Title: An iPSC patient specific model of CFH (Y402H) polymorphism displays characteristic features of AMD and indicates a beneficial role for UV light exposure

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Abstract

Age related macular degeneration (AMD) is the most common cause of blindness, accounting for 8.7% of all blindness globally. Vision loss is caused ultimately by apoptosis of the retinal pigment epithelium (RPE) and overlying photoreceptors. Treatments are evolving for the wet form of the disease, however these do not exist for the dry form. Complement factor H (*CFH*) polymorphism in exon 9 (Y402H) has shown a strong association with susceptibility to AMD resulting in complement activation, recruitment of phagocytes, retinal pigment epithelium (RPE) damage and visual decline. We have derived and characterised induced pluripotent stem cell (iPSCs) lines from two patients without AMD and low risk genotype and two patients with advanced AMD and high risk genotype and generated RPE cells that show local secretion of several proteins involved in the complement pathway including factor H (FH), factor I (FI) and factor H like 1 (FHL-1). The iPSC RPE cells derived from high risk patients mimic several key features of AMD including increased inflammation and cellular stress, accumulation of lipid droplets, impaired autophagy and deposition of "drüsen" like deposits. The low and high risk RPE cells respond differently to intermittent exposure to UV light which leads to an improvement in cellular and functional phenotype only in the high risk AMD-RPE cells. Taken together our data indicate that the patient specific iPSC model provides a robust platform for understanding the role of complement activation in AMD, evaluating new therapies based on complement modulation and drug testing.

Introduction

Age related macular degeneration (AMD) is the most common cause of blindness in the developed world, affecting one in three people by age 75 and is characterised by a loss of central vision, affecting the macular area of the retina. It accounts for 50% of blind and partially sighted registration with an estimated prevalence of ~600,000 significantly visually impaired people in the UK and over 8 million worldwide (1-4). 70,000 new diagnoses are made every year in UK and 13% of people aged over 80 are affected by late stage AMD. The number of AMD affected people in UK is expected to rise to 1.3 M by 2050 with healthcare costs rising to 16.4 billion during 2010-2020 (5). Visual loss associated with AMD is caused by apoptosis of the retinal pigment epithelium (RPE) and overlying photoreceptors. AMD occurs in two forms: "dry" AMD where cellular debris, called drüsen accumulates between the RPE and Bruch's membrane (BrM), appearing as yellow specks on the retina. "Wet" AMD is usually characterised by aberrant blood vessel growth and encroachment from the choroid underneath the retina although it can also originate from the inner retinal vasculature. Treatments are evolving for wet AMD including anti-VEGF treatments, photodynamic and laser therapy (6-8); however there are no effective treatments to prevent progression of the underlying disease process and advanced dry AMD.

AMD is a multifactorial progressive disease with a complex interaction between environmental, metabolic, hereditary factors and chronic innate immune activation (9). A variety of alleles and haplotypes associated with early and late AMD have been identified from genome wide association studies (GWAS) (10-13), but the precise roles of these genes and the mechanisms by which they increase disease risk are ill defined. One of the most significant genetic findings for AMD has been the complement factor H (*CFH*) polymorphisms. Complement Factor H (FH) protein functions by limiting the formation of C3 convertase of the complement system and by promoting the degradation of C3b to iC3b. Failure to control the activity of C3 convertase results in overproduction of C3b and C3a causing a shift in the complement cascade to its terminal lytic pathway. A significantly

deleterious consequence of this is the formation of the anaphylotoxin, C5a and the membrane attack complex both of which deliver potent inflammatory signals. The T>C substitution in exon 9 (Y402H) of the *CFH* gene is strongly associated with susceptibility to AMD and has led to recognition of the importance of complement activation in AMD pathogenesis (10). There is now evidence from large case-control association studies to confirm association with a variety of other complement cascade genes including *CFHR1-3, CFI*, *CFB, C3,* and *C9* (10, 11). The polymorphisms within the 10q26 gene loci containing the *PIEKHA1/HTRA1/ARMS2* genes have also consistently demonstrated strong associations with AMD in GWAS (10, 12). In addition to data gathered from large genetic cohorts, biochemical and molecular studies have provided substantial evidence to support an important role for complement activation in AMD. This is illustrated by the presence of activators and regulators of the complement system in drüsen (14) and the increased expression of membrane attack complex (MAC) proteins in choriocapillaris and BrM of aged individuals as well as those with the Y402H polymorphism (15-17).

The Y402H polymorphism can confer >5 fold increase risk of developing AMD and is present in approximately 30% of people of European descent. Although FH protein is synthesised by the choroid, it is not able to diffuse passively through BrM into the retina; however its alternatively spliced, truncated form, named FH-like protein 1 (FHL-1), is able to do so (18). FHL-1 retains all the necessary domains for complement regulation and binds to BrM through interactions with heparan sulphate (18-20). The Y402H polymorphism affects the ability of both FH and FHL-1 to bind to heparan sulphate (21). Furthermore, FH and lipoproteins compete for binding to heparan sulphate in BrM (22), thus it has been suggested that impaired binding of FH/FHL-1 to heparan sulphate in individuals with Y402H polymorphism results in fewer binding sites for FH/FHL-1, increased C3b depositions, lipoprotein accumulation and failure to regulate complement activation, leading to recruitment of mononuclear phagocytes, RPE damage, and visual function decline.

Recent advances in the field of induced pluripotency have permitted generation of patient specific induced pluripotent stem cells (iPSCs) which have the ability to differentiate into cells of any tissue type including photoreceptors and RPE (23). The ability to produce large quantities of functional patient-specific retinal cells from iPSCs offers an unparalleled chance to elucidate disease mechanisms and evaluate new therapeutic agents. Since the pathogenesis of AMD is largely unknown, creating a disease model using iPSC technology could be a valuable tool to address fundamental questions about disease biology as well as creating a biological tool to perform drug discovery and toxicity screening. The validity of this approach has been illustrated by two recent publications reporting derivation of iPSCs from AMD patients with *ARMS2/HTRA1* high risk genotypes displaying reduced SOD2 defence, rendering RPE more susceptible to oxidative damage (24, 25). We focused on derivation and characterisation of iPSC from individuals homozygous for the low and high risk *CFH* (Y402H) polymorphism. When compared to iPSC-RPE derived from age matched control low risk individuals, the high risk iPSC-RPE cells show a range of cellular, ultrastructural and functional deficiencies that mimic several key features of AMD including increased inflammation, hallmarks of cellular stress, accumulation of lipid droplets and deposition of "drüsen" like deposits. Exposure to intermittent ultra violet light (UV) elicited different responses from low and high risk RPE cells and in the latter revealed an improvement in the cellular and ultrastructural features associated with AMD. Together our data, suggest that the patient specific iPSC disease modelling provides a robust tool to assess potential therapeutic agents to treat AMD before long expensive trials.

