Molecular Vision 2026; 32:196-208 <http://www.molvis.org/molvis/v32/196>
Received 12 February 2025 | Accepted 10 June 2026 | Published 12 June 2026

Assessing mitochondrial phenotypes in a cybrid model of dry age-related macular degeneration

Jacob Dohl,[1] Nyan Lin Zaw,[2] Jett Nguyen,[2] Gordon Burns,[2] Brandon Park,[2] Shari Atilano,[2] Kevin Schneider,[2] Marilyn Chwa,[2] Maria Cristina Kenney[1,2]

[1]Department of Pathology and Laboratory Medicine, University of California Irvine, Irvine, CA; [2]Department of Ophthalmology, Gavin Herbert Eye Institute, University of California Irvine, Irvine, CA

Purpose: Dry age-related macular degeneration (DAMD) is the leading cause of vision loss in developed countries, yet there are no FDA-approved treatments currently available. Mitochondria play a significant role in the pathology of DAMD; the retinal pigment epithelium cells of patients with DAMD exhibit mitochondrial dysfunction, elevated levels of mitochondrial DNA lesions, and increased mitochondrial reactive oxygen species. Investigations into the mitochondrial contributions to DAMD are complex as human tissue is challenging to acquire, and animal models do not fully recapitulate disease phenotypes. Cytoplasmic hybrid (cybrid) cells, formed by depleting the mitochondria of an immortalized cell line and fusing with patient platelets, are a possible model for mitochondrial studies on DAMD. This study evaluates if cybrid models of DAMD recapitulate the mitochondrial hallmarks of the disease, including mitochondrial dysfunction, decreased mitochondrial protein levels, lipid accumulation, and mitochondrial DNA lesions.
Methods: The mitochondrial functions of five healthy and five DAMD cybrid cell lines were compared based on mitochondrial oxygen consumption rates, membrane potential, and protein expression. Secondary factors of mitochondrial dysfunction, including lipid accumulation and mitochondrial DNA stress response, were also examined.
Results: Compared to healthy control cybrid lines, we found no alterations in bioenergetics, protein levels, and lipid accumulation in DAMD cybrid lines. Mitochondrial DNA stress responses were aberrant in DAMD cybrids compared to healthy controls, suggesting some conserved mitochondrial dysfunction.
Conclusions: Taken together, this study suggests that these DAMD cybrids do not fully recapitulate DAMD mitochondrial pathology, though this is limited to the study population of males with the H mtDNA haplogroup. However, there may be a niche for cybrid cell lines in investigating mitochondrial DNA phenotypes in patients with DAMD. This is likely because DAMD is a multifactorial disease, dependent upon an individual's genetics and the retinal microenvironment.

Age-related macular degeneration (AMD) is a leading cause of vision loss globally, affecting ~150 million people in 2020, with that number expected to increase by nearly 50% by 204 [1,2]. AMD is divided into two classifications: neovascular AMD (nAMD) and Dry AMD (DAMD). nAMD is an uncommon but severe form of AMD, resulting from neovascularization from the choroid into the retina near the macula; this releases plasma and whole blood into the eye, causing central vision loss and blindness if untreated [3,4]. Following years of study, nAMD is well characterized, and robust therapies for intervention center around the suppression of VEGF and neovascularization signaling proteins [5,6]. DAMD pathology is characterized by localized atrophy of the macular Retinal Pigment Epithelium (RPE), a key player in retinal metabolism and photoreceptor support; the loss of these RPE cells inevitably leads to photoreceptor cell death and visual abnormalities, including central vision loss [7-10].

Correspondence to: Jacob Dohl, Department of Pathology and Laboratory Medicine, University of California, Irvine, CA 92697; email: jdohl@uci.edu

Clinically, DAMD is characterized by the presence of drusen, yellowish lipid and protein deposits that form under the RPE [11-13]. Drusen are hypothesized to be expelled metabolic byproducts and cellular waste, and their accumulation is linked to RPE metabolic dysregulation [14,15]. Indeed, DAMD is associated with several metabolic and mitochondrial hallmarks, including elevated reactive oxygen species (ROS), decreased mitochondrial amount, disruption of mitochondrial cristae, and altered metabolism, with a shift toward glycolysis [16-18]. As RPE cells depend heavily on mitochondria for energy production in the retinal microenvironment, these disruptions in normal RPE function may serve as targets to prevent disease progression [19-21]. Despite our current understanding of DAMD, no FDA-approved therapies for the disease are available [22,23]. Therefore, additional work is urgent to determine the underlying causes behind DAMD and generate targets for treatment.

As metabolic and mitochondrial hallmarks are evident in DAMD, significant work has been performed to determine if mitochondrial therapies could improve DAMD outcomes. Despite this, investigating the mitochondrial hallmarks of DAMD involves several challenges. Studies using human

eyes, both in vitro and in vivo, are subject to individual variability in environmental exposures and genetics; additionally, eye donation for research has decreased in recent years, limiting supply [24]. Non-human primate models of DAMD rarely develop DAMD spontaneously, requiring intervention to induce disease phenotypes [25-27]. The aging DAMD primate models also require 20–40 years to develop fully, limiting high throughput research. Rodents lack a macula and, therefore, cannot develop DAMD, requiring significant intervention to mimic the disease [28,29]. Therefore, our group developed a cytoplasmic hybrid (cybrid) cell line using mitochondria derived from patients with DAMD and healthy controls as a novel form of studying DAMD.

A cybrid cell line is a fusion between a mitochondrially depleted (ρ^0) cell and mitochondria from a donor cell [30,31]. Our laboratory generates ρ^0 ARPE-19 cells through ethidium bromide treatment; ethidium bromide intercalates into genomic and mitochondrial DNA (mtDNA), decreasing the replication rate [32,33]. Genomic DNA can overcome this limitation, typically through base excision repair; however, mtDNA lacks similar mechanisms, resulting in mtDNA and, eventually, total mitochondrial depletion [34-37]. The resultant ρ^0 ARPE-19 cells are then fused with mitochondria from a donor cell, typically platelets. These platelets, lacking a nucleus but abundant in mitochondria, are placed in culture with the ρ^0 cells and polyethylene glycol, forcing cellular fusion [38-40]. The resulting cell population is pruned, creating an immortalized line with mitochondria unique to the donor. Our laboratory utilizes platelets from patients with and without DAMD, creating cybrid lines that emulate patient mitochondrial processes without requiring tissue harvest or repeated blood draws.

Cybrid cell lines offer a unique way to test hypotheses related to AMD's mitochondrial aspects. Our laboratory has successfully studied nAMD using cybrid models, including epigenetic alterations, VEGF expression, and the effect of mitochondria-derived peptides on nAMD cybrid viability [41-44]. This project attempts to validate that cybrid cell lines can recapitulate the mitochondrial hallmarks of DAMD to determine if cybrids are a suitable model for the mitochondrial phenotypes of the disease. We interrogated key findings in the previous literature surrounding DAMD, including mitochondrial respiration, mitochondrial membrane potential ($\Delta\Psi m$), lipid raft accumulation, mitochondrial protein contents, and mtDNA copy number.

METHODS

Human subjects: The University of California, Irvine's Institutional Review Board approved research with human subjects (Approval #2003–3131). All participants provided informed consent, and clinical investigations were performed according to the tenets of the Declaration of Helsinki.

Cell culture: Cybrids were generated as previously described [45]. In brief, cybrid cells were prepared through polyethylene glycol fusion of ρ^0 ARPE-19 cells with platelets derived from healthy or DAMD patients. Cybrids from passages 5 to 10 were used during this study. All cybrids used for this study belonged to the 'H' mtDNA haplogroup and were males from 67 to 89 (Average age of DAMD patients: 82±4.6, Healthy Patients: 78.2±7.02, p value 0.39; Shown in Table 1). Our ARPE-19 cells have been validated using RPE-specific markers such as Bestrophin 1, Cellular retinaldehyde binding protein-1, and Keratin-18 [46]. Cybrid status and donor mtDNA involvement were confirmed using allelic discrimination, Sanger Sequencing, and Next-Generation Sequencing.

Culture conditions: Cybrid cells were cultured in Dulbecco's Modified Eagle Medium (DMEM)-F12 Medium (Cat. # 10–092CM, ThermoFisher Scientific, Pittsburgh, PA). Our media is supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 µg/ml streptomycin, and 50 ug/ml gentamycin. All cells are cultured at 37 °C with 5% CO_2 in a humidified incubator.

