Retinal Basal Laminar Deposits in Complement fH/fP Mouse Model of Dense Deposit Disease

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Retina

Dense deposit disease (DDD) and C3 glomerulonephritis (C3GN) are rare forms of glomerulonephritis that affect children and young adults. Both diseases result from abnormal regulation of the alternative pathway of the complement cascade and are now classified under the heading of "C3 glomerulopathies." DDD (also known as membranoproliferative glomerulonephritis type II) is characterized by electron-dense deposits in the glomerular basement membrane (GBM) as well as drusen in Bruch’s membrane (BrM). There are currently no mechanism-directed therapies to treat affected patients, approximately 50% of whom progress to having end-stage renal disease within 10 years of diagnosis. The composition of drusen in patients with DDD is similar to that in patients with age-related macular degeneration (AMD) including AMD. Among several proteins that regulate AP, complement factor H (fH) and factor properdin (fP) regulate complement activation; fH inhibits alternative pathway (AP) activation, whereas fP promotes it. We report pathologic changes in eyes of an fH and fP double-mutant mouse, which we previously showed have dense deposits in the GBM and early mortality from nephropathy.

Methods. fHm/m, fP−/−, and fHm/m/fP−/− mice were generated on a C57BL/6–129J background. Fundus imaging at 8 weeks of age was followed by analysis via light and electron microscopy. Retinal function was assessed by electroretinography (ERG). Complement levels and localization were tested by immunohistochemistry and ELISA. Retinas of fHm/m/fP−/− mice treated with intraperitoneal injections of an anti-C5 antibody were compared to those of age- and genotype-matched mice injected with an isotype control antibody.

Results. fHm/m/fP−/− mice suffered early-onset retinal hypopigmented spots detected using in vivo retinal photography, and histologic examination showed basal laminar deposits (BLamD), degeneration of the photoreceptors, and RPE vacuolization. ERG showed diminished retinal function. The anti-C5 antibody was retina-protective.

Conclusions. This unique mouse represents a new model of complement-mediated rapid-onset DDD, and could be useful in exploring the pathologic changes associated with BLamD in age-related macular degeneration.

Keywords: factor H, properdin, retinal degeneration, dense deposit disease

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 Unlike any other complement protein, is produced primarily by leukocytes instead of hepatocytes. Accordingly, IF levels are elevated in inflammatory microenvironments. Properdin-deficient mice have been used as models to study arthritis, ischemic-reperfusion injury, abdominal aortic aneurysm, asthma, septic and nonseptic shock, and C3 glomerulopathy. Previously, we created an fH mutant mouse by inserting two stop codons at the beginning of SCR 19 of the fH gene to selectively disrupt function of the C-terminal domain of fH to model aHUS-related mutations in this region. Unexpectedly, our fH mutant mouse had impaired fH activity in the fluid phase and on the cell surface due to expression of only a small amount of the truncated fH protein in the plasma, and a nonlethal form of C3 glomerulopathy developed instead of aHUS. When this fH mutant mouse was rendered deficient in fP either by genetic deletion or by antibody neutralization, paradoxically a more severe and lethal form of C3 glomerulopathy developed showing kidney injury on electron microscopy similar to human DDD. We characterized pathologic changes in the retina of this mouse model, finding rapid-onset hypopigmented spots representing RPE degeneration. Because this unique mouse model exhibits retinal manifestations similar to those in DDD, it could be useful in testing new therapies for DDD and retinopathies containing BLamD.

**MATERIALS AND METHODS**

**Animals**

Generation of fHm/fP, fP−/− and fHm/fP−/− on a C57BL/6–129J mixed background were described previously. All mice were housed in a UPenn nonbarrier facility maintained at 21-23°C, a 12-hour/12-hour light-dark cycle, and free access to food and water. All experiments used age-matched littermates as controls. Mice were negative for the rd1 and rd8 mutations. Experimental procedures were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmology and Vision Research. All protocols were approved by the animal care review board of the University of Pennsylvania. Most fHm/fP−/− mice had to be euthanized between 8 and 12 weeks due to renal failure. However, all mice used in this study were not critically moribund.