Results

Generation of iPSCs from high risk AMD donors and unaffected controls

To investigate how the Y402H polymorphism in *CFH* leads to the pathology associated with AMD, DNA was extracted from donor cell fibroblasts and sequenced to detect single

nucleotide polymorphisms in the *CFH, HTRA1* and *ARMS2* genes (**Supplementary Figure 1, A**). The two homozygous low risk donors were selected on the basis of low risk for all three SNP's rs11200638 (*HTRA1*), rs1061170 (*CFH*) and rs10490924 (*ARMS2*) and no clinical manifestation of AMD. The high risk donors were specifically selected as having advanced AMD with unilateral wet AMD and reticular pseudodrüsen (a known high risk feature for both types of advanced AMD) in their fellow eyes (**Supplementary Figure 1, B-C'**) and high risk SNP for *CFH* and low risk *HTRA1* and *ARMS2*. The high risk *CFH* in combination with low risk *HTRA1* polymorphism has been consistently associated with central drüsen formation in the older age group (26).

iPSCs were generated from dermal fibroblasts using non-integrative Sendai viral vectors expressing Yamanaka reprogramming transgenes. Between twenty and thirty clones were generated from each donor. At least three clones from each individual were expanded, adapted to feeder free culture conditions and thoroughly characterised using well established tests of pluripotency including expression of markers by immunocytochemistry (SSEA4 & OCT4, **Supplementary Figure 2, A**), RT-PCR (*NANOG, KLF4, C-MYC & SOX2*) **Supplementary Figure 2, B**) and flow cytometry (SSEA4 & OCT4, **Supplementary Figure 2, C**), ability to differentiate into all three germ layers *in vitro* (SMA, TUJ1 & AFP; **Supplementary Figure 2, D**) and *in vivo* (**Supplementary Figure 2, E)**, clearance of Sendai Transgenes (**Supplementary Figure 2, F**) and genetic identity to parent fibroblasts (**Supplementary Figure 2, G**). One clone from each patient was further selected for differentiation studies. CytoSNP analysis indicated no chromosomal re-arrangements, losses or duplications. Our RT-PCR analysis also indicated that *CFH* and *FHL-1* are not expressed in iPSCs (**Supplementary Figure 3, A**).

Establishing iPSC-RPE from high risk AMD patients and unaffected controls

iPSCs were differentiated to RPE using a defined serum and feeder free protocol described in the methods section. The RPE patches were mechanically isolated and expanded on laminin coated trans-well inserts or tissue culture plates. Hexagonal cells with pigmentation both visible macro and microscopically (**Figure 1, A, B**), which expressed the putative RPE cell marker ZO-1, CRALBP and BEST1 (**Figure 1, C**). Polarity in the RPE cells is important for their physiological function, we checked the presence of Na$^+$ K$^+$-ATPase in both low and high risk iPSC-RPE cells and showed apical localisation in both (**Figure 1, D**). iPSC-RPE cells also secreted pigment epithelium-derived factor (PEDF) also known as serpin F1 (SERPINF1) in a physiologically similar fashion to adult RPE (27) (**Figure 1, E**) all cultures. RPE cells form a tight barrier in the retina which can be measured by trans-epithelial resistance (TER). We observed no significant differences in TER between RPE derived from high or low risk AMD individuals (**Figure 1, F**). Phagocytosis assays also indicated that iPSC-RPE were able to phagocytose bovine rod outer segments with no differences observed between low and high risk AMD donors (data not shown).

Expression of Complement Factor H, Factor H like protein 1, Factor I and C3b in iPSC-RPE cells.

Expression of, FH/FHL-1 (**Figure 2, A**), FI (**Figure 2, B**) and C3 proteins (**Figure 2, C**) were detected in RPE conditioned supernatants derived from both high and low risk cultures, indicating that iPSC-RPE cells secrete the main component and regulators of the alternative complement pathway. Importantly the excreted proteins FH and FI were shown to be functional, indicated by the breakdown of C3b to iC3b (**Figure 2, D**). *CFH, FHL-1*, *CFI* and *C3 expression* were also confirmed at the mRNA level utilising qRT-PCR (**Figure 3, A, B, C, D & E**). *CFI, CFH* and *FHL-1* were all upregulated in high risk iPSC-RPE cells *(***Figure 3, A, B, C***)*, a trend also observed in iPSC-RPE derived from HTRA1/ARMS2 risk genotypes (25). Interestingly C3 was down regulated in high-risk iPSC-RPE, while C5 showed no significant

difference (**Figure 3, C, D**). Together these results add to the evidence for local complement synthesis in the eye as previously documented in the literature (28, 29).

Gene expression profiles of cytokines in AMD and control iPSC-RPE

High-risk iPSC-RPE had reduced gene expression of mitochondrial Superoxide dismutase 2, (*SOD2*) (**Figure 3, F**)*,* which acts to transform superoxide, a toxic by-product of oxidative phosphorylation, into less harmful hydrogen peroxide and diatomic oxygen. It has also been reported that ARMS2/HTRA1 polymorphism leads to compromised superoxide dismutase 2 response (23) while knockout of *SOD2* in mice is used as an early model of AMD (30). *APOE* (Apolipoprotein E), a transporter of lipoproteins and fat-soluble vitamins, and *TNF-α* (Tumour necrosis factor) a cytokine involved in systemic inflammation and implicated in downregulation of *OTX2* (31), showed increased expression in high risk donors (**Figure 3, G, H**), however Interleukin-18 (*IL18*), a cytokine which can suppress *VEGF* expression and has been associated with AMD (32), showed no significant difference between genotypes (**Figure 3, I**). No significant changes between genotypes were observed for vascular endothelial growth factor (*VEGF*) expression (**Figure 3, J**). Interleukin 1β (IL1β) has a broad spectrum of mediation in cellular function and has been implicated as an effector of the inflammatory response (33). We noted a significant difference between genotypes with high-risk donors expressing *IL1β* at a higher level than low risk RPE (**Figure 3, K**). Elevated levels of *IL-6* are found in the vitreal fluid of AMD patients and have been used as predictors of AMD progression (34), however we did not detect any difference in IL6 between genotypes suggesting that the release of this cytokine is likely from another source such as microglia (**Figure 3, L**). Orthodenticle homeobox 2 (*OTX2*) controls essential, homeostatic RPE genes. There was a slight decrease (although not significant) in *OTX2* in high-risk donors, perhaps linked to *TNF-α* expression as previously stated (**Figure 3, M**). *RPE65* expression remained constant in both genotypes (**Figure 3, N**).