Bioenergetics assay: Bioenergetics were analyzed using a Seahorse XF96 Analyzer (Agilent Technologies, Santa Clara, CA) to determine oxygen consumption rate (OCR). A cellular response curve of 30 K, 40 K, 60 K, 80 K, 100 K, and 120 K cells per well was performed to determine optimal seeding density, which was ultimately determined to be 120 K cells per well. Additionally, drug optimization tests were performed, and 1 µmol/l for oligomycin, 1 µmol/l for carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), and 0.5 µmol/l of rotenone+0.5 µmol/l of antimycin A (R+A) were determined to be the optimal concentration for a cellular response. Healthy and DAMD cybrids were plated and allowed to rest for 24 h before beginning the assay. Seahorse analyses were performed in Seahorse XF Base Media supplemented with glucose (25 mM/l), sodium pyruvate (1 mM/l), and glutamine (2 mM/l). Measurements were performed three times at five-minute baseline increments, followed by a fresh media addition and six additional measurements. This was followed by sequential oligomycin, FCCP, and R+A treatment with three measurements each for a total of eighteen measurements. Raw OCR data was normalized to cell count. In brief, following the assay, cells were fixed with 4% paraformaldehyde for 20 min at room temperature and then washed with

Table 1. Patient information.

Cybrid cell line	Haplogroup	Age	Sex	Group
13–128	H7e	86	M	DAMD
14–132	H3an	78	M	Healthy
14–143	H1c1	84	M	DAMD
15–161	H5a1	67	M	Healthy
15–171	HV5a	87	M	DAMD
17- 193	H	89	M	Healthy
17–201	H	77	M	Healthy
17–203	H	76	M	DAMD
18–221	H	77	M	DAMD
18–241	H	80	M	Healthy

Cybrid cell lines were generated using patient platelets. Demographics of these patients, including age, sex, and mitochondrial haplogroup, are listed above.

DMEM before being incubated with BioTracker 488 Nuclear Green Dye (Cat. # SCT120, Millipore Sigma, Temecula, CA) for 30 min. Cells were washed with live cell imaging solution (Cat. #A59688DJ, ThermoFisher Scientific) and imaged using a Sartorius Incucyte® S3 Live-Cell Analysis System (Sartorius, Göttingen, Germany) at 4x resolution. Quantification of Green Count was determined using the IncuCyte Software 2019B with a −15 edge and Max Area of 1500.

Mitochondrial membrane potential assay: ΔΨm was determined using a Biotium JC-1 Assay (Cat. #30001, Biotium, Fremont, CA) performed according to the recommended protocol. In brief, cybrids were plated in a 96-well plate at 40,000 cells/well and incubated overnight before testing. Media was removed, and cells were incubated with 100 ulnof the JC-1 working reagent for 15 min at 37 °C. The reagent was removed, and cells were washed with Live Cell Imaging Solution (Cat. #A59688DJ, ThermoFisher Scientific). Fluorescent intensity was determined using a SpectraMax Gemini XPS (Molecular Devices, San Jose, CA). Red fluorescence was measured at an excitation of 550 nm and emission of 600 nm, while green fluorescence was measured at an excitation of 485 nm and emission of 535 nm. ΔΨm was quantified through the ratio of red fluorescence/green fluorescence. Ten uM FCCP, a mitochondrial uncoupler, was used as a negative control for ΔΨm.

Western blotting: Cybrid cells were incubated with ice-cold RIPA lysis buffer (Cat. # 89,900, Life Technologies, Carlsbad, CA) containing protease inhibitors. Protein concentrations were determined using a Pierce BCA Protein Assay Kit (Cat. # 23225, ThermoFisher Scientific). Thirty ug of protein was loaded into 4%–20% polyacrylamide gels (Cat. # 4,561,096, Bio-Rad Laboratories) and separated using SDS–PAGE electrophoresis. Western blotting was performed using Total OXPHOS Human WB Antibody Cocktail at a 1:300 dilution (Cat. # ab110411, Abcam, Cambridge, UK), TOM20 at a 1:1000 dilution (Cat. # D8T4N, Cell Signaling, Danvers, MA), and Actin at a 1:5,000 dilution (Cat. # 13E5, Cell Signaling) as primary antibodies. For the Total OXPHOS Human WB Antibody Cocktail, IRDye 800CW Goat anti-Mouse IgG (Cat. #925–32210, Li-Cor Bio, Lincoln, NE) was used as a secondary antibody at a concentration of 1:10,000. REVERT 700 Total Protein Stain (Cat. # 926–11021, Li-CorBio) was the total protein stain loading control. Blots were imaged on a LICOR Odyssey CLx (LICOR Bio) using Image Studio software version 5.2 (LICOR Bio). Blots were analyzed using Emperia Studio software version 2.1 (LICOR Bio). For TOM20 and Actin, secondary anti-rabbit and anti-mouse horseradish peroxidase-conjugated antibodies were used at a concentration of 1:10,000. Protein expression was visualized using SuperSignal West Femto Maximum Sensitivity Substrate (Cat. # 34,095, ThermoFisher Scientific) with a ChemiDoc MP Imaging System (Bio-Rad Laboratories, Hercules, CA). Western blot densitometries were performed with Image Lab software version 6.1 (Bio-Rad Laboratories). Actin was used as a loading control.

BODIPY staining: Lipid aggregates were determined using 4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene (BODIPY), a neutral compound with high lipophilicity [47]. BODIPY staining was performed based on the protocol from Qui et al. [48] In brief, cells were plated at 10,000 cells per coverslip in a 6-well plate and allowed to incubate overnight before treatment. Oleic acid stimulates cellular triglyceride synthesis, increasing lipid raft accumulation within the cell membrane. Therefore, as a positive control, cells were pretreated

overnight with 60 uM oleic acid conjugated to BSA, with unconjugated BSA acting as a vehicle control. The next day, cells were washed and incubated with 6 uM BODIPY for 15 min. Cells were washed and fixed in 4% paraformaldehyde for 20 min at room temperature. Coverslips were mounted on microscope slides using Fluoroshield Mounting Medium with DAPI (Cat. # ab104139, Abcam). Slides were allowed to harden in the dark for three days before imaging.

Fluorescent imaging and analysis: Imaging was performed on a Leica (Leica Microsystems, Wetzlar, Germany) Stellaris 8 confocal microscope. BODIPY staining was visualized using the BODIPY FL setting (Excitation- 488 nm; Emission- 550 nm), while DAPI was visualized using the DAPI (dsDNA) setting (Excitation- 405 nm; Emission- 450 nm). Lipid raft punctae were identified and defined using the Intermodes thresholding algorithm in FIJI/ImageJ and counted using the Analyze Particles function. Particle size was thresholded from 10- ∞ with the Pixel Units option on. Nuclei were manually counted, and the punctae per cell were calculated for each analyzed image.

Mitochondrial DNA copy number qPCR analysis: Cybrid lines were treated with 1 uM FCCP for four hours per day for 0, 1, 2, or 3 days before collection to stimulate mitochondrial stress and metabolic insult. Healthy and DAMD cybrids had total DNA extracted using a PureLink Genomic DNA Kit (Cat. # K182002, ThermoFisher Scientific) and quantified using a NanoDrop 1000 Spectrophotometer. DNA amounts were normalized, and quantitative PCR was performed using TaqMan gene expression assays for 18S and MT-ND2 (Cat. # 4,331,182, ThermoFisher Scientific) genes and TaqMan Gene Expression Master Mix (Cat. # 4,369,016, ThermoFisher Scientific). Relative mtDNA copy number was determined using ΔCt. Triplicates were used for each sample.

Statistical analysis: All experiments were independently replicated in triplicate. Values are presented as mean ±SD. Mean group differences were determined using the Student *t* test, with values of $p \leq 0.05$ being considered statistically significant.

RESULTS

Bioenergetics are unchanged between age-related macular degeneration and healthy cybrids: RPE cells from patients with DAMD are notably deficient in energy production, and previous studies show a metabolic shift from oxidative phosphorylation to glycolysis [17,18]. We performed a Seahorse bioenergetic analysis to investigate this trend in DAMD cybrids. DAMD cybrids exhibited no change in cellular metabolism compared to healthy cybrids (Figure 1A). This trend occurred across basal respiration, maximal respiration, and respiratory control ratio (RCR; Figure 1B,C,E). Interestingly, when taken as a percentage of basal respiration, all lines showed a 200% increase in maximal respiration, suggesting a conserved mitochondrial respiratory capacity (Figure 1D). Proton leak, an indicator of mitochondrial membrane permeability and dysfunction, was similarly unchanged (Figure 1F). Bioenergetic studies were confirmed by examining ΔΨm, as alterations in ΔΨm can correlate with energy production. Similarly, JC-1 assays showed no difference in ΔΨm between healthy and DAMD cybrids (Figure 1G).

Lipid accumulation is unchanged in age-related macular degeneration cybrids: Lipid accumulation is well characterized in DAMD, with drusen partially made up of lipid deposits [49]. Additionally, transmission electron microscopy of ex vivo DAMD retinas shows lipid droplets and accumulation in the RPE, suggesting that drusen may begin as accumulations within the cell [16,18]. To determine if DAMD cybrids exhibit lipid accumulation within their membranes, cybrids were stained with BODIPY, and lipid rafts were visualized using fluorescent microscopy and then counted with ImageJ. Oleic acid, which stimulates cellular triglyceride synthesis, was used as a positive control (Figure 2B). There were no differences in lipid raft accumulation in DAMD cybrids compared to healthy cybrid lines (Figure 2C).

