiography, are routinely used to diagnose and monitor retinal disease progression (Kurzawa-Akanbi et al., 2021). In the advent of artificial intelligence, this advanced analytical method can help with collating different clinical characteristics and imaging data to identify clinically relevant associations to diagnose and manage ophthalmic conditions, and there is currently a lot of research activity in this area. Groups around the world reported using machine learning approaches to detect AMD and monitor disease progression through the assessment of drusen, the earliest biomarker of retinal tissue metabolic anomalies, using OCT images (Kapoor et al., 2019). Predicting the disease course and impact of AMD on vision (i.e., the progression to advanced late stages or the effect of specific therapies), is a particular challenge. To address this need, several machine learning programmes to predict the conversion of intermediate AMD to either geographic atrophy or neovascular AMD using OCT images are currently under development (Schmidt-Erfurth et al., 2018).

Nevertheless, other convergent approaches, e.g., using biofluid markers, may also be beneficial and could be integrated into machine learning algorithms to identify meaningful associations that are otherwise difficult to disentangle by traditional analytical methods (Pucchio et al., 2022). A systematic review by Pucchio et al. collated over 250 markers with a significant link to AMD and over 70 metabolic pathways associated with AMD development that were identified across 26 studies. Various biofluids, such as plasma, serum, aqueous humour, and vitreous humour were subjected to metabolic, lipid and proteomic profiles analyses, to name the most frequent approaches. A notable observation was a significant variability in metabolic profiles uncovered, with many biomarkers showing conflicting patterns across studies, even including those most frequently reported, such as HDL-cholesterol, c-reactive protein (CRP) and total serum triglycerides. Similarly, several studies reported markers specific to neovascular AMD, however none of these studies were confirmed by other reports. The biological pathways that were associated with AMD by more than one study were oxidative stress, the glycerophospholipid pathway, 2-oxocarboxylic acid mechanism, ABC transportation, protein digestion and absorption, and mineral absorption (Pucchio et al., 2022). While there is significant variability in AMD biomarker discovery, whether arising from biological or experimental differences, there is an emerging pattern of altered lipid metabolism and inflammation associating with AMD phenotype. Importantly, both mechanisms find confirmation in human donor tissue reports, for example in the significant lipid and complement component of drusen, and in *in vitro* patient-derived iPSC studies (Saini et al., 2017; Senabouth et al., 2022; Wang et al., 2010).

For IRDs where heterogenous genetic mutations are the cause of the disease, such as RP, patient-specific iPSC retinal models have been shown to be useful in the evaluation of the pathogenicity of specific gene mutations and therefore identifying new genetic biomarkers. As an example, a homozygous pathogenic insertion in male germ cell-associated kinase (*MAK*) gene was shown to be the cause of RP (Tucker et al., 2011). This was achieved through inducing retinal differentiation of iPSCs derived from the affected proband and a patient with *MAK*-unrelated retinitis pigmentosa. Subsequent molecular analyses of the retinal precursors in comparison to undifferentiated iPSCs showed that the pathogenic mechanism was through the prevention of a retina specific transcript isoform expression. Another example is that of structural variants that cause dominant RP mapped to a locus on chromosome 17, RP17. Here, the authors used Hi-C to study the effect of the genomic rearrangement on patient derived retinal organoids to show that the structural variant caused disruption of a topological associated domain (TAD) that insulated retinal enhancers from other genes in the region, leading to ectopic expression of other genes in the region (de Bruijn et al., 2020). Patient specific iPSCs are therefore a vital tool in dissecting highly genetically heterogenous disorders where access to the affected tissue for molecular studies is difficult.

The discovery of new disease-specific biomarkers is highly reliant on understanding disease pathobiology. As outlined in the previous section, iPSC-based disease modelling constitutes a platform for elucidation of disease mechanisms and identification of markers associated with the earliest stages of cellular dysmetabolism. Translating research findings and novel biomarkers for clinical use at scale is complex, and each candidate biomarker needs to be validated in patient studies, and assays

detecting the particular biomarker(s) must be evaluated and validated for specificity and sensitivity to be considered for adoption (Lambert et al., 2016). Biomarkers acquired from non- or minimally-invasively obtained biofluids are also a sought-after solution, which adds another layer of complexity to validating markers identified through *in vitro* studies of retinal cell models. As previously discussed, due to the biological and technical heterogeneity impacting results, further efforts are required to elucidate retinal pathobiology and to evaluate potential biomarkers in large, well-defined patient cohorts. In our labs, we have focused on understanding the biology of RPE cells carrying the common *CFH* high risk polymorphism predisposing to AMD (Cerniauskas et al., 2020; Hallam et al., 2017). We recently explored the role of EV-mediated signalling in intercellular communication between RPE and other retinal cells to understand its potential involvement in disease propagation and potential utility as a biomarker source (Kurzawa-Akanbi et al., 2022). We corroborated findings from the environmental exposure models and showed that EVs secreted by cells in a pathological state had altered contents to those produced by unaffected cells. Importantly these cargoes were active in biological pathways associated with AMD, such as oxidative stress, angiogenesis and actin cytoskeleton signalling (epithelial-mesenchymal transition), and are known structural contents of drusen (various proteins and lipids), indicating their putative utility as liquid biopsies (Flores-Bellver et al., 2021; Kurzawa-Akanbi et al., 2022).

Additional evidence in support of the role of paracrine signalling in AMD pathology, but not specific to EV-mediated signalling, came from an elegant study by Manian et al. showing that RPE-secreted factors are independently sufficient to induce angiogenesis and choriocapillaris atrophy. Strikingly, AMD patient serum exposure induced features consistent with choroidal neovascularisation, underscoring the altered composition of the biofluid in disease and implying the presence of disease associated markers (Manian et al., 2021). To further underscore the role of RPE secreted factors in retinal disease pathogenesis, a recent study showed an increased secretion of enzymes with elastase activity, i.e. elastin degrading enzymes, by iPSC-derived AMD RPE to the apical compartment (Navneet et al., 2023). Elastin is a major structural component of BrM, and a compromised integrity of the elastin layer has been linked to AMD and particularly choroidal neovascularisation (Navneet and Rohrer, 2022). The experimental findings of abnormally increased RPE secretion of elastase activity correlate with independent studies showing increased levels of elastin fragments and elastin autoantibodies in serum from wet AMD patients, as well as genetic findings of a link of a genetic variant near *MMP9* (matrix metalloproteinase with elastase activity) with neovascular AMD (Fritsche et al., 2016; Morohoshi et al., 2012; Sivaprasad et al., 2005). These results suggest a mechanistic link of abnormal elastin turnover with AMD and candidate biomarkers for neovascular AMD.

EVs present a novel tool for disease biomarker identification, as they encapsulate a portfolio of molecules, including those associated with the pathological state of cells (Kalluri and LeBleu, 2020). As EVs are present in various biofluids, including ocular fluids (vitreous humour, aqueous humour, tears) and blood, and cross the blood retina barrier, they present an emerging opportunity for the discovery of biomarkers with high sensitivity and specificity to the disease status and less variability across the population, as encountered by previous studies. The analysis of EVs could also potentially enable early diagnosis before any visual symptoms emerge and monitor the effectiveness of therapeutics (Chatterjee and Singh, 2023). Their potential utility could be in predicting the evolution of AMD and the risk of progression to the sight threatening stages of either geographic atrophy and/or neovascularisation, addressing the evident healthcare challenge.

Certainly, bringing EV-based diagnostics to the clinics will require an orchestrated effort in evaluating different biofluids as a source of biomarkers in large sample cohorts. As an example, Kang et al. presented a comprehensive comparative proteomic analysis of aqueous humour and aqueous humour-derived EVs from AMD patients' samples, including those before and after anti-VEGF treatment, and ARPE-19 conditioned media and isolated EVs, to identify disease associated pathways relevant to *in vivo* pathology, and potential disease biomarkers (Kang et al., 2014). Despite a significant variability in individual datasets, the authors identified several proteins, including cathepsin D, that were increased in abundance in the patients' aqueous humour samples, and their levels decreased after treatment. Although these results require verification in larger cohorts and it is unclear from this study whether utilising EVs offers benefits over the analysis of unprocessed biofluid; nevertheless, this integrated approach demonstrates a practical solution in biomarker and disease mechanisms identification, especially with the availability of patient-derived iPSCs for biomarker evaluation. Understanding the role of EVs in retinal homeostasis and whether (and how) EVs could be used as a diagnostic tool in retinal disorders remain open questions. Nonetheless, mounting evidence suggests a crucial role for these particles in intercellular communication and disease propagation and must be evaluated as a potential tool for tackling the growing prevalence of retinal disorders. This progress in vision research, together with the remarkable advancement of computing science, will undoubtedly have a positive impact on the development of modern technologies for better patient diagnostics and management.

## 7. Toxicity screens

Over 90% of drugs fail in human clinical trials using preclinical models (Sun et al., 2022). In this regard, patient-derived organoids and RPE cells offer a valuable screening platform for preclinical testing (Rawal et al., 2021). Due to the spatial organisation of multiple organ-specific cell types, organoids have huge potential as a test system for characterising the permeability of histological barriers, deposition, drug metabolism, alteration of intercellular communications, and intercellular environment reactions (Hikage et al., 2019; Kruk and Auersperg, 1992; Leite et al., 2016; Lu et al., 2017; Nikonorova et al., 2023; Park et al., 2019; Pinto et al., 2021; Spencer et al., 2020; Sugiyama et al., 2007; Takezawa et al., 1993; Walsh et al., 2014; Zhang et al., 2020). Assessing retinal drug toxicity has become important as different molecules and antibodies have become available for the treatment of neurodegenerative disorders (Poovaiah et al., 2018; Yu and Watts, 2013). 3D PSC-derived retinal organoids and RPEs largely recapitulate the structure and function of native retina and RPE respectively, and thus provide the missing link between compound screening and clinical trials (Cowan et al., 2020; Kokkinaki et al., 2011). Since retinal organoids and RPE cells can be easily derived in scalable platforms from patients with various age related and inherited conditions, studies that focus on correlation between genetic mutations and response to various drugs can be performed with ease (Kokkinaki et al., 2011; Sharma et al., 2021). As such they provide the *in vitro* disease models that recapitulate diversity of the human disease, avoiding generation of animal models with targeted mutations as in current practice. In the most recent report from The Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), 2.9 million animals have been used in EU since 1997 and a further 1.3 million animals are being used for reproductive toxicity tests, developmental toxicity tests, and repeated-dose toxicity tests for human health (Knight et al., 2023). Approximately 2000 animals are needed in the pre-clinical development phase of a single pharmaceutical product. The precise number of animals used for testing retinal toxicity is unclear, however the Home Office UK data for testing performed on sensory organs in animals in 2012 details use of 40,541 mice, 2484 rats and 504 other mammals. Sensory organs comprise skin, eyes and ears so if we assume an equal split between these, then the application of retinal organoids and RPE cells has the potential to reduce a maximum of 14,500 animal procedures a year.

To date, relatively few studies have used retinal organoids for drug toxicity assessment, mainly restricted to candidate molecules. For example, toxicity of G418 was observed in patient derived retinal organoids tested for readthrough for a PTC in RP2 (Schwarz et al.,

2017). In a proof-of-concept study, Dorgau et al. used retinal organoids to quantify toxicities of already licensed drugs with known responses in the human and animal retina *in vivo* through single cell RNA-Seq and immunofluorescence assays. The authors compared the impact of retinotoxic (dixogin, thioridazine, sildenafil, ethanol, and methanol) to benign molecules (flurbiprofen and ketorolac) to characterise their retinal toxicity (Dorgau et al., 2022). Digoxin, for example, a medicine used to treat heart failure and arrhythmias that has been linked to visual phenomena such as yellow-green discolouration, was found to cause multiple detrimental effects including cell death of photoreceptors, horizontal, amacrine, and retinal ganglion-cells, disruption of Müller glia cells, as well as activation of stress and apoptotic signalling mechanisms. The broad impact of digoxin was attributed to the inhibitory effect of this molecule on ATP-dependent sodium/potassium pumps which are expressed in many retinal cell types (Wetzel et al., 1999). Similarly the PDE5 inhibitor, sildenafil, used to manage erectile dysfunction and pulmonary hypertension, can cause transient blue/-green colour vision disturbances and was shown to result in the death or dysfunction of various retinal cell types including photoreceptors following its incubation with retinal organoids (Dorgau et al., 2022). This phenomenon has been attributed to cross-reactivity with PDE6 expressed in photoreceptors wherein it contributes to the maintenance of ion homeostasis during phototransduction (Tzoumas et al., 2020). Patients treated with the antipsychotic thioridazine may suffer from severe vision loss due to disorganisation of photoreceptor outer segments, followed by loss of RPE cells and choriocapillaris (Miller et al., 1982). This has been attributed to thioridazine-mediated inhibition of oxidative phosphorylation that results in abnormal rhodopsin synthesis. Accordingly, treatment of retinal organoids with thioridazine caused a disruption of the putative outer nuclear layer and a significant reduction in the number of photoreceptors, especially rods, horizontal and retinal ganglion cell death as well disorganisation of Müller glia cells. Although the study of Dorgau et al. was the first to show the utility of retinal organoids as toxicity assessment tools, a small number of subsequent publications are now using those to assess the toxicity of various substances including pesticides, flames retardants and other typical environmental pollutants and combined application of TNF and HBEGF (Gong et al., 2023; Volkner et al., 2022; Wang et al., 2023).

In parallel to the work carried out with retinal organoids, PSC-derived RPE cells have also been used in small proof-of-concept studies to evaluate the cytotoxicity of the recombinant tissue plasminogen activator (rtPA), which is used to treat various forms of thrombosis by dissolving blood clots. Work done in rabbits has shown that rtPA application leads to loss of photoreceptors and RPE toxicity, by enhancing *N*-methyl-D-aspartate signalling and apoptosis (Kumada et al., 2005; Nicole et al., 2001). Both the PSC-derived RPE and ARPE19 cells were exposed to various doses of rtPA, showing the sustained exposure to 100 μg/ml induced prolonged cytotoxic effects only in the PSC-derived RPE cells, but not ARPE19. Moreover, the response of the PSC-derived RPE cells was similar to human foetal RPE, demonstrating that PSC-derived RPE cells present a suitable platform for toxicity studies. This paved the way for further investigations into the cytotoxicity of RB chemotherapeutics (melphalan, topotecan and TW-37) or immunosuppressive corticosteroid-sparing agents (cyclosporin A, sirolimus, tacrolimus and lefluonomide and its active metabolite teriflunomide), which are used to treat inflammation in a range of disorders including ocular disease, often through intraocular injections (Cerna--Chavez et al., 2023; Oliveira et al., 2021).

It is notable that PSC-derived retinal organoids and RPE cells are now manufactured commercially as a drug development/toxicity assessment tool [Building functional in vitro models to improve clinical translation (newcellsbiotech.co.uk); NeuroS - Creative Biolabs (creative-biolabs.com)]. Their availability together with improvements in robotics, automated organoid imaging (3D Cell Model Landing Page (moleculardevices.com)), and machine learning should accelerate their application in drug discovery/repurposing and toxicity studies (Fujimura et al., 2023; Kegeles et al., 2020).

### 7.1. Current challenges in using PSC-derived RPE and retinal organoids in toxicity testing

So far, the retinal organoids and RPE models used in early toxicity screens are relatively simple as they do not include immune cells or inner/outer retina blood barriers that are present *in vivo* and are critically involved in drug absorption, metabolism, and flux across the retina blood barriers. While great advances have been made in the recreation of the oBRB (reviewed in **section 3.4**), vascularised retinal organoids with incorporated human microglia cells, which also recapitulate the RPE-photoreceptor interactions have not been reported yet. The lack of this complex microenvironment limits the nutrient availability, metabolite removal, leading to hypoxia and necrosis in the centre of the organoids. Furthermore, both the RPE and retinal organoids transcriptionally and functionally still resemble foetal tissue counterparts (Hallam et al., 2018). Importantly, current protocols do not generate standardised retinal organoids, hence sample heterogeneity can lead to spurious results.

Differentiation of retinal organoids mimics the timeline of human retinal development, often taking up to one year to generate mature organoids. This can pose a significant barrier for large scale application because of time and cost implications. Despite worldwide efforts, accelerated generation of mature human retinal organoids has not been achieved, hence many laboratories have surpassed this difficulty by setting up continuous batches of organoids, which allows on demand experiments. An important challenge for both iPSC-derived RPE cells and retinal organoids, is the intra line and intra-clonal variability with regard to differentiation efficiency, attributed to differences in epigenetic memory, signalling pathway activity and method of differentiation (Cooke et al., 2023; Hallam et al., 2018; Hiler et al., 2015; Leach et al., 2016). Most labs bypass this challenge by generating several iPSC lines from the same donor and isogenic controls, which are then pre-screened for differentiation efficiency, that further adds to the costs and timing needed to generate retinal organoids from many individuals. Large scale iPSC derivation initiatives such as HipSci and StemBancc, coupled with worldwide iPSC banking efforts, provide unique resources for the research and pharma community to select appropriate number of cell lines, so adequately powered experiments that consider these variations can be conducted (Streeter et al., 2017). Ultimately, PSC-derived retinal organoids and RPE drug toxicity screening platforms should aim to successfully meet the following: (1) scalability, standardization and reproducibility of outputs to ensure endorsement by academic, industrial sector and the regulatory agencies; (2) equivalence to adult tissue function and structure, encompassing and retaining key cellular interactions, appropriate microenvironment and mechanical properties to ensure optimal clinical translatability; and (3) automated data acquisition and analysis to enable high throughput testing and extensive implementation in preclinical studies.

## 8. Conclusions

The vast knowledge of retinal development acquired from various animal models has been instrumental for directing PSC differentiation to RPE cells and light-responsive retinal organoids, which largely mimic the structure and function of the native RPE and neural retina *in vivo*. These features have rendered PSC-derived retinal tissues an outstanding tool for retinal congenital disease modelling studies, drug development and testing new experimental therapies for devastating conditions that have no cure. Promising developments in transplantation studies show that PSC-derived photoreceptors engraft in animal models of retinal degeneration, make synaptic connections with interneurons, and partially restore vision, opening an exciting path towards cell-based transplantation for patients with advanced retinal degeneration. To fully translate these developments into clinical practice, it is necessary

to develop more complex physiological models that fully capture the cellular interactions between neural retina, RPE and choriocapillaris at scale, and invest in automated data acquisition and analyses tools that allow robust and reproducible assessment of retinal function.

## Declaration of interest

MKA is an inventor on patents describing the use of extracellular vesicles in the detection of neurodegenerative disorders in biofluids; she is an academic founder and chief scientific officer of ESP Diagnostics Ltd.

NT - none.

JCSC - none.

RG - none.

DS is a consultant for Alcon, DORC, BVI, Gyroscope, Roche, Alimera, Complement therapeutics, Eyepoint.

MEC is a consultant for Gensight, Prime Medicine and has served as a consultant and Scientific Advisory Board member for ProQR.

LA is chief scientific director of Newcells Biotech Ltd.

ML is a co-founder of Newcells Biotech Ltd.

## CRediT authorship contribution statement

**Marzena Kurzawa-Akanbi:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Nikolaos Tzoumas:** Writing – review & editing, Writing – original draft. **Julio C. Corral-Serrano:** Writing – review & editing, Writing – original draft, Visualization. **Rosellina Guarascio:** Writing – review & editing, Writing – original draft, Visualization. **David H. Steel:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Michael E. Cheetham:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Lyle Armstrong:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Majlinda Lako:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Data availability

No data was used for the research described in the article.

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## References

Achberger, K., Probst, C., Haderspeck, J., Bolz, S., Rogal, J., Chuchuy, J., Nikolova, M., Cora, V., Antkowiak, L., Haq, W., Shen, N., Schenke-Layland, K., Ueffing, M., Liebau, S., Loskill, P., 2019. Merging organoid and organ-on-a-chip technology to generate complex multi-layer tissue models in a human retina-on-a-chip platform. Elife 8, e46188. https://doi.org/10.7554/eLife.46188.

Afanasyeva, T.A.V., Athanasiou, D., Perdigao, P.R.L., Whiting, K.R., Duijkers, L., Astuti, G.D.N., Bennett, J., Garanto, A., van der Spuy, J., Roepman, R., Cheetham, M. E., Collin, R.W.J., 2023. CRISPR-Cas9 correction of a nonsense mutation in LCA5 rescues lebercilin expression and localization in human retinal organoids. Mol. Ther. Methods Clin. Dev. 29, 522–531. https://doi.org/10.1016/j.omtm.2023.05.012.

Afshar, A.R., Pekmezci, M., Bloomer, M.M., Cadenas, N.J., Stevers, M., Banerjee, A., Roy, R., Olshen, A.B., Van Ziffle, J., Onodera, C., Devine, W.P., Grenert, J.P., Bastian, B.C., Solomon, D.A., Damato, B.E., 2020. Next-generation sequencing of retinoblastoma identifies pathogenic alterations beyond RB1 inactivation that correlate with aggressive histopathologic features. Ophthalmology 127, 804–813. https://doi.org/10.1016/j.ophtha.2019.12.005.

Age-Related Eye Disease Study Research Group, 2001. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no.8. Arch. Ophthalmol 119, 1417–1436. https://doi.org/10.1001/archopht.119.10.1417.

Aisenbrey, S., Zhang, M., Bacher, D., Yee, J., Brunken, W.J., Hunter, D.D., 2006. Retinal pigment epithelial cells synthesize laminins, including laminin 5, and adhere to them through alpha3- and alpha6-containing integrins. Invest. Ophthalmol. Vis. Sci. 47, 5537–5544. https://doi.org/10.1167/iovs.05-1590.

Ajioka, I., Martins, R.A., Bayazitov, I.T., Donovan, S., Johnson, D.A., Frase, S., Cicero, S. A., Boyd, K., Zakharenko, S.S., Dyer, M.A., 2007. Differentiated horizontal interneurons clonally expand to form metastatic retinoblastoma in mice. Cell 131, 378–390. https://doi.org/10.1016/j.cell.2007.09.036.

Allikmets, R., Singh, N., Sun, H., Shroyer, N.F., Hutchinson, A., Chidambaram, A., Gerrard, B., Baird, L., Stauffer, D., Peiffer, A., Rattner, A., Smallwood, P., Li, Y., Anderson, K.L., Lewis, R.A., Nathans, J., Leppert, M., Dean, M., Lupski, J.R., 1997. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat. Genet. 15, 236–246. https://doi.org/10.1038/ng0397-236.

Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., Liu, D.R., 2019. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576, 149–157. https://doi.org/10.1038/s41586-019-1711-4.

Arzalluz-Luque, A., Cabrera, J.L., Skottman, H., Benguria, A., Bolinches-Amoros, A., Cuenca, N., Lupo, V., Dopazo, A., Tarazona, S., Delas, B., Carballo, M., Pascual, B., Hernan, I., Erceg, S., Lukovic, D., 2021. Mutant PRPF8 causes widespread splicing changes in spliceosome components in retinitis pigmentosa patient iPSC-derived RPE cells. Front. Neurosci. 15, 636969 https://doi.org/10.3389/fnins.2021.636969.

Baala, L., Audollent, S., Martinovic, J., Ozilou, C., Babron, M.C., Sivanandamoorthy, S., Saunier, S., Salomon, R., Gonzales, M., Rattenberry, E., Esculpavit, C., Toutain, A., Moraine, C., Parent, P., Marcorelles, P., Dauge, M.C., Roume, J., Le Merrer, M., Meiner, V., Meir, K., Menez, F., Beaufrere, A.M., Francannet, C., Tantau, J., Sinico, M., Dumez, Y., MacDonald, F., Munnich, A., Lyonnet, S., Gubler, M.C., Genin, E., Johnson, C.A., Vekemans, M., Encha-Razavi, F., Attie-Bitach, T., 2007. Pleiotropic effects of CEP290 (NPHP6) mutations extend to Meckel syndrome. Am. J. Hum. Genet. 81, 170–179. https://doi.org/10.1086/519494.

Banskota, S., Raguram, A., Suh, S., Du, S.W., Davis, J.R., Choi, E.H., Wang, X., Nielsen, S. C., Newby, G.A., Randolph, P.B., Osborn, M.J., Musunuru, K., Palczewski, K., Liu, D. R., 2022. Engineered virus-like particles for efficient in vivo delivery of therapeutic proteins. Cell 185,. https://doi.org/10.1016/j.cell.2021.12.021, 250-265.e16.

Barbelanne, M., Song, J., Ahmadzai, M., Tsang, W.Y., 2013. Pathogenic NPHP5 mutations impair protein interaction with Cep290, a prerequisite for ciliogenesis. Hum. Mol. Genet. 22, 2482–2494. https://doi.org/10.1093/hmg/ddt100.

Bassuk, A.G., Zheng, A., Li, Y., Tsang, S.H., Mahajan, V.B., 2016. Precision medicine: genetic repair of retinitis pigmentosa in patient-derived stem cells. Sci. Rep. 6, 19969 https://doi.org/10.1038/srep19969.

Ben-David, U., Arad, G., Weissbein, U., Mandefro, B., Maimon, A., Golan-Lev, T., Narwani, K., Clark, A.T., Andrews, P.W., Benvenisty, N., Carlos Biancotti, J., 2014. Aneuploidy induces profound changes in gene expression, proliferation and tumorigenicity of human pluripotent stem cells. Nat. Commun. 5, 4825. https://doi.org/10.1038/ncomms5825.

Benedicto, I., Lehmann, G.L., Ginsberg, M., Nolan, D.J., Bareja, R., Elemento, O., Salfati, Z., Alam, N.M., Prusky, G.T., Llanos, P., Rabbany, S.Y., Maminishkis, A., Miller, S.S., Rafii, S., Rodriguez-Boulan, E., 2017. Concerted regulation of retinal pigment epithelium basement membrane and barrier function by angiocrine factors. Nat. Commun. 8, 15374 https://doi.org/10.1038/ncomms15374.

Berger, W., Kloeckener-Gruissem, B., Neidhardt, J., 2010. The molecular basis of human retinal and vitreoretinal diseases. Prog. Retin. Eye Res. 29, 335–375. https://doi.org/10.1016/j.preteyeres.2010.03.004.