To further probe into the differences between high and low risk RPE cells, we performed RNA-seq studies which identified 41 genes residing in the 0.3% percentile and 99.7% percentile, equating to a -5.532 fold change or greater and 5.66 or greater respectively (**Supplementary Figure 3, C**). This analysis revealed the upregulated expression of *CGA* (Glycoprotein hormones α-chain) in RPE cells derived from high-risk donors (**Supplementary Figure 3, C**). Currently there is no documented expression of CGA in RPE cells, however the expression its of receptor (GNRHR, Gonadotropin-releasing hormone receptor) has been detected in retinal tissues (35). Recoverin (*RCVRN)* expression, which marks photoreceptor precursors, was also upregulated in high-risk donors (**Supplementary Figure 3, C).** It is of interest to note that expression of *RCVRN* has been observed in RPE cells which were induced to transdifferentiate to photoreceptors via overexpression of *Neurogenin* 1 or 3 (34). RPE cells undergoing this transition have been noted to retain pigmentation while displaying an elongated cell body and *RCVRN* expression in aPVMD2-ngn1 mouse (36, 37). It is currently unclear whether increased *RCVRN* expression in high risk AMD-RPE is related to cell fate changes, or to an impaired wound healing response and epithelial to mesenchymal transition which has already been reported in AMD-RPE (38). One family of genes was disproportionately represented in the low risk donor including members of the Wnt and Cadherin signalling pathways [Protocadherin gamma-A3 (PCDHGA3), Protocadherin beta-8 (PCDHB8), Protocadherin gamma-A6 (PCDHGA6) & Putative protocadherin beta-18 (PCDHB18P)], implying that low risk donors may find it easier to form cell-cell junctions when compared to high risk RPE, thus corroborating previously published data showing disrupted cell to cell junctions and induction of AMD associated pathological changes in light exposed RPE cells (39).

When all genes with > 1.5 fold expression changes between the high and low risk iPSC RPE were analysed using Enrichr, (OMIM disease) (40), macular degeneration, diabetes mellitus type 2 and protein glycosylation disorder diseases were found to be overrepresented in the

RNA-seq dataset (*p=0.05475*, Supplementary **Figure 3 B**). Glycosyltransferases are responsible for post-translational glycol modification of proteins, and this is considered a location specific modification as the enzymes required are normally compartmentalised. Glycosylation status is suggested to be important for efficient transport/diffusion of FHL-1 though BrM, FHL-1 is normally non-glycosylated and passes easily (41), while glycosylated CFH does not. Interfering with this status could be detrimental to location and diffusion characteristics. Additionally, advanced glycosylation end products (AGE) are a classical indication of an aged RPE cell layer (42). Together these data suggests that the *in vitro* iPSC-RPE model we have created mimics the disease at the molecular level.

C5b-9 deposition and APOE co-localisation in AMD iPSC-RPE

Many investigations have described the proteomic and lipid composition of drüsen (43). APOE is ubiquitously associated with drüsen formation and shown to comprise 36% of all proteins found extracellularly (43). The terminal complement complex (TCC) (C5b-C6-C7-C8-$C9_n$, [C5b-9]) is comprised of five proteins, C5b, C6, C7, and C8, with the fifth, C9 forming a transmembrane ring structure. We found the presence of aggregates that either contained ApoE, C5b-9 or both proteins in low and high risk iPSC-RPE; however the size of deposits containing both ApoE and C5b 9 was larger in the high risk RPE (**Figure 4, A-D**). Significantly, larger lipid globules were also detected in high risk donors compared with low risk (**Figure 4, E & F**).

Ultrastructural changes to AMD iPSC-RPE

Transmission electron microscopy (TEM) showed that the length of microvilli was reduced in RPE derived from the high risk donors (**Figure 5, A**). The mitochondrial number also decreased (**Figure 5, C**), however the area covered by them was slightly larger in high risk

donors (**Figure 5, B**) suggestive of fewer but larger mitochondria which could be the result of age related mitochondrial dysfunction or stress (44). Long range PCR assays indicated the absence of mtDNA deletions in the fibroblasts and RPE derived from both low and high risk individuals (data not shown). We also observed the formation of asymmetric vacuoles (marked with red stars) almost exclusively in RPE generated from high-risk donors (**Figure 5, D, E & F**). These vacuoles, which are indicative of "adaptive survival" in response to environmental or oxidative stress, have also been observed in a *SOD2* knockdown mouse model of early AMD (28). They have the potential to lead to vacuolation-mediated cell death, however our flow cytometric analysis did not indicate significant changes in apoptosis between low and high risk AMD iPSC-RPE (data not shown).

Autophagy is upregulated in high risk AMD iPSC-RPE cells

Due to the increased lipid build-up and ultrastructural changes we suspected that autophagy may have a role in AMD pathogenesis. It has also been documented previously that dysregulated autophagy may sensitise RPE cells to oxidative stress (45). Autophagy is associated with intra/inter cellular waste removal and is upregulated during nutrient starvation and general stress response. In donor fibroblasts no difference in expression of two key autophagy markers, LC3 puncta and p62 aggregates was observed between low and high risk donors (**Figure 6 A, B, C, D**), however p62 intensity was higher in low risk donors (**Figure 6, E**), and localised to the nuclei. In the corresponding iPSC-RPE, LC3 puncta and p62 aggregates were greatly upregulated in high risk RPE (**Figure 6 F, G, H, I**) along with the intensity of p62, which was also increased (**Figure 6, J**), which potentially suggests a block in autophagy in high risk RPE-iPSC.

The response of low and high risk AMD-RPE cells to UV exposure

UV can induce the generation of ROS derived from diatomic oxygen (O_2), superoxide anion (O_2-), hydroxyl and peroxyl radicals, resulting in DNA damage. The retina is highly susceptible to photochemical damage due to continuous light and UV exposure. This photochemical induction is exacerbated by the retinal oxygen tension (70 mmHg), which is higher than many other tissues, thereby increasing the probability of ROS formation. Although the relationship between UV light exposure and AMD is unclear, epidemiological evidence indicates an association between the severity of light exposure and the occurrence of AMD (46). Light in the visible UV spectrum (441 nm) is deleterious for RPE cells, being the most energetic radiation reaching the macula and causes photo-oxidation generating reactive photoproducts including N-retinylidene-N-retinylethanolamine (A2E), DNA oxidation, and cell apoptosis (47, 48). Drüsen and outer segments are composed largely of lipids (polyunsaturated fatty acids) and are particularly vulnerable to photo-oxidation leading to a chain reaction mechanism of lipid peroxidation and peroxide organic free radical production (49).