**Fundus Imaging**

Mice were anesthetized with a single intraperitoneal injection of ketamine (80 mg/kg) xylazine (40 mg/kg), and acepromazine (2 mg/kg). Pupils were dilated with 1% tropicamide (Mydriacyl; Alcon, Fort Worth, TX, US). After pupils were dilated, mice were placed on a padded metal stage. Color and autofluorescence images then were acquired using a fundus camera (Micron III; Phoenix Research Laboratories, Inc., Pleasanton, CA, USA).

**Morphologic Analysis**

Electron microscopy on retinal samples was performed as described previously. After enucleation, eyes were fixed in 1% osmium tetroxide/0.1 mol/L sodium cacodylate buffer, dehydrated, and embedded in EMBed-812 (Electron Microscopy Sciences, Hatfield, PA, USA). Ultrathin sections (60–80 nm thick) were stained and examined with a JEOL1010 transmission electron microscope (JEOL Ltd., Tokyo, Japan).

**Immunofluorescence**

After the globes were fixed in 4% paraformaldehyde, eyes were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek, Torrance, CA, USA). Immunofluorescence was performed on sections 10 µm thick as described previously. The sections then were dehydrated in increasing concentrations of ethanol, infiltrated overnight, and embedded the next day in a water-soluble, 2-hydroxyethyl methacrylate (HEMA) plastic resin (JB4 Solution A, Polysciences, Inc., Warrington, PA, USA). For standard histology, 3-µm thick plastic sections were cut in the sagittal plane and toluidine blue-stained as described previously. The number of nuclei per column of outer nuclear layer (ONL) PRs was counted in triplicate at 200 µm intervals from the optic nerve head (ONH) to 1800 µm from the ONH, using image analysis software (ImagePro Plus 4.1; Media Cybernetics, Silver Spring, MD, USA) to calculate distances from manually set lengths.

**Electroretinography (ERG)**

ERG recordings were performed as described previously. In brief, mice were dark-adapted overnight and then anesthetized with a mixture delivering (in mg/kg body weight) 100 ketamine, 2 xylazine, and 2 acepromazine. After pupils were dilated, and mice were placed on a stage maintained at 57°C. The ERG apparatus was modified by the manufacturer for experiments with mice by
RESULTS

**fHM/m/FP** Mice had Hypopigmented Lesions Detected by Fundus Photography at 8 Weeks of Age

To profile ophthalmic changes, we performed fundus photography of mice at 8 weeks of age. No apparent pathology was noted on fundus photographs of wild type (WT; *n* = 8, Fig. 1A), *fHM/m* (*n* = 8, Fig. 1B), and *FP*/*FP* (*n* = 8, Fig. 1C) mice. However, hypopigmented spots were observed in *fHM/m/FP*/*FP* mice (*n* = 16; Fig. 1D, white arrowheads). Hypopigmented spots were visible as early as age 4 weeks. The numbers of hypopigmented lesions then were quantified and analyzed. Interestingly, male *fHM/m/FP*/*FP* mice (*n* = 8) had significantly higher numbers of lesions than female *fHM/m/FP*/*FP* mice (*n* = 8) and other genotypes (*n* = 8 in WT, *fHM/m*, and *FP*/*FP*). Plastic sections revealed RPE vacuolization at the locations corresponding to hypopigmented lesions displayed on fundus images (Fig. G, red frames) *P* < 0.01; ***P* < 0.001. Scale Bars: mean ± SEM. IPL: inner plexiform layer; INL, inner nuclear layer.

**C5 ELISA**

To measure levels of intact C5, 96-well ELISA plates were coated with 5 μg/ml anti-mouse C5 monoclonal antibody (mAb) BB5.1 in 0.1 M carbonate buffer overnight at 4°C. Plates were washed after each step with PBS/0.5% Tween-20 (PBS-T). Plates then were blocked with 1% BSA-PBS for 1 hour at room temperature (RT) followed by addition of serially diluted mouse plasma samples in 1% BSA-PBS starting at 1:10. After incubation with plasma for 1 hour at RT, the plate was washed three times with PBS-T and then incubated with horseradish peroxidase goat polyclonal anti-human C5 Ab (cross-reacts with mouse C5, 1:1000; Quidel, CA, USA) for 1 hour at RT. After washing, the plate was developed using OptEIA substrate (BD Biosciences, San Jose, CA, USA).