Bharti, K., den Hollander, A.I., Lakkaraju, A., Sinha, D., Williams, D.S., Finnemann, S.C., Bowes-Rickman, C., Malek, G., D'Amore, P.A., 2022. Cell culture models to study retinal pigment epithelium-related pathogenesis in age-related macular degeneration. Exp. Eye Res. 222, 109170 https://doi.org/10.1016/j.exer.2022.109170.

Bhattacharya, S., Yin, J., Huo, W., Chaum, E., 2022. Modeling of mitochondrial bioenergetics and autophagy impairment in MELAS-mutant iPSC-derived retinal pigment epithelial cells. Stem Cell Res. Ther. 13, 260. https://doi.org/10.1186/s13287-022-02937-6.

Biesemeier, A., Taubitz, T., Julien, S., Yoeruek, E., Schraermeyer, U., 2014. Choriocapillaris breakdown precedes retinal degeneration in age-related macular degeneration. Neurobiol. Aging 35, 2562–2573. https://doi.org/10.1016/j.neurobiolaging.2014.05.003.

Bohm, K.J., Gobin, Y.P., Francis, J.H., McInerney, G., Dabo-Trubelja, A., Dalecki, P.H., Marr, B.P., Abramson, D.H., 2018. Choroidal infarction following ophthalmic artery chemotherapy. Int. J. Retina Vitreous 4, 16. https://doi.org/10.1186/s40942-018-0119-x.

Boldt, K., Mans, D.A., Won, J., van Reeuwijk, J., Vogt, A., Kinkl, N., Letteboer, S.J., Hicks, W.L., Hurd, R.E., Naggert, J.K., Texier, Y., den Hollander, A.I., Koenekoop, R. K., Bennett, J., Cremers, F.P., Gloeckner, C.J., Nishina, P.M., Roepman, R., Ueffing, M., 2011. Disruption of intraflagellar protein transport in photoreceptor cilia causes Leber congenital amaurosis in humans and mice. J. Clin. Invest. 121, 2169–2180. https://doi.org/10.1172/JCI45627.

Boon, N., Lu, X., Andriessen, C.A., Moustakas, I., Buck, T.M., Freund, C., Arendzen, C.H., Böhringer, S., Boon, C.J.F., Mei, H., Wijnholds, J., 2023. AAV-mediated gene augmentation therapy of CRB1 patient-derived retinal organoids restores the histological and transcriptional retinal phenotype. Stem Cell Reports 18, 1123–1137. https://doi.org/10.1016/j.stemcr.2023.03.014.

Bowles, E., Corson, T.W., Bayani, J., Squire, J.A., Wong, N., Lai, P.B., Gallie, B.L., 2007. Profiling genomic copy number changes in retinoblastoma beyond loss of RB1. Genes Chromosomes Cancer 46, 118–129. https://doi.org/10.1002/gcc.20383.

Brydon, E.M., Bronstein, R., Buskin, A., Lako, M., Pierce, E.A., Fernandez-Godino, R., 2019. AAV-mediated gene augmentation therapy restores critical functions in mutant PRPF31(+/-) iPSC-derived RPE cells. Mol. Ther. Methods Clin. Dev. 15, 392–402. https://doi.org/10.1016/j.omtm.2019.10.014.

Buchholz, D.E., Hikita, S.T., Rowland, T.J., Friedrich, A.M., Hinman, C.R., Johnson, L.V., Clegg, D.O., 2009. Derivation of functional retinal pigmented epithelium from induced pluripotent stem cells. Stem Cells 27, 2427–2434. https://doi.org/10.1002/stem.189.

Bujakowska, K.M., Liu, Q., Pierce, E.A., 2017. Photoreceptor cilia and retinal ciliopathies. Cold Spring Harb. Perspect. Biol. 9, a028274. https://doi.org/10.1101/cshperspect.a028274.

Burnight, E.R., Giacalone, J.C., Cooke, J.A., Thompson, J.R., Bohrer, L.R., Chirco, K.R., Drack, A.V., Fingert, J.H., Worthington, K.S., Wiley, L.A., Mullins, R.F., Stone, E.M., Tucker, B.A., 2018. CRISPR-Cas9 genome engineering: treating inherited retinal degeneration. Prog. Retin. Eye Res. 65, 28–49. https://doi.org/10.1016/j.preteyeres.2018.03.003.

Burnight, E.R., Wiley, L.A., Drack, A.V., Braun, T.A., Anfinson, K.R., Kaalberg, E.E., Halder, J.A., Affatigato, L.M., Mullins, R.F., Stone, E.M., Tucker, B.A., 2014. CEP290 gene transfer rescues Leber congenital amaurosis cellular phenotype. Gene Ther. 21, 662–672. https://doi.org/10.1038/gt.2014.39.

Buskin, A., Zhu, L., Chichagova, V., Basu, B., Mozaffari-Jovin, S., Dolan, D., Droop, A., Collin, J., Bronstein, R., Mehrotra, S., Farkas, M., Hilgen, G., White, K., Pan, K.T., Treumann, A., Hallam, D., Bialas, K., Chung, G., Mellough, C., Ding, Y., Krasnogor, N., Przyborski, S., Zwolinski, S., Al-Aama, J., Alharthi, S., Xu, Y., Wheway, G., Szymanska, K., McKibbin, M., Inglehearn, C.F., Elliott, D.J., Lindsay, S., Ali, R.R., Steel, D.H., Armstrong, L., Sernagor, E., Urlaub, H., Pierce, E., Luhrmann, R., Grellscheid, S.N., Johnson, C.A., Lako, M., 2018. Disrupted alternative splicing for genes implicated in splicing and ciliogenesis causes PRPF31 retinitis pigmentosa. Nat. Commun. 9, 4234. https://doi.org/10.1038/s41467-018-06448-y.

Cahan, P., Daley, G.Q., 2013. Origins and implications of pluripotent stem cell variability and heterogeneity. Nat. Rev. Mol. Cell Biol. 14, 357–368. https://doi.org/10.1038/nrm3584.

Cai, H., Gong, J., Abriola, L., Hoyer, D., Nyscf Global Stem Cell Array, T., Noggle, S., Paull, D., Del Priore, L.V., Fields, M.A., 2019. High-throughput screening identifies compounds that protect RPE cells from physiological stressors present in AMD. Exp. Eye Res. 185, 107641 https://doi.org/10.1016/j.exer.2019.04.009.

Califf, R.M., 2018. Biomarker definitions and their applications. Exp. Biol. Med. (Maywood) 243, 213–221. https://doi.org/10.1177/1535370217750088.

Capowski, E.E., Samimi, K., Mayerl, S.J., Phillips, M.J., Pinilla, I., Howden, S.E., Saha, J., Jansen, A.D., Edwards, K.L., Jager, L.D., Barlow, K., Valiauga, R., Erlichman, Z., Hagstrom, A., Sinha, D., Sluch, V.M., Chamling, X., Zack, D.J., Skala, M.C., Gamm, D.M., 2019. Reproducibility and staging of 3D human retinal organoids across multiple pluripotent stem cell lines. Development 146, dev171686. https://doi.org/10.1242/dev.171686.

Capowski, E.E., Wright, L.S., Liang, K., Phillips, M.J., Wallace, K., Petelinsek, A., Hagstrom, A., Pinilla, I., Borys, K., Lien, J., Min, J.H., Keles, S., Thomson, J.A., Gamm, D.M., 2016. Regulation of WNT signaling by VSX2 during optic vesicle patterning in human induced pluripotent stem cells. Stem Cells 34, 2625–2634. https://doi.org/10.1002/stem.2414.

Carelli, V., Hirano, M., Enriquez, J.A., Chinnery, P.F., 2022. Implications of mitochondrial DNA mutations in human induced pluripotent stem cells. Nat. Rev. Genet. 23, 69–70. https://doi.org/10.1038/s41576-021-00430-z.

Carr, A.J., Vugler, A.A., Hikita, S.T., Lawrence, J.M., Gias, C., Chen, L.L., Buchholz, D.E., Ahmado, A., Semo, M., Smart, M.J., Hasan, S., da Cruz, L., Johnson, L.V., Clegg, D. O., Coffey, P.J., 2009. Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. PLoS One 4, e8152. https://doi.org/10.1371/journal.pone.0008152.

Cereso, N., Pequignot, M.O., Robert, L., Becker, F., De Luca, V., Nabholz, N., Rigau, V., De Vos, J., Hamel, C.P., Kalatzis, V., 2014. Proof of concept for AAV2/5-mediated gene therapy in iPSC-derived retinal pigment epithelium of a choroideremia patient. Mol. Ther. Methods Clin. Dev. 1, 14011 https://doi.org/10.1038/mtm.2014.11.

Cerna-Chavez, R., Rozanska, A., Poretti, G.L., Benvenisty, N., Parulekar, M., Lako, M., 2023. Retinal pigment epithelium exhibits gene expression and phagocytic activity alterations when exposed to retinoblastoma chemotherapeutics. Exp. Eye Res. 233, 109542 https://doi.org/10.1016/j.exer.2023.109542.

Cerniauskas, E., Kurzawa-Akanbi, M., Xie, L., Hallam, D., Moya-Molina, M., White, K., Steel, D., Doherty, M., Whitfield, P., Al-Aama, J., Armstrong, L., Kavanagh, D., Lambris, J.D., Korolchuk, V.I., Harris, C., Lako, M., 2020. Complement modulation reverses pathology in Y402H-retinal pigment epithelium cell model of age-related macular degeneration by restoring lysosomal function. Stem Cells Transl. Med. 9, 1585–1603. https://doi.org/10.1002/sctm.20-0211.

Chang, Y.C., Chang, W.C., Hung, K.H., Yang, D.M., Cheng, Y.H., Liao, Y.W., Woung, L.C., Tsai, C.Y., Hsu, C.C., Lin, T.C., Liu, J.H., Chiou, S.H., Peng, C.H., Chen, S.J., 2014. The generation of induced pluripotent stem cells for macular degeneration as a drug screening platform: identification of curcumin as a protective agent for retinal pigment epithelial cells against oxidative stress. Front. Aging Neurosci. 6, 191. https://doi.org/10.3389/fnagi.2014.00191.

Chang, Y.J., Jenny, L.A., Li, Y.S., Cui, X., Kong, Y., Li, Y., Sparrow, J.R., Tsang, S.H., 2023. CRISPR editing demonstrates rs10490924 raised oxidative stress in iPSC-derived retinal cells from patients with ARMS2/HTRA1-related AMD. Proc. Natl. Acad. Sci. U S A 120, e2215005120. https://doi.org/10.1073/pnas.2215005120.

Chatterjee, A., Singh, R., 2023. Extracellular vesicles: an emerging player in retinal homeostasis. Front. Cell Dev. Biol. 11, 1059141 https://doi.org/10.3389/fcell.2023.1059141.

Chawla, B., Singh, R., 2017. Recent advances and challenges in the management of retinoblastoma. Indian J. Ophthalmol. 65, 133–139. https://doi.org/10.4103/ijo.IJO_883_16.

Chen, H.Y., Kelley, R.A., Li, T., Swaroop, A., 2021. Primary cilia biogenesis and associated retinal ciliopathies. Semin. Cell Dev. Biol. 110, 70–88. https://doi.org/10.1016/j.semcdb.2020.07.013.

Chen, H.Y., Swaroop, M., Papal, S., Mondal, A.K., Song, H.B., Campello, L., Tawa, G.J., Regent, F., Shimada, H., Nagashima, K., de Val, N., Jacobson, S.G., Zheng, W., Swaroop, A., 2023a. Reserpine maintains photoreceptor survival in retinal ciliopathy by resolving proteostasis imbalance and ciliogenesis defects. Elife 12, e83205. https://doi.org/10.7554/eLife.83205.

Chen, J.-R., Chen, C., Chen, J., Ji, Y., Lian, Y., Zhang, J., Yu, J., Li, X.-Y., Qu, J., Guan, M.-X., 2023b. Nuclear modifier YARS2 allele correction restored retinal ganglion cells-specific deficiencies in Leber's hereditary optic neuropathy. Hum. Mol. Genet. 32, 1539–1551. https://doi.org/10.1093/hmg/ddad001.

Chen, J., Riazifar, H., Guan, M.-X., Huang, T., 2016. Modeling autosomal dominant optic atrophy using induced pluripotent stem cells and identifying potential therapeutic targets. Stem Cell Res. Ther. 7, 2. https://doi.org/10.1186/s13287-015-0264-1.

Chen, L., Perera, N.D., Karoukis, A.J., Feathers, K.L., Ali, R.R., Thompson, D.A., Fahim, A. T., 2022. Oxidative stress differentially impacts apical and basolateral secretion of angiogenic factors from human iPSC-derived retinal pigment epithelium cells. Sci. Rep. 12, 12694 https://doi.org/10.1038/s41598-022-16701-6.

Chen, X., Liu, Y., Sheng, X., Tam, P.O., Zhao, K., Chen, X., Rong, W., Liu, Y., Liu, X., Pan, X., Chen, L.J., Zhao, Q., Vollrath, D., Pang, C.P., Zhao, C., 2014. PRPF4 mutations cause autosomal dominant retinitis pigmentosa. Hum. Mol. Genet. 23, 2926–2939. https://doi.org/10.1093/hmg/ddu005.

Chichagova, V., Georgiou, M., Carter, M., Dorgau, B., Hilgen, G., Collin, J., Queen, R., Chung, G., Ajeian, J., Moya-Molina, M., Kustermann, S., Pognan, F., Hewitt, P., Schmitt, M., Sernagor, E., Armstrong, L., Lako, M., 2023. Incorporating microglia-like cells in human induced pluripotent stem cell-derived retinal organoids. J. Cell Mol. Med. 27, 435–445. https://doi.org/10.1111/jcmm.17670.

Chichagova, V., Hallam, D., Collin, J., Buskin, A., Saretzki, G., Armstrong, L., Yu-Wai-Man, P., Lako, M., Steel, D.H., 2017. Human iPSC disease modelling reveals functional and structural defects in retinal pigment epithelial cells harbouring the m.3243A > G mitochondrial DNA mutation. Sci. Rep. 7, 12320 https://doi.org/10.1038/s41598-017-12396-2.

Chichagova, V., Hilgen, G., Ghareeb, A., Georgiou, M., Carter, M., Sernagor, E., Lako, M., Armstrong, L., 2020. Human iPSC differentiation to retinal organoids in response to IGF1 and BMP4 activation is line- and method-dependent. Stem Cells 38, 195–201. https://doi.org/10.1002/stem.3116.

Chivers, M., Li, N., Pan, F., Wieffer, H., Slowik, R., Leartsakulpanitch, J., 2021. The burden of X-linked retinitis pigmentosa on patients and society: a Narrative literature review. Clinicoecon. Outcomes Res. 13, 565–572. https://doi.org/10.2147/CEOR.S297287.

Choi, E.H., Daruwalla, A., Suh, S., Leinonen, H., Palczewski, K., 2021. Retinoids in the visual cycle: role of the retinal G protein-coupled receptor. J. Lipid Res. 62, 100040 https://doi.org/10.1194/jlr.TR120000850.

Choudhary, P., Booth, H., Gutteridge, A., Surmacz, B., Louca, I., Steer, J., Kerby, J., Whiting, P.J., 2017. Directing differentiation of pluripotent stem cells toward retinal pigment epithelium lineage. Stem Cells Transl. Med. 6, 490–501. https://doi.org/10.5966/sctm.2016-0088.

Choudhary, P., Whiting, P.J., 2016. A strategy to ensure safety of stem cell-derived retinal pigment epithelium cells. Stem Cell Res. Ther. 7, 127. https://doi.org/10.1186/s13287-016-0380-6.

Ciampi, L., Mantica, F., Lopez-Blanch, L., Permanyer, J., Rodriguez-Marin, C., Zang, J., Cianferoni, D., Jimenez-Delgado, S., Bonnal, S., Miravet-Verde, S., Ruprecht, V., Neuhauss, S.C.F., Banfi, S., Carrella, S., Serrano, L., Head, S.A., Irimia, M., 2022. Specialization of the photoreceptor transcriptome by Srrm3-dependent microexons is required for outer segment maintenance and vision. Proc. Natl. Acad. Sci. U S A 119, e2117090119. https://doi.org/10.1073/pnas.2117090119.

Cideciyan, A.V., Jacobson, S.G., 2019. Leber congenital amaurosis (LCA): potential for improvement of vision. Invest. Ophthalmol. Vis. Sci. 60, 1680–1695. https://doi.org/10.1167/iovs.19-26672.

Cideciyan, A.V., Rachel, R.A., Aleman, T.S., Swider, M., Schwartz, S.B., Sumaroka, A., Roman, A.J., Stone, E.M., Jacobson, S.G., Swaroop, A., 2011. Cone photoreceptors

are the main targets for gene therapy of NPHP5 (IQCB1) or NPHP6 (CEP290) blindness: generation of an all-cone Nphp6 hypomorph mouse that mimics the human retinal ciliopathy. Hum. Mol. Genet. 20, 1411–1423. https://doi.org/10.1093/hmg/ddr022.

Clark, S.J., Bishop, P.N., Day, A.J., 2010a. Complement factor H and age-related macular degeneration: the role of glycosaminoglycan recognition in disease pathology. Biochem. Soc. Trans. 38, 1342–1348. https://doi.org/10.1042/BST0381342.

Clark, S.J., Higman, V.A., Mulloy, B., Perkins, S.J., Lea, S.M., Sim, R.B., Day, A.J., 2006. His-384 allotypic variant of factor H associated with age-related macular degeneration has different heparin binding properties from the non-disease-associated form. J. Biol. Chem. 281, 24713–24720. https://doi.org/10.1074/jbc.M605083200.

Clark, S.J., Perveen, R., Hakobyan, S., Morgan, B.P., Sim, R.B., Bishop, P.N., Day, A.J., 2010b. Impaired binding of the age-related macular degeneration-associated complement factor H 402H allotype to Bruch's membrane in human retina. J. Biol. Chem. 285, 30192–30202. https://doi.org/10.1074/jbc.M110.103986.

Clarke, A.R., Maandag, E.R., van Roon, M., van der Lugt, N.M.T., van der Valk, M., Hooper, M.L., Berns, A., te Rielef, H., 1992. Requirement for a functional Rb-1 gene in murine development. Nature 359, 328–330. https://doi.org/10.1038/359328a0.

Comitato, A., Spampanato, C., Chakarova, C., Sanges, D., Bhattacharya, S.S., Marigo, V., 2007. Mutations in splicing factor PRPF3, causing retinal degeneration, form detrimental aggregates in photoreceptor cells. Hum. Mol. Genet. 16, 1699–1707. https://doi.org/10.1093/hmg/ddm118.

Cooke, J.A., Voigt, A.P., Collingwood, M.A., Stone, N.E., Whitmore, S.S., DeLuca, A.P., Burnight, E.R., Anfinson, K.R., Vakulskas, C.A., Reutzel, A.J., Daggett, H.T., Andorf, J.L., Stone, E.M., Mullins, R.F., Tucker, B.A., 2023. Propensity of patient-derived iPSCs for retinal differentiation: implications for autologous cell replacement. Stem Cells Transl. Med. 12, 365–378. https://doi.org/10.1093/stcltm/szad028.

Copland, D.A., Theodoropoulou, S., Liu, J., Dick, A.D., 2018. A perspective of AMD through the eyes of immunology. Invest. Ophthalmol. Vis. Sci. 59, AMD83–AMD92. https://doi.org/10.1167/iovs.18-23893.

Coppieters, F., Lefever, S., Leroy, B.P., De Baere, E., 2010. CEP290, a gene with many faces: mutation overview and presentation of CEP290base. Hum. Mutat. 31, 1097–1108. https://doi.org/10.1002/humu.21337.

Cora, V., Haderspeck, J., Antkowiak, L., Mattheus, U., Neckel, P.H., Mack, A.F., Bolz, S., Ueffing, M., Pashkovskaia, N., Achberger, K., Liebau, S., 2019. A cleared view on retinal organoids. Cells 8, 391. https://doi.org/10.3390/cells8050391.

Corral-Serrano, J.C., Lamers, I.J.C., van Reeuwijk, J., Duijkers, L., Hoogendoorn, A.D.M., Yildirim, A., Argyrou, N., Ruigrok, R.A.A., Letteboer, S.J.F., Butcher, R., van Essen, M.D., Sakami, S., van Beersum, S.E.C., Palczewski, K., Cheetham, M.E., Liu, Q., Boldt, K., Wolfrum, U., Ueffing, M., Garanto, A., Roepman, R., Collin, R.W.J., 2020. PCARE and WASF3 regulate ciliary F-actin assembly that is required for the initiation of photoreceptor outer segment disk formation. Proc. Natl. Acad. Sci. U S A 117, 9922–9931. https://doi.org/10.1073/pnas.1903125117.

Corral-Serrano, J.C., Sladen, P.E., Ottaviani, D., Rezek, O.F., Athanasiou, D., Jovanovic, K., van der Spuy, J., Mansfield, B.C., Cheetham, M.E., 2023. Eupatilin improves cilia defects in human CEP290 ciliopathy models. Cells 12, 1575. https://doi.org/10.3390/cells12121575.

Cowan, C.S., Renner, M., De Gennaro, M., Gross-Scherf, B., Goldblum, D., Hou, Y., Munz, M., Rodrigues, T.M., Krol, J., Szikra, T., Cuttat, R., Waldt, A., Papasaikas, P., Diggelmann, R., Patino-Alvarez, C.P., Galliker, P., Spirig, S.E., Pavlinic, D., Gerber-Hollbach, N., Schuierer, S., Srdanovic, A., Balogh, M., Panero, R., Kusnyerik, A., Szabo, A., Stadler, M.B., Orgul, S., Picelli, S., Hasler, P.W., Hierlemann, A., Scholl, H.P.N., Roma, G., Nigsch, F., Roska, B., 2020. Cell types of the human retina and its organoids at single-cell resolution. Cell 182. https://doi.org/10.1016/j.cell.2020.08.013, 1623-1640 e1634.

Curcio, C.A., Medeiros, N.E., Millican, C.L., 1996. Photoreceptor loss in age-related macular degeneration. Invest. Ophthalmol. Vis. Sci. 37, 1236–1249. https://iovs.arvojournals.org/article.aspx?articleid=2180378.

Dalvin, L.A., Ancona-Lezama, D., Lucio-Alvarez, J.A., Masoomian, B., Jabbour, P., Shields, C.L., 2018. Ophthalmic vascular events after primary unilateral intra-arterial chemotherapy for retinoblastoma in early and recent eras. Ophthalmology 125, 1803–1811. https://doi.org/10.1016/j.ophtha.2018.05.013.

Danese, A., Patergnani, S., Maresca, A., Peron, C., Raimondi, A., Caporali, L., Marchi, S., La Morgia, C., Del Dotto, V., Zanna, C., Iannielli, A., Segnali, A., Di Meo, I., Cavaliere, A., Lebiedzinska-Arciszewska, M., Wieckowski, M.R., Martinuzzi, A., Moraes-Filho, M.N., Salomao, S.R., Berezovsky, A., Belfort, R., Buser, C., Ross-Cisneros, F.N., Sadun, A.A., Tacchetti, C., Broccoli, V., Giorgi, C., Tiranti, V., Carelli, V., Pinton, P., 2022. Pathological mitophagy disrupts mitochondrial homeostasis in Leber's hereditary optic neuropathy. Cell Rep. 40, 111124 https://doi.org/10.1016/j.celrep.2022.111124.

de Bruijn, S.E., Fiorentino, A., Ottaviani, D., Fanucchi, S., Melo, U.S., Corral-Serrano, J.C., Mulders, T., Georgiou, M., Rivolta, C., Pontikos, N., Arno, G., Roberts, L., Greenberg, J., Albert, S., Gilissen, C., Aben, M., Rebello, G., Mead, S., Raymond, F.L., Corominas, J., Smith, C.E.L., Kremer, H., Downes, S., Black, G.C., Webster, A.R., Inglehearn, C.F., van den Born, L.I., Koenekoop, R.K., Michaelides, M., Ramesar, R.S., Hoyng, C.B., Mundlos, S., Mhlanga, M.M., Cremers, F.P.M., Cheetham, M.E., Roosing, S., Hardcastle, A.J., 2020. Structural variants create new topological-associated domains and ectopic retinal enhancer-gene contact in dominant retinitis pigmentosa. Am. J. Hum. Genet. 107, 802–814. https://doi.org/10.1016/j.ajhg.2020.09.002.

de Jong, S., Koolen, L., Vázquez-Domínguez, I., de Breuk, A., Albert, S., Hoyng, C.B., Katti, S., den Hollander, A.I., Garanto, A., 2022. Generation of an iPSC line (SCTCi014-A) and isogenic control line (SCTCi014-A-1) from an age-related macular degeneration patient carrying the variant c.355G>A in the CFI gene. Stem Cell Res. 62, 102797 https://doi.org/10.1016/j.scr.2022.102797.

De Masi, C., Spitalieri, P., Murdocca, M., Novelli, G., Sangiuolo, F., 2020. Application of CRISPR/Cas9 to human-induced pluripotent stem cells: from gene editing to drug discovery. Hum. Genomics 14, 25. https://doi.org/10.1186/s40246-020-00276-2.

den Hollander, A.I., Koenekoop, R.K., Mohamed, M.D., Arts, H.H., Boldt, K., Towns, K.V., Sedmak, T., Beer, M., Nagel-Wolfrum, K., McKibbin, M., Dharmaraj, S., Lopez, I., Ivings, L., Williams, G.A., Springell, K., Woods, C.G., Jafri, H., Rashid, Y., Strom, T.M., van der Zwaag, B., Gosens, I., Kersten, F.F., van Wijk, E., Veltman, J.A., Zonneveld, M.N., van Beersum, S.E., Maumenee, I.H., Wolfrum, U., Cheetham, M.E., Ueffing, M., Cremers, F.P., Inglehearn, C.F., Roepman, R., 2007. Mutations in LCA5, encoding the ciliary protein lebercilin, cause Leber congenital amaurosis. Nat. Genet. 39, 889–895. https://doi.org/10.1038/ng2066.

den Hollander, A.I., Koenekoop, R.K., Yzer, S., Lopez, I., Arends, M.L., Voesenek, K.E., Zonneveld, M.N., Strom, T.M., Meitinger, T., Brunner, H.G., Hoyng, C.B., van den Born, L.I., Rohrschneider, K., Cremers, F.P., 2006. Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. Am. J. Hum. Genet. 79, 556–561. https://doi.org/10.1086/507318.