To investigate whether UV exposure acts to exacerbate the gene expression, functional or structural defects observed in RPE cells derived from AMD patients, we exposed iPSC-RPE cells continuously to 0.0045 mW/cm^2 of 390-410nm light for 1 hour each day for 5 days which resulted in an increase in the concentration of intracellular reactive oxygen species and decreased mitochondrial membrane potential (data not shown). Pigmentation levels can affect the absorption of UV light, however pigmentation levels between low and high risk donors did not differ significantly (p=0.5; **Supplementary figure 3, D**). *IL6* expression, a cytokine previously associated with AMD (50), was increased in response to UV in both low and high risk iPSC-RPE cells (**Figure 7, A**). *SOD2, VEGF, IL18, CFH* and *FHL-1* expression increased only in the high risk RPE cells (**Figure 7, B, C, D, E**), suggesting inflamasome activation. The expression of *CFI* was upregulated only in the low risk iPSC-RPE cells (**Figure 7, F**). No change in *APOE, TNF-α, IL1β, CFH, C3, C5, RPE65 or OTX2* expression was observed in both high and low risk iPSC-RPE upon UV treatment (**Figure 7, G, H, I, J,**

K, L, M, N). Mitochondrial area decreased in both low and high-risk iPSC-RPE when exposed to 390-410nm UV light (**Figure 7, P**), while the overall number of mitochondria remained similar (**Figure 7, Q**). While there was no significant increase in the number of vacuoles or drüsen-like deposits in low risk iPSC-RPE cells, in the high risk iPSC-RPE both parameters decreased significantly (below levels observed in the low risk iPSC-RPE cultures; **Figure 7, R** and **7, S and Supplementary Figure 3 E**), which we hypothesise is possibly due to increased expression of *SOD2* and other protective complement proteins (FHL-1, CFH) in response to UV exposure. Photobiomodulation or optogenetics has shown that specific wavelengths of light exhibit physiological effects on biological systems. Near-infra-red for example enhances mitochondrial activity via activation of cytochrome oxidase [a photoacceptor, due to four redox active metal centres, presumed to convert bosons (photons) to fermions (electrons and positrons)], increases in electrons leads accelerated electron transport and increased generation of ATP (51). Currently a photoacceptor, such as cytochrome oxidase, for 390-410nm light has yet to be identified, traditionally studies have focused on the "optical window" biased on absorption and scattering of light in tissues, which is higher for both in the blue end of the spectrum, however the optical properties of the cornea decrease this scattering effect making blue light physiologically relevant to the retina. Additionally microvilli length in high-risk donor cells increased in response to UV (**Figure 7, O**). Together our data suggest that low and high risk iPSC-RPE cells respond differently to UV light exposure and set precedence for using iPSC-RPE disease modelling as a platform for testing existing and new therapeutic regimes.

Discussion

To date there are no effective treatments that target the underlying disease process in AMD. Availability of patient specific models which can generate large numbers of RPE cells would provide a significant advance for a better understanding of AMD physiopathology, the contribution of environmental, lifestyle and dietary factors and drug testing. The advent of iPSC technology has made the *in vitro* modelling of many inherited diseases possible; however to date this method has predominantly been viewed as useful to physiopathologies that manifest early during development or in childhood. Three recent studies have implemented premature aging approaches to model Parkinson's disease (52) and AMD (23) using iPSC, paving the way for modelling of complex age related disease.

In this manuscript, we investigated whether iPSC could be used to provide a disease modelling tool which mimics an AMD phenotype in the laboratory in the absence and presence of stress stimuli. Our rationale was to focus on patients phenotyped with a significant risk factor for AMD, such as Y402H polymorphism in the *CFH* gene. Using the iPSC derived RPE cells generated from low and high risk donors in the absence of any stress stimuli, we have been able to confirm several key cellular features of AMD as follows: (*i*) increased expression of inflammatory markers (for example *IL1β*); (*ii*) lower expression of the protective oxidative stress markers (*SOD2); (iii)* increased number of stress vacuoles (and their surface area); (*iv*) increased accumulation of lipid droplets and (*v*) increased expression of LC3 vesicles and higher p62 expression/aggregate suggestive of impaired autophagy. Most importantly, we were able to identify the formation of deposits comprising of components including Apolipoprotein E and C5b-9, in keeping with drüsen formation. These deposits occupied a significantly higher volume in the RPE derived from high-risk lines. The presence of drüsen and its larger volume in RPE derived from the high risk iPSC lines, together with confirmation of key molecular features observed in previous AMD studies,

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suggest that this iPSC model closely mimics the disease phenotype observed in AMD patients.

The complement proteins associated with AMD (FH, FI, FHR1, FHR3, C2, C3) are plasma proteins predominantly produced in the liver; however biosynthesis at extrahepatic sites is now well recognised (17, 18). As with the blood-brain barrier, the blood-retinal barrier limits access to circulating plasma proteins and it has been suggested that local complement synthesis may be required for its effects in such areas. Indeed, it has been shown that choroid and RPE as well as cultured unstimulated RPE cells produce transcripts for most classical (CP) and alternative (AP) pathway complement genes (17, 18). Data summarised in this manuscript indicate that iPSC derived RPE cells express the active complement proteins and are able to modulate their expression in response to stress stimuli without having to rely on secretion from the choroid and diffusion through the BrM as previously suggested (18).

In view of this local complement regulation by RPE cells themselves, we were interested to assess the response of low and high risk RPE responses to stress stimuli. Since a trend towards an association between severity of light exposure and AMD has been suggested by epidemiological studies, we exposed the iPSC-derived RPE to repeated doses of UV for five consecutive days. Pigmentation level between the two groups was not significantly different. The low risk RPE cells responded by increasing the expression of inflammatory marker *IL6* and complement factor I *(CFI).* More significant changes were observed in high risk RPE cells, which upregulated the expression of protective oxidative stress defence protein, *SOD2* as well as complement factor H (*CFH*) and its truncated form, *FHL-1*, in addition to showing an improved ultrastructural (increased microvilli length, reduced number of stress vacuoles and lower mitochondrial area) and functional (lower volume of drüsen like deposits) properties. These results indicate that the low and high-risk AMD-RPE cells respond very differently to UV exposure and moreover this provides evidence for UV mediated functional

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and cellular improvement of AMD-associated cellular changes in high-risk AMD-RPE cells. These intriguing results which we attribute to increased *SOD2* expression need to be validated in a larger number of cell lines derived from additional high risk donors over longer intervals and with different UV doses. They do however highlight an important role for increased oxidative stress defence as a potential therapeutic strategy for AMD, corroborating recent data obtained with the HTRA1/ARMS2-iPSC model and exposure to nicotinamide (25).