**Anti-C5 Antibody Treatment**

*fHM/m/FP*/*FP* mice were treated systemically by intraperitoneal administration of an anti-C5 mAb (BB5.1). 1 mg twice weekly for 12 weeks starting at 4 weeks of age. All mice weighed 20-25 g during injections. Mice in the control group were treated with an isotype control mAb (purified from MoPC-31C hybridoma; American Type Culture Collection [ATCC], Manassas, VA, USA) at the same dosage and frequency but only for 4 weeks due to early mortality from kidney disease.

**Statistical Analysis**

The means ± SEM were calculated for each comparison pair. Statistical analyses for hypopigmented spot counting, ERG and C5 ELISA were performed in GraphPad Prism 6.0 (San Diego, CA, USA) using the 1-way ANOVA with a Tukey post hoc test comparing the mean of each group with the mean of every other group. ONL thickness (nuclei) in plastic sections were compared using 1-way ANOVA with post hoc pairwise comparisons using Bonferroni adjustment. *P* < 0.05 was considered statistically significant.
Male fHm/m/fP mice (5.70 ± 1.70) was much higher than that of females (2.35 ± 0.29), although there was larger variation in severity scores within the male group.

Histology showed PR death and RPE degeneration in fHm/m/fP mice at 8 weeks of age.

Morphologic analysis was performed at 8 weeks of age. Plastic sections of WT (Fig. 4A), fHm/m (Fig. 4B), and fP−/− (Fig. 4C) mice revealed normal retinal morphology. In contrast, sections from fHm/m/fP−/− mice revealed ONL thinning (Fig. 4D), and disrupted inner and outer segments (Fig. 4E) in fHm/m/fP−/− mice. Finally, ONL thickness measurement via counting of PR

**FIGURE 2.** EM analysis on WT and fHm/m/fP−/− retinas. Low (left column) and high (right column) magnification EMs compare (A, B) WT to (C–J) fHm/m/fP−/− mice. (C) fHm/m/fP−/− mice exhibited hypertrophic RPE cells (arrowheads) with subretinal debris (arrows). (D) Some RPE cells in fHm/m/fP−/− mice contained more melanosomes compared to WT, and also contained vacuoles (asterisks). (E) Other RPE cells had fewer melanosomes compared to WT. (F) Some RPE cells contained melanosomes aggregated (arrow). (G) Extruded PR nuclei migrating toward the RPE were noted (arrow). (H, J) Multiple vacuoles (asterisks) were found in the RPE cells. (D, E, H, J) Green arrowheads indicate the basolateral RPE plasma membrane. Black arrowheads indicate the elastic layer of BrM. (A, C, E, G, I) Scale bar: 10 μm. (B, D, F, H, J) Scale bar: 1.5 μm. CC, choriocapillaris; BI, basal infolding; Nu, nucleus.