Deng, W.-L., Gao, M.-L., Lei, X.-L., Lv, J.-N., Zhao, H., He, K.-W., Xia, X.-X., Li, L.-Y., Chen, Y.-C., Li, Y.-P., Pan, D., Xue, T., Jin, Z.-B., 2018. Gene correction reverses ciliopathy and photoreceptor loss in iPSC-derived retinal organoids from retinitis pigmentosa patients. Stem Cell Reports 10, 1267–1281. https://doi.org/10.1016/j.stemcr.2018.02.003.

Deng, X., Iwagawa, T., Fukushima, M., Watanabe, S., 2020. Characterization of human-induced pluripotent stem cells carrying homozygous RB1 gene deletion. Genes Cells 25, 510–517. https://doi.org/10.1111/gtc.12771.

Diakatou, M., Dubois, G., Erkilic, N., Sanjurjo-Soriano, C., Meunier, I., Kalatzis, V., 2021. Allele-specific knockout by CRISPR/Cas to treat autosomal dominant retinitis pigmentosa caused by the G56R mutation in NR2E3. Int. J. Mol. Sci. 22, 2607. https://doi.org/10.3390/ijms22052607.

Dimaras, H., Khetan, V., Halliday, W., Orlic, M., Prigoda, N.L., Piovesan, B., Marrano, P., Corson, T.W., Eagle Jr., R.C., Squire, J.A., Gallie, B.L., 2008. Loss of RB1 induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. Hum. Mol. Genet. 17, 1363–1372. https://doi.org/10.1093/hmg/ddn024.

DiMauro, S., Davidzon, G., 2005. Mitochondrial DNA and disease. Ann. Med. 37, 222–232. https://doi.org/10.1080/07853890510007368.

DiStefano, T., Chen, H.Y., Panebianco, C., Kaya, K.D., Brooks, M.J., Gieser, L., Morgan, N.Y., Pohida, T., Swaroop, A., 2018. Accelerated and improved differentiation of retinal organoids from pluripotent stem cells in rotating-wall vessel bioreactors. Stem Cell Reports 10, 300–313. https://doi.org/10.1016/j.stemcr.2017.11.001.

Dodson, K.H., Echevarria, F.D., Li, D., Sappington, R.M., Edd, J.F., 2015. Retina-on-a-chip: a microfluidic platform for point access signaling studies. Biomed. Microdevices 17, 114. https://doi.org/10.1007/s10544-015-0019-x.

Dommering, C.J., Mol, B.M., Moll, A.C., Burton, M., Cloos, J., Dorsman, J.C., Meijers-Heijboer, H., van der Hout, A.H., 2014. RB1 mutation spectrum in a comprehensive nationwide cohort of retinoblastoma patients. J. Med. Genet. 51, 366–374. https://doi.org/10.1136/jmedgenet-2014-102264.

Dorgau, B., Georgiou, M., Chaudhary, A., Moya-Molina, M., Collin, J., Queen, R., Hilgen, G., Davey, T., Hewitt, P., Schmitt, M., Kustermann, S., Pognan, F., Steel, D.H., Sernagor, E., Armstrong, L., Lako, M., 2022. Human retinal organoids provide a suitable tool for toxicological investigations: a comprehensive validation using drugs and compounds affecting the retina. Stem Cells Transl. Med. 11, 159–177. https://doi.org/10.1093/stcltm/szab010.

Doudna, J.A., Charpentier, E., 2014. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346, 1258096. https://doi.org/10.1126/science.1258096.

Dubaic, M., Peskova, L., Hampl, M., Weissova, K., Celiker, C., Shylo, N.A., Hruba, E., Kavkova, M., Zikmund, T., Weatherbee, S.D., Kaiser, J., Barta, T., Buchtova, M., 2023. Role of ciliopathy protein TMEM107 in eye development: insights from a mouse model and retinal organoid. Life Sci. Alliance 6, e202302073. https://doi.org/10.26508/lsa.202302073.

Dulla, K., Aguila, M., Lane, A., Jovanovic, K., Parfitt, D.A., Schulkens, I., Chan, H.L., Schmidt, I., Beumer, W., Vorthoren, L., Collin, R.W.J., Garanto, A., Duijkers, L., Brugulat-Panes, A., Semo, M., Vugler, A.A., Biasutto, P., Adamson, P., Cheetham, M.E., 2018. Splice-modulating oligonucleotide QR-110 restores CEP290 mRNA and function in human c.2991+1655A>G LCA10 models. Mol. Ther. Nucleic Acids 12, 730–740. https://doi.org/10.1016/j.omtn.2018.07.010.

Dulla, K., Slijkerman, R., van Diepen, H.C., Albert, S., Dona, M., Beumer, W., Turunen, J.J., Chan, H.L., Schulkens, I.A., Vorthoren, L., den Besten, C., Buil, L., Schmidt, I., Miao, J., Venselaar, H., Zang, J., Neuhauss, S.C.F., Peters, T., Broekman, S., Pennings, R., Kremer, H., Platenburg, G., Adamson, P., de Vrieze, E., van Wijk, E., 2021. Antisense oligonucleotide-based treatment of retinitis pigmentosa caused by USH2A exon 13 mutations. Mol. Ther. 29, 2441–2455. https://doi.org/10.1016/j.ymthe.2021.04.024.

Duong, T.T., Vasireddy, V., Ramachandran, P., Herrera, P.S., Leo, L., Merkel, C., Bennett, J., Mills, J.A., 2018. Use of induced pluripotent stem cell models to probe the pathogenesis of Choroideremia and to develop a potential treatment. Stem Cell Res. 27, 140–150. https://doi.org/10.1016/j.scr.2018.01.009.

Dyer, M.A., 2016. Lessons from retinoblastoma: implications for cancer, development, evolution, and regenerative medicine. Trends Mol. Med. 22, 863–876. https://doi.org/10.1016/j.molmed.2016.07.010.

Dyka, F.M., Boye, S.L., Chiodo, V.A., Hauswirth, W.W., Boye, S.E., 2014. Dual adeno-associated virus vectors result in efficient in vitro and in vivo expression of an oversized gene, MYO7A. Hum. Gene Ther. Methods 25, 166–177. https://doi.org/10.1089/hgtb.2013.212.

Dyson, N.J., 2016. RB1: a prototype tumor suppressor and an enigma. Genes Dev. 30, 1492–1502. https://doi.org/10.1101/gad.282145.116.
Ebeling, M.C., Geng, Z., Kapphahn, R.J., Roehrich, H., Montezuma, S.R., Dutton, J.R., Ferrington, D.A., 2021. Impaired mitochondrial function in iPSC-retinal pigment epithelium with the complement factor H polymorphism for age-related macular degeneration. Cells 10, 789. https://doi.org/10.3390/cells10040789.
Ebeling, M.C., Geng, Z., Stahl, M.R., Kapphahn, R.J., Roehrich, H., Montezuma, S.R., Ferrington, D.A., Dutton, J.R., 2022. Testing mitochondrial-targeted drugs in iPSC-RPE from patients with age-related macular degeneration. Pharmaceuticals 15, 62. https://doi.org/10.3390/ph15010062.
Eiraku, M., Sasai, Y., 2011. Mouse embryonic stem cell culture for generation of three-dimensional retinal and cortical tissues. Nat. Protoc. 7, 69–79. https://doi.org/10.1038/nprot.2011.429.
Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T., Sasai, Y., 2011. Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature 472, 51–56. https://doi.org/10.1038/nature09941.
Estrada-Cuzcano, A., Koenekoop, R.K., Coppieters, F., Kohl, S., Lopez, I., Collin, R.W., De Baere, E.B., Roeleveld, D., Marek, J., Bernd, A., Rohrschneider, K., van den Born, L.I., Meire, F., Maumenee, I.H., Jacobson, S.G., Hoyng, C.B., Zrenner, E., Cremers, F.P., den Hollander, A.I., 2011. IQCB1 mutations in patients with leber congenital amaurosis. Invest. Ophthalmol. Vis. Sci. 52, 834–839. https://doi.org/10.1167/iovs.10-5221.
Evans, M.J., Kaufman, M.H., 1981. Establishment in culture of pluripotential cells from mouse embryos. Nature 292, 154–156. https://doi.org/10.1038/292154a0.
Faber, S., Mercey, O., Junger, K., Garanto, A., May-Simera, H., Ueffing, M., Collin, R.W., Boldt, K., Guichard, P., Hamel, V., Roepman, R., 2023. Gene augmentation of LCA5-associated Leber congenital amaurosis ameliorates bulge region defects of the photoreceptor ciliary axoneme. JCI Insight 8, e169162. https://doi.org/10.1172/jci.insight.169162.
Fabian, I.D., Shah, V., Kapelushnik, N., Naeem, Z., Onadim, Z., Price, E.A., Duncan, C., Stansfield, D., Sagoo, M.S., Reddy, M.A., 2020. Number, frequency and time interval of examinations under anesthesia in bilateral retinoblastoma. Graefes Arch. Clin. Exp. Ophthalmol. 258, 879–886. https://doi.org/10.1007/s00417-019-04589-4.
Farnoodian, M., Bose, D., Khristov, V., Susaimanickam, P.J., Maddileti, S., Mariappan, I., Abu-Asab, M., Campos, M., Villasmil, R., Wan, Q., Maminishkis, A., McGaughey, D., Barone, F., Gundry, R.L., Riordon, D.R., Boheler, K.R., Sharma, R., Bharti, K., 2022. Cell-autonomous lipid-handling defects in Stargardt iPSC-derived retinal pigment epithelium cells. Stem Cell Reports 17, 2438–2450. https://doi.org/10.1016/j.stemcr.2022.10.001.
FDA-NIH Biomarker Working Group, 2016. BEST (Biomarkers, EndpointS, and other Tools) Resource. https://www.ncbi.nlm.nih.gov/books/NBK326791/.
Ferguson, L.R., Balaiya, S., Mynampati, B.K., Sambhav, K., Chalam, K.V., 2015. Deprivation of bFGF promotes spontaneous differentiation of human embryonic stem cells into retinal pigment epithelial cells. J. Stem Cells 10, 159–170. https://www.ncbi.nlm.nih.gov/pubmed/27125061.
Fields, M.A., Del Priore, L.V., Adelman, R.A., Rizzolo, L.J., 2020. Interactions of the choroid, Bruch's membrane, retinal pigment epithelium, and neurosensory retina collaborate to form the outer blood-retinal-barrier. Prog. Retin. Eye Res. 76, 100803 https://doi.org/10.1016/j.preteyeres.2019.100803.
Fligor, C.M., Lavekar, S.S., Harkin, J., Shields, P.K., VanderWall, K.B., Huang, K.C., Gomes, C., Meyer, J.S., 2021. Extension of retinofugal projections in an assembled model of human pluripotent stem cell-derived organoids. Stem Cell Reports 16, 2228–2241. https://doi.org/10.1016/j.stemcr.2021.05.009.
Flores-Bellver, M., Mighty, J., Aparicio-Domingo, S., Li, K.V., Shi, C., Zhou, J., Cobb, H., McGrath, P., Michelis, G., Lenhart, P., Bilousova, G., Heissel, S., Rudy, M.J., Coughlan, C., Goodspeed, A.E., Becerra, S.P., Redenti, S., Canto-Soler, M.V., 2021. Extracellular vesicles released by human retinal pigment epithelium mediate increased polarised secretion of drusen proteins in response to AMD stressors. J. Extracell. Vesicles 10, e12165. https://doi.org/10.1002/jev2.12165.
Floros, V.I., Pyle, A., Dietmann, S., Wei, W., Tang, W.C.W., Irie, N., Payne, B., Capalbo, A., Noli, L., Coxhead, J., Hudson, G., Crosier, M., Strahl, H., Khalaf, Y., Saitou, M., Ilic, D., Surani, M.A., Chinnery, P.F., 2018. Segregation of mitochondrial DNA heteroplasmy through a developmental genetic bottleneck in human embryos. Nat. Cell Biol. 20, 144–151. https://doi.org/10.1038/s41556-017-0017-8.
Foltz, L.P., Clegg, D.O., 2017. Rapid, directed differentiation of retinal pigment epithelial cells from human embryonic or induced pluripotent stem cells. J. Vis. Exp., 56274 https://doi.org/10.3791/56274.
Fritsche, L.G., Igl, W., Bailey, J.N., Grassmann, F., Sengupta, S., Bragg-Gresham, J.L., Burdon, K.P., Hebbring, S.J., Wen, C., Gorski, M., Kim, I.K., Cho, D., Zack, D., Souied, E., Scholl, H.P., Bala, E., Lee, K.E., Hunter, D.J., Sardell, R.J., Mitchell, P., Merriam, J.E., Cipriani, V., Hoffman, J.D., Schick, T., Lechanteur, Y.T., Guymer, R. H., Johnson, M.P., Jiang, Y., Stanton, C.M., Buitendijk, G.H., Zhan, X., Kwong, A.M., Boleda, A., Brooks, M., Gieser, L., Ratnapriya, R., Branham, K.E., Foerster, J.R., Heckenlively, J.R., Othman, M.I., Vote, B.J., Liang, H.H., Souzeau, E., McAllister, I. L., Isaacs, T., Hall, J., Lake, S., Mackey, D.A., Constable, I.J., Craig, J.E., Kitchner, T. E., Yang, Z., Su, Z., Luo, H., Chen, D., Ouyang, H., Flagg, K., Lin, D., Mao, G., Ferreyra, H., Stark, K., von Strachwitz, C.N., Wolf, A., Brandl, C., Rudolph, G., Olden, M., Morrison, M.A., Morgan, D.J., Schu, M., Ahn, J., Silvestri, G., Tsironi, E. E., Park, K.H., Farrer, L.A., Orlin, A., Brucker, A., Li, M., Curcio, C.A., Mohand-Said, S., Sahel, J.A., Audo, I., Benchaboune, M., Cree, A.J., Rennie, C.A., Goverdhan, S.V., Grunin, M., Hagbi-Levi, S., Campochiaro, P., Katsanis, N., Holz, F. G., Blond, F., Blanche, H., Deleuze, J.F., Igo Jr., R.P., Truitt, B., Peachey, N.S., Meuer, S.M., Myers, C.E., Moore, E.L., Klein, R., Hauser, M.A., Postel, E.A., Courtenay, M.D., Schwartz, S.G., Kovach, J.L., Scott, W.K., Liew, G., Tan, A.G., Gopinath, B., Merriam, J.C., Smith, R.T., Khan, J.C., Shahid, H., Moore, A.T., McGrath, J.A., Laux, R., Brantley Jr., M.A., Agarwal, A., Ersoy, L., Caramoy, A., Langmann, T., Saksens, N.T., de Jong, E.K., Hoyng, C.B., Cain, M.S., Richardson, A. J., Martin, T.M., Blangero, J., Weeks, D.E., Dhillon, B., van Duijn, C.M., Doheny, K. F., Romm, J., Klaver, C.C., Hayward, C., Gorin, M.B., Klein, M.L., Baird, P.N., den Hollander, A.I., Fauser, S., Yates, J.R., Allikmets, R., Wang, J.J., Schaumberg, D.A., Klein, B.E., Hagstrom, S.A., Chowers, I., Lotery, A.J., Leveillard, T., Zhang, K., Brilliant, M.H., Hewitt, A.W., Swaroop, A., Chew, E.Y., Pericak-Vance, M.A., DeAngelis, M., Stambolian, D., Haines, J.L., Iyengar, S.K., Weber, B.H., Abecasis, G. R., Heid, I.M., 2016. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat. Genet. 48, 134–143. https://doi.org/10.1038/ng.3448.
Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, D., Joung, J.K., Sander, J.D., 2013. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat. Biotechnol. 31, 822–826. https://doi.org/10.1038/nbt.2623.
Fuhrmann, S., 2010. Eye morphogenesis and patterning of the optic vesicle. Curr. Top. Dev. Biol. 93, 61–84. https://doi.org/10.1016/B978-0-12-385044-7.00003-5.
Fuhrmann, S., Levine, E.M., Reh, T.A., 2000. Extraocular mesenchyme patterns the optic vesicle during early eye development in the embryonic chick. Development 127, 4599–4609. https://doi.org/10.1242/dev.127.21.4599.
Fujimura, Y., Sakai, I., Shioka, I., Takata, N., Hashimoto, A., Funatomi, T., Okuda, S., 2023. Machine learning-based estimation of spatial gene expression pattern during ESC-derived retinal organoid development. Sci. Rep. 13, 22781 https://doi.org/10.1038/s41598-023-49758-y.
Gammage, P.A., Moraes, C.T., Minczuk, M., 2018. Mitochondrial genome engineering: the revolution may not Be CRISPR-ized. Trends Genet. 34, 101–110. https://doi.org/10.1016/j.tig.2017.11.001.
Gao, M.-L., Lei, X.-L., Han, F., He, K.-W., Jin, S.-Q., Zhang, Y.-Y., Jin, Z.-B., 2020. Patient-specific retinal organoids recapitulate disease features of late-onset retinitis pigmentosa. Front. Cell Dev. Biol. 8, 128. https://doi.org/10.3389/fcell.2020.00128.
Garanto, A., van Beersum, S.E., Peters, T.A., Roepman, R., Cremers, F.P., Collin, R.W., 2013. Unexpected CEP290 mRNA splicing in a humanized knock-in mouse model for Leber congenital amaurosis. PLoS One 8, e79369. https://doi.org/10.1371/journal.pone.0079369.
Garita-Hernandez, M., Routet, F., Guibbal, L., Khabou, H., Toualbi, L., Riancho, L., Reichman, S., Duebel, J., Sahel, J.A., Goureau, O., Dalkara, D., 2020. AAV-mediated gene delivery to 3D retinal organoids derived from human induced pluripotent stem cells. Int. J. Mol. Sci. 21, 994. https://doi.org/10.3390/ijms21030994.
Gasparini, S.J., Llonch, S., Borsch, O., Ader, M., 2019. Transplantation of photoreceptors into the degenerative retina: current state and future perspectives. Prog. Retin. Eye Res. 69, 1–37. https://doi.org/10.1016/j.preteyeres.2018.11.001.
Georgiannakis, A., Burgoyne, T., Lueck, K., Futter, C., Greenwood, J., Moss, S.E., 2015. Retinal pigment epithelial cells mitigate the effects of complement attack by endocytosis of C5b-9. J. Immunol. 195, 3382–3389. https://doi.org/10.4049/jimmunol.1500937.
Georgiou, M., Yang, C., Atkinson, R., Pan, K.T., Buskin, A., Molina, M.M., Collin, J., Al-Aama, J., Goertler, F., Ludwig, S.E.J., Davey, T., Luhrmann, R., Nagaraja-Grellscheid, S., Johnson, C.A., Ali, R., Armstrong, L., Korolchuk, V., Urlaub, H., Mozaffari-Jovin, S., Lako, M., 2022. Activation of autophagy reverses progressive and deleterious protein aggregation in PRPF31 patient-induced pluripotent stem cell-derived retinal pigment epithelium cells. Clin. Transl. Med. 12, e759. https://doi.org/10.1002/ctm2.759.
Germain, P.L., Testa, G., 2017. Taming human genetic variability: transcriptomic meta-analysis guides the experimental design and interpretation of iPSC-based disease modeling. Stem Cell Reports 8, 1784–1796. https://doi.org/10.1016/j.stemcr.2017.05.012.
Golestaneh, N., Chu, Y., Xiao, Y.Y., Stoleru, G.L., Theos, A.C., 2017. Dysfunctional autophagy in RPE, a contributing factor in age-related macular degeneration. Cell Death Dis. 8, e2537 https://doi.org/10.1038/cddis.2016.453.
Gong, Y., Ge, L., Li, Q., Gong, J., Chen, M., Gao, H., Kang, J., Yu, T., Li, J., Xu, H., 2023. Ethanol causes cell death and neuronal differentiation defect during initial neurogenesis of the neural retina by disrupting calcium signaling in human retinal organoids. Stem Cell Rev. Rep. 19, 2790–2806. https://doi.org/10.1007/s12015-023-10604-3.
Gonzalez-Cordero, A., Goh, D., Kruczek, K., Naeem, A., Fernando, M., kleine Holthaus, S.-M., Takaaki, M., Blackford, S.J.I., Kloc, M., Agundez, L., Sampson, R.D., Borooah, S., Ovando-Roche, P., Mehat, M.S., West, E.L., Smith, A.J., Pearson, R.A., Ali, R.R., 2018. Assessment of AAV vector tropisms for mouse and human pluripotent stem cell–derived RPE and photoreceptor cells. Hum. Gene Ther. 29, 1124–1139. https://doi.org/10.1089/hum.2018.027.
Gonzalez-Cordero, A., Kruczek, K., Naeem, A., Fernando, M., Kloc, M., Ribeiro, J., Goh, D., Duran, Y., Blackford, S.J.I., Abelleira-Hervas, L., Sampson, R.D., Shum, I.O., Branch, M.J., Gardner, P.J., Sowden, J.C., Bainbridge, J.W.B., Smith, A.J., West, E.L., Pearson, R.A., Ali, R.R., 2017. Recapitulation of human retinal development from human pluripotent stem cells generates transplantable populations of cone photoreceptors. Stem Cell Reports 9, 820–837. https://doi.org/10.1016/j.stemcr.2017.07.022.
Gore, A., Li, Z., Fung, H.L., Young, J.E., Agarwal, S., Antosiewicz-Bourget, J., Canto, I., Giorgetti, A., Israel, M.A., Kiskinis, E., Lee, J.H., Loh, Y.H., Manos, P.D., Montserrat, N., Panopoulos, A.D., Ruiz, S., Wilbert, M.L., Yu, J., Kirkness, E.F., Izpisua Belmonte, J.C., Rossi, D.J., Thomson, J.A., Eggan, K., Daley, G.Q., Goldstein, L.S., Zhang, K., 2011. Somatic coding mutations in human induced pluripotent stem cells. Nature 471, 63–67. https://doi.org/10.1038/nature09805.
Gorman, G.S., Schaefer, A.M., Ng, Y., Gomez, N., Blakely, E.L., Alston, C.L., Feeney, C., Horvath, R., Yu-Wai-Man, P., Chinnery, P.F., Taylor, R.W., Turnbull, D.M., McFarland, R., 2015. Prevalence of nuclear and mitochondrial DNA mutations