Several clinical trials attempting to inhibit the complement pathway have been completed or are under way including FCFD4514S (anti-CFD [complement factor D]), LFG 316 (anti-C5), ARC1905 (anti-C5), catalyst protease (anti-C3) and eculizimab (anti-C5) (53, 54). In particular, Lampalizumab (FCFD4514S) has been shown to reduce the geographic atrophy (GA) enlargement in phase II trials of dry AMD in patients who also have a complement factor I (*CFI*) polymorphism, indicating that inhibition of complement is a promising approach. Nonetheless, human clinical trials are complex, expensive and require prolonged periods to assess the long-term effect of a therapy in large numbers of patients with specific phenotypes to provide a consistent end point. The assessment is further complicated by differing progression rates in patients and the uncertain choice of disease endpoints to assess progression (55). This is a significant problem and can lead to trials having negative but disputed conclusions (e.g. the COMPLETE study on Eculizimab for dry AMD) (56). A robust and well characterised *in vitro* model such as the one described herein provides an efficient tool to assess potential therapeutic agents to treat AMD (such as complement pathway modulation), to better understand disease physiopathology and to test/repurpose drugs.

Materials and methods

Human donors

Written informed consent was obtained from each donor, all samples were obtained as part of a NHS research ethics committee approved biobank for fibroblasts from patients with retinal disease (ethics number 11/NE/0294) and based at the Institute of Genetic Medicine, Newcastle University and adhered to the tenets set forth in the Declaration of Helsinki.

iPSC generation

Dermal fibroblasts were isolated from skin biopsies taken from patients with wet AMD and age matched donors with no clinical or genotypic indication of ocular disease (**Supplementary figure 1, A** and **B**). iPSCs were generated by Sendai viral transduction of the transcription factors *OCT4, SOX2, KLF4, and c-MYC* (Thermo Fisher, CytoTune2-iPS Reprogramming Kit, Waltham, Massachusetts, USA) following manufacturer's instructions. iPSCs were maintained in defined conditions in mTeSR1 (Stem Cell technologies, Vancouver, Canada) on growth factor reduced Matrigel (BD Biosciences, San Jose, USA).

iPSC characterisation

The pluripotency of iPSC lines was confirmed with immuno-fluorescence, flow cytometry and RT-PCR. Cells were fixed with 4% paraformaldehyde for 15 minutes and stained with primary antibodies OCT4 and SSEA4 (Abcam, UK). Primary antibodies were detected using Alexa Fluor secondary antibodies (**Supplementary figure 4, B**). Nuclei were stained using 4',6-diamidino-2-phenylindole (DAPI). Images were captured using a fluorescence microscope (Leica Axiovert, Germany). iPSC were assessed for their propensity to generate all three germ layers using primary antibodies against AFP, TUJ1 and SMA, primary antibodies were detected using Alexa Fluor secondary antibodies (3-Germ Layer Immunocytochemistry Kit Invitrogen, USA). RT-PCR, was used to detect mRNA transcripts of key pluripotency transcription factors, *NANOG, KLF4, c-MYC & SOX2* (**Supplementary**

figure 4, C) and Sendai clearance (**Supplementary figure 5, A**). RNA was isolated from each iPSC line, RNA was isolated using a column biased method (Promega, USA) and one thousand ng of total RNA was reverse transcribed (GoScript Reverse Transcription System, Promega, USA). Standard RT-PCR was performed and PCR products electrophoresed on a 2% agarose gel.

Directly conjugated antibodies against NANOG (Cell Signalling, USA) and TRA-1-60 (Millipore, UK) (**Supplementary figure 4, B**) were used detect the percentage population of pluripotent cells using BD FACS Canto II (BD Biosciences, San Jose, USA).

Karyotyping

All cell lines were karyotyped using Illumina CytoSNP analysis and the BlueFuse Multi 4.3 software (Illumina, San Diego, United States) according to standard protocols of the manufacturer.

Generation of iPSC-RPE

iPSC were allowed to reach one hundred percent confluency, at which point the medium was switched from mTeSR1 to RPE differentiation medium (AdRPMI, Thermo Fisher Scientific, B-27® Supplement (Thermo Fisher Scientific), 10% Knock-Out Serum Replacement (Thermo Fisher Scientific), 1% 100 X GlutaMAX (Thermo Fisher Scientific), and 1% Penicillin-Streptomycin Solution (Thermo Fisher Scientific). Cells were cultured for 16 days with medium replenished daily for the first 14 days. On day 16 the differentiation medium was supplemented with 2 µM Purmorphamine (Stemcell technologies, Vancouver, Canada) until day 21. Medium was replenished twice a week for the next 3-4 months. RPE patches were mechanically picked and placed in TryPLE (10X) (Invitrogen, USA) for 30 minutes to dissociate the cells, agitated by gentle pipetting at 10, 20 and 30 minutes. Cells were sieved using a 100µm cell strainer and re-plated at $4.5x\ 10^5$ cells per cm^2 on 24 well plates or 0.33 cm^2 PET hanging cell culture inserts (Merck Millipore; Billerica, United States) coated with PLO/laminin (50ng/µl) (Sigma-Aldrich, USA).

Western Blotting

Supernatant was collected from apical portion of RPE cultures grown on transwell inserts incubated with DMEM/F12 for 4 days, soluble proteins were resolved on BioRad® 4–20% Tris-Glycine pre-cast gels non-reduced (Bio-Rad, USA) and transferred to Nitrocellulose Pre-Cut Blotting Membranes (Thermo Fisher, USA). Blocking and antibody incubations were performed using 2% milk solutions. Membranes were incubated with primary antibody at 4°C overnight with gentle agitation. Primary antibodies were detected using applicable horseradish peroxidase (HRP) - conjugated secondary antibodies (**Supplementary Figure 4, B**). Detection of HRP-labelled secondary antibody was performed with ECL SuperSignal Substrate (Pierce Biotechnology, USA). Bands were identified by autoradiography with Carestream Kodak Biomax XAR film (Sigma-Aldrich, USA) and developed.