**FIGURE 3.** High magnification (>20,000) EM images of RPE and BrM. Normal morphology in (A) WT (B) fHm/m mice, and (C) fP−/− mice. In contrast, (D–F) fHm/m/fP−/− mice exhibited basal laminar deposits (arrowheads) located between the basolateral RPE plasma membrane (green arrowheads) and elastic layer of BrM (black arrowheads), as well as (E) BrM thickening. Scale bar: 1.5 μm. (G) Pathologic severity score calculations (maximum score = 12). WT mice (n = 4) exhibited little, if any, pathology (severity score 0.02 ± 0.02). fHm/m mice (n = 5) exhibited mild pathology (1.73 ± 0.58), as did fP−/− mice (n = 3; 0.97 ± 0.07). fHm/fP−/− female mice (n = 4) had somewhat worse degeneration than fHm/m and fP−/− mice (2.35 ± 0.29). However, fHm/m/fP−/− male mice (n = 4) had considerably greater degeneration and much wider variability in scores than all three previous groups (5.70 ± 1.70). *P < 0.05, ***P < 0.001. Severity scores are presented as mean ± SEM.
Morphometric analysis of plastic sections from male mouse retinas. Photomicrographs of plastic sections from (A) WT, (B) fH/m, and (C) fP−/− mice revealed normal retinal histology. In contrast, sections from (D–F) fH/m/fP−/− mice demonstrated (D) ONL thinning, and (E) disrupted IS and OS. (F) ONL thickness was measured via counting of PR nuclei per column. Measurements were made in triplicate at intervals of 200 μm for a total range of 2000 μm on either side of the optic nerve (reference point 0 on the x-axis). There were no significant differences among WT (n = 3), fH/m (n = 3), and fP−/− (n = 3) mice. In comparison, the ONL in fH/m/fP−/− mice (n = 3) was significantly thinner. Asterisks represent significant differences between fH/m/fP−/− and WT mice. *P < 0.05. (A–D) Scale bar: 25 μm. (E) Scale bar: 50 μm. GCL, ganglion cell layer.
nuclei revealed a significantly thinner ONL in fHm/m/fP−/−C0 mice compared to other genotypes (Fig. 4F).

Fluorescence Photomicrographs Showed Increased C3 Staining in fHm/m/fP−/− Mice

Previously, deposition of C3 in the kidneys of fHm/m mice was found in mesangium and along the capillary loops of glomeruli. However, C3 staining was brighter and restricted to the capillary loops in glomeruli of fHm/m/fP−/− mice. To determine whether the retina also had C3 deposits, we performed immunolabeling for C3 in the retina (Fig. 5).

Compared to WT, fHm/m, and fP−/− mice (Figs. 5A–C), we detected prominent sub-RPE C3 deposition in fHm/m/fP−/− mice (Figs. 5D, 5E, 5K, 5L). Choroidal labeling also was observed in some fHm/m/fP−/− mice (Fig. 5D), while in other mice patches of PR outer segments also were stained (Fig. 5E). Higher magnification images that were costained with an antibody against RPE65 further highlighted the sub-RPE C3 deposition in fHm/m/fP−/− mice (Figs. 5H–K). Pixel density analysis revealed a significantly increased C3 signal in the sub-RPE/BrM space of fHm/m/fP−/− mice (Fig. 5G). Double-labeling with anti-rhodopsin antibody, which labels the PR outer segments (IS/OS; Fig. 5M), confirmed C3 deposits in the sub-

Figure 5. Fluorescence photomicrographs showed more C3 staining in fHm/m/fP−/− mice. Relative to (A) WT, (B) fHm/m, (C) fP−/−, and (F) labeling with isotype control antibody in fHm/m/fP−/− retinas, the C3 signal in (D, E) fHm/m/fP−/− mice was more striking and localized in (D) sub-RPE areas, choroid, and (E) PR outer segments. (G) Mean pixel density of fluorescence showed that C3 signal in the sub-RPE/BrM region was significantly higher in fHm/m/fP−/− (n = 5) than in other genotype (n = 2 in WT, fHm/m, and fP−/−) mice. *P < 0.05; **P < 0.01. Scale bars: mean ± SEM. (H–K) Higher magnification images with RPE65 staining showed that the C3 signal is more prominent under the RPE cells in (K) fHm/m/fP−/− compared to (H) WT, (I) fHm/m, and (J) fP−/− mice. In contrast with rhodopsin signal in PR OS (M), C3 is localized in the OS and sub-RPE region (L, N). (A–F, L–N) Scale bar: 100 μm. (H–K) Scale bar: 50 μm.
higher serum C5 levels than fHm/m mice (anti-C5 antibody (0.001).<ref>

Differences in amplitude for rod-b and rod-a waves between male fHm/m/fP and WT mice were statistically significant. (D) male fHm/m/fP (n = 9) and female (n = 7) mice had significantly higher serum C5 levels than fHm/m mice (n = 4), and there was no significant difference in C5 levels between fHm/m/fP/male and WT mice (n = 4). (E) In fHm/m/fP/mice that received systemic anti-C5 antibody treatment (n = 5), the numbers of hypopigmented spots on fundus imaging were significantly decreased compared to control mice treated with control antibody (n = 4). (F) ERG amplitudes were higher in fHm/m/fP/mice treated with anti-C5 antibody (n = 8) compared to control antibody (n = 5), and the rod-a wave amplitude was significantly protected. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bars: mean ± SEM.