Mitochondrial mass and electron transport chain contents are unchanged in dry age-related macular degeneration cybrids: Despite consistent bioenergetics, we next assessed overall mitochondrial mass and mitochondrial ETC protein expression to detect any underlying abnormalities that may suggest mitochondrial dysfunction. Using TOM20, a ubiquitously expressed mitochondrial transporter subunit, overall mitochondrial mass was not different between DAMD and healthy cybrids (Figure 3G). ETC complexes II-V were analyzed and similarly showed no differences in expression level, suggesting no underlying mitochondrial stress (Figure 3B-E).

Mitochondrial DNA stress response is aberrant in some dry age-related macular degeneration cybrids but unchanged overall: mtDNA replication is significantly elevated during the mitochondrial stress response to ensure an adequate supply of mitochondrial proteins to sustain energy production within the cell [50,51]. To determine if this protective pathway is functional in DAMD, cybrid lines were treated with low-dose FCCP for 4 h per day for up to three days. Healthy cybrid mtDNA levels correlate positively with days treated with FCCP (Figure 4A). DAMD cybrids showed a less consistent response, and their mtDNA levels are not correlated with FCCP treatment (Figure 4B). When compared, there is no difference in mtDNA levels between healthy and

DAMD cybrids, though they gradually differ as days progress (Figure 4C).

DISCUSSION

The goal of this study was to develop an in vitro model of DAMD for mitochondrial research. This model would couple the ease of use of ARPE-19 cells with the applicability of human-derived mitochondria, providing more insight into human responses to DAMD than typical in vitro studies. As previously stated, mitochondrial and bioenergetic dysfunction play a significant role in DAMD pathology. Therefore, a cybrid model that can recapitulate the mitochondrial phenotypes of DAMD RPE would be a valuable approach for future research into DAMD pathology. This study evaluated the viability of cybrid cell lines as a vector to study DAMD. Currently, there are limited mechanisms by which researchers can explore DAMD, especially in a human context.

Human in vivo studies of DAMD are limited to imaging of the retina, through processes including optical coherence tomography and fundus imaging, and therefore lack the ability to interrogate intracellular events [22]. Post-mortem studies of ex vivo retinas are a powerful tool for investigating DAMD. However, these are limited by supply and do little to aid in diagnosis and disease prevention. Retinal organoids are a recently developed tool for investigating DAMD in an in vitro setting. However, these require long culture periods to develop and do not naturally develop DAMD pathology, limiting their applicability [52-54]. Consequently, many researchers have turned to animal models of DAMD to investigate the disease's pathology further.

Animal models of DAMD are typically divided into non-human primate and rodent models. Non-human primates are the animal model most closely related to humans; however, using them for DAMD research can be challenging. Rhesus macaques develop human-like drusen, even having structural similarities [25]. However, these monkeys rarely spontaneously develop late-stage DAMD or wet AMD, calling into question the model's accuracy [26,27]. Additionally, working with primates is a slow, laborious, and expensive prospect, with an aging model taking 20–40 years before results can show promise. Rodent models have long been a staple for in vivo research; however, rodents lack a macula, making investigations into a localized disease challenging [28]. Despite this, there are still several rodent models which attempt to model DAMD despite lacking a macula; $Cfh^{-/-}$ mice and CFH Y402H mice utilize transgenic alterations of the CFH pathway to model the complement alterations seen in humans; $Ccl2^{-/-}$ and $Ccr2^{-/-}$ mice are used to investigate the role of chemokines and macrophages in DAMD; and $SOD1^{-/-}$ and Cigarette smoke/hydroquinone$^{+/-}$ high-fat diet$^{+/-}$ blue light mice are important in determining the role that oxidative stress plays in DAMD development [29].

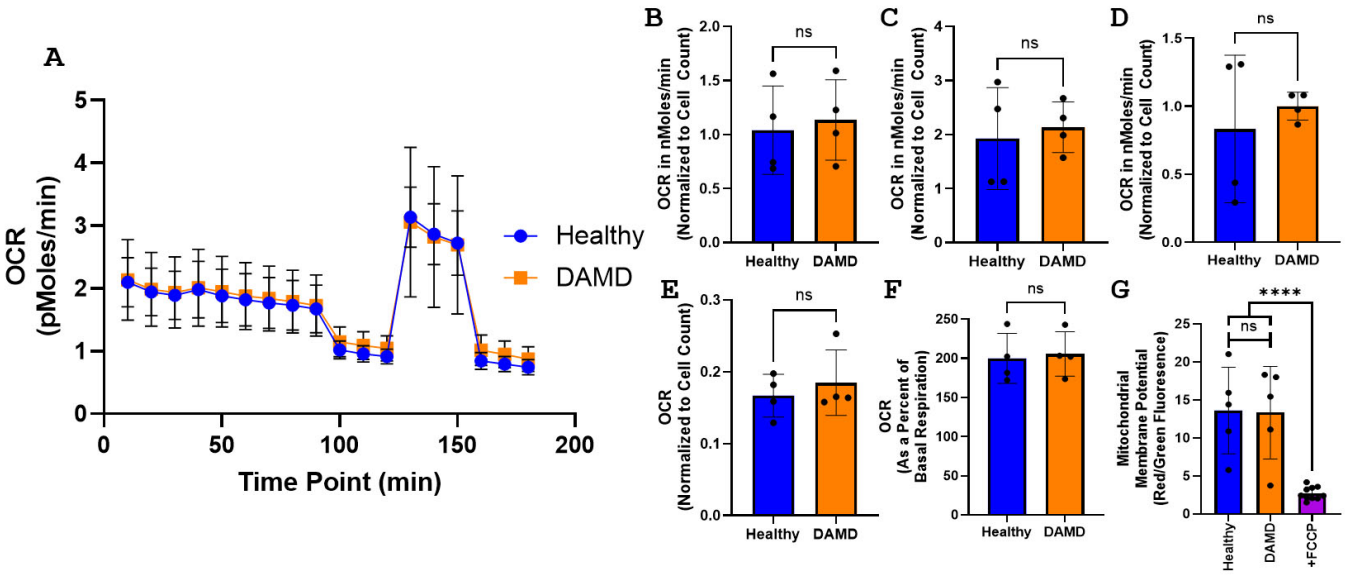

Figure 1. Healthy and DAMD cybrids show no differences in mitochondrial bioenergetics. Bioenergetic analysis of healthy and DAMD cybrids (**A**) showed no difference in basal (**B**) or maximal OCR (**C**), RCR (**D**), or proton leak (**E**). Interestingly, maximal respiration was always nearly 200% of basal OCR, suggesting conserved mitochondrial efficiency (**F**). ΔΨm was assessed with a JC1 assay and showed no difference between healthy and DAMD cybrids (G). Ten uM FCCP was used as a negative control. Healthy Cybrids: n=4, DAMD Cybrids: n=4.