DNA extraction and sequencing

DNA was extracted using a column biased method (Qiagen, Germany), sequence tagged PCR was performed using 100ng of DNA. Sanger sequencing was performed (GATC biotech, Germany) and results were interpreted using finch TV (Geospiza, USA).

Quantitative RT-PCR

RNA was extracted from frozen cell pellets using ReliaPrep™ RNA Cell Miniprep System as per the manufactures instructions. RNA quantification was performed with a NanoDrop™2000 spectrophotometer (Thermo, USA). We ensured that the 260/280 ratio and concentration was between 1.7–2.1 and yields of >250ng/µl respectively. cDNA synthesis was performed using Promega GoScript™ Reverse Transcription System as per the manufactures instructions. All experiments were performed using a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, UK), using SYBR green reaction technology (Promega, UK). Cycle parameters are as follows: 40 cycles of 95°C for 15 seconds and 60°C for 1 minute, finalising with a Melt Curve Stage. The Livak method ($\Delta\Delta C_t$) was used (57), C_t results of the target genes were normalised to the C_t of the reference gene *GAPDH*

(ΔC_t), the ΔC_t obtained then normalised to the ΔC_t of the calibrator, yielding the ($\Delta\Delta C_t$), finally the fold difference in expression was determined ($2^{-\Delta\Delta C_t}$). A list of the primers used can be found in (**Supplementary figure 5, B**).

RNA Sequencing

RNA was extracted using RNeasy Micro Kit (Qiagen)) according to the manufactures instructions, from six cell culture inserts, three of each genotype. cDNA was generated using SMART-Seq v4 Ultra Low Input RNA Kit (Clontech, USA).. Sequencing was carried out on a NextSeq 500 (Illumina).75 bp paired-end sequencing was carried out using a NextSeq 500 High Output v2 Kit (150 cycles) (Illumina). . RNA-seq data were processed and analysed to identify differentially expressed RNA. The quality of sequencing reads was firstly checked with FastQC (Version 0.11.2) (58). Poly-N tails were trimmed off from reads with an in house Perl script. Low quality bases (Q < 30) and standard Illumina (Illumina, Inc. California, U.S.) paired-end sequencing adaptors on 3' ends of reads were trimmed off using autoadapt (Version 0.2) and only those that were at least 20bp in length after trimming were kept. The high quality reads were then mapped to the human reference genome hg38 with STAR (Version 2.5.0c) (59). Reads mapped to genes were then counted with HTSeq-count (Version 0.6.1) (60) according to annotations from GENCODE (Version 24) (61). Differentially expressed genes were identified with Bionconductor (Version 3.2) package DESeq2 (Version 1.10.1) (62). Genes differentially expressed in the 99.73 percentile, whereby genes that lie three standard deviations from the mean (μ- 3σ) were selected ($\geq$5 fold change). This gene list was queried against the PANTHER (protein annotation through evolutionary relationship) classification system to highlight disproportionally expressed pathways. RNA-seq data is deposited into GEO (accession number GSE91087). A list of the overrepresented glycogenesis genes can be found in Table 6.

Pigment Bleaching

Post fixation and prior to immunocytochemistry, RPE cells were bleached using a Melanin Bleach Kit (Polysciences, USA) to remove pigmentation, as melanosomes can cause excessive auto-fluorescence. Pre-treatment Solution A was added for 5 minutes at room temperature, the solution was removed and cells washed two times with PBS. Pre-treatment Solution B was then added for 1-3 minutes until pigmentation was removed. This solution was removed and cells washed again with PBS.

Phagocytosis of rod outer segments

Bovine POS were obtained from InVision BioResources (Seattle, United States). Prior to performing the assay, POS were FITC labelled using the following procedure. The POS were centrifuged at 4500 × g for 4 minutes. They were then re-suspended in AdRPMI, 10% FBS with 0.4 mg/ml FITC (Sigma) and incubated for 1 hour at room temperature protected from light. This was followed by another centrifugation for 4 minutes at 4500 × g. POS were washed three times with PBS and then re-suspended in 2.5% sucrose (Sigma) in PBS and stored in -80°C until further use. For phagocytosis experiments, normal RPE cell medium was changed to the POS medium (AdRPMI, B27, 10% FBS). Once thawed, POS were re-suspended in POS medium. 1×10^6 FITC-labelled POS were added per cell culture insert for 4 hours at 37°C. In parallel negative control experiments were performed where cells were kept for the same duration but at 4°C. The incubations were followed by two cell washes with PBS. Cells were then dissociated with TrypLE™ Select Enzyme (10X) (Thermo Fisher Scientific) and washed. They were then re-suspended in 2% FBS solution in PBS with the addition of DRAQ5 (1:400, BioStatus; Shepshed, United Kingdom) for 5 minutes. Extracellular fluorescence was quenched with 0.2% Trypan Blue Stain (Thermo Fisher Scientific) for 10 minutes. Cells were then washed at least three times with PBS and re-suspended in 2% FBS solution in PBS. Cells were analysed on a BD™ LSR II flow cytometer (BD Biosciences; Franklin Lakes, United States), collecting at least 10,000 events

per sample. The data was analysed on BD FACSDiva software (BD Biosciences), $\% = \left(\frac{FITC\ positive}{Total\ number\ of\ cells}\right) \times 100$.

Immunofluorescence

Cells were washed once with PBS, followed by fixation with 4% paraformaldehyde for 15 minutes at room temperature. Cells were then washed once with PBS, followed by blocking in 0.25% Triton-X-100 (Sigma) and 5% NGS (Thermo Scientific) in PBS for 1 hour. This solution was removed and replaced with antibody diluent (0.25% Triton-X-100, 1% BSA in PBS) with applicable antibody dilution; [Anti-ZO1, rabbit (Invitrogen; dilution 1:200), Anti-C5b-9 (Dako 1:200), Anti-APOE (Merck Millipore 1:1000), Anti-LC3 (Cell Signalling 1:250), Anti-p62 (1:500)] (**Supplementary Figure 4, B**) at 4°C overnight. Cells were washed 3 times in PBS followed by incubation with secondary antibodies [Cy™3 AffiniPure Goat Anti-Rabbit IgG (Jackson ImmunoResearch (West Grove, United States); dilution 1:1000), Anti-mouse IgG–FITC antibody (Sigma; dilution 1:1000) Donkey anti-Goat 488 (1:500, Abcam) Donkey anti mouse 647 (Abcam 1:500)] for 1 hour at room temperature. Cells were washed as stated previously and mounted in Vecta sheild (Vector Labs, USA) with Hoechst 33342 (1:1000, Thermo Scientific), TO-PRO-3 (1:1000, Thermo Scientific) or DAPI (Sysmex, 1:1000). C5b-9 and APOE signals were detected using a Nikon A1R confocal (resonant, invert) (Nikon, Japan), 45-50µm optimally sampled. Image processing was performed using Huygens Essential (Germany) co-localisation with the threshold set to 1%.