FIGURE 6. ERG and hypopigmented spots in different groups of mice. (A) rod-b, (B) rod-a, and (C) cone b-wave amplitudes were lower in male fHm/m/fP (n = 8) compared to female (n = 8), fHm/m (n = 8), fP (n = 4), and WT (n = 8) mice. Differences in amplitude for rod-b and rod-a waves between male fHm/m/fP and WT mice were statistically significant. (D) male fHm/m/fP (n = 9) and female (n = 7) mice had significantly higher serum C5 levels than fHm/m mice (n = 4), and there was no significant difference in C5 levels between fHm/m/fP/male and WT mice (n = 4). (E) In fHm/m/fP/mice that received systemic anti-C5 antibody treatment (n = 5), the numbers of hypopigmented spots on fundus imaging were significantly decreased compared to control mice treated with control antibody (n = 4). (F) ERG amplitudes were higher in fHm/m/fP/mice treated with anti-C5 antibody (n = 8) compared to control antibody (n = 5), and the rod-a wave amplitude was significantly protected. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bars: mean ± SEM.

RPE region and in small patches of outer segments (Figs. 5L–N).

ERG Showed Reduced Amplitudes in fHm/m/fP Mouse

We performed ERG to assess retinal PR function. The rod-b (Fig. 6A), rod-a (Fig. 6B), and cone b-wave (Fig. 6C) amplitudes were lower in male fHm/m/fP mice relative to females and to the other genotypes. The reduction relative to WT was significant in rod-b and rod-a waves (Figs. 6A, 6B; **P < 0.01).

Properdin Deficiency Increases Plasma C5 Levels in fHm/m Mice and Anti-C5 Antibody can Rescue the Retinal Degeneration

Previously, we found a significant increase of intact C3 and C5 in the plasma of fHm/m/fP/mice compared to fHm/m mice, indicating that P deficiency partially decreased AP and terminal complement consumption.50,51 Restoration of plasma C5 in fHm/m/fP/mice was confirmed in the current study (Fig. 6D). Male (n = 9) and female (n = 7) fHm/m/fP/mice had significantly higher serum C5 levels than fHm/m mice (n = 4, **P < 0.01, ***P < 0.001). There was no significant difference in C5 levels between fHm/m/fP/male and fHm/m/fP/female mice (n = 4), whereas levels in fHm/m/fP/female mice were significantly reduced compared to WT mice (P < 0.01). In a previous study of lethal glomerulonephritis in fHm/m/fP mice, we demonstrated that blocking C5 with a monoclonal antibody starting at 4 weeks of age prevented renal disease and death.56 To determine if rapid onset retinopathy in fHm/m/fP mice also is mediated by C5, we treated a group of fHm/m/fP mice with either an anti-C5 mAb (n = 8) or an isotype control mAb (n = 4). Specifically, the anti-C5 mAb binds to and inhibits cleavage of intact C5 into C5a and C5b. Each mouse (weighing 20–25 g) received 1 mg of mAb per injection, given that previous studies with the BB5.1 clone showed that 1 mg reduced up to 80% of serum hemolytic activity.57 Treatment started at 4 weeks of age, continuing for 12 weeks in the anti-C5 mAb group but only 4 weeks in the control mAb group due to early mortality. We found that fHm/m/fP mice treated with the anti-C5 mAb had fewer hypopigmented lesions on fundus photography compared to control antibody-treated mice (Fig. 6E). This significant difference was even more impressive given that the anti-C5 mAb group was 8 weeks older than the control mAb group due to early mortality. We found that fHm/m/fP mice treated with the anti-C5 mAb had fewer hypopigmented lesions on fundus photography compared to control antibody-treated mice (Fig. 6E). This significant difference was even more impressive given that the anti-C5 mAb group was 8 weeks older than the control mAb group, potentially allowing for more age-dependent retinal degeneration. Correspondingly, ERG amplitudes also were higher in fHm/m/fP/mice treated with anti-C5 mAb (Fig. 6F). In particular, the difference in rod-a wave amplitudes was significant.