related to adult mitochondrial disease. Ann. Neurol. 77, 753–759. https://doi.org/10.1002/ana.24362.
Graziotto, J.J., Farkas, M.H., Bujakowska, K., Deramaudt, B.M., Zhang, Q., Nandrot, E.F., Inglehearn, C.F., Bhattacharya, S.S., Pierce, E.A., 2011. Three gene-targeted mouse models of RNA splicing factor RP show late-onset RPE and retinal degeneration. Invest. Ophthalmol. Vis. Sci. 52, 190–198. https://doi.org/10.1167/iovs.10-5194.
Graziotto, J.J., Inglehearn, C.F., Pack, M.A., Pierce, E.A., 2008. Decreased levels of the RNA splicing factor Prpf3 in mice and zebrafish do not cause photoreceptor degeneration. Invest. Ophthalmol. Vis. Sci. 49, 3830–3838. https://doi.org/10.1167/iovs.07-1483.
Griesenbach, U., Inoue, M., Meng, C., Farley, R., Chan, M., Newman, N.K., Brum, A., You, J., Kerton, A., Shoemark, A., Boyd, A.C., Davies, J.C., Higgins, T.E., Gill, D.R., Hyde, S.C., Innes, J.A., Porteous, D.J., Hasegawa, M., Alton, E.W., 2012. Assessment of F/HN-pseudotyped lentivirus as a clinically relevant vector for lung gene therapy. Am. J. Respir. Crit. Care Med. 186, 846–856. https://doi.org/10.1164/rccm.201206-1056OC.
Grigoryan, E.N., 2022. Self-organization of the retina during eye development, retinal regeneration in vivo, and in retinal 3D organoids in vitro. Biomedicines 10, 1458. https://doi.org/10.3390/biomedicines10061458.
Grindley, J.C., Davidson, D.R., Hill, R.E., 1995. The role of Pax-6 in eye and nasal development. Development 121, 1433–1442. https://doi.org/10.1242/dev.121.5.1433.
Grönlund, M.A., Honarvar, A.K.S., Andersson, S., Moslemi, A.R., Oldfors, A., Holme, E., Tulinius, M., Darin, N., 2010. Ophthalmological findings in children and young adults with genetically verified mitochondrial disease. Br. J. Ophthalmol. 94, 121. https://doi.org/10.1136/bjo.2008.154187.
Guo, G., Stirparo, G.G., Strawbridge, S.E., Spindlow, D., Yang, J., Clarke, J., Dattani, A., Yanagida, A., Li, M.A., Myers, S., Ozel, B.N., Nichols, J., Smith, A., 2021. Human naive epiblast cells possess unrestricted lineage potential. Cell Stem Cell 28,. https://doi.org/10.1016/j.stem.2021.02.025, 1040-1056.e6.
Guo, Y., Wang, P., Ma, J.H., Cui, Z., Yu, Q., Liu, S., Xue, Y., Zhu, D., Cao, J., Li, Z., Tang, S., Chen, J., 2019. Modeling retinitis pigmentosa: retinal organoids generated from the iPSCs of a patient with the USH2A mutation show early developmental abnormalities. Front. Cell. Neurosci. 13, 361. https://doi.org/10.3389/fncel.2019.00361.
Gupta, T., Kapoor, K., Sahni, D., Singh, B., 2016. Mapping the time line of development in each layer of human foetal retina. J. Clin. Diagn. Res. 10, AC04-AC07 https://doi.org/10.7860/JCDR/2016/14936.7372.
Hallam, D., Collin, J., Bojic, S., Chichagova, V., Buskin, A., Xu, Y., Lafage, L., Otten, E.G., Anyfantis, G., Mellough, C., Przyborski, S., Alharthi, S., Korolchuk, V., Lotery, A., Saretzki, G., McKibbin, M., Armstrong, L., Steel, D., Kavanagh, D., Lako, M., 2017. An induced pluripotent stem cell patient specific model of complement factor H (Y402H) polymorphism displays characteristic features of age-related macular degeneration and indicates a beneficial role for UV light exposure. Stem Cells 35, 2305–2320. https://doi.org/10.1002/stem.2708.
Hallam, D., Hilgen, G., Dorgau, B., Zhu, L., Yu, M., Bojic, S., Hewitt, P., Schmitt, M., Uteng, M., Kustermann, S., Steel, D., Nicholds, M., Thomas, R., Treumann, A., Porter, A., Sernagor, E., Armstrong, L., Lako, M., 2018. Human-induced pluripotent stem cells generate light responsive retinal organoids with variable and nutrient-dependent efficiency. Stem Cells 36, 1535–1551. https://doi.org/10.1002/stem.2883.
Hamalainen, R.H., Manninen, T., Koivumaki, H., Kislin, M., Otonkoski, T., Suomalainen, A., 2013. Tissue- and cell-type-specific manifestations of heteroplasmic mtDNA 3243A>G mutation in human induced pluripotent stem cell-derived disease model. Proc. Natl. Acad. Sci. U S A 110, E3622–E3630. https://doi.org/10.1073/pnas.1311660110.
Hamilton, R.D., Foss, A.J., Leach, L., 2007. Establishment of a human in vitro model of the outer blood-retinal barrier. J. Anat. 211, 707–716. https://doi.org/10.1111/j.1469-7580.2007.00812.x.
Hammadi, S., Tzoumas, N., Ferrara, M., Meschede, I.P., Lo, K., Harris, C., Lako, M., Steel, D.H., 2023. Bruch's membrane: a key consideration with complement-based therapies for age-related macular degeneration. J. Clin. Med. 12, 2870. https://doi.org/10.3390/jcm12082870.
Hartong, D.T., Berson, E.L., Dryja, T.P., 2006. Retinitis pigmentosa. Lancet 368, 1795–1809. https://doi.org/10.1016/S0140-6736(06)69740-7.
Harvey, J.P., Sladen, P.E., Yu-Wai-Man, P., Cheetham, M.E., 2022. Induced pluripotent stem cells for inherited optic neuropathies—disease modeling and therapeutic development. J. Neuroophthalmol. 42, 35–44. https://doi.org/10.1097/wno.0000000000001375.
Hashimoto, M., Bacman, S.R., Peralta, S., Falk, M.J., Chomyn, A., Chan, D.C., Williams, S.L., Moraes, C.T., 2015. MitoTALEN: a general approach to reduce mutant mtDNA loads and restore oxidative phosphorylation function in mitochondrial diseases. Mol. Ther. 23, 1592–1599. https://doi.org/10.1038/mt.2015.126.
Helou, J., Otto, E.A., Attanasio, M., Allen, S.J., Parisi, M.A., Glass, I., Utsch, B., Hashmi, S., Fazzi, E., Omran, H., O'Toole, J.F., Sayer, J.A., Hildebrandt, F., 2007. Mutation analysis of NPHP6/CEP290 in patients with Joubert syndrome and Senior-Loken syndrome. J. Med. Genet. 44, 657–663. https://doi.org/10.1136/jmg.2007.052027.
Herbert, A.P., Deakin, J.A., Schmidt, C.Q., Blaum, B.S., Egan, C., Ferreira, V.P., Pangburn, M.K., Lyon, M., Uhrin, D., Barlow, P.N., 2007. Structure shows that a glycosaminoglycan and protein recognition site in factor H is perturbed by age-related macular degeneration-linked single nucleotide polymorphism. J. Biol. Chem. 282, 18960–18968. https://doi.org/10.1074/jbc.M609636200.
Hikage, F., Atkins, S., Kahana, A., Smith, T.J., Chun, T.H., 2019. HIF2A-LOX pathway promotes fibrotic tissue remodeling in thyroid-associated orbitopathy. Endocrinology 160, 20–35. https://doi.org/10.1210/en.2018-00272.
Hiler, D., Chen, X., Hazen, J., Kupriyanov, S., Carroll, P.A., Qu, C., Xu, B., Johnson, D., Griffiths, L., Frase, S., Rodriguez, A.R., Martin, G., Zhang, J., Jeon, J., Fan, Y., Finkelstein, D., Eisenman, R.N., Baldwin, K., Dyer, M.A., 2015. Quantification of retinogenesis in 3D cultures reveals epigenetic memory and higher efficiency in iPSCs derived from rod photoreceptors. Cell Stem Cell 17, 101–115. https://doi.org/10.1016/j.stem.2015.05.015.
Hirami, Y., Osakada, F., Takahashi, K., Okita, K., Yamanaka, S., Ikeda, H., Yoshimura, N., Takahashi, M., 2009. Generation of retinal cells from mouse and human induced pluripotent stem cells. Neurosci. Lett. 458, 126–131. https://doi.org/10.1016/j.neulet.2009.04.035.
Hoang, Q.V., Linsenmeier, R.A., Chung, C.K., Curcio, C.A., 2002. Photoreceptor inner segments in monkey and human retina: mitochondrial density, optics, and regional variation. Vis. Neurosci. 19, 395–407. https://doi.org/10.1017/S0952523802194028.
Holz, F.G., Strauss, E.C., Schmitz-Valckenberg, S., van Lookeren Campagne, M., 2014. Geographic atrophy: clinical features and potential therapeutic approaches. Ophthalmology 121, 1079–1091. https://doi.org/10.1016/j.ophtha.2013.11.023.
Homma, K., Toda, E., Osada, H., Nagai, N., Era, T., Tsubota, K., Okano, H., Ozawa, Y., 2021. Taurine rescues mitochondria-related metabolic impairments in the patient-derived induced pluripotent stem cells and epithelial-mesenchymal transition in the retinal pigment epithelium. Redox Biol. 41, 101921 https://doi.org/10.1016/j.redox.2021.101921.
Hoon, M., Okawa, H., Della Santina, L., Wong, R.O., 2014. Functional architecture of the retina: development and disease. Prog. Retin. Eye Res. 42, 44–84. https://doi.org/10.1016/j.preteyeres.2014.06.003.
Hsu, P.D., Scott, D.A., Weinstein, J.A., Ran, F.A., Konermann, S., Agarwala, V., Li, Y., Fine, E.J., Wu, X., Shalem, O., Cradick, T.J., Marraffini, L.A., Bao, G., Zhang, F., 2013. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat. Biotechnol. 31, 827–832. https://doi.org/10.1038/nbt.2647.
Huang, K.-C., Wang, M.-L., Chen, S.-J., Kuo, J.-C., Wang, W.-J., Nhi Nguyen, P.N., Wahlin, K.J., Lu, J.-F., Tran, A.A., Shi, M., Chien, Y., Yarmishyn, A.A., Tsai, P.-H., Yang, T.-C., Jane, W.-N., Chang, C.-C., Peng, C.-H., Schlaeger, T.M., Chiou, S.-H., 2019. Morphological and molecular defects in human three-dimensional retinal organoid model of X-linked juvenile retinoschisis. Stem Cell Reports 13, 906–923. https://doi.org/10.1016/j.stemcr.2019.09.010.
Hui, F., Tang, J., Williams, P.A., McGuinness, M.B., Hadoux, X., Casson, R.J., Coote, M., Trounce, I.A., Martin, K.R., van Wijngaarden, P., Crowston, J.G., 2020. Improvement in inner retinal function in glaucoma with nicotinamide (vitamin B3) supplementation: a crossover randomized clinical trial. Clin. Exp. Ophthalmol. 48, 903–914. https://doi.org/10.1111/ceo.13818.
Hung, S.S., Chrysostomou, V., Li, F., Lim, J.K., Wang, J.H., Powell, J.E., Tu, L., Daniszewski, M., Lo, C., Wong, R.C., Crowston, J.G., Pebay, A., King, A.E., Bui, B.V., Liu, G.S., Hewitt, A.W., 2016. AAV-mediated CRISPR/Cas gene editing of retinal cells in vivo. Invest. Ophthalmol. Vis. Sci. 57, 3470–3476. https://doi.org/10.1167/iovs.16-19316.
Idelson, M., Alper, R., Obolensky, A., Ben-Shushan, E., Hemo, I., Yachimovich-Cohen, N., Khaner, H., Smith, Y., Wiser, O., Gropp, M., Cohen, M.A., Even-Ram, S., Berman-Zaken, Y., Matzrafi, L., Rechavi, G., Banin, E., Reubinoff, B., 2009. Directed differentiation of human embryonic stem cells into functional retinal pigment epithelium cells. Cell Stem Cell 5, 396–408. https://doi.org/10.1016/j.stem.2009.07.002.
Iglesias, A., Anyane-Yeboa, K., Wynn, J., Wilson, A., Truitt Cho, M., Guzman, E., Sisson, R., Egan, C., Chung, W.K., 2014. The usefulness of whole-exome sequencing in routine clinical practice. Genet. Med. 16, 922–931. https://doi.org/10.1038/gim.2014.58.
Jacks, T., Fazeli, A., Schmitt, E.M., Bronson, R.T., Goodell, M.A., Weinberg, R.A., 1992. Effects of an Rb mutation in the mouse. Nature 359, 295–300. https://doi.org/10.1038/359295a0.
Jaffe, G.J., Westby, K., Csaky, K.G., Mones, J., Pearlman, J.A., Patel, S.S., Joondeph, B.C., Randolph, J., Masonson, H., Rezaei, K.A., 2021. C5 inhibitor avacincaptad pegol for geographic atrophy due to age-related macular degeneration: a randomized pivotal phase 2/3 trial. Ophthalmology 128, 576–586. https://doi.org/10.1016/j.ophtha.2020.08.027.
Jang, H., Jo, D.H., Cho, C.S., Shin, J.H., Seo, J.H., Yu, G., Gopalappa, R., Kim, D., Cho, S.-R., Kim, J.H., Kim, H.H., 2022. Application of prime editing to the correction of mutations and phenotypes in adult mice with liver and eye diseases. Nat. Biomed. Eng. 6, 181–194. https://doi.org/10.1038/s41551-021-00788-9.
Jia, R., Meng, X., Chen, S., Zhang, F., Du, J., Liu, X., Yang, L., 2023. AAV-mediated gene-replacement therapy restores viability of BCD patient iPSC derived RPE cells and vision of Cyp4v3 knockout mice. Hum. Mol. Genet. 32, 122–138. https://doi.org/10.1093/hmg/ddac181.
Jin, Z.-B., Okamoto, S., Xiang, P., Takahashi, M., 2012. Integration-free induced pluripotent stem cells derived from retinitis pigmentosa patient for disease modeling. Stem Cells Transl. Med. 1, 503–509. https://doi.org/10.5966/sctm.2012-0005.
Johnson, C.A., Malicki, J.J., 2019. The nuclear arsenal of cilia. Dev. Cell 49, 161–170. https://doi.org/10.1016/j.devcel.2019.03.009.
Johnson, L.V., Forest, D.L., Banna, C.D., Radeke, C.M., Maloney, M.A., Hu, J., Spencer, C. N., Walker, A.M., Tsie, M.S., Bok, D., Radeke, M.J., Anderson, D.H., 2011. Cell culture model that mimics drusen formation and triggers complement activation associated with age-related macular degeneration. Proc. Natl. Acad. Sci. U S A 108, 18277–18282. https://doi.org/10.1073/pnas.1109703108.
Kallman, A., Capowski, E.E., Wang, J., Kaushik, A.M., Jansen, A.D., Edwards, K.L., Chen, L., Berlinicke, C.A., Joseph Phillips, M., Pierce, E.A., Qian, J., Wang, T.H., Gamm, D.M., Zack, D.J., 2020. Investigating cone photoreceptor development using

patient-derived NRL null retinal organoids. Commun. Biol. 3, 82. https://doi.org/10.1038/s42003-020-0808-5.
Kalluri, R., LeBleu, V.S., 2020. The biology, function, and biomedical applications of exosomes. Science 367, eaau6977. https://doi.org/10.1126/science.aau6977.
Kaltak, M., de Bruijn, P., Piccolo, D., Lee, S.E., Dulla, K., Hoogenboezem, T., Beumer, W., Webster, A.R., Collin, R.W.J., Cheetham, M.E., Platenburg, G., Swildens, J., 2023. Antisense oligonucleotide therapy corrects splicing in the common Stargardt disease type 1-causing variant ABCA4 c.5461-10T>C. Mol. Ther. Nucleic Acids 31, 674–688. https://doi.org/10.1016/j.omtn.2023.02.020.
Kandoi, S., Martinez, C., Chen, K.X., Reddy, L.V.K., Mehine, M., Mansfield, B.C., Duncan, J.L., Lamba, D.A., 2023. Disease modeling and pharmacological rescue of autosomal dominant Retinitis Pigmentosa associated with RHO copy number variation. PREPRINT available at medRxiv 2023. https://doi.org/10.1101/2023.02.27.23286248, 02.27.23286248.
Kang, G.Y., Bang, J.Y., Choi, A.J., Yoon, J., Lee, W.C., Choi, S., Yoon, S., Kim, H.C., Baek, J.H., Park, H.S., Lim, H.J., Chung, H., 2014. Exosomal proteins in the aqueous humor as novel biomarkers in patients with neovascular age-related macular degeneration. J. Proteome Res. 13, 581–595. https://doi.org/10.1021/pr400751k.
Kapoor, R., Whigham, B.T., Al-Aswad, L.A., 2019. Artificial intelligence and optical coherence tomography imaging. Asia Pac. J. Ophthalmol. (Phila) 8, 187–194. https://doi.org/10.22608/APO.201904.
Karjosukarso, D.W., Bukkems, F., Duijkers, L., Leijsten, N., Collin, R.W.J., 2023. Generation of three isogenic control lines from patient-derived iPSCs carrying bi-allelic ABCA4 variants underlying Stargardt disease. Stem Cell Res. 71, 103169 https://doi.org/10.1016/j.scr.2023.103169.
Kawasaki, H., Suemori, H., Mizuseki, K., Watanabe, K., Urano, F., Ichinose, H., Haruta, M., Takahashi, M., Yoshikawa, K., Nishikawa, S., Nakatsuji, N., Sasai, Y., 2002. Generation of dopaminergic neurons and pigmented epithelia from primate ES cells by stromal cell-derived inducing activity. Proc. Natl. Acad. Sci. U S A 99, 1580–1585. https://doi.org/10.1073/pnas.032662199.
Kegeles, E., Naumov, A., Karpulevich, E.A., Volchkov, P., Baranov, P., 2020. Convolutional neural networks can predict retinal differentiation in retinal organoids. Front. Cell. Neurosci. 14, 171. https://doi.org/10.3389/fncel.2020.00171.
Khabou, H., Garita-Hernandez, M., Chaffiol, A., Reichman, S., Jaillard, C., Brazhnikova, E., Bertin, S., Forster, V., Desrosiers, M., Winckler, C., Goureau, O., Picaud, S., Duebel, J., Sahel, J.A., Dalkara, D., 2018. Noninvasive gene delivery to foveal cones for vision restoration. JCI Insight 3, e96029. https://doi.org/10.1172/jci.insight.96029.
Khan, J.C., Thurlby, D.A., Shahid, H., Clayton, D.G., Yates, J.R., Bradley, M., Moore, A.T., Bird, A.C., Genetic Factors in, A.M.D.S, 2006. Smoking and age related macular degeneration: the number of pack years of cigarette smoking is a major determinant of risk for both geographic atrophy and choroidal neovascularisation. Br. J. Ophthalmol. 90, 75–80. https://doi.org/10.1136/bjo.2005.073643.
Khan, M., Arno, G., Fakin, A., Parfitt, D.A., Dhooge, P.P.A., Albert, S., Bax, N.M., Duijkers, L., Niblock, M., Hau, K.L., Bloch, E., Schiff, E.R., Piccolo, D., Hogden, M.C., Hoyng, C.B., Webster, A.R., Cremers, F.P.M., Cheetham, M.E., Garanto, A., Collin, R. W.J., 2020. Detailed phenotyping and therapeutic strategies for intronic ABCA4 variants in Stargardt disease. Mol. Ther. Nucleic Acids 21, 412–427. https://doi.org/10.1016/j.omtn.2020.06.007.
Khanna, H., 2015. Photoreceptor sensory cilium: traversing the ciliary gate. Cells 4, 674–686. https://doi.org/10.3390/cells4040674.
Kim, S.A., Kim, S.J., Choi, Y.A., Yoon, H.J., Kim, A., Lee, J., 2020. Retinal VEGFA maintains the ultrastructure and function of choriocapillaris by preserving the endothelial PLVAP. Biochem. Biophys. Res. Commun. 522, 240–246. https://doi.org/10.1016/j.bbrc.2019.11.085.
Kim, Y.J., Kim, S., Jung, Y., Jung, E., Kwon, H.J., Kim, J., 2018. Eupatilin rescues ciliary transition zone defects to ameliorate ciliopathy-related phenotypes. J. Clin. Invest. 128, 3642–3648. https://doi.org/10.1172/JCI99232.
Kivela, T., 2009. The epidemiological challenge of the most frequent eye cancer: retinoblastoma, an issue of birth and death. Br. J. Ophthalmol. 93, 1129–1131. https://doi.org/10.1136/bjo.2008.150292.
Kivela, T.T., Hadjistilianou, T., 2017. Neonatal retinoblastoma. Asia Pac. J. Oncol. Nurs. 4, 197–204. https://doi.org/10.4103/apjon.apjon_18_17.
Kleinstiver, B.P., Prew, M.S., Tsai, S.Q., Topkar, V.V., Nguyen, N.T., Zheng, Z., Gonzales, A.P., Li, Z., Peterson, R.T., Yeh, J.R., Aryee, M.J., Joung, J.K., 2015. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature 523, 481–485. https://doi.org/10.1038/nature14592.
Klimanskaya, I., Hipp, J., Rezai, K.A., West, M., Atala, A., Lanza, R., 2004. Derivation and comparative assessment of retinal pigment epithelium from human embryonic stem cells using transcriptomics. Cloning Stem Cells 6, 217–245. https://doi.org/10.1089/clo.2004.6.217.
Klimczak, R.R., Koerber, J.T., Dalkara, D., Flannery, J.G., Schaffer, D.V., 2009. A novel adeno-associated viral variant for efficient and selective intravitreal transduction of rat Müller cells. PLoS One 4, e7467. https://doi.org/10.1371/journal.pone.0007467.
Klingeborn, M., Dismuke, W.M., Skiba, N.P., Kelly, U., Stamer, W.D., Bowes Rickman, C., 2017. Directional exosome proteomes reflect polarity-specific functions in retinal pigmented epithelium monolayers. Sci. Rep. 7, 4901. https://doi.org/10.1038/s41598-017-05102-9.
Knight, J., Hartung, T., Rovida, C., 2023. 4.2 million and counting... The animal toll for REACH systemic toxicity studies. ALTEX 40, 389–407. https://doi.org/10.14573/altex.2303201.
Knudson Jr., A.G., 1971. Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. U S A 68, 820–823. https://doi.org/10.1073/pnas.68.4.820.
Kokkinaki, M., Sahibzada, N., Golestaneh, N., 2011. Human induced pluripotent stem-derived retinal pigment epithelium (RPE) cells exhibit ion transport, membrane potential, polarized vascular endothelial growth factor secretion, and gene expression pattern similar to native RPE. Stem Cells 29, 825–835. https://doi.org/10.1002/stem.635.
Kooi, I.E., Mol, B.M., Massink, M.P., Ameziane, N., Meijers-Heijboer, H., Dommering, C. J., van Mil, S.E., de Vries, Y., van der Hout, A.H., Kaspers, G.J., Moll, A.C., Te Riele, H., Cloos, J., Dorsman, J.C., 2016a. Somatic genomic alterations in retinoblastoma beyond RB1 are rare and limited to copy number changes. Sci. Rep. 6, 25264 https://doi.org/10.1038/srep25264.
Kooi, I.E., Mol, B.M., Massink, M.P., de Jong, M.C., de Graaf, P., van der Valk, P., Meijers-Heijboer, H., Kaspers, G.J., Moll, A.C., Te Riele, H., Cloos, J., Dorsman, J.C., 2016b. A meta-analysis of retinoblastoma copy numbers refines the list of possible driver genes involved in tumor progression. PLoS One 11, e0153323. https://doi.org/10.1371/journal.pone.0153323.
Kosanke, M., Davenport, C., Szepes, M., Wiehlmann, L., Kohrn, T., Dorda, M., Gruber, J., Menge, K., Sievert, M., Melchert, A., Gruh, I., Göhring, G., Martin, U., 2021. iPSC culture expansion selects against putatively actionable mutations in the mitochondrial genome. Stem Cell Reports 16, 2488–2502. https://doi.org/10.1016/j.stemcr.2021.08.016.
Kosmaoglou, M., Schwarz, N., Bett, J.S., Cheetham, M.E., 2008. Molecular chaperones and photoreceptor function. Prog. Retin. Eye Res. 27, 434–449. https://doi.org/10.1016/j.preteyeres.2008.03.001.
Krausova, M., Kreplova, M., Banik, P., Cvackova, Z., Kubovciak, J., Modrak, M., Zudova, D., Lindovsky, J., Kubik-Zahorodna, A., Palkova, M., Kolar, M., Prochazka, J., Sedlacek, R., Stanek, D., 2023. Retinitis pigmentosa-associated mutations in mouse Prpf8 cause misexpression of circRNAs and degeneration of cerebellar granule cells. Life Sci. Alliance 6, e202201855. https://doi.org/10.26508/lsa.202201855.
Kruczek, K., Qu, Z., Gentry, J., Fadl, B.R., Gieser, L., Hiriyanna, S., Batz, Z., Samant, M., Samanta, A., Chu, C.J., Campello, L., Brooks, B.P., Wu, Z., Swaroop, A., 2021. Gene therapy of dominant CRX-leber congenital amaurosis using patient stem cell-derived retinal organoids. Stem Cell Reports 16, 252–263. https://doi.org/10.1016/j.stemcr.2020.12.018.
Kruczek, K., Qu, Z., Welby, E., Shimada, H., Hiriyanna, S., English, M.A., Zein, W.M., Brooks, B.P., Swaroop, A., 2022. In vitro modeling and rescue of ciliopathy associated with IQCB1/NPHP5 mutations using patient-derived cells. Stem Cell Reports 17, 2172–2186. https://doi.org/10.1016/j.stemcr.2022.08.006.
Kruk, P.A., Auersperg, N., 1992. Human ovarian surface epithelial cells are capable of physically restructuring extracellular matrix. Am. J. Obstet. Gynecol. 167, 1437–1443. https://doi.org/10.1016/s0002-9378(11)91730-x.
Kumada, M., Niwa, M., Hara, A., Matsuno, H., Mori, H., Ueshima, S., Matsuo, O., Yamamoto, T., Kozawa, O., 2005. Tissue type plasminogen activator facilitates NMDA-receptor-mediated retinal apoptosis through an independent fibrinolytic cascade. Invest. Ophthalmol. Vis. Sci. 46, 1504–1507. https://doi.org/10.1167/iovs.04-0595.
Kurzawa-Akanbi, M., Lotery, A., Steel, D.H., Lako, M., 2021. Age-related macular degeneration - biomarkers and therapies. Regen. Med. 16, 431–434. https://doi.org/10.2217/rme-2021-0059.
Kurzawa-Akanbi, M., Whitfield, P., Burte, F., Bertelli, P.M., Pathak, V., Doherty, M., Hilgen, B., Gliaudelyte, L., Platt, M., Queen, R., Coxhead, J., Porter, A., Oberg, M., Fabrikova, D., Davey, T., Beh, C.S., Georgiou, M., Collin, J., Boczonadi, V., Hartlova, A., Taggart, M., Al-Aama, J., Korolchuk, V.I., Morris, C.M., Guduric-Fuchs, J., Steel, D.H., Medina, R.J., Armstrong, L., Lako, M., 2022. Retinal pigment epithelium extracellular vesicles are potent inducers of age-related macular degeneration disease phenotype in the outer retina. J. Extracell. Vesicles 11, e12295. https://doi.org/10.1002/jev2.12295.
Kuwahara, A., Ozone, C., Nakano, T., Saito, K., Eiraku, M., Sasai, Y., 2015. Generation of a ciliary margin-like stem cell niche from self-organizing human retinal tissue. Nat. Commun. 6, 6286. https://doi.org/10.1038/ncomms7286.
Lako, M., Dorgau, B., Collin, J., Rozanska, A., Zerti, D., Crossier, M., Hussain, R., Coxhead, J., Dhanaseelan, T., Patel, A., Sowden, J., FitzPatrick, D., Queen, R., 2023. Spatiotemporal single cell analyses reveal a transient population of retinal progenitor cells in the ciliary margin of developing human retina. PREPRINT (Version 1) available at Research Square. https://doi.org/10.21203/rs.3.rs-3160527/v1.
Lamba, D.A., Gust, J., Reh, T.A., 2009. Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx-deficient mice. Cell Stem Cell 4, 73–79. https://doi.org/10.1016/j.stem.2008.10.015.
Lamba, D.A., Karl, M.O., Ware, C.B., Reh, T.A., 2006. Efficient generation of retinal progenitor cells from human embryonic stem cells. Proc. Natl. Acad. Sci. U S A 103, 12769–12774. https://doi.org/10.1073/pnas.0601990103.
Lamba, D.A., McUsic, A., Hirata, R.K., Wang, P.R., Russell, D., Reh, T.A., 2010. Generation, purification and transplantation of photoreceptors derived from human induced pluripotent stem cells. PLoS One 5, e8763. https://doi.org/10.1371/journal.pone.0008763.
Lambacher, N.J., Bruel, A.L., van Dam, T.J., Szymanska, K., Slaats, G.G., Kuhns, S., McManus, G.J., Kennedy, J.E., Gaff, K., Wu, K.M., van der Lee, R., Burglen, L., Doummar, D., Riviere, J.B., Faivre, L., Attie-Bitach, T., Saunier, S., Curd, A., Peckham, M., Giles, R.H., Johnson, C.A., Huynen, M.A., Thauvin-Robinet, C., Blacque, O.E., 2016. TMEM107 recruits ciliopathy proteins to subdomains of the ciliary transition zone and causes Joubert syndrome. Nat. Cell Biol. 18, 122–131. https://doi.org/10.1038/ncb3273.
Lambert, N.G., ElShelmani, H., Singh, M.K., Mansergh, F.C., Wride, M.A., Padilla, M., Keegan, D., Hogg, R.E., Ambati, B.K., 2016. Risk factors and biomarkers of age-