In vitro **and** *in vivo* **3 germ layer differentiation**

In vitro: iPSCs were spontaneously differentiated to allow the emergence of cell types representative of the three embryonic germ layers. iPSCs were allowed to reach 80% confluency after which the media was switched to DMEM/F12 (Thermo Fisher Scientific), 20% Foetal bovine serum (FBS) (Thermo Fisher Scientific), 1% Penicillin–Streptomycin Solution (Thermo Fisher Scientific) and 1% MEM Non-essential Amino Acids Solution (Thermo Fisher Scientific). Medium was replaced daily for 3 weeks. 3-Germ Layer Immunocytochemistry Kit

(Thermo Fisher Scientific) was used to detect cells positive for markers of Mesoderm, Endoderm and Ectoderm. Briefly, media was removed from cellular monolayers, followed by a 15 minute incubation with fixative solution. Fixative was removed and Permeabilisation Solution was added for 15 minutes, this was then removed and replaced with blocking solution for 1 hour, after which the applicable antibody was added; Smooth muscle actin (SMA) (Mesoderm), alpha-fetoprotein (AFP) (endoderm) or Anti-beta III Tubulin (TUJI1) (Ectoderm). Cells were washed 3 times for 3 minutes in wash buffer. Secondary antibody was then added (**Supplementary Figure 4, B**), for 1 hour, after which the cells were washed as previously stated and 1–2 drops/mL of NucBlue was added. Plates were stored at 4°C prior to imaging on a Zeiss Axioplan microscope. All incubations occurred at room temperature unless otherwise stated.

Teratoma formation in immuno-deficient mice

All procedures were approved and conformed to institutional guidelines. $1x10^6$ iPSCs were injected subcutaneously into the right flank of adult NOD/SCID mice. All cells were co-transplanted in a 50µl Matrigel carrier, (BD Biosciences) to enhance teratomae formation. Mice were sacrificed after 70-90 days, and teratoma tissues extracted. Teratoma material for histological analysis was fixed in Bouins fixative [70% saturated picric acid (Sigma); 25% formaldehyde (37%/40%, Sigma); 5% glacial acetic acid (Sigma)] overnight. Tissues were processed then sectioned to 6µm then counterstained with either Haematoxylin and Eosin or Massons trichrome stain. Sections were assessed using bright field microscopy on an Axio Imager (Lecia, Germany).

Trans-epithelial resistance

Trans-epithelial resistance (TER) was measured with a Millicell ERS-2 Voltohmmeter (Merck Millipore). Firstly the electrical resistance of a blank cell culture insert with media in both apical and basal compartments was measured, after which inserts with cells were measured.

A minimum of two repeated measurements were made of each insert. TER was calculated using the following formula: $TER\ (\Omega cm^2) = \left(\frac{Average\ Blank}{Average\ Sample}\right) \times Area.$

Transmission electron microscopy

Cells were fixed with 2% glutaraldehyde and kept at 4°C. TEM including all the cell processing was performed at Newcastle University Electron Microscopy Research Services, Ultrathin sections were stained with heavy metal salts (uranyl acetate and lead citrate) and imaged on a Philips CM100 TEM.

Statistical analysis

Shapiro-Wilk test was used to determine normality, for normally distributed data sets, one-way ANOVA followed by Tukey's post-hoc test was used to analyse intergroup differences between samples. Two-way ANOVA was used to compare between samples and treatment groups. For non-normally distributed data Wilcoxon matched-pairs signed rank test was utilised in matched samples, while Mann Whitney test were used all other times. GraphPad Prism 7.0 (San Diego, USA) was used to perform all statistical analyses. Data are presented as mean ± S.D. and a confidence interval of 95% was set and p ≤0.05 was considered statistically significant.

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Figure Legends

Figure 1. Derivation of AMD-iPSC RPE. (A) Light microscopy images of iPSC-RPE cells derived from low risk donors [F018 and F116:402Y] donor, and high risk AMD patients [F180 and F181:402H]. Scale bar = 50 μm. (B) Flatbed scanned images of a representative well from 6 well plates, pigmented patches are clearly visible. Scale bar = 17.4 mm. (C) CRALBP/ ZO-1 and BEST1 immunostaining of representative iPSC-derived RPE cells, Scale = 25 μm. (D) Immunofluorescence representative images of iPSC-RPE and orthogonal images along X and Y axis (Green = Na$^+$ K$^+$-ATPase) (Blue = DAPI) Scale bar = 50 μm, A=apical, B=basal. (E) Pigment Epithelium-Derived Factor (PEDF) ELISA of apical and basal supernatant, concentration ng/ml. (F) Trans-epithelial Resistance (TER) data shown as Ωcm^2 (resistance).

Figure 2. Expression and secretion of complement proteins by iPSC-RPE by western blotting. (A) Western blot of protein excreted into the medium by RPE cells, Anti-FH ab shows a band visible at 150 kDa and FHL-1 visible at 37 kDa. (B) Anti-FI ab indicates a band at 65 kDa. (C) C3 is visible at 187 kDa, while C3b is also visible in the positive control at 90 kDa. (D) Western blot of a fluid phase tripartite co-factor assay probed with anti-C3 Ab. Supernatant from AMD patient iPSC-RPE cells cultured for 48 hrs in serum free media was incubated with purified C3b to test the activity of FI and FH previously demonstrated to be

secreted by these cells (A,B) (lane 6). Activity was confirmed by the generation of a 67-kDa α'1 chain and a 40-kDa α '2 chain. It is also observed that these cells had secreted C3 into the supernatant (C) (lane 5). The serum free media alone had no effect on C3 (lane 4). C3b/FH combined had minimal cleavage (lane 1) and C3b alone was not cleaved (lane 2). C3b/FH/FI combined were able to cleave C3b to the 67-kDa α'1 chain and a 40-kDa α '2 chain in the positive control (lane3).

Figure 3. Gene expression analysis in low and high risk AMD iPSC-RPE. Data is presented as mean +/- SEM, n=3.