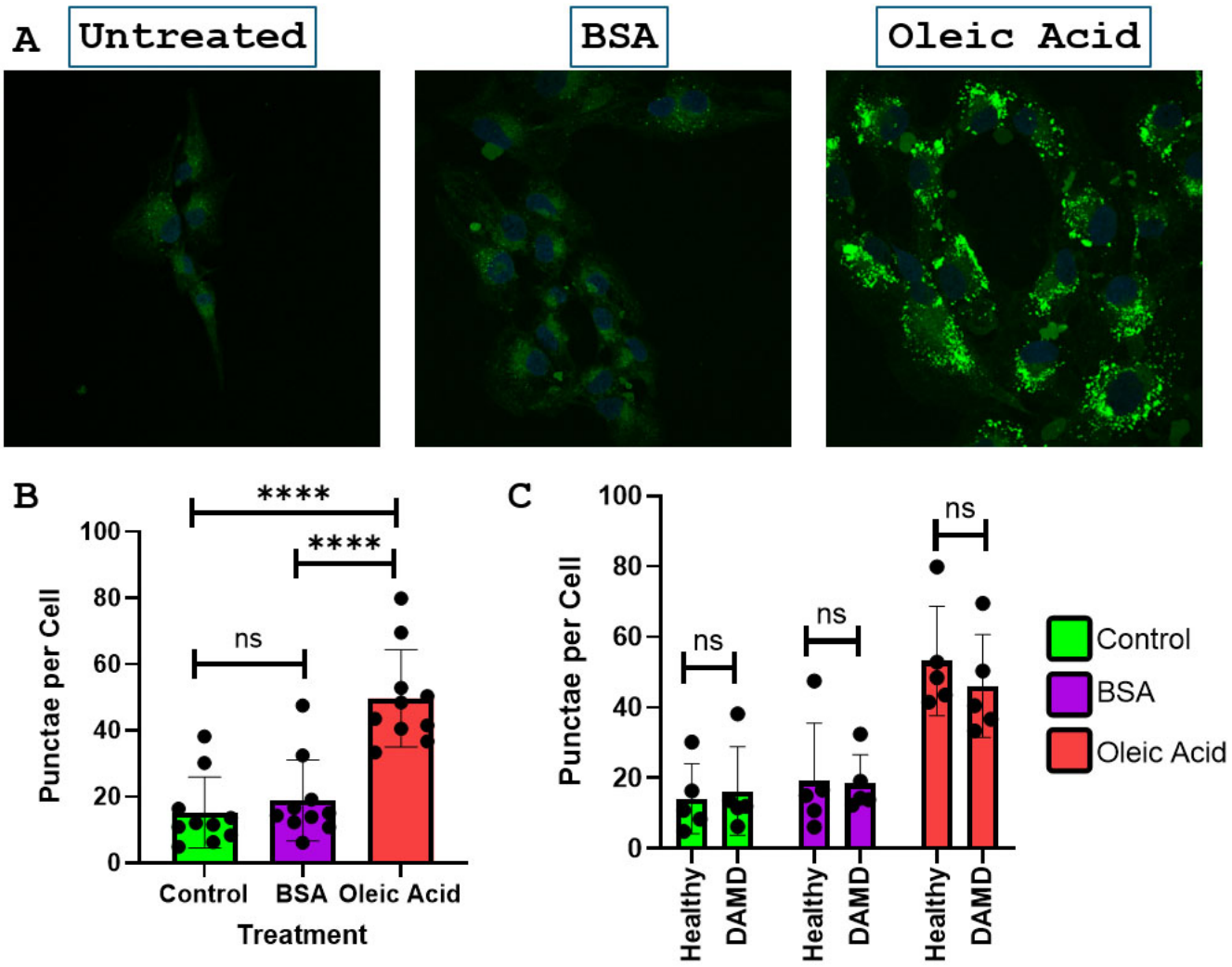

Figure 2. Lipid accumulation was similar between healthy and DAMD cybrids. Healthy and DAMD cybrids were treated with BODIPY, a fluorescent molecule that binds to pockets of lipid accumulation, to determine if lipid accumulation was altered in DAMD cybrids (**A**). Oleic acid bound to BSA (60 uM) was used as a positive control as it stimulates cellular triglyceride synthesis, while unbound BSA was used as vehicle control (**B**). No differences between healthy and DAMD cybrids were seen in trial conditions (**C**). Healthy Cybrids: n=5, DAMD Cybrids: n=5. .

Finally, in vitro models also play a role in DAMD research. Cultured primary RPE cells from human donors are an excellent way to stretch human donations beyond single-globe dissections. Fetal RPE cells and iPSC cells can both be induced to grow into an RPE cell model of AMD, which spontaneously produces drusen; however, this process requires significant time and testing to ensure that the cells growing have not become aberrant during the culturing and differentiation processes [55]. Porcine RPE cells may also provide a model of human AMD, producing drusen similar to those in human RPE, but they lack the human genome that the other options possess [56]. Immortalized human RPE cells (ARPE-19) may also provide a basic model of human RPE activity. Issues with in vitro models include the lack of a complex environment that indeed recapitulates the retinal microenvironment, the accumulation of mutations as passage number increases, and a minimal accumulation of drusen [28].

Due to the limitations of currently available models for DAMD research, there is a niche that DAMD cybrids can fill. Cybrids are a valuable model for exploring mitochondrial disorders as the conserved nuclear genotype between cell lines allows for controlled studies into how mitochondria drive phenotypic cellular changes. Establishing cybrids has improved over time, with the introduction of new techniques,

including the inhibition of mitochondrial DNA polymerase γ and the supplementation of dideoxynucleotides, making cybrid generation more accessible [57]. Once established, cybrids can be maintained for several passages with conventional cell culture practices and require minimal investment from the researcher. Therefore, this study aimed to evaluate the ability of cybrid cells to recapitulate a DAMD mitochondrial phenotype, as there is significant mitochondrial pathology in DAMD, despite it not being an explicit mitochondrial disease.

The established DAMD mitochondrial pathology involves progressive mitochondrial dysfunction in the RPE, which exacerbates cellular stressors and results in cell death [20,21]. Indeed, multiple studies have demonstrated that cultured RPE cells from AMD donors exhibit decreased mitochondrial function compared to those from healthy controls [18,58]. AMD RPE demonstrates decreased mitochondrial mass and ETC content, consistent with decreased mitochondrial output [59-61]. As mitochondrial production falters, lipid and lipofuscin deposits, hallmarks of DAMD progression, begin to accumulate, likely contributing to drusen formation. Supporting this idea, electron microscopy reveals intracellular lipid deposits in the RPE of people with DAMD [16,18,59]. mtDNA lesions are also notable hallmarks of DAMD progression, with damage accumulating with age and disease pathology [62-64].

To asses our cybrid model for its ability to recapitulate the mitochondrial pathology of DAMD, we phenotyped these differences in healthy and DAMD cybrids, including bioenergetics output, mitochondrial mass, and lipid accumulation. We found no difference in the bioenergetic output or ΔΨm in DAMD cybrids. Our results demonstrate that mitochondrial mass and ETC protein expression were unchanged. Lipid raft accumulation under control and stimulated conditions was consistent between healthy and DAMD cybrids. Perhaps the most interesting finding is that mtDNA alterations under stress conditions were consistent in healthy cybrids, increasing in response to mitochondrial stress, but showed individual variability in DAMD cybrids, with several lines showing decreases or no changes in mtDNA content.

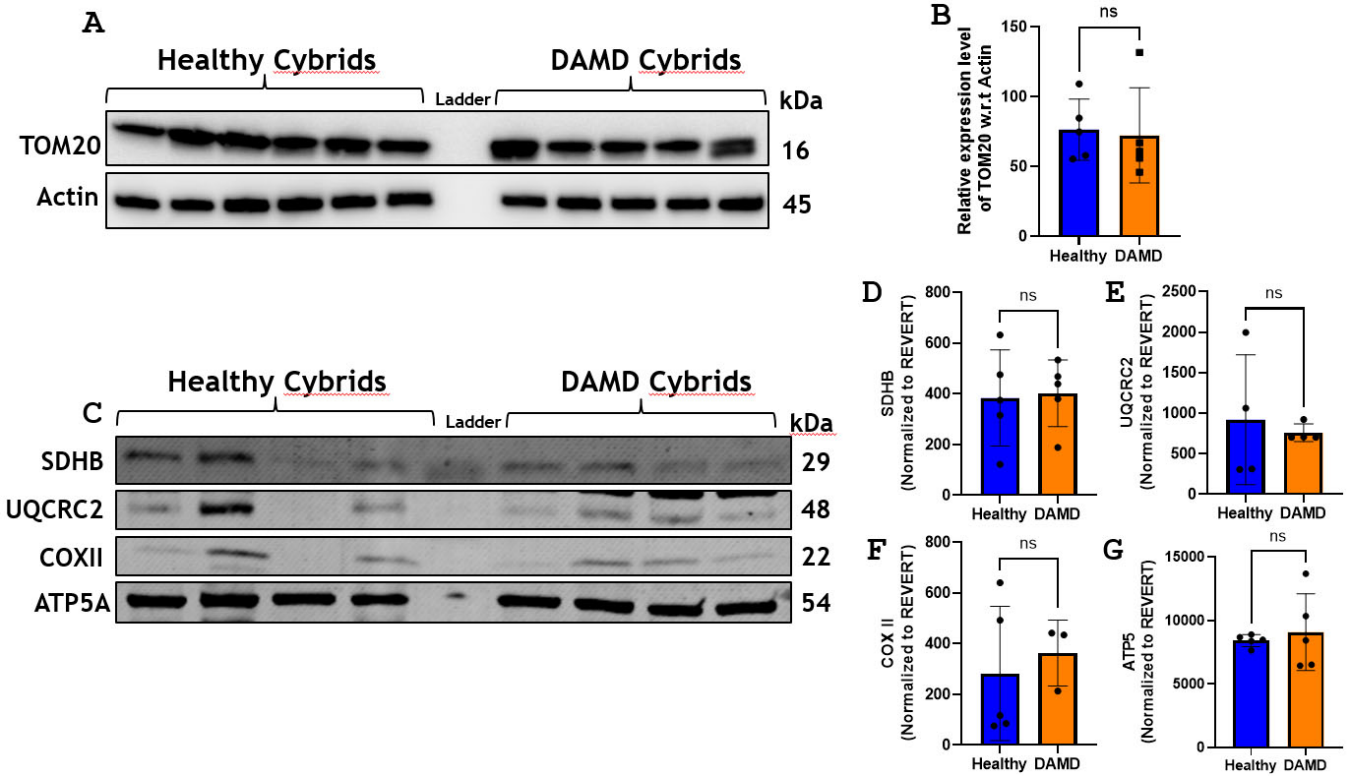

Figure 3. Mitochondrial mass and electron transport chain proteins were unchanged between healthy and DAMD cybrids. western blotting of TOM20 (**A**), a ubiquitously expressed transport protein subunit, was used to quantify total mitochondrial mass, which was unchanged between healthy and DAMD cybrids (**B**). To assess mitochondrial protein expression, western blotting of several ETC proteins was performed (**C**). There was no difference found between healthy and DAMD cybrids in protein expression of SDHB of complex II (**D**), UQCRC2 of complex III (**E**), COX II of complex IV (F), and ATP5 of complex V (G). Healthy Cybrids: n=5, DAMD Cybrids: n=5. .