related macular degeneration. Prog. Retin. Eye Res. 54, 64–102. https://doi.org/10.1016/j.preteyeres.2016.04.003.
Lamm, N., Ben-David, U., Golan-Lev, T., Storchová, Z., Benvenisty, N., Kerem, B., 2016. Genomic instability in human pluripotent stem cells arises from replicative stress and chromosome condensation defects. Cell Stem Cell 18, 253–261. https://doi.org/10.1016/j.stem.2015.11.003.
Lane, A., Jovanovic, K., Shortall, C., Ottaviani, D., Panes, A.B., Schwarz, N., Guarascio, R., Hayes, M.J., Palfi, A., Chadderton, N., Farrar, G.J., Hardcastle, A.J., Cheetham, M.E., 2020. Modeling and rescue of RP2 retinitis pigmentosa using iPSC-derived retinal organoids. Stem Cell Reports 15, 67–79. https://doi.org/10.1016/j.stemcr.2020.05.007.
Layer, P.G., Willbold, E., 1989. Embryonic chicken retinal cells can regenerate all cell layers in vitro, but ciliary pigmented cells induce their correct polarity. Cell Tissue Res. 258, 233–242. https://doi.org/10.1007/BF00239443.
Layer, P.G., Willbold, E., 1993. Histogenesis of the avian retina in reaggregation culture: from dissociated cells to laminar neuronal networks. Int. Rev. Cytol. 146, 1–47. https://doi.org/10.1016/s0074-7696(08)60378-2.
Layer, P.G., Willbold, E., 1994. Regeneration of the avian retina by retinospheroid technology. Prog. Retin. Eye Res. 13, 197–230. https://doi.org/10.1016/1350-9462(94)90010-8.
Leach, L.L., Buchholz, D.E., Nadar, V.P., Lowenstein, S.E., Clegg, D.O., 2015. Canonical/beta-catenin Wnt pathway activation improves retinal pigmented epithelium derivation from human embryonic stem cells. Invest. Ophthalmol. Vis. Sci. 56, 1002–1013. https://doi.org/10.1167/iovs.14-15835.
Leach, L.L., Clegg, D.O., 2015. Concise review: making stem cells retinal: methods for deriving retinal pigment epithelium and implications for patients with ocular disease. Stem Cells 33, 2363–2373. https://doi.org/10.1002/stem.2010.
Leach, L.L., Croze, R.H., Hu, Q., Nadar, V.P., Clevenger, T.N., Pennington, B.O., Gamm, D.M., Clegg, D.O., 2016. Induced pluripotent stem cell-derived retinal pigmented epithelium: a comparative study between cell lines and differentiation methods. J. Ocul. Pharmacol. Therapeut. 32, 317–330. https://doi.org/10.1089/jop.2016.0022.
Lee, E.J., Diaz-Aguilar, M.S., Min, H., Choi, J., Valdez Duran, D.A., Grandjean, J.M., Wiseman, R.L., Kroeger, H., Lin, J.H., 2023a. Mitochondria and endoplasmic reticulum stress in retinal organoids from patients with vision loss. Am. J. Pathol. 193, 1721–1739. https://doi.org/10.1016/j.ajpath.2022.12.002.
Lee, E.Y.H.P., Chang, C.-Y., Hu, N., Wang, Y.-C.J., Lai, C.-C., Herrup, K., Lee, W.-H., Bradley, A., 1992. Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. Nature 359, 288–294. https://doi.org/10.1038/359288a0.
Lee, S., Lee, H., Baek, G., Kim, J.S., 2023b. Precision mitochondrial DNA editing with high-fidelity DddA-derived base editors. Nat. Biotechnol. 41, 378–386. https://doi.org/10.1038/s41587-022-01486-w.
Lees, J.G., Gardner, D.K., Harvey, A.J., 2017. Pluripotent stem cell metabolism and mitochondria: beyond ATP. Stem Cells Int. 2017, 2874283 https://doi.org/10.1155/2017/2874283.
Leibowitz, M.L., Papathanasiou, S., Doerfler, P.A., Blaine, L.J., Sun, L., Yao, Y., Zhang, C. Z., Weiss, M.J., Pellman, D., 2021. Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. Nat. Genet. 53, 895–905. https://doi.org/10.1038/s41588-021-00838-7.
Leitch, C.C., Zaghloul, N.A., Davis, E.E., Stoetzel, C., Diaz-Font, A., Rix, S., Alfadhel, M., Lewis, R.A., Eyaid, W., Banin, E., Dollfus, H., Beales, P.L., Badano, J.L., Katsanis, N., 2008. Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome. Nat. Genet. 40, 443–448. https://doi.org/10.1038/ng.97.
Leite, S.B., Roosens, T., El Taghdouini, A., Mannaerts, I., Smout, A.J., Najimi, M., Sokal, E., Noor, F., Chesne, C., van Grunsven, L.A., 2016. Novel human hepatic organoid model enables testing of drug-induced liver fibrosis in vitro. Biomaterials 78, 1–10. https://doi.org/10.1016/j.biomaterials.2015.11.026.
Lenahan, C., Sanghavi, R., Huang, L., Zhang, J.H., 2020. Rhodopsin: a potential biomarker for neurodegenerative diseases. Front. Neurosci. 14, 326. https://doi.org/10.3389/fnins.2020.00326.
Lenis, T.L., Hu, J., Ng, S.Y., Jiang, Z., Sarfare, S., Lloyd, M.B., Esposito, N.J., Samuel, W., Jaworski, C., Bok, D., Finnemann, S.C., Radeke, M.J., Redmond, T.M., Travis, G.H., Radu, R.A., 2018. Expression of ABCA4 in the retinal pigment epithelium and its implications for Stargardt macular degeneration. Proc. Natl. Acad. Sci. U S A 115, E11120–E11127. https://doi.org/10.1073/pnas.1802519115.
Leong, Y.C., Di Foggia, V., Pramod, H., Bitner-Glindzicz, M., Patel, A., Sowden, J.C., 2022. Molecular pathology of Usher 1B patient-derived retinal organoids at single cell resolution. Stem Cell Reports 17, 2421–2437. https://doi.org/10.1016/j.stemcr.2022.09.006.
Leung, A., Sacristan-Reviriego, A., Perdigao, P.R.L., Sai, H., Georgiou, M., Kalitzeos, A., Carr, A.F., Coffey, P.J., Michaelides, M., Bainbridge, J., Cheetham, M.E., van der Spuy, J., 2022. Investigation of PTC124-mediated translational readthrough in a retinal organoid model of AIPL1-associated Leber congenital amaurosis. Stem Cell Reports 17, 2187–2202. https://doi.org/10.1016/j.stemcr.2022.08.005.
Levin, L.A., Nilsson, S.F.E., Ver Hoeve, J., Wu, S., Kaufman, P.L., Alm, A., 2011. Adler's Physiology of the Eye, eleventh ed. Elsevier (Chapter 4).
Li, Y.P., Wang, Y.T., Wang, W., Zhang, X., Shen, R.J., Jin, K., Jin, L.W., Jin, Z.B., 2022. Second hit impels oncogenesis of retinoblastoma in patient-induced pluripotent stem cell-derived retinal organoids: direct evidence for Knudson's theory. PNAS Nexus 1, pgac162. https://doi.org/10.1093/pnasnexus/pgac162.
Liang, Y., Tan, F., Sun, X., Cui, Z., Gu, J., Mao, S., Chan, H.F., Tang, S., Chen, J., 2022. Aberrant retinal pigment epithelial cells derived from induced pluripotent stem cells of a retinitis pigmentosa patient with the PRPF6 mutation. Int. J. Mol. Sci. 23, 9049. https://doi.org/10.3390/ijms23169049.
Liao, D.S., Grossi, F.V., El Mehdi, D., Gerber, M.R., Brown, D.M., Heier, J.S., Wykoff, C. C., Singerman, L.J., Abraham, P., Grassmann, F., Nuernberg, P., Weber, B.H.F., Deschatelets, P., Kim, R.Y., Chung, C.Y., Ribeiro, R.M., Hamdani, M., Rosenfeld, P.J., Boyer, D.S., Slakter, J.S., Francois, C.G., 2020. Complement C3 inhibitor pegcetacoplan for geographic atrophy secondary to age-related macular degeneration: a randomized phase 2 trial. Ophthalmology 127, 186–195. https://doi.org/10.1016/j.ophtha.2019.07.011.
Lin, H., Xu, H., Liang, F.-Q., Liang, H., Gupta, P., Havey, A.N., Boulton, M.E., Godley, B. F., 2011. Mitochondrial DNA damage and repair in RPE associated with aging and age-related macular degeneration. Invest. Ophthalmol. Vis. Sci. 52, 3521–3529. https://doi.org/10.1167/iovs.10-6163.
Linder, B., Dill, H., Hirmer, A., Brocher, J., Lee, G.P., Mathavan, S., Bolz, H.J., Winkler, C., Laggerbauer, B., Fischer, U., 2011. Systemic splicing factor deficiency causes tissue-specific defects: a zebrafish model for retinitis pigmentosa. Hum. Mol. Genet. 20, 368–377. https://doi.org/10.1093/hmg/ddq473.
Liu, H., Zhang, Y., Zhang, Y.Y., Li, Y.P., Hua, Z.Q., Zhang, C.J., Wu, K.C., Yu, F., Zhang, Y., Su, J., Jin, Z.B., 2020. Human embryonic stem cell-derived organoid retinoblastoma reveals a cancerous origin. Proc. Natl. Acad. Sci. U S A 117, 33628–33638. https://doi.org/10.1073/pnas.2011780117.
Liu, S., Mozaffari-Jovin, S., Wollenhaupt, J., Santos, K.F., Theuser, M., Dunin-Horkawicz, S., Fabrizio, P., Bujnicki, J.M., Luhrmann, R., Wahl, M.C., 2015. A composite double-/single-stranded RNA-binding region in protein Prp3 supports tri-snRNP stability and splicing. Elife 4, e07320. https://doi.org/10.7554/eLife.07320.
Liu, S., Rauhut, R., Vornlocher, H.P., Luhrmann, R., 2006. The network of protein-protein interactions within the human U4/U6.U5 tri-snRNP. RNA 12, 1418–1430. https://doi.org/10.1261/rna.55406.
Liu, X., Bulgakov, O.V., Wen, X.H., Woodruff, M.L., Pawlyk, B., Yang, J., Fain, G.L., Sandberg, M.A., Makino, C.L., Li, T., 2004. AIPL1, the protein that is defective in Leber congenital amaurosis, is essential for the biosynthesis of retinal rod cGMP phosphodiesterase. Proc. Natl. Acad. Sci. U S A 101, 13903–13908. https://doi.org/10.1073/pnas.0405160101.
Lopes, V.S., Boye, S.E., Louie, C.M., Boye, S., Dyka, F., Chiodo, V., Fofo, H., Hauswirth, W.W., Williams, D.S., 2013. Retinal gene therapy with a large MYO7A cDNA using adeno-associated virus. Gene Ther. 20, 824–833. https://doi.org/10.1038/gt.2013.3.
Lu, W., Rettenmeier, E., Paszek, M., Yueh, M.F., Tukey, R.H., Trottier, J., Barbier, O., Chen, S., 2017. Crypt organoid culture as an in vitro model in drug metabolism and cytotoxicity studies. Drug Metab. Dispos. 45, 748–754. https://doi.org/10.1124/dmd.117.075945.
Lu, Y., Shiau, F., Yi, W., Lu, S., Wu, Q., Pearson, J.D., Kallman, A., Zhong, S., Hoang, T., Zuo, Z., Zhao, F., Zhang, M., Tsai, N., Zhuo, Y., He, S., Zhang, J., Stein-O'Brien, G.L., Sherman, T.D., Duan, X., Fertig, E.J., Goff, L.A., Zack, D.J., Handa, J.T., Xue, T., Bremner, R., Blackshaw, S., Wang, X., Clark, B.S., 2020. Single-cell analysis of human retina identifies evolutionarily conserved and species-specific mechanisms controlling development. Dev. Cell 53,. https://doi.org/10.1016/j.devcel.2020.04.009, 473-491.e9.
Ludwig, A.L., Mayerl, S.J., Gao, Y., Banghart, M., Bacig, C., Fernandez Zepeda, M.A., Zhao, X., Gamm, D.M., 2023. Re-formation of synaptic connectivity in dissociated human stem cell-derived retinal organoid cultures. Proc. Natl. Acad. Sci. U S A 120, e2213418120. https://doi.org/10.1073/pnas.2213418120.
Lukovic, D., Artero Castro, A., Kaya, K.D., Munezero, D., Gieser, L., Davo-Martinez, C., Corton, M., Cuenca, N., Swaroop, A., Ramamurthy, V., Ayuso, C., Erceg, S., 2020. Retinal organoids derived from hiPSCs of an AIPL1-LCA patient maintain cytoarchitecture despite reduced levels of mutant AIPL1. Sci. Rep. 10, 5426. https://doi.org/10.1038/s41598-020-62047-2.
MacCarthy, A., Birch, J.M., Draper, G.J., Hungerford, J.L., Kingston, J.E., Kroll, M.E., Stiller, C.A., Vincent, T.J., Murphy, M.F., 2009. Retinoblastoma: treatment and survival in Great Britain 1963 to 2002. Br. J. Ophthalmol. 93, 38–39. https://doi.org/10.1136/bjo.2008.139626.
Maddalena, A., Tornabene, P., Tiberi, P., Minopoli, R., Manfredi, A., Mutarelli, M., Rossi, S., Simonelli, F., Naggert, J.K., Cacchiarelli, D., Auricchio, A., 2018. Triple vectors expand AAV transfer capacity in the retina. Mol. Ther. 26, 524–541. https://doi.org/10.1016/j.ymthe.2017.11.019.
Majamaa, K., Moilanen, J.S., Uimonen, S., Remes, A.M., Salmela, P.I., Kärppä, M., Majamaa-Voltti, K.A.M., Rusanen, H., Sorri, M., Peuhkurinen, K.J., Hassinen, I.E., 1998. Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population. Am. J. Hum. Genet. 63, 447–454. https://doi.org/10.1086/301959.
Makarov, E.M., Makarova, O.V., Achsel, T., Luhrmann, R., 2000. The human homologue of the yeast splicing factor prp6p contains multiple TPR elements and is stably associated with the U5 snRNP via protein-protein interactions. J. Mol. Biol. 298, 567–575. https://doi.org/10.1006/jmbi.2000.3685.
Makarova, O.V., Makarov, E.M., Liu, S., Vornlocher, H.P., Luhrmann, R., 2002. Protein 61K, encoded by a gene (PRPF31) linked to autosomal dominant retinitis pigmentosa, is required for U4/U6*U5 tri-snRNP formation and pre-mRNA splicing. EMBO J. 21, 1148–1157. https://doi.org/10.1093/emboj/21.5.1148.
Maminishkis, A., Chen, S., Jalickee, S., Banzon, T., Shi, G., Wang, F.E., Ehalt, T., Hammer, J.A., Miller, S.S., 2006. Confluent monolayers of cultured human fetal retinal pigment epithelium exhibit morphology and physiology of native tissue. Invest. Ophthalmol. Vis. Sci. 47, 3612–3624. https://doi.org/10.1167/iovs.05-1622.
Manian, K.V., Galloway, C.A., Dalvi, S., Emanuel, A.A., Mereness, J.A., Black, W., Winschel, L., Soto, C., Li, Y., Song, Y., DeMaria, W., Kumar, A., Slukvin, I., Schwartz, M.P., Murphy, W.L., Anand-Apte, B., Chung, M., Benoit, D.S.W., Singh, R., 2021. 3D iPSC modeling of the retinal pigment epithelium-choriocapillaris complex

identifies factors involved in the pathology of macular degeneration. Cell Stem Cell 28,. https://doi.org/10.1016/j.stem.2021.02.006, 846-862.e8.
Marc, R.E., Anderson, J.R., Jones, B.W., Sigulinsky, C.L., Lauritzen, J.S., 2014. The AII amacrine cell connectome: a dense network hub. Front. Neural Circuits 8, 104. https://doi.org/10.3389/fncir.2014.00104.
Markus, A., Shamul, A., Chemla, Y., Farah, N., Shaham, L., Goldstein, R.S., Mandel, Y., 2019. An optimized protocol for generating labeled and transplantable photoreceptor precursors from human embryonic stem cells. Exp. Eye Res. 180, 29–38. https://doi.org/10.1016/j.exer.2018.11.013.
Martin, G.R., 1981. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc. Natl. Acad. Sci. U S A 78, 7634–7638. https://doi.org/10.1073/pnas.78.12.7634.
Martin, R.M., Ikeda, K., Cromer, M.K., Uchida, N., Nishimura, T., Romano, R., Tong, A.J., Lemgart, V.T., Camarena, J., Pavel-Dinu, M., Sindhu, C., Wiebking, V., Vaidyanathan, S., Dever, D.P., Bak, R.O., Laustsen, A., Lesch, B.J., Jakobsen, M.R., Sebastiano, V., Nakauchi, H., Porteus, M.H., 2019. Highly efficient and marker-free genome editing of human pluripotent stem cells by CRISPR-Cas9 RNP and AAV6 donor-mediated homologous recombination. Cell Stem Cell 24,. https://doi.org/10.1016/j.stem.2019.04.001, 821-828.e5.
Mathers, P.H., Grinberg, A., Mahon, K.A., Jamrich, M., 1997. The Rx homeobox gene is essential for vertebrate eye development. Nature 387, 603–607. https://doi.org/10.1038/42475.
May-Simera, H., Nagel-Wolfrum, K., Wolfrum, U., 2017. Cilia - the sensory antennae in the eye. Prog. Retin. Eye Res. 60, 144–180. https://doi.org/10.1016/j.preteyeres.2017.05.001.
May-Simera, H.L., Wan, Q., Jha, B.S., Hartford, J., Khristov, V., Dejene, R., Chang, J., Patnaik, S., Lu, Q., Banerjee, P., Silver, J., Insinna-Kettenhofen, C., Patel, D., Lotfi, M., Malicdan, M., Hotaling, N., Maminishkis, A., Sridharan, R., Brooks, B., Miyagishima, K., Gunay-Aygun, M., Pal, R., Westlake, C., Miller, S., Sharma, R., Bharti, K., 2018. Primary cilium-mediated retinal pigment epithelium maturation is disrupted in ciliopathy patient cells. Cell Rep. 22, 189–205. https://doi.org/10.1016/j.celrep.2017.12.038.
McClements, M.E., Steward, H., Atkin, W., Goode, E.A., Gandara, C., Chichagova, V., MacLaren, R.E., 2022. Tropism of AAV vectors in photoreceptor-like cells of human iPSC-derived retinal organoids. Transl. Vis. Sci. Technol. 11, 3. https://doi.org/10.1167/tvst.11.4.3.
Megaw, R., Abu-Arafeh, H., Jungnickel, M., Mellough, C., Gurniak, C., Witke, W., Zhang, W., Khanna, H., Mill, P., Dhillon, B., Wright, A.F., Lako, M., Ffrench-Constant, C., 2017. Gelsolin dysfunction causes photoreceptor loss in induced pluripotent cell and animal retinitis pigmentosa models. Nat. Commun. 8, 271. https://doi.org/10.1038/s41467-017-00111-8.
Mekhoubad, S., Bock, C., de Boer, A.S., Kiskinis, E., Meissner, A., Eggan, K., 2012. Erosion of dosage compensation impacts human iPSC disease modeling. Cell Stem Cell 10, 595–609. https://doi.org/10.1016/j.stem.2012.02.014.
Mellough, C.B., Bauer, R., Collin, J., Dorgau, B., Zerti, D., Dolan, D.W.P., Jones, C.M., Izuogu, O.G., Yu, M., Hallam, D., Steyn, J.S., White, K., Steel, D.H., Santibanez-Koref, M., Elliott, D.J., Jackson, M.S., Lindsay, S., Grellscheid, S., Lako, M., 2019. An integrated transcriptional analysis of the developing human retina. Development 146 (2), dev169474. https://doi.org/10.1242/dev.169474.
Mellough, C.B., Collin, J., Sernagor, E., Wride, N.K., Steel, D.H., Lako, M., 2014. Lab generated retina: realizing the dream. Vis. Neurosci. 31, 317–332. https://doi.org/10.1017/S095252381400008X.
Mellough, C.B., Sernagor, E., Moreno-Gimeno, I., Steel, D.H., Lako, M., 2012. Efficient stage-specific differentiation of human pluripotent stem cells toward retinal photoreceptor cells. Stem Cells 30, 673–686. https://doi.org/10.1002/stem.1037.
Meyer, J.S., Howden, S.E., Wallace, K.A., Verhoeven, A.D., Wright, L.S., Capowski, E.E., Pinilla, I., Martin, J.M., Tian, S., Stewart, R., Pattnaik, B., Thomson, J.A., Gamm, D. M., 2011. Optic vesicle-like structures derived from human pluripotent stem cells facilitate a customized approach to retinal disease treatment. Stem Cells 29, 1206–1218. https://doi.org/10.1002/stem.674.
Meyer, J.S., Shearer, R.L., Capowski, E.E., Wright, L.S., Wallace, K.A., McMillan, E.L., Zhang, S.C., Gamm, D.M., 2009. Modeling early retinal development with human embryonic and induced pluripotent stem cells. Proc. Natl. Acad. Sci. U S A 106, 16698–16703. https://doi.org/10.1073/pnas.0905245106.
Miesfeld, J.B., Brown, N.L., 2019. Eye organogenesis: a hierarchical view of ocular development. Curr. Top. Dev. Biol. 132, 351–393. https://doi.org/10.1016/bs.ctdb.2018.12.008.
Mill, P., Christensen, S.T., Pedersen, L.B., 2023. Primary cilia as dynamic and diverse signalling hubs in development and disease. Nat. Rev. Genet. 24, 421–441. https://doi.org/10.1038/s41576-023-00587-9.
Miller 3rd, F.S., Bunt-Milam, A.H., Kalina, R.E., 1982. Clinical-ultrastructural study of thioridazine retinopathy. Ophthalmology 89, 1478–1488. https://doi.org/10.1016/s0161-6420(82)34613-8.
Miller, J.C., Tan, S., Qiao, G., Barlow, K.A., Wang, J., Xia, D.F., Meng, X., Paschon, D.E., Leung, E., Hinkley, S.J., Dulay, G.P., Hua, K.L., Ankoudinova, I., Cost, G.J., Urnov, F. D., Zhang, H.S., Holmes, M.C., Zhang, L., Gregory, P.D., Rebar, E.J., 2011. A TALE nuclease architecture for efficient genome editing. Nat. Biotechnol. 29, 143–148. https://doi.org/10.1038/nbt.1755.
Mischler, A., Karakis, V., Mahinthakumar, J., Carberry, C.K., San Miguel, A., Rager, J.E., Fry, R.C., Rao, B.M., 2021. Two distinct trophectoderm lineage stem cells from human pluripotent stem cells. J. Biol. Chem. 296, 100386 https://doi.org/10.1016/j.jbc.2021.100386.
Mitchell, P., Liew, G., Gopinath, B., Wong, T.Y., 2018. Age-related macular degeneration. Lancet 392, 1147–1159. https://doi.org/10.1016/S0140-6736(18)31550-2.
Molins, B., Fuentes-Prior, P., Adán, A., Antón, R., Arostegui, J.I., Yagüe, J., Dick, A.D., 2016. Complement factor H binding of monomeric C-reactive protein downregulates proinflammatory activity and is impaired with at risk polymorphic CFH variants. Sci. Rep. 6, 22889 https://doi.org/10.1038/srep22889.
Mookherjee, S., Chen, H.Y., Isgrig, K., Yu, W., Hiriyanna, S., Levron, R., Li, T., Colosi, P., Chien, W., Swaroop, A., Wu, Z., 2018. A CEP290 C-terminal domain complements the mutant CEP290 of Rd16 mice in trans and rescues retinal degeneration. Cell Rep. 25, https://doi.org/10.1016/j.celrep.2018.09.043, 611-623.e6.
Moraes, C.T., Ciacci, F., Silvestri, G., Shanske, S., Sciacco, M., Hirano, M., Schon, E.A., Bonilla, E., DiMauro, S., 1993. Atypical clinical presentations associated with the MELAS mutation at position 3243 of human mitochondrial DNA. Neuromuscul. Disord. 3, 43–50. https://doi.org/10.1016/0960-8966(93)90040-Q.
Morohoshi, K., Patel, N., Ohbayashi, M., Chong, V., Grossniklaus, H.E., Bird, A.C., Ono, S. J., 2012. Serum autoantibody biomarkers for age-related macular degeneration and possible regulators of neovascularization. Exp. Mol. Pathol. 92, 64–73. https://doi.org/10.1016/j.yexmp.2011.09.017.
Mulfaul, K., Mullin, N.K., Giacalone, J.C., Voigt, A.P., M, R.D., Stone, E.M., Tucker, B.A., Mullins, R.F., 2022. Local factor H production by human choroidal endothelial cells mitigates complement deposition: implications for macular degeneration. J. Pathol. 257, 29–38. https://doi.org/10.1002/path.5867.
Mullins, R.F., Dewald, A.D., Streb, L.M., Wang, K., Kuehn, M.H., Stone, E.M., 2011. Elevated membrane attack complex in human choroid with high risk complement factor H genotypes. Exp. Eye Res. 93, 565–567. https://doi.org/10.1016/j.exer.2011.06.015.
Munier, F.L., Beck-Popovic, M., Balmer, A., Gaillard, M.C., Bovey, E., Binaghi, S., 2011. Occurrence of sectoral choroidal occlusive vasculopathy and retinal arteriolar embolization after superselective ophthalmic artery chemotherapy for advanced intraocular retinoblastoma. Retina 31, 566–573. https://doi.org/10.1097/IAE.0b013e318203c101.
Murphy, D., Cieply, B., Carstens, R., Ramamurthy, V., Stoilov, P., 2016. The Musashi 1 controls the splicing of photoreceptor-specific exons in the vertebrate retina. PLoS Genet. 12, e1006256 https://doi.org/10.1371/journal.pgen.1006256.
Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., Saito, K., Yonemura, S., Eiraku, M., Sasai, Y., 2012. Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell Stem Cell 10, 771–785. https://doi.org/10.1016/j.stem.2012.05.009.
Navneet, S., Brandon, C., Simpson, K., Rohrer, B., 2023. Exploring the therapeutic potential of elastase inhibition in age-related macular degeneration in mouse and human. Cells 12, 1308. https://doi.org/10.3390/cells12091308.
Navneet, S., Rohrer, B., 2022. Elastin turnover in ocular diseases: a special focus on age-related macular degeneration. Exp. Eye Res. 222, 109164 https://doi.org/10.1016/j.exer.2022.109164.
Naylor, A., Hopkins, A., Hudson, N., Campbell, M., 2019. Tight junctions of the outer blood retina barrier. Int. J. Mol. Sci. 21, 211. https://doi.org/10.3390/ijms21010211.
Nazlamova, L., Villa Vasquez, S.S., Lord, J., Karthik, V., Cheung, M.K., Lakowski, J., Wheway, G., 2022. Microtubule modification defects underlie cilium degeneration in cell models of retinitis pigmentosa associated with pre-mRNA splicing factor mutations. Front. Genet. 13, 1009430 https://doi.org/10.3389/fgene.2022.1009430.
Nelson, J.W., Randolph, P.B., Shen, S.P., Everette, K.A., Chen, P.J., Anzalone, A.V., An, M., Newby, G.A., Chen, J.C., Hsu, A., Liu, D.R., 2022. Engineered pegRNAs improve prime editing efficiency. Nat. Biotechnol. 40, 402–410. https://doi.org/10.1038/s41587-021-01039-7.
Neumann, C.J., Nuesslein-Volhard, C., 2000. Patterning of the zebrafish retina by a wave of sonic hedgehog activity. Science 289, 2137–2139. https://doi.org/10.1126/science.289.5487.2137.
Ng, E.S.Y., Kady, N., Hu, J., Dave, A., Jiang, Z., Pei, J., Gorin, M.B., Matynia, A., Radu, R. A., 2022. Membrane attack complex mediates retinal pigment epithelium cell death in Stargardt macular degeneration. Cells 11, 3462. https://doi.org/10.3390/cells11213462.
Nicole, O., Docagne, F., Ali, C., Margaill, I., Carmeliet, P., MacKenzie, E.T., Vivien, D., Buisson, A., 2001. The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. Nat. Med. 7, 59–64. https://doi.org/10.1038/83358.
Nie, Z., Wang, C., Chen, J., Ji, Y., Zhang, H., Zhao, F., Zhou, X., Guan, M.-X., 2022. Abnormal morphology and function in retinal ganglion cells derived from patients-specific iPSCs generated from individuals with Leber's hereditary optic neuropathy. Hum. Mol. Genet. 32, 231–243. https://doi.org/10.1093/hmg/ddac190.
Nikonorova, V.G., Chrishtop, V.V., Mironov, V.A., Prilepskii, A.Y., 2023. Advantages and potential benefits of using organoids in nanotoxicology. Cells 12, 610. https://doi.org/10.3390/cells12040610.
Ning, K., Luo, Z., Kowal, T.J., Tran, M., Majumder, R., Jarin, T.M., Wu, A.Y., Goldberg, J. L., Sun, Y., 2023. Characterization of primary cilia formation in human ESC-derived retinal organoids. Stem Cells Int. 2023, 6494486 https://doi.org/10.1155/2023/6494486.
Norrie, J.L., Nityanandam, A., Lai, K., Chen, X., Wilson, M., Stewart, E., Griffiths, L., Jin, H., Wu, G., Orr, B., Tran, Q., Allen, S., Reilly, C., Zhou, X., Zhang, J., Newman, K., Johnson, D., Brennan, R., Dyer, M.A., 2021. Retinoblastoma from human stem cell-derived retinal organoids. Nat. Commun. 12, 4535. https://doi.org/10.1038/s41467-021-24781-7.
Nottrott, S., Urlaub, H., Luhrmann, R., 2002. Hierarchical, clustered protein interactions with U4/U6 snRNA: a biochemical role for U4/U6 proteins. EMBO J. 21, 5527–5538. https://doi.org/10.1093/emboj/cdf544.
Nunnari, J., Suomalainen, A., 2012. Mitochondria: in sickness and in health. Cell 148, 1145–1159. https://doi.org/10.1016/j.cell.2012.02.035.