Figure 4. Drüsen like deposits form in high risk iPSC-RPE. (A) Volume of APOE and C5b-9 co-expressing deposits in low and high risk iPSC-RPE, dashed black line represents clinically significant drüsen size. High risk donor iPSC-RPE cells accumulate larger deposits than low risk, n=3. (B) Low risk iPSC-RPE example of F116, Blue = DAPI, Red = C5b-9 and Green = APOE. Scale bar = 50 µm. (C) High risk iPSC-RPE example of F181, Blue = DAPI, Red = C5b-9 and Green = APOE. Scale bar = 50 µm. (D) Secondary antibody only control, Blue = DAPI, Red = C5b-9 and Green = APOE. Scale bar = 50 µm. (E) Oil red O staining, high risk donor iPSC-RPE contained larger lipid globules than low risk donors. (F) Examples of low and high risk donor Oil red O staining. Scale bar = 200 µm.

Figure 5. High risk iPSC-RPE show ultrastructural changes in TEM analysis. (A) Microvilli length (calculated per field of view: 100 µm^2) is decreased in high risk donor iPSC-RPE, n=3. (B) Mitochondrial area (calculated per field of view: 100 µm^2) was increased in high risk donor iPSC-RPE, p=0.0417, n=3. (C) Mitochondrial number (calculated per field of view: 100 µm^2) was decreased in high risk donor iPSC-RPE, p=0.0008, n=3. (D) The number of vacuole structures (calculated per field of view: 100 µm^2) was greatly increased in high risk donor iPSC-RPE, p=0.0055, n=3. I Example of low risk iPSC-RPE cells, left hand side = F018, right hand side = F116, Scale bar = 2 µm. (F) Example of high risk iPSC-RPE cells,

left hand side = F180, right hand side = F181, Red Asterisk indicate vacuoles. Scale bar = 2 µm.

Figure 6. Autophagy is blocked in high risk iPSC-RPE cells but not in high risk dermal fibroblasts. (A) Example fluorescence staining of low risk fibroblasts Blue = DAPI, Green = LC3 and Red = p62. Scale bar = 10 µm. (B) Example fluorescence staining of high risk fibroblasts Blue = DAPI, Green = LC3 and Red = p62. Scale bar = 10 µm. (C) The number of LC3 vesicles per cell did not differ between genotypes in iPSC-RPE cells. (D) Number of p62 positive aggregates per cell was not affected by genotype (E). The intensity of p62 decreases in high risk AMD iPSC-RPE cells. (F) Example fluorescence staining of low risk iPSC-RPE Blue = TO-PRO-3, Green = LC3 and Red = p62. Scale bar = 10 µm. (G) Example fluorescence staining of high risk iPSC-RPE Blue = TO-PRO-3, Green = LC3 and Red = p62. Scale bar = 10 µm. (H) LC3 aggregates were significantly increased in high risk iPSC-RPE (I) p62 aggregates per cell were significantly increased in high risk (J) p62 intensity also increased.

Figure 7. The response of low and high risk RPE to intermittent UV exposure. (A-N) Quantitative RT-PCR expression data shown as fold change in relation to low risk control, data are presented as mean +/ SEM, n=3, * $p< 0.5$. (O) Average length of microvilli per field of view (100 µm^2), UV exposure increased microvilli length in high risk iPSC-RPE cells, $p<0.0001$. (P) Average mitochondrial area per field of view (100 µm^2), UV exposure decreased mitochondrial area in both low and high risk iPSC-RPE ($p=0.0003$ & $p=0.0002$). (Q) Average mitochondrial number per field of view (100 µm^2), UV exposure did not influence the average number of mitochondria ($p=0.9789$). (R) Average vacuole number (calculated per field of view: 100 µm^2), UV exposure decreased the number of vacuoles in high risk cells, $p=0.0038$. (S) "Drüsen" area, UV exposure decreased the deposition of C5b-9 and APOE in high risk iPSC-RPE cells, * $p=0.0002$.

Supplementary Figure 1

(A) Table showing donor ID age and gender and Sanger sequencing reads of rs1061170, rs11200638, rs10490924. (B) Spectral domain optical coherence tomography (OCT) and infrared fundal images of homozygous Y402H AMD Donors. Both patients had unilateral wet AMD in the left eye (C and C) and reticular drüsen in their fellow eyes (B' and C'). OCTs show retinal pigment epithelial detachment and choroidal neovascularisation (*), subretinal fluid (+) and intraretinal fluid (arrow) in the eyes affected by wet AMD. The reticular drüsen are seen as a stippled appearance on the infrared images in the macular areas of the fellow eyes.

Supplementary figure 2

(A) Immunofluorescence of high and low risk iPSC, Green = OCT4, Red = SSEA4 and Blue = DAPI. Scale bar = 200 µm. (B) RT-PCR of all iPSC lines compared to H9 cells. *NANOG, KLF4, c-MYC* and *SOX2* were tested. *GAPDH* and *RLP13A* were controls. (C) Example of flow cytometry of iPSC line F180, showing TRA-1-60 and NANOG, populations consisted of over 85% dual positive cells in all lines tested. (D) Immunocytochemistry showing the presence of three germ layers from in vitro differentiation of iPSC. A representative example from a high risk AMD-iPSC is shown. Scale bar = 200 µm. (E) Teratoma staining, cartilage was stained with Weigerts iron Haematoxylin, Nuclei- brown-black, Cartilage- pale blue, and Cytoplasm- pink. Mayers' Haematoxylin (Haemalum) and Eosin was used for all other staining. (F) Sendai clearance, polycystronic primers spanning Sendai genome and transcription factor were used to detect absence of residue viral induction elements. (G) CytoSNP, 70,000 SNPs from across the genome were compared in iPSC against parent fibroblasts to determine any chromosomal alterations.

Supplementary figure 3

(A) RT-PCR of *CFH* and *FHL-1* in iPSCs. (B) Enrichr results queried against OMIM disease database. (C) RNA sequencing result of downregulated and upregulated genes residing in the 0.3% percentile and 99.7% percentile were selected, equating to -5.532 fold change or greater and 5.66 or greater respectfully. Gene list shown ranked in order of highest to lowest expressed. (D) Example images and histogram of integrated density (ImageJ) of iPSC-RPE cells in transwell inserts exposed to UV, *p=0.5*. (E) Example images of low and high risk iPSC-RPE cells after UV exposure, scale bar = 2 µm.

Supplementary figure 4

(A) Sequencing primers for SNP's rs1061170, rs11200638 & rs10490924. Lower case nucleotides indicate sequencing tag. (B) Primary and secondary antibody list. (C) Pluripotency PCR primers.

Supplementary figure 5

(A) Sendai clearance test primers (B) qPCR primer list.