When mitochondria are exposed to oxidative stress and metabolic insult, mtDNA replication is induced to preserve an intact mitochondrial genome and provide additional genetic material to produce necessary proteins [50,65,66]. This phenomenon is proposed as an anti-apoptotic mitochondrial adaptation, and it is most apparent in cancer cells exposed to chemotherapeutic agents [51]. Alterations suggest a mitochondrial inability to adapt to and combat oxidative and metabolic stressors. DAMD cybrid mtDNA levels do not respond to the administration of stress treatment, suggesting maladaptive changes compared to healthy controls. This result is consistent with the pathology of DAMD and suggests that cybrid models retain specific mitochondrial phenotypes. Previous studies on mtDNA mutations in DAMD have shown an elevation in mtDNA single-nucleotide polymorphisms (SNPs) in all regions of the mtDNA genome; notably, however, is the finding by Udar et al. in 2009, which showed that people with DAMD had higher SNPs in the mitochondrial genome control region [62,67,68]. The mtDNA control region is a 1122-base-pair-long non-coding area of DNA which contains the Heavy-strand origin of replication and the origins of transcription for both strands [69-72]. Therefore, it may be that conserved mutations within the mtDNA control region limit the DAMD cybrids from replicating mtDNA following stress responses, although this hypothesis must be confirmed experimentally.

While these results show a limited impact of the cybrid model of DAMD metabolic phenotypes, this study has notable limitations that impact its generalizability and robustness. First, cybrid subjects were restricted to men of the H mtDNA haplogroup. As a preliminary study, we sought to minimize confounding factors within this study, which leads to limitations in the scope of the translatability of the results to women and other haplogroups. Second, the limited sample size, with five subjects in each group, reduces statistical power. Human variability also generally requires higher sample sizes to detect significant changes

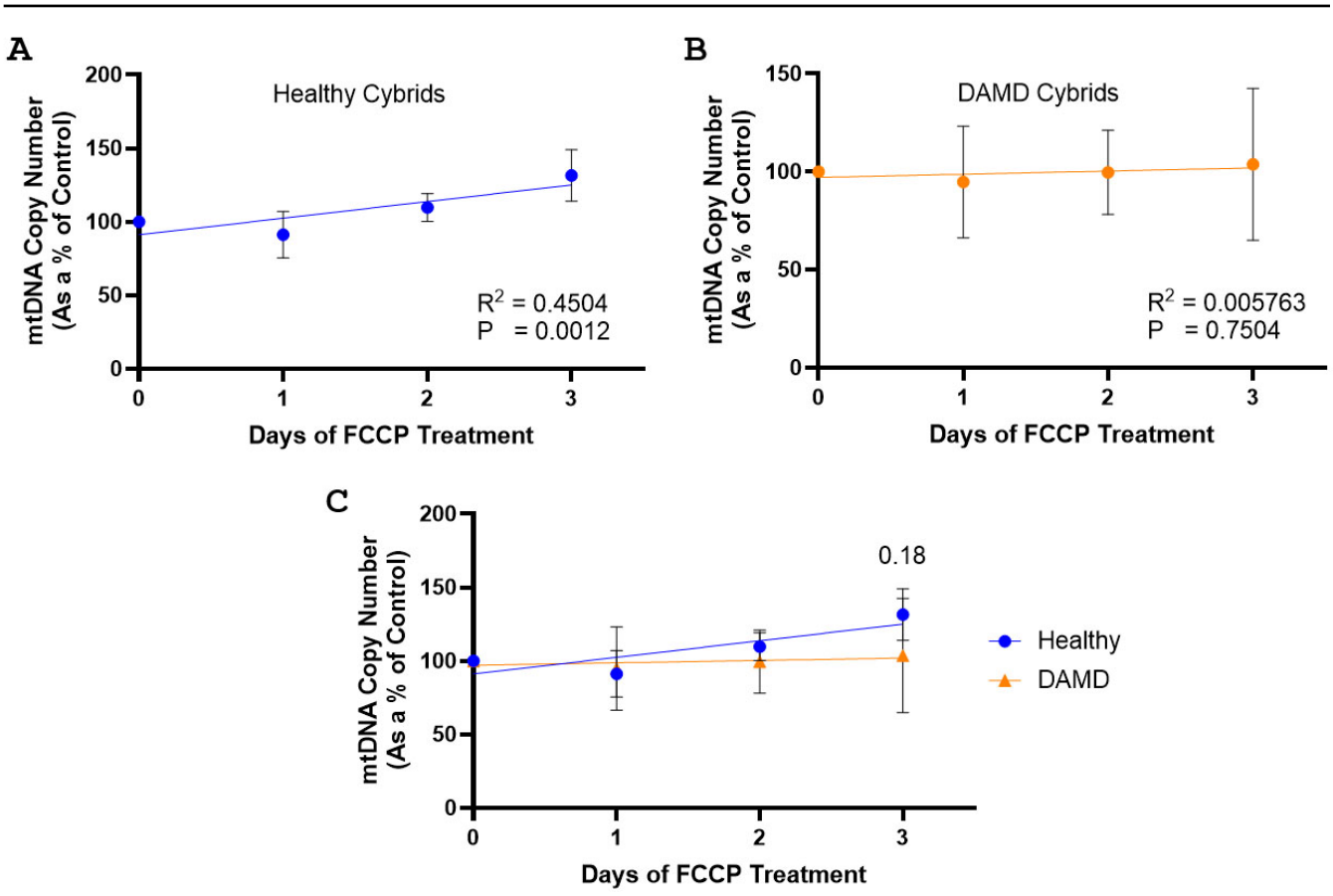

Figure 4. mtDNA levels increase in response to stress in healthy cybrids but not in DAMD cybrids. Healthy and DAMD cybrids were treated with 1 uM FCCP for 4 h daily for 0, 1, 2, and 3 days to simulate metabolic insult and bioenergetic stress conditions. Healthy cybrid mtDNA levels significantly correlated with metabolic stress (p=0.0012), while DAMD cybrid mtDNA showed no correlation (p=0.75). However, when compared, there was no significant difference in the absolute increase in mtDNA between healthy and DAMD cybrids (C). Healthy Cybrids: n=5, DAMD Cybrids: n=5. .

in phenotypes, prompting further questions of robustness. Third is the use of ARPE-19 cells as the ρ0 parental cell line. While ARPE-19 cells are optimal cybrid parental cells due to their hardiness and ease of use, they lack several key RPE proteins expressed in vivo, are transcriptionally different than RPE cells, and may exhibit chromosomal abnormalities in culture [57,73]. ARPE-19 cells are therefore not able to fully recapitulate normal RPE pathology, which must be taken into consideration when assessing these findings. Future studies interested in generating a cybrid model of DAMD mitochondrial pathology should utilize larger sample sizes and explore other cellular vehicles, such as induced pluripotent stem cells (iPSCs), to enhance statistical power and applicability. It would be important in future model development to consider elucidating the contributions of sex and mtDNA haplogroup on DAMD mitochondrial pathology, as these are essential in understanding the underlying disease pathology. However, results from our study will assist with future studies that can use our outcomes for subsequent power analyses.

Additionally, in vitro studies are notorious for failing to accurately replicate the microenvironment of the system they are simulating, with this study being no exception. In this study, DMEM/F12 media supplemented with FBS and antibiotics were used to ensure consistency with previous studies on ARPE-19 cells and to facilitate accessibility for future researchers. However, this approach differs significantly from the natural environment of the retina. Extracellularly, the metabolic microenvironment of the retina is complex. The RPE takes glucose from the choroid, passes it unused to the photoreceptors for glycolytic use, and then resorbs leftover lactic acid for oxidative phosphorylation [74-76]. Future studies may consider modifying the in vitro metabolic microenvironment to more closely resemble the retinal microenvironment, perhaps by reducing glucose levels, supplementing with lactate, or co-culturing with iPSC-derived photoreceptors [77,78].