O'Brien, J.M., 2001. Retinoblastoma: clinical presentation and the role of neuroimaging. AJNR Am. J. Neuroradiol. 22, 427–429. https://www.ajnr.org/content/22/3/427.long.

O'Rahilly, R., 1975. The prenatal development of the human eye. Exp. Eye Res. 21, 93–112. https://doi.org/10.1016/0014-4835(75)90075-5.

Oliveira, C.R., Paiva, M.R.B., Ribeiro, M.C.S., Andrade, G.F., Carvalho, J.L., Gomes, D.A., Nehemy, M., Fialho, S.L., Silva-Cunha, A., Goes, A.M., 2021. Human stem cell-derived retinal pigment epithelial cells as a model for drug screening and pre-clinical assays compared to ARPE-19 cell line. Int. J. Stem Cells 14, 74–84. https://doi.org/10.15283/ijsc20094.

Osakada, F., Ikeda, H., Mandai, M., Wataya, T., Watanabe, K., Yoshimura, N., Akaike, A., Sasai, Y., Takahashi, M., 2008. Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. Nat. Biotechnol. 26, 215–224. https://doi.org/10.1038/nbt1384.

Otsuka, Y., Imamura, K., Oishi, A., Kondo, T., Suga, M., Yada, Y., Shibukawa, R., Okanishi, Y., Sagara, Y., Tsukita, K., Tsujikawa, A., Inoue, H., 2022. One-step induction of photoreceptor-like cells from human iPSCs by delivering transcription factors. iScience 25, 103987. https://doi.org/10.1016/j.isci.2022.103987.

Ovando-Roche, P., West, E.L., Branch, M.J., Sampson, R.D., Fernando, M., Munro, P., Georgiadis, A., Rizzi, M., Kloc, M., Naeem, A., Ribeiro, J., Smith, A.J., Gonzalez-Cordero, A., Ali, R.R., 2018. Use of bioreactors for culturing human retinal organoids improves photoreceptor yields. Stem Cell Res. Ther. 9, 156. https://doi.org/10.1186/s13287-018-0907-0.

Paek, J., Park, S.E., Lu, Q., Park, K.T., Cho, M., Oh, J.M., Kwon, K.W., Yi, Y.S., Song, J.W., Edelstein, H.I., Ishibashi, J., Yang, W., Myerson, J.W., Kiseleva, R.Y., Aprelev, P., Hood, E.D., Stambolian, D., Seale, P., Muzykantov, V.R., Huh, D., 2019. Microphysiological engineering of self-assembled and perfusable microvascular beds for the production of vascularized three-dimensional human microtissues. ACS Nano 13, 7627–7643. https://doi.org/10.1021/acsnano.9b00686.

Pandey, A.N., 2014. Retinoblastoma: an overview. Saudi J. Ophthalmol. 28, 310–315. https://doi.org/10.1016/j.sjopt.2013.11.001.

Panthagani, J., Montecinos, P., Lopez, J.P., Parulekar, M., 2020. Cystoid macular edema following intravitreal chemotherapy treatment for retinoblastoma. Pediatr. Blood Cancer 67, e28348. https://doi.org/10.1002/pbc.28348.

Papasaikas, P., Tejedor, J.R., Vigevani, L., Valcarcel, J., 2015. Functional splicing network reveals extensive regulatory potential of the core spliceosomal machinery. Mol. Cell 57, 7–22. https://doi.org/10.1016/j.molcel.2014.10.030.

Parfitt, D.A., Lane, A., Ramsden, C.M., Carr, A.F., Munro, P.M., Jovanovic, K., Schwarz, N., Kanuga, N., Muthiah, M.N., Hull, S., Gallo, J.M., da Cruz, L., Moore, A. T., Hardcastle, A.J., Coffey, P.J., Cheetham, M.E., 2016. Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. Cell Stem Cell 18, 769–781. https://doi.org/10.1016/j.stem.2016.03.021.

Park, E., Kim, H.K., Jee, J., Hahn, S., Jeong, S., Yoo, J., 2019. Development of organoid-based drug metabolism model. Toxicol. Appl. Pharmacol. 385, 114790 https://doi.org/10.1016/j.taap.2019.114790.

Park, J.-C., Park, M.J., Lee, S.-Y., Kim, D., Kim, K.-T., Jang, H.-K., Cha, H.-J., 2023. Gene editing with 'pencil' rather than 'scissors' in human pluripotent stem cells. Stem Cell Res. Ther. 14, 164. https://doi.org/10.1186/s13287-023-03394-5.

Parravano, M., Costanzo, E., Scondotto, G., Trifiro, G., Virgili, G., 2021. Anti-VEGF and other novel therapies for neovascular age-related macular degeneration: an update. BioDrugs 35, 673–692. https://doi.org/10.1007/s40259-021-00499-2.

Payne, B.A.I., Wilson, I.J., Yu-Wai-Man, P., Coxhead, J., Deehan, D., Horvath, R., Taylor, R.W., Samuels, D.C., Santibanez-Koref, M., Chinnery, P.F., 2013. Universal heteroplasmy of human mitochondrial DNA. Hum. Mol. Genet. 22, 384–390. https://doi.org/10.1093/hmg/dds435.

Pennesi, M.E., Neuringer, M., Courtney, R.J., 2012. Animal models of age related macular degeneration. Mol. Aspects Med. 33, 487–509. https://doi.org/10.1016/j.mam.2012.06.003.

Perales-Clemente, E., Cook, A.N., Evans, J.M., Roellinger, S., Secreto, F., Emmanuele, V., Oglesbee, D., Mootha, V.K., Hirano, M., Schon, E.A., Terzic, A., Nelson, T.J., 2016. Natural underlying mtDNA heteroplasmy as a potential source of intra-person hiPSC variability. EMBO J. 35, 1979–1990. https://doi.org/10.15252/embj.201694892.

Perdigao, P.R.L., Ollington, B., Sai, H., Leung, A., Sacristan-Reviriego, A., van der Spuy, J., 2023. Retinal organoids from an AIPL1 CRISPR/Cas9 knockout cell line successfully recapitulate the molecular features of LCA4 disease. Int. J. Mol. Sci. 24, 5912. https://doi.org/10.3390/ijms24065912.

Petit, L., Ma, S., Cheng, S.Y., Gao, G., Punzo, C., 2017. Rod outer segment development influences AAV-mediated photoreceptor transduction after subretinal injection. Hum. Gene Ther. 28, 464–481. https://doi.org/10.1089/hum.2017.020.

Phillips, M.J., Capowski, E.E., Petersen, A., Jansen, A.D., Barlow, K., Edwards, K.L., Gamm, D.M., 2018. Generation of a rod-specific NRL reporter line in human pluripotent stem cells. Sci. Rep. 8, 2370. https://doi.org/10.1038/s41598-018-20813-3.

Pinto, G., Saenz-de-Santa-Maria, I., Chastagner, P., Perthame, E., Delmas, C., Toulas, C., Moyal-Jonathan-Cohen, E., Brou, C., Zurzolo, C., 2021. Patient-derived glioblastoma stem cells transfer mitochondria through tunneling nanotubes in tumor organoids. Biochem. J. 478, 21–39. https://doi.org/10.1042/BCJ20200710.

Plaza Reyes, A., Petrus-Reurer, S., Antonsson, L., Stenfelt, S., Bartuma, H., Panula, S., Mader, T., Douagi, I., Andre, H., Hovatta, O., Lanner, F., Kvanta, A., 2016. Xeno-free and defined human embryonic stem cell-derived retinal pigment epithelial cells functionally integrate in a large-eyed preclinical model. Stem Cell Reports 6, 9–17. https://doi.org/10.1016/j.stemcr.2015.11.008.

Plaza Reyes, A., Petrus-Reurer, S., Padrell Sanchez, S., Kumar, P., Douagi, I., Bartuma, H., Aronsson, M., Westman, S., Lardner, E., Andre, H., Falk, A., Nandrot, E.F., Kvanta, A., Lanner, F., 2020. Identification of cell surface markers and establishment of monolayer differentiation to retinal pigment epithelial cells. Nat. Commun. 11, 1609. https://doi.org/10.1038/s41467-020-15326-5.

Poovaiah, N., Davoudi, Z., Peng, H., Schlichtmann, B., Mallapragada, S., Narasimhan, B., Wang, Q., 2018. Treatment of neurodegenerative disorders through the blood-brain barrier using nanocarriers. Nanoscale 10, 16962–16983. https://doi.org/10.1039/c8nr04073g.

Porter, F.D., Drago, J., Xu, Y., Cheema, S.S., Wassif, C., Huang, S.P., Lee, E., Grinberg, A., Massalas, J.S., Bodine, D., Alt, F., Westphal, H., 1997. Lhx2, a LIM homeobox gene, is required for eye, forebrain, and definitive erythrocyte development. Development 124, 2935–2944. https://doi.org/10.1242/dev.124.15.2935.

Potter, V.L., Moye, A.R., Robichaux, M.A., Wensel, T.G., 2021. Super-resolution microscopy reveals photoreceptor-specific subciliary location and function of ciliopathy-associated protein CEP290. JCI Insight 6, e145256. https://doi.org/10.1172/jci.insight.145256.

Prada, C., Puga, J., Perez-Mendez, L., Lopez, R., Ramirez, G., 1991. Spatial and temporal patterns of neurogenesis in the chick retina. Eur. J. Neurosci. 3, 559–569. https://doi.org/10.1111/j.1460-9568.1991.tb00843.x.

Prigione, A., Lichtner, B., Kuhl, H., Struys, E.A., Wamelink, M., Lehrach, H., Ralser, M., Timmermann, B., Adjaye, J., 2011. Human induced pluripotent stem cells harbor homoplasmic and heteroplasmic mitochondrial DNA mutations while maintaining human embryonic stem cell–like metabolic reprogramming. Stem Cells 29, 1338–1348. https://doi.org/10.1002/stem.683.

Pucchio, A., Krance, S.H., Pur, D.R., Miranda, R.N., Felfeli, T., 2022. Artificial intelligence analysis of biofluid markers in age-related macular degeneration: a systematic review. Clin. Ophthalmol. 16, 2463–2476. https://doi.org/10.2147/OPTH.S377262.

Pucci, F., Gardano, L., Harrington, L., 2013. Short telomeres in ESCs lead to unstable differentiation. Cell Stem Cell 12, 479–486. https://doi.org/10.1016/j.stem.2013.01.018.

Pulagam, L.P., Palczewski, K., 2010. Phototransduction: rhodopsin. In: Dartt, D.A. (Ed.), Encyclopedia of the Eye. Academic Press, Oxford, pp. 403–412.

Quartilho, A., Simkiss, P., Zekite, A., Xing, W., Wormald, R., Bunce, C., 2016. Leading causes of certifiable visual loss in England and Wales during the year ending 31 March 2013. Eye 30, 602–607. https://doi.org/10.1038/eye.2015.288.

Quinn, P.M., Buck, T.M., Mulder, A.A., Ohonin, C., Alves, C.H., Vos, R.M., Bialecka, M., van Herwaarden, T., van Dijk, E.H.C., Talib, M., Freund, C., Mikkers, H.M.M., Hoeben, R.C., Goumans, M.J., Boon, C.J.F., Koster, A.J., Chuva de Sousa Lopes, S.M., Jost, C.R., Wijnholds, J., 2019. Human iPSC-derived retinas recapitulate the fetal CRB1 CRB2 complex formation and demonstrate that photoreceptors and Müller glia are targets of AAV5. Stem Cell Reports 12, 906–919. https://doi.org/10.1016/j.stemcr.2019.03.002.

Rabinowicz, R., Barchana, M., Liphshiz, I., Linn, S., Futerman, B., Ben-Arush, M.W., 2013. Cancer incidence and survival among infants in Israel, 1998-2007. Pediatr. Hematol. Oncol. 30, 646–654. https://doi.org/10.3109/08880018.2013.813099.

Rachel, R.A., Yamamoto, E.A., Dewanjee, M.K., May-Simera, H.L., Sergeev, Y.V., Hackett, A.N., Pohida, K., Munasinghe, J., Gotoh, N., Wickstead, B., Fariss, R.N., Dong, L., Li, T., Swaroop, A., 2015. CEP290 alleles in mice disrupt tissue-specific cilia biogenesis and recapitulate features of syndromic ciliopathies. Hum. Mol. Genet. 24, 3775–3791. https://doi.org/10.1093/hmg/ddv123.

Ramamurthy, V., Niemi, G.A., Reh, T.A., Hurley, J.B., 2004. Leber congenital amaurosis linked to AIPL1: a mouse model reveals destabilization of cGMP phosphodiesterase. Proc. Natl. Acad. Sci. U S A 101, 13897–13902. https://doi.org/10.1073/pnas.0404197101.

Ramsden, C.M., Nommiste, B., A, R.L., Carr, A.F., Powner, M.B., M, J.K.S., Chen, L.L., Muthiah, M.N., Webster, A.R., Moore, A.T., Cheetham, M.E., da Cruz, L., Coffey, P.J., 2017. Rescue of the MERTK phagocytic defect in a human iPSC disease model using translational read-through inducing drugs. Sci. Rep. 7, 51. https://doi.org/10.1038/s41598-017-00142-7.

Rath, P.P., Jenkins, S., Michaelides, M., Smith, A., Sweeney, M.G., Davis, M.B., Fitzke, F. W., Bird, A.C., 2008. Characterisation of the macular dystrophy in patients with the A3243G mitochondrial DNA point mutation with fundus autofluorescence. Br. J. Ophthalmol. 92, 623. https://doi.org/10.1136/bjo.2007.131177.

Rawal, P., Tripathi, D.M., Ramakrishna, S., Kaur, S., 2021. Prospects for 3D bioprinting of organoids. Biodes. Manuf. 4, 627–640. https://doi.org/10.1007/s42242-020-00124-1.

Ray, P., Luo, X., Rao, E.J., Basha, A., Woodruff 3rd, E.A., Wu, J.Y., 2010. The splicing factor Prp31 is essential for photoreceptor development in Drosophila. Protein Cell 1, 267–274. https://doi.org/10.1007/s13238-010-0035-9.

Regent, F., Chen, H.Y., Kelley, R.A., Qu, Z., Swaroop, A., Li, T., 2020. A simple and efficient method for generating human retinal organoids. Mol. Vis. 26, 97–105. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7051860/.

Regent, F., Morizur, L., Lesueur, L., Habeler, W., Plancheron, A., Ben M'Barek, K., Monville, C., 2019. Automation of human pluripotent stem cell differentiation toward retinal pigment epithelial cells for large-scale productions. Sci. Rep. 9, 10646 https://doi.org/10.1038/s41598-019-47123-6.

Reichenbach, A., Bringmann, A., 2013. New functions of Müller cells. Glia 61, 651–678. https://doi.org/10.1002/glia.22477.

Reichman, S., Terray, A., Slembrouck, A., Nanteau, C., Orieux, G., Habeler, W., Nandrot, E.F., Sahel, J.A., Monville, C., Goureau, O., 2014. From confluent human iPS cells to self-forming neural retina and retinal pigmented epithelium. Pro. Natl. Acad. Sci. U S A 111, 8518–8523. https://doi.org/10.1073/pnas.1324212111.

RetNet - Retinal Information Network, August 2023. https://web.sph.uth.edu/RetNet/.

Richard-Parpaillon, L., Heligon, C., Chesnel, F., Boujard, D., Philpott, A., 2002. The IGF pathway regulates head formation by inhibiting Wnt signaling in Xenopus. Dev. Biol. 244, 407–417. https://doi.org/10.1006/dbio.2002.0605.

Rio Frio, T., Wade, N.M., Ransijn, A., Berson, E.L., Beckmann, J.S., Rivolta, C., 2008. Premature termination codons in PRPF31 cause retinitis pigmentosa via haploinsufficiency due to nonsense-mediated mRNA decay. J. Clin. Invest. 118, 1519–1531. https://doi.org/10.1172/JCI34211.

Rius, R., Cowley, M.J., Riley, L., Puttick, C., Thorburn, D.R., Christodoulou, J., 2019. Biparental inheritance of mitochondrial DNA in humans is not a common phenomenon. Genet. Med. 21, 2823–2826. https://doi.org/10.1038/s41436-019-0568-0.

Rodrigues, A., Slembrouck-Brec, A., Nanteau, C., Terray, A., Tymoshenko, Y., Zagar, Y., Reichman, S., Xi, Z., Sahel, J.A., Fouquet, S., Orieux, G., Nandrot, E.F., Byrne, L.C., Audo, I., Roger, J.E., Goureau, O., 2022. Modeling PRPF31 retinitis pigmentosa using retinal pigment epithelium and organoids combined with gene augmentation rescue. NPJ Regen. Med. 7, 39. https://doi.org/10.1038/s41536-022-00235-6.

Rodriguez-Galindo, C., Wilson, M.W., Chantada, G., Fu, L., Qaddoumi, I., Antoneli, C., Leal-Leal, C., Sharma, T., Barnoya, M., Epelman, S., Pizzarello, L., Kane, J.R., Barfield, R., Merchant, T.E., Robison, L.L., Murphree, A.L., Chevez-Barrios, P., Dyer, M.A., O'Brien, J., Ribeiro, R.C., Hungerford, J., Helveston, E.M., Haik, B.G., Wilimas, J., 2008. Retinoblastoma: one world, one vision. Pediatrics 122, e763–e770. https://doi.org/10.1542/peds.2008-0518.

Rodriguez-Navarro, J.A., Cuervo, A.M., 2010. Autophagy and lipids: tightening the knot. Semin. Immunopathol. 32, 343–353. https://doi.org/10.1007/s00281-010-0219-7.

Rofagha, S., Bhisitkul, R.B., Boyer, D.S., Sadda, S.R., Zhang, K., Group, S.-U.S., 2013. Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP). Ophthalmology 120, 2292–2299. https://doi.org/10.1016/j.ophtha.2013.03.046.

Roosing, S., Thiadens, A.A., Hoyng, C.B., Klaver, C.C., den Hollander, A.I., Cremers, F.P., 2014. Causes and consequences of inherited cone disorders. Prog. Retin. Eye Res. 42, 1–26. https://doi.org/10.1016/j.preteyeres.2014.05.001.

Rowland, T.J., Blaschke, A.J., Buchholz, D.E., Hikita, S.T., Johnson, L.V., Clegg, D.O., 2013. Differentiation of human pluripotent stem cells to retinal pigmented epithelium in defined conditions using purified extracellular matrix proteins. J . Tissue Eng. Regen. Med. 7, 642–653. https://doi.org/10.1002/term.1458.

Rowland, T.J., Buchholz, D.E., Clegg, D.O., 2012. Pluripotent human stem cells for the treatment of retinal disease. J. Cell. Physiol. 227, 457–466. https://doi.org/10.1002/jcp.22814.

Rozanska, A., Cerna-Chavez, R., Queen, R., Collin, J., Zerti, D., Dorgau, B., Beh, C.S., Davey, T., Coxhead, J., Hussain, R., Al-Aama, J., Steel, D.H., Benvenisty, N., Armstrong, L., Parulekar, M., Lako, M., 2022. pRB-depleted pluripotent stem cell retinal organoids recapitulate cell state transitions of retinoblastoma development and suggest an important role for pRB in retinal cell differentiation. Stem Cells Transl. Med. 11, 415–433. https://doi.org/10.1093/stcltm/szac008.

Ruan, G.X., Barry, E., Yu, D., Lukason, M., Cheng, S.H., Scaria, A., 2017. CRISPR/Cas9-Mediated genome editing as a therapeutic approach for Leber congenital amaurosis 10. Mol. Ther. 25, 331–341. https://doi.org/10.1016/j.ymthe.2016.12.006.

Russell, S., Bennett, J., Wellman, J.A., Chung, D.C., Yu, Z.F., Tillman, A., Wittes, J., Pappas, J., Elci, O., McCague, S., Cross, D., Marshall, K.A., Walshire, J., Kehoe, T.L., Reichert, H., Davis, M., Raffini, L., George, L.A., Hudson, F.P., Dingfield, L., Zhu, X., Haller, J.A., Sohn, E.H., Mahajan, V.B., Pfeifer, W., Weckmann, M., Johnson, C., Gewaily, D., Drack, A., Stone, E., Wachtel, K., Simonelli, F., Leroy, B.P., Wright, J.F., High, K.A., Maguire, A.M., 2017. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet 390, 849–860. https://doi.org/10.1016/S0140-6736(17)31868-8.

Russell, S.R., Drack, A.V., Cideciyan, A.V., Jacobson, S.G., Leroy, B.P., Van Cauwenbergh, C., Ho, A.C., Dumitrescu, A.V., Han, I.C., Martin, M., Pfeifer, W.L., Sohn, E.H., Walshire, J., Garafalo, A.V., Krishnan, A.K., Powers, C.A., Sumaroka, A., Roman, A.J., Vanhonsebrouck, E., Jones, E., Nerinckx, F., De Zaeytijd, J., Collin, R. W.J., Hoyng, C., Adamson, P., Cheetham, M.E., Schwartz, M.R., den Hollander, W., Asmus, F., Platenburg, G., Rodman, D., Girach, A., 2022. Intravitreal antisense oligonucleotide sepofarsen in Leber congenital amaurosis type 10: a phase 1b/2 trial. Nat. Med. 28, 1014–1021. https://doi.org/10.1038/s41591-022-01755-w.