Despite these limitations, it is essential to highlight the information that negative data can provide and the new hypotheses that stem from this work. Perhaps the most important finding is that this work highlights that DAMD is a multifactorial disease, with mitochondrial alterations being only a subset of the pathology. Intracellularly, the connection between DAMD and genetic factors, specifically the CFH3, HTRA1, and APOE genes, is well established and has been extensively studied [4,23,79]. A cybrid model, which controls for nuclear genetic variation, cannot assess the nuanced relationship between genetic variation and mitochondrial dysfunction. A compelling hypothesis is that the intact ARPE-19 genome removes the negative nuclear influences that the donor's nucleus had on the mitochondria. Furthermore, the ARPE-19 genome may activate compensatory mechanisms for mitochondrial regulation in the transplanted mitochondria; together, this would abolish DAMD phenotypes. Future investigations detailing transcriptomic and epigenomic alterations in ARPE-19 cells, donor cells, and the resulting cybrids would provide essential insight into the nuclear regulatory mechanisms of mitochondrial function. Future findings may suggest that nuclear-mitochondrial crosstalk is essential for maintaining or disrupting mitochondrial phenotypes in DAMD.

Additionally, while mitochondrial function is aberrant in DAMD pathology, this pathology may be ameliorated outside of the context of the retinal microenvironment. It is notable that the mitochondria used for cybrid generation come from patient-derived platelets and are therefore not subjected to the same stressors as those within the retina. This may be explored in future studies, which could involve taking platelet mitochondria and retinal mitochondria from post-mortem donors and generating cybrids to compare mitochondrial function. This would clarify the role of cybrids as vectors for niche mitochondrial analysis and whether it is mitochondrial origin or cybridization that alters mitochondrial function.

Finally, it is well documented that the inflammatory response is an integral part of DAMD pathology, including the complement system, cytokine production, and microglial activation [80]. Mitochondria are often activated during inflammatory responses, elevating ETC activity and producing additional ATP to supply the cell with energy [81]. However, this can be a vicious cycle, where elevated mitochondrial activity leads to increased ROS levels and oxidation events, which can further stimulate inflammatory processes and cell death [81]. While RPE mitochondria are subject to these stressors consistently, an in vitro model of DAMD is separated from these complex systems. Therefore, the lack of an inflammatory system may allow mitochondria to decrease activity and return to a less pathological state. Future studies may coculture RPE cybrid cells with microglia or add exogenous cytokines to attempt to recreate an inflammatory system and elucidate the mitochondrial reaction to these events.

This work aimed to develop a cybrid model of DAMD mitochondrial phenotypes, as traditional models of DAMD investigation have difficulty interrogating mitochondrial contributions to disease pathology. A cybrid model would thereby enhance the study of the disease and make research of this kind more accessible to other researchers. This study found that our cybrid model of DAMD did not fully recapitulate the phenotypes observed in the literature, except mtDNA

instability following stress responses, but was limited in its scope and generalizability. Despite the limited success of this study, we propose new hypotheses for exploration, namely how the cybrid generation process affects mitochondria from different tissue types, how nuclear regulation may influence mitochondrial compensation and epigenetics, and whether inflammatory processes are key to understanding mitochondrial dysfunction in DAMD, in the hopes that this may still contribute to our current understanding of cybrid cells and DAMD.

ACKNOWLEDGMENTS

The authors would like to acknowledge Mithalesh Singh for his contributions to the Kenney Lab and for advising on aspects of this work. We would also like to thank Dr. Anthony Nesburn for his support during a significant period of turmoil for himself and the laboratory. In memoriam of Dr. Maria Cristina Kenney, M.D. Ph.D. CONFLICTS OF INTEREST: The authors declare no conflicts of interest. FINANCIAL DISCLOSURE: This work was supported by the Discovery Eye Foundation, Polly and Michael Smith, Iris and the B. Gerald Cantor Foundation and National Eye Institute R01 EY027363. NSF Graduate Research Fellowship DGE-1839285 also gave support. We acknowledge the support of the Institute for Clinical and Translational Science at the University of California, Irvine. Supported in part by an Unrestricted Departmental Grant from Research to Prevent Blindness.

REFERENCES

1. Schultz NM, Braunack-Mayer L, Schwartz J, Gaspar L. The Patient Experience: Symptoms and Impact of Dry Age-Related Macular Degeneration. Ophthalmol Ther 2021; 10:151-64. [PMID: 33512689].

2. Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen J, Leasher J, Limburg H, Naidoo K, Pesudovs K, Silvester A, Stevens GA, Tahhan N, Wong T, Taylor H, Arditi A, Barkana Y, Bozkurt B, Bron A, Budenz D, Cai F, Casson R, Chakravarthy U, Choi J, Congdon N, Dana R, Dandona R, Dandona L, Dekaris I, Del Monte M, Deva J, Dreer L, Ellwein L, Frazier M, Frick K, Friedman D, Furtado J, Gao H, Gazzard G, George R, Gichuhi S, Gonzalez V, Hammond B, Hartnett ME, He M, Hejtmancik J, Hirai F, Huang J, Ingram A, Javitt J, Joslin C, Khairallah M, Khanna R, Kim J, Lambrou G, Lansingh VC, Lanzetta P, Lim J, Mansouri K, Mathew A, Morse A, Munoz B, Musch D, Nangia V, Palaiou M, Parodi MB, Pena FY, Peto T, Quigley H, Raju M, Ramulu P, Reza D, Robin A, Rossetti L, Saaddine J, Sandar M, Serle J, Shen T, Shetty R, Sieving P, Silva JC, Sitorus RS, Stambolian D, Tejedor J, Tielsch J, Tsilimbaris M, van Meurs J, Varma R, Virgili G, Wang YX, Wang NL, West S, Wiedemann P, Wormald R, Zheng Y. Global causes of blindness and distance vision impairment 1990–2020: a systematic review and meta-analysis. Lancet Glob Heal. 2017;5.

3. Wolf AT, Harris A, Oddone F, Siesky B, Verticchio Vercellin A, Ciulla TA. Disease progression pathways of wet AMD: opportunities for new target discovery. Expert Opin Ther Targets 2022; 26:5-12. [PMID: 35060431].

4. Amini MA, Karbasi A, Vahabirad M, Khanaghaei M, Alizamir A. Mechanistic Insight into Age-Related Macular Degeneration (AMD): Anatomy, Epidemiology, Genetics, Pathogenesis, Prevention, Implications, and Treatment Strategies to Pace AMD Management. Chonnam Med J 2023; 59:143-59. [PMID: 37840684].

5. Al-Khersan H, Hussain RM, Ciulla TA, Dugel PU. Innovative therapies for neovascular age-related macular degeneration. Expert Opin Pharmacother 2019; 20:1879-91. [PMID: 31298960].

6. Smith AG, Kaiser PK. Emerging treatments for wet age-related macular degeneration. Expert Opin Emerg Drugs 2014; 19:157-64. [PMID: 24555421].

7. Patel PN, Patel PA, Land MR, Bakerkhatib-Taha I, Ahmed H, Sheth V. Targeting the Complement Cascade for Treatment of Dry Age-Related Macular Degeneration. Biomedicines 2022; 10:1884-[PMID: 36009430].

8. Wu A, Lu R, Lee E. Tissue engineering in age-related macular degeneration: a mini-review. J Biol Eng 2022; 16:11-Internet[PMID: 35578246].

9. Wang Y, Fung NSK, Lam WC, Lo ACY. mTOR Signalling Pathway: A Potential Therapeutic Target for Ocular Neurodegenerative Diseases. Antioxidants (Basel) 2022; 11:11-[PMID: 35883796].

10. Tong Y, Zhang Z, Wang S. Role of Mitochondria in Retinal Pigment Epithelial Aging and Degeneration. Front Aging 2022; 3:926627[PMID: 35912040].

11. Monge M, Araya A, Wu L. Subretinal drusenoid deposits: An update. Taiwan J Ophthalmol 2022; 12:138-46. [PMID: 35813798].

12. Nashine S. Potential Therapeutic Candidates for Age-Related Macular Degeneration (AMD). Cells 2021; 10:10-[PMID: 34572131].

13. Romero-Vazquez S, Llorens V, Soler-Boronat A, Figueras-Roca M, Adan A, Molins B. Interlink between Inflammation and Oxidative Stress in Age-Related Macular Degeneration: Role of Complement Factor H. Biomedicines 2021; 9:9-[PMID: 34209418].

14. Yao J, Jia L, Khan N, Lin C, Mitter SK, Boulton ME, Dunaief JL, Klionsky DJ, Guan JL, Thompson DA, Zacks DN. Deletion of autophagy inducer RB1CC1 results in degeneration of the retinal pigment epithelium. Autophagy 2015; 11:939-53. [PMID: 26075877].

15. Mitter SK, Song C, Qi X, Mao H, Rao H, Akin D, Lewin A, Grant M, Dunn W Jr, Ding J, Bowes Rickman C, Boulton M. Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD. Autophagy 2014; 10:1989-2005. [PMID: 25484094].

16. Bianchi E, Scarinci F, Ripandelli G, Feher J, Pacella E, Magliulo G, Gabrieli CB, Plateroti R, Plateroti P, Mignini F, Artico M. Retinal pigment epithelium, age-related macular degeneration and neurotrophic keratouveitis. Int J Mol Med 2013; 31:232-42. [PMID: 23128960].

17. Zhang M, Jiang N, Chu Y, Postnikova O, Varghese R, Horvath A, Cheema AK, Golestaneh N. Dysregulated metabolic pathways in age-related macular degeneration. Sci Rep 2020; 10:2464-[PMID: 32051464].

18. Golestaneh N, Chu Y, Xiao YY, Stoleru GL, Theos AC. Dysfunctional autophagy in RPE, a contributing factor in age-related macular degeneration. Cell Death Dis 2017; 8:e2537[PMID: 28055007].

19. Kanow MA, Giarmarco MM, Jankowski CSR, Tsantilas K, Engel AL, Du J, Linton JD, Farnsworth CC, Sloat SR, Rountree A, Sweet IR, Lindsay KJ, Parker ED, Brockerhoff SE, Sadilek M, Chao JR, Hurley JB. Biochemical adaptations of the retina and retinal pigment epithelium support a metabolic ecosystem in the vertebrate eye. Elife 2017; 6:e28899[PMID: 28901286].

20. Fisher CR, Ferrington DA. Perspective on AMD Pathobiology: A Bioenergetic Crisis in the RPE. Invest Ophthalmol Vis Sci 2018; 59:AMD41-7. [PMID: 30025108].

21. Ebeling MC, Polanco JR, Qu J, Tu C, Montezuma SR, Ferrington DA. Improving retinal mitochondrial function as a treatment for age-related macular degeneration. Redox Biol 2020; 34:101552[PMID: 32446621].

22. Deng Y, Qiao L, Du M, Qu C, Wan L, Li J, Huang L. Age-related macular degeneration: Epidemiology, genetics, pathophysiology, diagnosis, and targeted therapy. Genes Dis 2021; 9:62-79. [PMID: 35005108].

23. Cabral de Guimaraes TA, Daich Varela M, Georgiou M, Michaelides M. Treatments for dry age-related macular degeneration: therapeutic avenues, clinical trials and future directions. Br J Ophthalmol 2022; 106:297-304. [PMID: 33741584].

24. Williams AM, Stamer WD, Allingham RR. Increasing the Availability and Quality of Donor Eyes for Research. JAMA Ophthalmol 2016; 134:351-2. [PMID: 26768017].

25. Gouras P, Ivert L, Landauer N, Mattison JA, Ingram DK, Neuringer M. Drusenoid maculopathy in rhesus monkeys (Macaca mulatta): effects of age and gender. Graefes Arch Clin Exp Ophthalmol 2008; 246:1395-402. [PMID: 18709381].

26. Bellhorn RW, King CD, Aguirre G, Ripps H, Siegel I, Tsai H. Pigment abnormalities in the macula of rhesus monkeys: clinical observations. Invest Ophthalmol Vis Sci 1981; 21:.

27. Francis PJ, Appukuttan B, Simmons E, Landauer N, Stoddard J, Hamon S, Ott J, Ferguson B, Klein M, Stout JT, Neuringer M. Rhesus monkeys and humans share common susceptibility genes for age-related macular disease. Hum Mol Genet 2008; 17:2673-80. [PMID: 18535016].

28. Malek G, Busik J, Grant MB, Choudhary M. Models of retinal diseases and their applicability in drug discovery. Expert Opin Drug Discov 2018; 13:359-77. [PMID: 29382242].

29. Pennesi ME, Neuringer M, Courtney RJ. Animal models of age related macular degeneration. Mol Aspects Med 2012; 33:487-509. [PMID: 22705444].

30. King MP, Attardi G. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. Science 1989; 246:500-3. [PMID: 2814477].

31. King MP, Attardi G. Isolation of human cell lines lacking mitochondrial DNA. Methods Enzymol 1996; 264:304-13. [PMID: 8965704].

32. Schubert S, Heller S, Löffler B, Schäfer I, Seibel M, Villani G, Seibel P. Generation of Rho Zero Cells: Visualization and Quantification of the mtDNA Depletion Process. Int J Mol Sci 2015; 16:9850-65. [PMID: 25941929].

33. Galindo-Murillo R, Cheatham TE. Ethidium bromide interactions with DNA: an exploration of a classic DNA-ligand complex with unbiased molecular dynamics simulations. Nucleic Acids Res 2021; 49:3735-47. [PMID: 33764383].

34. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen 2017; 58:235-63. [PMID: 28485537].

35. Warren EB, Aicher AE, Fessel JP, Konradi C. Mitochondrial DNA depletion by ethidium bromide decreases neuronal mitochondrial creatine kinase: Implications for striatal energy metabolism. PLoS One 2017; 12:e0190456[PMID: 29287112].

36. Kai Y, Takamatsu C, Tokuda K, Okamoto M, Irita K, Takahashi S. Rapid and random turnover of mitochondrial DNA in rat hepatocytes of primary culture. Mitochondrion 2006; 6:299-304. [PMID: 17098481].

37. Kao LP, Ovchinnikov D, Wolvetang E. The effect of ethidium bromide and chloramphenicol on mitochondrial biogenesis in primary human fibroblasts. Toxicol Appl Pharmacol 2012; 261:42-9. [PMID: 22712077].

38. Melchinger H, Jain K, Tyagi T, Hwa J. Role of Platelet Mitochondria: Life in a Nucleus-Free Zone. Front Cardiovasc Med 2019; 6:153-[PMID: 31737646].

39. Wojcieszyn JW, Schlegel RA, Lumley-Sapanski K, Jacobson KA. Studies on the mechanism of polyethylene glycol-mediated cell fusion using fluorescent membrane and cytoplasmic probes. J Cell Biol 1983; 96:151-9. [PMID: 6826645].

40. Yang J, Shen MH. Polyethylene glycol-mediated cell fusion. Methods Mol Biol 2006; 325:59-66. [PMID: 16761719].

41. Nashine S, Kenney MC. Effects of Humanin G (HNG) on angiogenesis and neurodegeneration markers in Age-related Macular Degeneration (AMD). Mitochondrion 2024; 74:101818[PMID: 38029849].

42. Nashine S, Cohen P, Wan J, Kenney MC. Effect of Humanin G (HNG) on inflammation in age-related macular degeneration (AMD). Aging (Albany NY) 2022; 14:4247-69. [PMID: 35576057].

43. Nashine S, Nesburn AB, Kuppermann BD, Kenney MC. Age-related macular degeneration (AMD) mitochondria modulate epigenetic mechanisms in retinal pigment epithelial cells. Exp Eye Res 2019; 189:107701[PMID: 31226340].

44. Costa RD, Thomaz Neto FJ, Moustafa MT, Atilano SR, Chwa M, Cáceres-Del-Carpi J, Mohamed MH, Kenney MC, Kuppermann BD. The role of mitochondrial genes on nuclear gene expression in neovascular age related macular degeneration: analysis of nuclear VEGF gene expression after ranibizumab treatment in cytoplasmic hybrid retinal pigment epithelial cell lines correlated with clinical evolution. Int J Retina Vitreous 2023; 9:44-[PMID: 37491310].

45. . MC K. M C, SR A, JM P, P F, C R, D M, T H, G W, K S, AB N, DS B, BD K, SM J, MV M, DC W, N U. Mitochondrial DNA variants mediate energy production and expression levels for CFH, C3 and EFEMP1 genes: implications for age-related macular degeneration. PLoS One 2013; 8:.

46. Moustafa MT, Ramirez C, Schneider K, Atilano SR, Limb GA, Kuppermann BD, Kenney MC. Protective Effects of Memantine on Hydroquinone-Treated Human Retinal Pigment Epithelium Cells and Human Retinal Müller Cells. J Ocul Pharmacol Ther 2017; 33:610-9. [PMID: 28961056].

47. Kowada T, Maeda H, Kikuchi K. BODIPY-based probes for the fluorescence imaging of biomolecules in living cells. Chem Soc Rev 2015; 44:4953-72. [PMID: 25801415].

48. Qiu B, Simon MC. BODIPY 493/503 Staining of Neutral Lipid Droplets for Microscopy and Quantification by Flow Cytometry. Bio Protoc 2016; 6:6-[PMID: 28573161].

49. Somasundaran S, Constable IJ, Mellough CB, Carvalho LS. Retinal pigment epithelium and age-related macular degeneration: A review of major disease mechanisms. Clin Exp Ophthalmol 2020; 48:1043-56. [PMID: 32710488].

50. Hori A, Yoshida M, Shibata T, Ling F. Reactive oxygen species regulate DNA copy number in isolated yeast mitochondria by triggering recombination-mediated replication. Nucleic Acids Res 2009; 37:749-61. [PMID: 19074198].

51. Mei H, Sun S, Bai Y, Chen Y, Chai R, Li H. Reduced mtDNA copy number increases the sensitivity of tumor cells to chemotherapeutic drugs. Cell Death Dis 2015; 6:e1710-1710. [PMID: 25837486].