Saha, A., Capowski, E., Fernandez Zepeda, M.A., Nelson, E.C., Gamm, D.M., Sinha, R., 2022. Cone photoreceptors in human stem cell-derived retinal organoids demonstrate intrinsic light responses that mimic those of primate fovea. Cell Stem Cell 29,. https://doi.org/10.1016/j.stem.2022.01.002, 460-471.e3.

Saini, J.S., Corneo, B., Miller, J.D., Kiehl, T.R., Wang, Q., Boles, N.C., Blenkinsop, T.A., Stern, J.H., Temple, S., 2017. Nicotinamide ameliorates disease phenotypes in a human iPSC model of age-related macular degeneration. Cell Stem Cell 20,. https://doi.org/10.1016/j.stem.2016.12.015, 635-647.e7.

Sakamoto, T., Sakamoto, H., Murphy, T.L., Spee, C., Soriano, D., Ishibashi, T., Hinton, D. R., Ryan, S.J., 1995. Vessel formation by choroidal endothelial cells in vitro is modulated by retinal pigment epithelial cells. Arch. Ophthalmol. 113, 512–520. https://doi.org/10.1001/archopht.1995.01100040134039.

Sanchez-Bellver, L., Toulis, V., Marfany, G., 2021. On the wrong track: alterations of ciliary transport in inherited retinal dystrophies. Front. Cell Dev. Biol. 9, 623734 https://doi.org/10.3389/fcell.2021.623734.

Sandoe, J., Eggan, K., 2013. Opportunities and challenges of pluripotent stem cell neurodegenerative disease models. Nat. Neurosci. 16, 780–789. https://doi.org/10.1038/nn.3425.

Sanjurjo-Soriano, C., Erkilic, N., Damodar, K., Boukhaddaoui, H., Diakatou, M., Garita-Hernandez, M., Mamaeva, D., Dubois, G., Jazouli, Z., Jimenez-Medina, C., Goureau, O., Meunier, I., Kalatzis, V., 2022. Retinoic acid delays initial photoreceptor differentiation and results in a highly structured mature retinal organoid. Stem Cell Res. Ther. 13, 478. https://doi.org/10.1186/s13287-022-03146-x.

Sanjurjo-Soriano, C., Jimenez-Medina, C., Erkilic, N., Cappellino, L., Lefevre, A., Nagel-Wolfrum, K., Wolfrum, U., Van Wijk, E., Roux, A.F., Meunier, I., Kalatzis, V., 2023. USH2A variants causing retinitis pigmentosa or Usher syndrome provoke differential retinal phenotypes in disease-specific organoids. HGG Adv. 4, 100229 https://doi.org/10.1016/j.xhgg.2023.100229.

Sarkar, S., Rubinsztein, D.C., 2008. Huntington's disease: degradation of mutant huntingtin by autophagy. FEBS J. 275, 4263–4270. https://doi.org/10.1111/j.1742-4658.2008.06562.x.

Sato, S., Frederiksen, R., Cornwall, M.C., Kefalov, V.J., 2017. The retina visual cycle is driven by cis retinol oxidation in the outer segments of cones. Vis. Neurosci. 34, E004. https://doi.org/10.1017/s0952523817000013.

Sayer, J.A., Otto, E.A., O'Toole, J.F., Nurnberg, G., Kennedy, M.A., Becker, C., Hennies, H.C., Helou, J., Attanasio, M., Fausett, B.V., Utsch, B., Khanna, H., Liu, Y., Drummond, I., Kawakami, I., Kusakabe, T., Tsuda, M., Ma, L., Lee, H., Larson, R.G., Allen, S.J., Wilkinson, C.J., Nigg, E.A., Shou, C., Lillo, C., Williams, D.S., Hoppe, B., Kemper, M.J., Neuhaus, T., Parisi, M.A., Glass, I.A., Petry, M., Kispert, A., Gloy, J., Ganner, A., Walz, G., Zhu, X., Goldman, D., Nurnberg, P., Swaroop, A., Leroux, M.R., Hildebrandt, F., 2006. The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. Nat. Genet. 38, 674–681. https://doi.org/10.1038/ng1786.

Schaffer, S.W., Jong, C.J., Ito, T., Azuma, J., 2014. Role of taurine in the pathologies of MELAS and MERRF. Amino Acids 46, 47–56. https://doi.org/10.1007/s00726-012-1414-8.

Schaffert, N., Hossbach, M., Heintzmann, R., Achsel, T., Luhrmann, R., 2004. RNAi knockdown of hPrp31 leads to an accumulation of U4/U6 di-snRNPs in Cajal bodies. EMBO J. 23, 3000–3009. https://doi.org/10.1038/sj.emboj.7600296.

Schick, R., Farah, N., Markus, A., Korngreen, A., Mandel, Y., 2020. Electrophysiologic characterization of developing human embryonic stem cell-derived photoreceptor precursors. Invest. Ophthalmol. Vis. Sci. 61, 44. https://doi.org/10.1167/iovs.61.11.44.

Schmidt-Erfurth, U., Waldstein, S.M., Klimscha, S., Sadeghipour, A., Hu, X., Gerendas, B. S., Osborne, A., Bogunovic, H., 2018. Prediction of individual disease conversion in early AMD using artificial intelligence. Invest. Ophthalmol. Vis. Sci. 59, 3199–3208. https://doi.org/10.1167/iovs.18-24106.

Scholefield, J., Harrison, P.T., 2021. Prime editing – an update on the field. Gene Ther. 28, 396–401. https://doi.org/10.1038/s41434-021-00263-9.

Schrier, S.A., Falk, M.J., 2011. Mitochondrial disorders and the eye. Curr. Opin. Ophthalmol. 22, 325–331. https://doi.org/10.1097/ICU.0b013e328349419d.

Schwarz, N., Carr, A.J., Lane, A., Moeller, F., Chen, L.L., Aguila, M., Nommiste, B., Muthiah, M.N., Kanuga, N., Wolfrum, U., Nagel-Wolfrum, K., da Cruz, L., Coffey, P. J., Cheetham, M.E., Hardcastle, A.J., 2015. Translational read-through of the RP2 Arg120stop mutation in patient iPSC-derived retinal pigment epithelium cells. Hum. Mol. Genet. 24, 972–986. https://doi.org/10.1093/hmg/ddu509.

Schwarz, N., Lane, A., Jovanovic, K., Parfitt, D.A., Aguila, M., Thompson, C.L., da Cruz, L., Coffey, P.J., Chapple, J.P., Hardcastle, A.J., Cheetham, M.E., 2017. Arl3 and RP2 regulate the trafficking of ciliary tip kinesins. Hum. Mol. Genet. 26, 2480–2492. https://doi.org/10.1093/hmg/ddx143.

Senabouth, A., Daniszewski, M., Lidgerwood, G.E., Liang, H.H., Hernandez, D., Mirzaei, M., Keenan, S.N., Zhang, R., Han, X., Neavin, D., Rooney, L., Lopez Sanchez, M.I.G., Gulluyan, L., Paulo, J.A., Clarke, L., Kearns, L.S., Gnanasambandapillai, V., Chan, C.L., Nguyen, U., Steinmann, A.M., McCloy, R.A., Farbehi, N., Gupta, V.K., Mackey, D.A., Bylsma, G., Verma, N., MacGregor, S., Watt, M.J., Guymer, R.H., Powell, J.E., Hewitt, A.W., Pebay, A., 2022. Transcriptomic and proteomic retinal pigment epithelium signatures of age-related macular degeneration. Nat. Commun. 13, 4233. https://doi.org/10.1038/s41467-022-31707-4.

Sercel, A.J., Carlson, N.M., Patananan, A.N., Teitell, M.A., 2021. Mitochondrial DNA dynamics in reprogramming to pluripotency. Trends Cell Biol. 31, 311–323. https://doi.org/10.1016/j.tcb.2020.12.009.

Shaheen, R., Almoisheer, A., Faqeih, E., Babay, Z., Monies, D., Tassan, N., Abouelhoda, M., Kurdi, W., Al Mardawi, E., Khalil, M.M., Seidahmed, M.Z., Alnemer, M., Alsahan, N., Sogaty, S., Alhashem, A., Singh, A., Goyal, M., Kapoor, S., Alomar, R., Ibrahim, N., Alkuraya, F.S., 2015. Identification of a novel MKS locus defined by TMEM107 mutation. Hum. Mol. Genet. 24, 5211–5218. https://doi.org/10.1093/hmg/ddv242.

Sharma, R., George, A., Nimmagadda, M., Ortolan, D., Karla, B.S., Qureshy, Z., Bose, D., Dejene, R., Liang, G., Wan, Q., Chang, J., Jha, B.S., Memon, O., Miyagishima, K.J., Rising, A., Lal, M., Hanson, E., King, R., Campos, M.M., Ferrer, M., Amaral, J., McGaughey, D., Bharti, K., 2021. Epithelial phenotype restoring drugs suppress macular degeneration phenotypes in an iPSC model. Nat. Commun. 12, 7293. https://doi.org/10.1038/s41467-021-27488-x.

Shen, J., Yang, X., Dong, A., Petters, R.M., Peng, Y.-W., Wong, F., Campochiaro, P.A., 2005. Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. J. Cell. Physiol. 203, 457–464. https://doi.org/10.1002/jcp.20346.

Shimada, H., Lu, Q., Insinna-Kettenhofen, C., Nagashima, K., English, M.A., Semler, E.M., Mahgerefteh, J., Cideciyan, A.V., Li, T., Brooks, B.P., Gunay-Aygun, M., Jacobson, S. G., Cogliati, T., Westlake, C.J., Swaroop, A., 2017. In vitro modeling using ciliopathy-patient-derived cells reveals distinct cilia dysfunctions caused by CEP290 mutations. Cell Rep. 20, 384–396. https://doi.org/10.1016/j.celrep.2017.06.045.

Shmookler Reis, R.J., Goldstein, S., 1983. Mitochondrial DNA in mortal and immortal human cells. Genome number, integrity, and methylation. J. Biol. Chem. 258, 9078–9085. https://doi.org/10.1016/S0021-9258(17)44633-3.

Shylo, N.A., Christopher, K.J., Iglesias, A., Daluiski, A., Weatherbee, S.D., 2016. TMEM107 is a critical regulator of ciliary protein composition and is mutated in orofaciodigital syndrome. Hum. Mutat. 37, 155–159. https://doi.org/10.1002/humu.22925.

Singh, H.P., Wang, S., Stachelek, K., Lee, S., Reid, M.W., Thornton, M.E., Craft, C.M., Grubbs, B.H., Cobrinik, D., 2018. Developmental stage-specific proliferation and retinoblastoma genesis in RB-deficient human but not mouse cone precursors. Proc. Natl. Acad. Sci. U S A 115, E9391–E9400. https://doi.org/10.1073/pnas.1808903115.

Singh, M.S., Park, S.S., Albini, T.A., Canto-Soler, M.V., Klassen, H., MacLaren, R.E., Takahashi, M., Nagiel, A., Schwartz, S.D., Bharti, K., 2020. Retinal stem cell transplantation: balancing safety and potential. Prog. Retin. Eye Res. 75, 100779 https://doi.org/10.1016/j.preteyeres.2019.100779.

Sinn, P.L., Arias, A.C., Brogden, K.A., McCray Jr., P.B., 2008. Lentivirus vector can be readministered to nasal epithelia without blocking immune responses. J. Virol. 82, 10684–10692. https://doi.org/10.1128/JVI.00227-08.

Sivaprasad, S., Chong, N.V., Bailey, T.A., 2005. Serum elastin-derived peptides in age-related macular degeneration. Invest. Ophthalmol. Vis. Sci. 46, 3046–3051. https://doi.org/10.1167/iovs.04-1277.

Sladen, P.E., Jovanovic, K., Guarascio, R., Ottaviani, D., Salsbury, G., Novoselova, T., Chapple, J.P., Yu-Wai-Man, P., Cheetham, M.E., 2022. Modelling autosomal dominant optic atrophy associated with OPA1 variants in iPSC-derived retinal ganglion cells. Hum. Mol. Genet. 31, 3478–3493. https://doi.org/10.1093/hmg/ddac128.

Sladen, P.E., Perdigao, P.R.L., Salsbury, G., Novoselova, T., van der Spuy, J., Chapple, J. P., Yu-Wai-Man, P., Cheetham, M.E., 2021. CRISPR-Cas9 correction of OPA1 c.1334G>A: p.R445H restores mitochondrial homeostasis in dominant optic atrophy patient-derived iPSCs. Mol. Ther. Nucleic Acids 26, 432–443. https://doi.org/10.1016/j.omtn.2021.08.015.

Smith, H.L., Li, W., Cheetham, M.E., 2015. Molecular chaperones and neuronal proteostasis. Semin. Cell Dev. Biol. 40, 142–152. https://doi.org/10.1016/j.semcdb.2015.03.003.

Sofat, R., Casas, J.P., Webster, A.R., Bird, A.C., Mann, S.S., Yates, J.R., Moore, A.T., Sepp, T., Cipriani, V., Bunce, C., Khan, J.C., Shahid, H., Swaroop, A., Abecasis, G., Branham, K.E., Zareparsi, S., Bergen, A.A., Klaver, C.C., Baas, D.C., Zhang, K., Chen, Y., Gibbs, D., Weber, B.H., Keilhauer, C.N., Fritsche, L.G., Lotery, A., Cree, A. J., Griffiths, H.L., Bhattacharya, S.S., Chen, L.L., Jenkins, S.A., Peto, T., Lathrop, M., Leveillard, T., Gorin, M.B., Weeks, D.E., Ortube, M.C., Ferrell, R.E., Jakobsdottir, J., Conley, Y.P., Rahu, M., Seland, J.H., Soubrane, G., Topouzis, F., Vioque, J., Tomazzoli, L., Young, I., Whittaker, J., Chakravarthy, U., de Jong, P.T., Smeeth, L., Fletcher, A., Hingorani, A.D., 2012. Complement factor H genetic variant and age-related macular degeneration: effect size, modifiers and relationship to disease subtype. Int. J. Epidemiol. 41, 250–262. https://doi.org/10.1093/ije/dyr204.

Song, M.J., Quinn, R., Nguyen, E., Hampton, C., Sharma, R., Park, T.S., Koster, C., Voss, T., Tristan, C., Weber, C., Singh, A., Dejene, R., Bose, D., Chen, Y.C., Derr, P., Derr, K., Michael, S., Barone, F., Chen, G., Boehm, M., Maminishkis, A., Singec, I., Ferrer, M., Bharti, K., 2023. Bioprinted 3D outer retina barrier uncovers RPE-dependent choroidal phenotype in advanced macular degeneration. Nat. Methods 20, 149–161. https://doi.org/10.1038/s41592-022-01701-1.

Sparrow, J.R., Hicks, D., Hamel, C.P., 2010. The retinal pigment epithelium in health and disease. Curr. Mol. Med. 10, 802–823. https://doi.org/10.2174/156652410793937813.

Spencer, C., Abend, S., McHugh, K.J., Saint-Geniez, M., 2017. Identification of a synergistic interaction between endothelial cells and retinal pigment epithelium. J. Cell Mol. Med. 21, 2542–2552. https://doi.org/10.1111/jcmm.13175.

Spencer, C.E., Flint, L.E., Duckett, C.J., Cole, L.M., Cross, N., Smith, D.P., Clench, M.R., 2020. Role of MALDI-MSI in combination with 3D tissue models for early stage efficacy and safety testing of drugs and toxicants. Expert Rev. Proteomics 17, 827–841. https://doi.org/10.1080/14789450.2021.1876568.

Sridevi Gurubaran, I., Viiri, J., Koskela, A., Hyttinen, J.M.T., Paterno, J.J., Kis, G., Antal, M., Urtti, A., Kauppinen, A., Felszeghy, S., Kaarniranta, K., 2020. Mitophagy in the retinal pigment epithelium of dry age-related macular degeneration investigated in the nfe2l2/PGC-1α-/- mouse model. Int. J. Mol. Sci. 21, 1976. https://doi.org/10.3390/ijms21061976.

Sridhar, A., Hoshino, A., Finkbeiner, C.R., Chitsazan, A., Dai, L., Haugan, A.K., Eschenbacher, K.M., Jackson, D.L., Trapnell, C., Bermingham-McDonogh, O., Glass, I., Reh, T.A., 2020. Single-cell transcriptomic comparison of human fetal retina, hPSC-derived retinal organoids, and long-term retinal cultures. Cell Rep. 30, https://doi.org/10.1016/j.celrep.2020.01.007, 1644-1659.e4.

Srimongkol, A., Laosillapacharoen, N., Saengwimol, D., Chaitankar, V., Rojanaporn, D., Thanomchard, T., Borwornpinyo, S., Hongeng, S., Kaewkhaw, R., 2023. Sunitinib efficacy with minimal toxicity in patient-derived retinoblastoma organoids. J. Exp. Clin. Cancer Res. 42, 39. https://doi.org/10.1186/s13046-023-02608-1.

Stacey, A.W., Bowman, R., Foster, A., Kivela, T.T., Munier, F.L., Cassoux, N., Fabian, I.D., Global Retinoblastoma Study, G., 2021. Incidence of retinoblastoma has increased: results from 40 European countries. Ophthalmology 128, 1369–1371. https://doi.org/10.1016/j.ophtha.2021.01.024.

Stathopoulos, C., Bartolini, B., Marie, G., Beck-Popovic, M., Saliou, G., Munier, F.L., 2021. Risk factors for acute choroidal ischemia after intra-arterial melphalan for retinoblastoma: the role of the catheterization approach. Ophthalmology 128, 754–764. https://doi.org/10.1016/j.ophtha.2020.09.021.

Stenkamp, D.L., 2015. Development of the vertebrate eye and retina. Prog. Mol. Biol. Transl. Sci. 134, 397–414. https://doi.org/10.1016/bs.pmbts.2015.06.006.

Sterneckert, J.L., Reinhardt, P., Scholer, H.R., 2014. Investigating human disease using stem cell models. Nat. Rev. Genet. 15, 625–639. https://doi.org/10.1038/nrg3764.

Stone, E.M., Cideciyan, A.V., Aleman, T.S., Scheetz, T.E., Sumaroka, A., Ehlinger, M.A., Schwartz, S.B., Fishman, G.A., Traboulsi, E.I., Lam, B.L., Fulton, A.B., Mullins, R.F., Sheffield, V.C., Jacobson, S.G., 2011. Variations in NPHP5 in patients with nonsyndromic Leber congenital amaurosis and Senior-Loken syndrome. Arch. Ophthalmol. 129, 81–87. https://doi.org/10.1001/archophthalmol.2010.330.

Strauss, D.G., Blinova, K., 2017. Clinical trials in a dish. Trends Pharmacol. Sci. 38, 4–7. https://doi.org/10.1016/j.tips.2016.10.009.

Strauss, O., 2005. The retinal pigment epithelium in visual function. Physiol. Rev. 85, 845–881. https://doi.org/10.1152/physrev.00021.2004.

Streeter, I., Harrison, P.W., Faulconbridge, A., The HipSci, C., Flicek, P., Parkinson, H., Clarke, L., 2017. The human-induced pluripotent stem cell initiative-data resources for cellular genetics. Nucleic Acids Res. 45, D691–D697. https://doi.org/10.1093/nar/gkw928.

Su, P.J., Liu, Z., Zhang, K., Han, X., Saito, Y., Xia, X., Yokoi, K., Shen, H., Qin, L., 2015. Retinal synaptic regeneration via microfluidic guiding channels. Sci. Rep. 5, 13591 https://doi.org/10.1038/srep13591.

Su, T., Liang, L., Zhang, L., Wang, J., Chen, L., Su, C., Cao, J., Yu, Q., Deng, S., Chan, H.F., Tang, S., Guo, Y., Chen, J., 2022. Retinal organoids and microfluidic chip-based approaches to explore the retinitis pigmentosa with USH2A mutations. Front. Bioeng. Biotechnol. 10, 939774 https://doi.org/10.3389/fbioe.2022.939774.

Sugiyama, Y., Koike, T., Shiojiri, N., 2007. Immunohistochemical analyses of cell-cell interactions during hepatic organoid formation from fetal mouse liver cells cultured in vitro. Histochem. Cell Biol. 128, 521–531. https://doi.org/10.1007/s00418-007-0339-x.

Sullivan, L.S., Bowne, S.J., Birch, D.G., Hughbanks-Wheaton, D., Heckenlively, J.R., Lewis, R.A., Garcia, C.A., Ruiz, R.S., Blanton, S.H., Northrup, H., Gire, A.I., Seaman, R., Duzkale, H., Spellicy, C.J., Zhu, J., Shankar, S.P., Daiger, S.P., 2006. Prevalence of disease-causing mutations in families with autosomal dominant retinitis pigmentosa: a screen of known genes in 200 families. Invest. Ophthalmol. Vis. Sci. 47, 3052–3064. https://doi.org/10.1167/iovs.05-1443.

Sun, D., Gao, W., Hu, H., Zhou, S., 2022. Why 90% of clinical drug development fails and how to improve it? Acta Pharm. Sin. B 12, 3049–3062. https://doi.org/10.1016/j.apsb.2022.02.002.

Takahashi, K., Yamanaka, S., 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676. https://doi.org/10.1016/j.cell.2006.07.024.

Takezawa, T., Mori, Y., Yonaha, T., Yoshizato, K., 1993. Characterization of morphology and cellular metabolism during the spheroid formation by fibroblasts. Exp. Cell Res. 208, 430–441. https://doi.org/10.1006/excr.1993.1265.

Tanackovic, G., Ransijn, A., Ayuso, C., Harper, S., Berson, E.L., Rivolta, C., 2011a. A missense mutation in PRPF6 causes impairment of pre-mRNA splicing and autosomal-dominant retinitis pigmentosa. Am. J. Hum. Genet. 88, 643–649. https://doi.org/10.1016/j.ajhg.2011.04.008.

Tanackovic, G., Ransijn, A., Thibault, P., Abou Elela, S., Klinck, R., Berson, E.L., Chabot, B., Rivolta, C., 2011b. PRPF mutations are associated with generalized defects in spliceosome formation and pre-mRNA splicing in patients with retinitis pigmentosa. Hum. Mol. Genet. 20, 2116–2130. https://doi.org/10.1093/hmg/ddr094.

Tanackovic, G., Rivolta, C., 2009. PRPF31 alternative splicing and expression in human retina. Ophthalmic Genet. 30, 76–83. https://doi.org/10.1080/13816810902744621.

Thomas, B.B., Lin, B., Martinez-Camarillo, J.C., Zhu, D., McLelland, B.T., Nistor, G., Keirstead, H.S., Humayun, M.S., Seiler, M.J., 2021. Co-Grafts of human embryonic stem cell derived retina organoids and retinal pigment epithelium for retinal reconstruction in immunodeficient retinal degenerate Royal College of Surgeons rats. Front. Neurosci. 15, 752958 https://doi.org/10.3389/fnins.2021.752958.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., Jones, J.M., 1998. Embryonic stem cell lines derived from human blastocysts. Science 282, 1145–1147. https://doi.org/10.1126/science.282.5391.1145.

Tomkiewicz, T.Z., Nieuwenhuis, S.E., Cremers, F.P.M., Garanto, A., Collin, R.W.J., 2022. Correction of the splicing defect caused by a recurrent variant in ABCA4 (c.769-784C>T) that underlies Stargardt disease. Cells 11, 3947. https://doi.org/10.3390/cells11243947.

Tornabene, P., Ferla, R., Llado-Santaeularia, M., Centrulo, M., Dell'Anno, M., Esposito, F., Marrocco, E., Pone, E., Minopoli, R., Iodice, C., Nusco, E., Rossi, S., Lyubenova, H., Manfredi, A., Di Filippo, L., Iuliano, A., Torella, A., Piluso, G., Musacchia, F., Surace, E.M., Cacchiarelli, D., Nigro, V., Auricchio, A., 2022. Therapeutic homology-independent targeted integration in retina and liver. Nat. Commun. 13, 1963. https://doi.org/10.1038/s41467-022-29550-8.

Tornabene, P., Trapani, I., Minopoli, R., Centrulo, M., Lupo, M., de Simone, S., Tiberi, P., Dell'Aquila, F., Marrocco, E., Iodice, C., Iuliano, A., Gesualdo, C., Rossi, S., Giaquinto, L., Albert, S., Hoyng, C.B., Polishchuk, E., Cremers, F.P.M., Surace, E.M., Simonelli, F., De Matteis, M.A., Polishchuk, R., Auricchio, A., 2019. Intein-mediated protein trans-splicing expands adeno-associated virus transfer capacity in the retina. Sci. Transl. Med. 11, eaav4523 https://doi.org/10.1126/scitranslmed.aav4523.

Torriano, S., Erkilic, N., Baux, D., Cereso, N., De Luca, V., Meunier, I., Moosajee, M., Roux, A.F., Hamel, C.P., Kalatzis, V., 2018. The effect of PTC124 on choroideremia fibroblasts and iPSC-derived RPE raises considerations for therapy. Sci. Rep. 8, 8234. https://doi.org/10.1038/s41598-018-26481-7.

Trapani, I., Colella, P., Sommella, A., Iodice, C., Cesi, G., de Simone, S., Marrocco, E., Rossi, S., Giunti, M., Palfi, A., Farrar, G.J., Polishchuk, R., Auricchio, A., 2014. Effective delivery of large genes to the retina by dual AAV vectors. EMBO Mol. Med. 6, 194–211. https://doi.org/10.1002/emmm.201302948.

Travis, G.H., Golczak, M., Moise, A.R., Palczewski, K., 2007. Diseases caused by defects in the visual cycle: retinoids as potential therapeutic agents. Annu. Rev. Pharmacol. Toxicol. 47, 469–512. https://doi.org/10.1146/annurev.pharmtox.47.120505.105225.