52. Hao X, Du L, Liu G, Li Z, Wang S. Human retinal organoids for modelling dry age-related macular degeneration and screening drugs. Genes Dis 2025; 12:101593[PMID: 40837411].

53. Xue Y, Lin B, Chen JT, Tang WC, Browne AW, Seiler MJ. The Prospects for Retinal Organoids in Treatment of Retinal Diseases. Asia Pac J Ophthalmol (Phila) 2022; 11:314-27. [PMID: 36041146].

54. Parween S, Howell A, Varghese S, Vergara MN. Human Retinal Organoid Model of Photoreceptor Cell Death Amenable to Drug Screening for AMD. Invest Ophthalmol Vis Sci 2024; 65:4508-4508. .

55. Johnson LV, Forest DL, Banna CD, Radeke CM, Maloney MA, Hu J, Spencer CN, Walker AM, Tsie MS, Bok D, Radeke MJ, Anderson DH. Cell culture model that mimics drusen formation and triggers complement activation associated with age-related macular degeneration. Proc Natl Acad Sci U S A 2011; 108:18277-82. [PMID: 21969589].

56. Pilgrim MG, Lengyel I, Lanzirotti A, Newville M, Fearn S, Emri E, Knowles JC, Messinger JD, Read RW, Guidry C, Curcio CA. Subretinal Pigment Epithelial Deposition of Drusen Components Including Hydroxyapatite in a Primary Cell Culture Model. Invest Ophthalmol Vis Sci 2017; 58:708-19. [PMID: 28146236].

57. Ablonczy Z, Dahrouj M, Tang PH, Liu Y, Sambamurti K, Marmorstein AD, Crosson CE. Human retinal pigment epithelium cells as functional models for the RPE in vivo. Invest Ophthalmol Vis Sci 2011; 52:8614-20. [PMID: 21960553].

58. Ferrington DA, Ebeling MC, Kapphahn RJ, Terluk MR, Fisher CR, Polanco JR, Roehrich H, Leary MM, Geng Z, Dutton JR, Montezuma SR. Altered bioenergetics and enhanced resistance to oxidative stress in human retinal pigment epithelial cells from donors with age-related macular degeneration. Redox Biol 2017; 13:255-65. [PMID: 28600982].

59. Feher J, Kovacs I, Artico M, Cavallotti C, Papale A, Balacco Gabrieli C. Mitochondrial alterations of retinal pigment epithelium in age-related macular degeneration. Neurobiol Aging 2006; 27:983-93. [PMID: 15979212].

60. Nordgaard CL, Karunadharma PP, Feng X, Olsen TW, Ferrington DA. Mitochondrial proteomics of the retinal pigment epithelium at progressive stages of age-related macular degeneration. Invest Ophthalmol Vis Sci 2008; 49:2848-55. [PMID: 18344451].

61. Nordgaard CL, Berg KM, Kapphahn RJ, Reilly C, Feng X, Olsen TW, Ferrington DA. Proteomics of the retinal pigment epithelium reveals altered protein expression at progressive stages of age-related macular degeneration. Invest Ophthalmol Vis Sci 2006; 47:815-22. [PMID: 16505012].

62. Karunadharma PP, Nordgaard CL, Olsen TW, Ferrington DA. Mitochondrial DNA damage as a potential mechanism for age-related macular degeneration. Invest Ophthalmol Vis Sci 2010; 51:5470-9. [PMID: 20505194].

63. Lin H, Xu H, Liang FQ, Liang H, Gupta P, Havey AN, Boulton ME, Godley BF. Mitochondrial DNA damage and repair in RPE associated with aging and age-related macular degeneration. Invest Ophthalmol Vis Sci 2011; 52:3521-9. [PMID: 21273542].

64. Terluk MR, Kapphahn RJ, Soukup LM, Gong H, Gallardo C, Montezuma SR, Ferrington DA. Investigating mitochondria as a target for treating age-related macular degeneration. J Neurosci 2015; 35:7304-11. [PMID: 25948278].

65. Wei YH, Lee CF, Lee HC, Ma YS, Wang CW, Lu CY, Pang CY. Increases of mitochondrial mass and mitochondrial genome in association with enhanced oxidative stress in human cells harboring 4,977 BP-deleted mitochondrial DNA. Ann N Y Acad Sci 2001; 928:97-112. [PMID: 11795533].

66. Moreno-Loshuertos R, Acín-Pérez R, Fernández-Silva P, Movilla N, Pérez-Martos A, Rodriguez de Cordoba S,

Gallardo ME, Enríquez JA. Differences in reactive oxygen species production explain the phenotypes associated with common mouse mitochondrial DNA variants. Nat Genet 2006; 38:1261-8. [PMID: 17013393].

67. Qu S, Lin H, Pfeiffer N, Grus FH. Age-Related Macular Degeneration and Mitochondria-Associated Autoantibodies: A Review of the Specific Pathogenesis and Therapeutic Strategies. Int J Mol Sci 2024; 25:1624-[PMID: 38338904].

68. Udar N, Atilano SR, Memarzadeh M, Boyer DS, Chwa M, Lu S, Maguen B, Langberg J, Coskun P, Wallace DC, Nesburn AB, Khatibi N, Hertzog D, Le K, Hwang D, Kenney MC. Mitochondrial DNA haplogroups associated with age-related macular degeneration. Invest Ophthalmol Vis Sci 2009; 50:2966-74. [PMID: 19151382].

69. D'Souza AR, Minczuk M. Mitochondrial transcription and translation: overview. Essays Biochem 2018; 62:309-20. [PMID: 30030363].

70. Vega Avalos JH, Hernández LE, Zuñiga LY, Sánchez-Parada MG, González Santiago AE, Román Pintos LM, Castañeda Arellano R, Hernández-Ortega LD, Mercado-Sesma AR, Orozco-Luna FJ, Baptista-Rosas RC. Mitochondrial Control Region Variants Related to Breast Cancer. Genes (Basel) 2022; 13:1962-[PMID: 36360199].

71. Falah M, Farhadi M, Kamrava SK, Mahmoudian S, Daneshi A, Balali M, Asghari A, Houshmand M. Association of genetic variations in the mitochondrial DNA control region with presbycusis. Clin Interv Aging 2017; 12:459-65. [PMID: 28424544].

72. Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. Nature 1981; 290:457-65. [PMID: 7219534].

73. Pfeffer BA, Fliesler SJ. Reassessing the suitability of ARPE-19 cells as a valid model of native RPE biology. Exp Eye Res 2022; 219:109046[PMID: 35314139].

74. Hurley JB. Retina Metabolism and Metabolism in the Pigmented Epithelium: A Busy Intersection. Annu Rev Vis Sci 2021; 7:665-92. [PMID: 34102066].

75. Swarup A, Samuels IS, Bell BA, Han JYS, Du J, Massenzio E, Abel ED, Boesze-Battaglia K, Peachey NS, Philp NJ. Modulating GLUT1 expression in retinal pigment epithelium decreases glucose levels in the retina: impact on photoreceptors and Müller glial cells. Am J Physiol Cell Physiol 2019; 316:C121-33. [PMID: 30462537].

76. Zhao C, Yasumura D, Li X, Matthes M, Lloyd M, Nielsen G, Ahern K, Snyder M, Bok D, Dunaief JL, LaVail MM, Vollrath D. mTOR-mediated dedifferentiation of the retinal pigment epithelium initiates photoreceptor degeneration in mice. J Clin Invest 2011; 121:369-83. [PMID: 21135502].

77. Ghareeb AE, Lako M, Steel DH. Coculture techniques for modeling retinal development and disease, and enabling regenerative medicine. Stem Cells Transl Med 2020; 9:1531-48. [PMID: 32767661].

78. Amirpour N, Nasr-Esfahani MH, Esfandiari E, Razavi S, Karamali F. Comparing Three Methods of Co-culture of Retinal Pigment Epithelium with Progenitor Cells Derived Human Embryonic Stem Cells. Int J Prev Med 2013; 4:1243-50. [PMID: 24404357].

79. van Asten F, Simmons M, Singhal A, Keenan TD, Ratnapriya R, Agrón E, Clemons TE, Swaroop A, Lu Z, Chew EY. Age-Related Eye Disease Study 2 Research Group. A Deep Phenotype Association Study reveals specific phenotype associations with genetic variants in age-related macular degeneration: Age-Related Eye Disease Study 2 (AREDS2) Report No. 14. Ophthalmology 2018; 125:559-68. [PMID: 29096998].

80. Tan W, Zou J, Yoshida S, Jiang B, Zhou Y. The Role of Inflammation in Age-Related Macular Degeneration. Int J Biol Sci 2020; 16:2989-3001. [PMID: 33061811].

81. López-Armada MJ, Riveiro-Naveira RR, Vaamonde-García C, Valcárcel-Ares MN. Mitochondrial dysfunction and the inflammatory response. Mitochondrion 2013; 13:106-18. [PMID: 23333405].