Tretiach, M., Madigan, M.C., Gillies, M.C., 2004. Conditioned medium from mixed retinal pigmented epithelium and Müller cell cultures reduces in vitro permeability

of retinal vascular endothelial cells. Br. J. Ophthalmol. 88, 957–961. https://doi.org/10.1136/bjo.2003.033894.
Truong, V., Viken, K., Geng, Z., Barkan, S., Johnson, B., Ebeling, M.C., Montezuma, S.R., Ferrington, D.A., Dutton, J.R., 2021. Automating human induced pluripotent stem cell culture and differentiation of iPSC-derived retinal pigment epithelium for personalized drug testing. SLAS Technol. 26, 287–299. https://doi.org/10.1177/2472630320972110.
Tucker, B.A., Mullins, R.F., Streb, L.M., Anfinson, K., Eyestone, M.E., Kaalberg, E., Riker, M.J., Drack, A.V., Braun, T.A., Stone, E.M., 2013. Patient-specific iPSC-derived photoreceptor precursor cells as a means to investigate retinitis pigmentosa. Elife 2, e00824. https://doi.org/10.7554/eLife.00824.
Tucker, B.A., Scheetz, T.E., Mullins, R.F., DeLuca, A.P., Hoffmann, J.M., Johnston, R.M., Jacobson, S.G., Sheffield, V.C., Stone, E.M., 2011. Exome sequencing and analysis of induced pluripotent stem cells identify the cilia-related gene male germ cell-associated kinase (MAK) as a cause of retinitis pigmentosa. Proc. Natl. Acad. Sci. U S A 108, E569–E576. https://doi.org/10.1073/pnas.1108918108.
Tzoumas, N., Farrah, T.E., Dhaun, N., Webb, D.J., 2020. Established and emerging therapeutic uses of PDE type 5 inhibitors in cardiovascular disease. Br. J. Pharmacol. 177, 5467–5488. https://doi.org/10.1111/bph.14920.
Tzoumas, N., Hallam, D., Harris, C.L., Lako, M., Kavanagh, D., Steel, D.H.W., 2021. Revisiting the role of factor H in age-related macular degeneration: insights from complement-mediated renal disease and rare genetic variants. Surv. Ophthalmol. 66, 378–401. https://doi.org/10.1016/j.survophthal.2020.10.008.
Tzoumas, N., Kavanagh, D., Cordell, H.J., Lotery, A.J., Patel, P.J., Steel, D.H., 2022. Rare complement factor I variants associated with reduced macular thickness and age-related macular degeneration in the UK Biobank. Hum. Mol. Genet. 31, 2678–2692. https://doi.org/10.1093/hmg/ddac060.
Tzoumas, N., Riding, G., Williams, M.A., Steel, D.H.W., 2023. Complement inhibitors for age-related macular degeneration. Cochrane Database Syst. Rev. 6, CD009300 https://doi.org/10.1002/14651858.CD009300.pub3.
Udry, F., Decembrini, S., Gamm, D.M., Déglon, N., Kostic, C., Arsenijevic, Y., 2020. Lentiviral mediated RPE65 gene transfer in healthy hiPSCs-derived retinal pigment epithelial cells markedly increased RPE65 mRNA, but modestly protein level. Sci. Rep. 10, 8890. https://doi.org/10.1038/s41598-020-65657-y.
Ule, J., Blencowe, B.J., 2019. Alternative splicing regulatory networks: functions, mechanisms, and evolution. Mol. Cell 76, 329–345. https://doi.org/10.1016/j.molcel.2019.09.017.
Urnov, F.D., Rebar, E.J., Holmes, M.C., Zhang, H.S., Gregory, P.D., 2010. Genome editing with engineered zinc finger nucleases. Nat. Rev. Genet. 11, 636–646. https://doi.org/10.1038/nrg2842.
Usui-Ouchi, A., Giles, S., Harkins-Perry, S., Mills, E.A., Bonelli, R., Wei, G., Ouchi, Y., Ebihara, N., Nakao, S., Friedlander, M., Eade, K.T., 2023. Integrating human iPSC-derived macrophage progenitors into retinal organoids to generate a mature retinal microglial niche. Glia 71, 2372–2382. https://doi.org/10.1002/glia.24428.
Valdes-Sanchez, L., Calado, S.M., de la Cerda, B., Aramburu, A., Garcia-Delgado, A.B., Massalini, S., Montero-Sanchez, A., Bhatia, V., Rodriguez-Bocanegra, E., Diez-Lloret, A., Rodriguez-Martinez, D., Chakarova, C., Bhattacharya, S.S., Diaz-Corrales, F.J., 2019. Retinal pigment epithelium degeneration caused by aggregation of PRPF31 and the role of HSP70 family of proteins. Mol. Med. 26, 1. https://doi.org/10.1186/s10020-019-0124-z.
Valente, E.M., Silhavy, J.L., Brancati, F., Barrano, G., Krishnaswami, S.R., Castori, M., Lancaster, M.A., Boltshauser, E., Boccone, L., Al-Gazali, L., Fazzi, E., Signorini, S., Louie, C.M., Bellacchio, E., International Joubert Syndrome Related Disorders Study, G., Bertini, E., Dallapiccola, B., Gleeson, J.G., 2006. Mutations in CEP290, which encodes a centrosomal protein, cause pleiotropic forms of Joubert syndrome. Nat. Genet. 38, 623–625. https://doi.org/10.1038/ng1805.
Vázquez-Domínguez, I., Duijkers, L., Fadaie, Z., Alaerds, E.C.W., Post, M.A., van Oosten, E.M., O'Gorman, L., Kwint, M., Koolen, L., Hoogendoorn, A.D.M., Kroes, H. Y., Gilissen, C., Cremers, F.P.M., Collin, R.W.J., Roosing, S., Garanto, A., 2022. The predicted splicing variant c.11+5G>A in RPE65 leads to a reduction in mRNA expression in a cell-specific manner. Cells 11, 3640. https://doi.org/10.3390/cells11223640.
Vergara, M.N., Flores-Bellver, M., Aparicio-Domingo, S., McNally, M., Wahlin, K.J., Saxena, M.T., Mumm, J.S., Canto-Soler, M.V., 2017. Three-dimensional automated reporter quantification (3D-ARQ) technology enables quantitative screening in retinal organoids. Development 144, 3698–3705. https://doi.org/10.1242/dev.146290.
Vithayathil, S.A., Ma, Y., Kaipparettu, B.A., 2012. Transmitochondrial cybrids: tools for functional studies of mutant mitochondria. Methods Mol. Biol. 837, 219–230. https://doi.org/10.1007/978-1-61779-504-6_15.
Völkner, M., Pavlou, M., Büning, H., Michalakis, S., Karl, M.O., 2021. Optimized adeno-associated virus vectors for efficient transduction of human retinal organoids. Hum. Gene Ther. 32, 694–706. https://doi.org/10.1089/hum.2020.321.
Volkner, M., Wagner, F., Steinheuer, L.M., Carido, M., Kurth, T., Yazbeck, A., Schor, J., Wieneke, S., Ebner, L.J.A., Del Toro Runzer, C., Taborsky, D., Zoschke, K., Vogt, M., Canzler, S., Hermann, A., Khattak, S., Hackermuller, J., Karl, M.O., 2022. HBEGF-TNF induce a complex outer retinal pathology with photoreceptor cell extrusion in human organoids. Nat. Commun. 13, 6183. https://doi.org/10.1038/s41467-022-33848-y.
Wahlin, K.J., Maruotti, J.A., Sripathi, S.R., Ball, J., Angueyra, J.M., Kim, C., Grebe, R., Li, W., Jones, B.W., Zack, D.J., 2017. Photoreceptor outer segment-like structures in long-term 3D retinas from human pluripotent stem cells. Sci. Rep. 7, 766. https://doi.org/10.1038/s41598-017-00774-9.
Wallace, D.C., Chalkia, D., 2013. Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. Cold Spring Harb. Perspect. Biol. 5, a021220. https://doi.org/10.1101/cshperspect.a021220.
Walsh, A.J., Cook, R.S., Sanders, M.E., Aurisicchio, L., Ciliberto, G., Arteaga, C.L., Skala, M.C., 2014. Quantitative optical imaging of primary tumor organoid metabolism predicts drug response in breast cancer. Cancer Res. 74, 5184–5194. https://doi.org/10.1158/0008-5472.Can-14-0663.
Wang, A.L., Lukas, T.J., Yuan, M., Du, N., Tso, M.O., Neufeld, A.H., 2009. Autophagy and exosomes in the aged retinal pigment epithelium: possible relevance to drusen formation and age-related macular degeneration. PLoS One 4, e4160. https://doi.org/10.1371/journal.pone.0004160.
Wang, L., Clark, M.E., Crossman, D.K., Kojima, K., Messinger, J.D., Mobley, J.A., Curcio, C.A., 2010. Abundant lipid and protein components of drusen. PLoS One 5, e10329. https://doi.org/10.1371/journal.pone.0010329.
Wang, L., Hiler, D., Xu, B., AlDiri, I., Chen, X., Zhou, X., Griffiths, L., Valentine, M., Shirinifard, A., Sablauer, A., Thiagarajan, S., Barabas, M.E., Zhang, J., Johnson, D., Frase, S., Dyer, M.A., 2018. Retinal cell type DNA methylation and histone modifications predict reprogramming efficiency and retinogenesis in 3D organoid cultures. Cell Rep. 22, 2601–2614. https://doi.org/10.1016/j.celrep.2018.01.075.
Wang, Y., Yin, N., Yang, R., Zhao, M., Li, S., Zhang, S., Zhao, Y., Faiola, F., 2023. Development of a simplified human embryonic stem cell-based retinal pre-organoid model for toxicity evaluations of common pollutants. Cutan. Ocul. Toxicol. 42, 264–272. https://doi.org/10.1080/15569527.2023.2249988.
Warda, O., Naeem, Z., Roelofs, K.A., Sagoo, M.S., Reddy, M.A., 2023. Retinoblastoma and vision. Eye 37, 797–808. https://doi.org/10.1038/s41433-021-01845-y.
Webb, J.L., Ravikumar, B., Atkins, J., Skepper, J.N., Rubinsztein, D.C., 2003. Alpha-Synuclein is degraded by both autophagy and the proteasome. J. Biol. Chem. 278, 25009–25013. https://doi.org/10.1074/jbc.M300227200.
Wei, W., Gaffney, D.J., Chinnery, P.F., 2021. Cell reprogramming shapes the mitochondrial DNA landscape. Nat. Commun. 12, 5241. https://doi.org/10.1038/s41467-021-25482-x.
Weismann, D., Hartvigsen, K., Lauer, N., Bennett, K.L., Scholl, H.P.N., Issa, P.C., Cano, M., Brandstätter, H., Tsimikas, S., Skerka, C., Superti-Furga, G., Handa, J.T., Zipfel, P.F., Witztum, J.L., Binder, C.J., 2011. Complement factor H binds malondialdehyde epitopes and protects from oxidative stress. Nature 478, 76–81. https://doi.org/10.1038/nature10449.
West, E.L., Majumder, P., Naeem, A., Fernando, M., O'Hara-Wright, M., Lanning, E., Kloc, M., Ribeiro, J., Ovando-Roche, P., Shum, I.O., Jumbu, N., Sampson, R., Hayes, M., Bainbridge, J.W.B., Georgiadis, A., Smith, A.J., Gonzalez-Cordero, A., Ali, R.R., 2022. Antioxidant and lipid supplementation improve the development of photoreceptor outer segments in pluripotent stem cell-derived retinal organoids. Stem Cell Reports 17, 775–788. https://doi.org/10.1016/j.stemcr.2022.02.019.
Wetzel, R.K., Arystarkhova, E., Sweadner, K.J., 1999. Cellular and subcellular specification of Na,K-ATPase alpha and beta isoforms in the postnatal development of mouse retina. J. Neurosci. 19, 9878–9889. https://doi.org/10.1523/JNEUROSCI.19-22-09878.1999.
Wheway, G., Parry, D.A., Johnson, C.A., 2014. The role of primary cilia in the development and disease of the retina. Organogenesis 10, 69–85. https://doi.org/10.4161/org.26710.
Wheway, G., Schmidts, M., Mans, D.A., Szymanska, K., Nguyen, T.T., Racher, H., Phelps, I.G., Toedt, G., Kennedy, J., Wunderlich, K.A., Sorusch, N., Abdelhamed, Z. A., Natarajan, S., Herridge, W., van Reeuwijk, J., Horn, N., Boldt, K., Parry, D.A., Letteboer, S.J.F., Roosing, S., Adams, M., Bell, S.M., Bond, J., Higgins, J., Morrison, E.E., Tomlinson, D.C., Slaats, G.G., van Dam, T.J.P., Huang, L., Kessler, K., Giessl, A., Logan, C.V., Boyle, E.A., Shendure, J., Anazi, S., Aldahmesh, M., Al Hazzaa, S., Hegele, R.A., Ober, C., Frosk, P., Mhanni, A.A., Chodirker, B.N., Chudley, A.E., Lamont, R., Bernier, F.P., Beaulieu, C.L., Gordon, P., Pon, R.T., Donahue, C., Barkovich, A.J., Wolf, L., Toomes, C., Thiel, C.T., Boycott, K.M., McKibbin, M., Inglehearn, C.F., Consortium, U.K., University of Washington Center for Mendelian, G., Stewart, F., Omran, H., Huynen, M.A., Sergouniotis, P.I., Alkuraya, F.S., Parboosingh, J.S., Innes, A.M., Willoughby, C.E., Giles, R.H., Webster, A.R., Ueffing, M., Blacque, O., Gleeson, J.G., Wolfrum, U., Beales, P.L., Gibson, T., Doherty, D., Mitchison, H.M., Roepman, R., Johnson, C.A., 2015. An siRNA-based functional genomics screen for the identification of regulators of ciliogenesis and ciliopathy genes. Nat. Cell Biol. 17, 1074–1087. https://doi.org/10.1038/ncb3201.
Wickramasinghe, V.O., Gonzalez-Porta, M., Perera, D., Bartolozzi, A.R., Sibley, C.R., Hallegger, M., Ule, J., Marioni, J.C., Venkitaraman, A.R., 2015. Regulation of constitutive and alternative mRNA splicing across the human transcriptome by PRPF8 is determined by 5' splice site strength. Genome Biol. 16, 201. https://doi.org/10.1186/s13059-015-0749-3.
Will, C.L., Luhrmann, R., 2011. Spliceosome structure and function. Cold Spring Harb. Perspect. Biol. 3, a003707 https://doi.org/10.1101/cshperspect.a003707.
Wong, R.C.B., Lim, S.Y., Hung, S.S.C., Jackson, S., Khan, S., Van Bergen, N.J., De Smit, E., Liang, H.H., Kearns, L.S., Clarke, L., Mackey, D.A., Hewitt, A.W., Trounce, I.A., Pebay, A., 2017. Mitochondrial replacement in an iPSC model of Leber's hereditary optic neuropathy. Aging (Albany NY) 9, 1341–1350. https://doi.org/10.18632/aging.101231.
Wonnapinij, P., Chinnery, P.F., Samuels, D.C., 2008. The distribution of mitochondrial DNA heteroplasmy due to random genetic drift. Am. J. Hum. Genet. 83, 582–593. https://doi.org/10.1016/j.ajhg.2008.10.007.
Wright, C., Mazzucco, A.E., Becker, S.M., Sieving, P.A., Tumminia, S.J., 2020. NEI-supported age-related macular degeneration research: past, present, and future. Transl. Vis. Sci. Technol. 9, 49. https://doi.org/10.1167/tvst.9.7.49.
Wu, Y.R., Wang, A.G., Chen, Y.T., Yarmishyn, A.A., Buddhakosai, W., Yang, T.C., Hwang, D.K., Yang, Y.P., Shen, C.N., Lee, H.C., Chiou, S.H., Peng, C.H., Chen, S.J., 2018. Bioactivity and gene expression profiles of hiPSC-generated retinal ganglion cells in MT-ND4 mutated Leber's hereditary optic neuropathy. Exp. Cell Res. 363, 299–309. https://doi.org/10.1016/j.yexcr.2018.01.020.

Wu, Z., Pang, N., Zhang, Y., Chen, H., Peng, Y., Fu, J., Wei, Q., 2020. CEP290 is essential for the initiation of ciliary transition zone assembly. PLoS Biol. 18, e3001034 https://doi.org/10.1371/journal.pbio.3001034.

Xi, Z., Vats, A., Sahel, J.-A., Chen, Y., Byrne, L.C., 2022. Gene augmentation prevents retinal degeneration in a CRISPR/Cas9-based mouse model of PRPF31 retinitis pigmentosa. Nat. Commun. 13, 7695. https://doi.org/10.1038/s41467-022-35361-8.

Xue, C., Greene, E.C., 2021. DNA repair pathway choices in CRISPR-cas9-mediated genome editing. Trends Genet. 37, 639–656. https://doi.org/10.1016/j.tig.2021.02.008.

Yanai, A., Laver, C.R.J., Gregory-Evans, C.Y., Liu, R.R., Gregory-Evans, K., 2015. Enhanced functional integration of human photoreceptor precursors into human and rodent retina in an ex vivo retinal explant model system. Tissue Eng. Part A 21, 1763–1771. https://doi.org/10.1089/ten.TEA.2014.0669.

Yang, C.P., Hung, I.J., Jaing, T.H., Shih, L.Y., Chang, W.H., 2005. Cancer in infants: a review of 82 cases. Pediatr. Hematol. Oncol. 22, 463–481. https://doi.org/10.1080/08880010591002233.

Yang, H., Beutler, B., Zhang, D., 2022. Emerging roles of spliceosome in cancer and immunity. Protein Cell 13, 559–579. https://doi.org/10.1007/s13238-021-00856-5.

Yang, J., Li, Y., Chan, L., Tsai, Y.T., Wu, W.H., Nguyen, H.V., Hsu, C.W., Li, X., Brown, L. M., Egli, D., Sparrow, J.R., Tsang, S.H., 2014a. Validation of genome-wide association study (GWAS)-identified disease risk alleles with patient-specific stem cell lines. Hum. Mol. Genet. 23, 3445–3455. https://doi.org/10.1093/hmg/ddu053.

Yang, L., Grishin, D., Wang, G., Aach, J., Zhang, C.Z., Chari, R., Homsy, J., Cai, X., Zhao, Y., Fan, J.B., Seidman, C., Seidman, J., Pu, W., Church, G., 2014b. Targeted and genome-wide sequencing reveal single nucleotide variations impacting specificity of Cas9 in human stem cells. Nat. Commun. 5, 5507. https://doi.org/10.1038/ncomms6507.

Yang, Q., Li, J., Zeng, S., Li, Z., Liu, X., Li, J., Zhou, W., Chai, Y., Zhou, D., 2023. Retinal organoid models show heterozygous rhodopsin mutation favors endoplasmic reticulum stress-induced apoptosis in rods. Stem Cell. Dev. 32, 681–692. https://doi.org/10.1089/scd.2023.0034.

Yang, T.-C., Yarmishyn, A.A., Yang, Y.-P., Lu, P.-C., Chou, S.-J., Wang, M.-L., Lin, T.-C., Hwang, D.-K., Chou, Y.-B., Chen, S.-J., Yu, W.-K., Wang, A.-G., Hsu, C.-C., Chiou, S.-H., 2020. Mitochondrial transport mediates survival of retinal ganglion cells in affected LHON patients. Hum. Mol. Genet. 29, 1454–1464. https://doi.org/10.1093/hmg/ddaa063.

Yang, Y., Wu, H., Kang, X., Liang, Y., Lan, T., Li, T., Tan, T., Peng, J., Zhang, Q., An, G., Liu, Y., Yu, Q., Ma, Z., Lian, Y., Soh, B.S., Chen, Q., Liu, P., Chen, Y., Sun, X., Li, R., Zhen, X., Liu, P., Yu, Y., Li, X., Fan, Y., 2018. Targeted elimination of mutant mitochondrial DNA in MELAS-iPSCs by mitoTALENs. Protein Cell 9, 283–297. https://doi.org/10.1007/s13238-017-0499-y.

Yanik, O., Gunduz, K., Yavuz, K., Tacyildiz, N., Unal, E., 2015. Chemotherapy in retinoblastoma: current approaches. Turk. J. Ophthalmol. 45, 259–267. https://doi.org/10.4274/tjo.06888.

Yin, H., Kanasty, R.L., Eltoukhy, A.A., Vegas, A.J., Dorkin, J.R., Anderson, D.G., 2014. Non-viral vectors for gene-based therapy. Nat. Rev. Genet. 15, 541–555. https://doi.org/10.1038/nrg3763.

Yiu, G., Tieu, E., Nguyen, A.T., Wong, B., Smit-McBride, Z., 2016. Genomic disruption of VEGF-A expression in human retinal pigment epithelial cells using CRISPR-cas9 endonuclease. Invest. Ophthalmol. Vis. Sci. 57, 5490–5497. https://doi.org/10.1167/iovs.16-20296.

Yoshida, T., Ozawa, Y., Suzuki, K., Yuki, K., Ohyama, M., Akamatsu, W., Matsuzaki, Y., Shimmura, S., Mitani, K., Tsubota, K., Okano, H., 2014. The use of induced pluripotent stem cells to reveal pathogenic gene mutations and explore treatments for retinitis pigmentosa. Mol. Brain 7, 45. https://doi.org/10.1186/1756-6606-7-45.

Young, R.W., 1967. The renewal of photoreceptor cell outer segments. J. Cell Biol. 33, 61–72. https://doi.org/10.1083/jcb.33.1.61.

Young, R.W., 1985. Cell differentiation in the retina of the mouse. Anat. Rec. 212, 199–205. https://doi.org/10.1002/ar.1092120215.

Young, R.W., 1987. Pathophysiology of age-related macular degeneration. Surv. Ophthalmol. 31, 291–306. https://doi.org/10.1016/0039-6257(87)90115-9.

Yu-Wai-Man, P., Votruba, M., Burté, F., La Morgia, C., Barboni, P., Carelli, V., 2016. A neurodegenerative perspective on mitochondrial optic neuropathies. Acta Neuropathol. 132, 789–806. https://doi.org/10.1007/s00401-016-1625-2.

Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., Slukvin, I.I., Thomson, J.A., 2007. Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917–1920. https://doi.org/10.1126/science.1151526.

Yu, Y.J., Watts, R.J., 2013. Developing therapeutic antibodies for neurodegenerative disease. Neurotherapeutics 10, 459–472. https://doi.org/10.1007/s13311-013-0187-4.

Yuan, L., Kawada, M., Havlioglu, N., Tang, H., Wu, J.Y., 2005. Mutations in PRPF31 inhibit pre-mRNA splicing of rhodopsin gene and cause apoptosis of retinal cells. J. Neurosci. 25, 748–757. https://doi.org/10.1523/JNEUROSCI.2399-04.2005.

Zambelli, F., Mertens, J., Dziedzicka, D., Sterckx, J., Markouli, C., Keller, A., Tropel, P., Jung, L., Viville, S., Van de Velde, H., Geens, M., Seneca, S., Sermon, K., Spits, C., 2018. Random mutagenesis, clonal events, and embryonic or somatic origin determine the mtDNA variant type and load in human pluripotent stem cells. Stem Cell Reports 11, 102–114. https://doi.org/10.1016/j.stemcr.2018.05.007.

Zarbin, M., Sugino, I., Townes-Anderson, E., 2019. Concise review: update on retinal pigment epithelium transplantation for age-related macular degeneration. Stem Cells Transl. Med. 8, 466–477. https://doi.org/10.1002/sctm.18-0282.

Zarbin, M.A., 2003. Analysis of retinal pigment epithelium integrin expression and adhesion to aged submacular human Bruch's membrane. Trans. Am. Ophthalmol. Soc. 101, 499–520. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1359002/.

Zhang, I., Lepine, P., Han, C., Lacalle-Aurioles, M., Chen, C.X., Haag, R., Durcan, T.M., Maysinger, D., 2020. Nanotherapeutic modulation of human neural cells and glioblastoma in organoids and monocultures. Cells 9, 2434. https://doi.org/10.3390/cells9112434.

Zhang, Q., Presswalla, F., Ali, R.R., Zacks, D.N., Thompson, D.A., Miller, J.M.L., 2021. Pharmacologic activation of autophagy without direct mTOR inhibition as a therapeutic strategy for treating dry macular degeneration. Aging (Albany NY) 13, 10866–10890. https://doi.org/10.18632/aging.202974.

Zhang, X.M., Yang, X.J., 2001. Regulation of retinal ganglion cell production by Sonic hedgehog. Development 128, 943–957. https://doi.org/10.1242/dev.128.6.943.

Zhong, X., Gutierrez, C., Xue, T., Hampton, C., Vergara, M.N., Cao, L.H., Peters, A., Park, T.S., Zambidis, E.T., Meyer, J.S., Gamm, D.M., Yau, K.W., Canto-Soler, M.V., 2014. Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. Nat. Commun. 5, 4047. https://doi.org/10.1038/ncomms5047.

Zhou, J., Hu, J., Wang, Y., Gao, S., 2023. Induction and application of human naive pluripotency. Cell Rep. 42, 112379 https://doi.org/10.1016/j.celrep.2023.112379.

Zhu, C.-C., Traboulsi, E.I., Parikh, S., 2017. Ophthalmological findings in 74 patients with mitochondrial disease. Ophthalmic Genet. 38, 67–69. https://doi.org/10.3109/13816810.2015.1130153.

Zimmermann, A., Preynat-Seauve, O., Tiercy, J.-M., Krause, K.-H., Villard, J., 2012. Haplotype-based banking of human pluripotent stem cells for transplantation: potential and limitations. Stem Cells Dev. 21, 2364–2373. https://doi.org/10.1089/scd.2012.0088.

Zuber, M.E., Gestri, G., Viczian, A.S., Barsacchi, G., Harris, W.A., 2003. Specification of the vertebrate eye by a network of eye field transcription factors. Development 130, 5155–5167. https://doi.org/10.1242/dev.00723.