DISCUSSION

We reported a mouse model of retinopathy seen in human DDD. Key changes in the eyes of our fHm/m/fP mice that
mirror human pathology include white spots on fundus imaging, PR death, and RPE pigmentary changes. In addition, we detected reductions in rod-b and -a wave amplitudes on ERG, which also is observed in patients with DDD when they suffer nyctalopia.38,39

The choriocapillary–BrM–RPE interface and the capillary tuft–GBM–epithelial interface are strikingly similar, which may explain why DDD patients sometimes have concurrent dense deposit pathology in the kidney and eye. 4 Patients typically do not experience visual symptoms until 10 to 15 years after diagnosis, when retinal atrophy and RPE clumping cause impairments in visual acuity and visual fields. Eventually, a large proportion of patients with DDD have multiple sub-RPE drusen that appear yellowish-white on funduscopy. 8,40,41 The drusen usually are found clustered in the macula, although larger soft drusen in other regions also can be present. These drusen are histopathologically similar to the GBM deposits. 3,4,8 Further loss of visual acuity or fields may result from additional complications, including subretinal neovascularization, retinal detachment, and central serous retinopathy.42,43

Ultrastructural analysis in our fH m/m/fP/C0/C0 mice revealed BLamD. These deposits form between the RPE and RPE basal lamina as discrete pockets in aging normal eyes, but as a continuous layer in AMD eyes.44-47 A continuous layer of BLamD under the fovea is considered by some investigators to be a histologic definition of AMD, 48 and has been associated with advanced AMD risk.45 The primary components of BLamD are fibrous long-spacing collagen, as well as esterified and unesterified cholesterols.44 It has been hypothesized that by separating the RPE from its basal lamina, BLamD may promote transport of membranous debris into the inner collagenous zone of BrM. This debris then may accumulate and facilitate formation of basal linear deposits and soft drusen.49

In addition, BLamD can contain complement components, including C3.46 Because we detected increased C3 immunolabeling in the BrM of fH m/m/fP/C0/C0 mice, complement activation and deposition is likely to contribute to BLamD formation in our model. Moreover, since C3 circulates in serum and is produced/secreted locally by the RPE,50–52 C3 from both sources may contribute to the observed deposits.

However, BLamD is not limited to AMD eyes. Although the presence of BLamD is 80% sensitive for AMD in eyes over 60 years old, it only has a specificity of 18%.53 Indeed, BLamD has been identified in various mouse models of aging as a more general marker of RPE stress. 54 Environmental stressors also have caused formation of BLamD, including cigarette smoke exposure55 and laser photochemical injury56 in mice on a high-fat diet. In humans, BLamD has been reported in other adult-onset forms of retinal degeneration, including Sorsby fundus dystrophy, late-onset retinal degeneration, and Malattia Leventinese/Doyne’s honeycomb retinal dystrophy.57–59 BLamD was reported previously in an fH knockout mouse model that exhibited pathologic changes in the kidney.60,61 We added to this observation by showing that the fH/fP double mutant mouse model of lethal DDD had significant BLamD. Further, while the fH knockout mice were characterized at 8 to 24

![Figure 7](https://example.com/f7.png)
months, our mice were studied at 8 weeks of age, showing accelerated degeneration.

The aforementioned pathologic changes in fH<sup>m/m</sup>/fP<sup>−/−</sup> mice resulted from modifications to the AP of complement that led to increased autologous complement-mediated tissue injury. The fH mutation impaired fH cell surface attachment, and in theory would render retinas more susceptible to complement-mediated damage. However, due to low expression of the truncated fH and consequent lack of sufficient AP complement regulation in the fluid phase, C3 and C5 were largely consumed, and this paradoxically protected the kidney and eye from more severe injury in fH<sup>m/m</sup> mice (Fig 7A). Previously, we found that C3 and C5 consumption was reduced in fH<sup>m/m</sup>/fP<sup>−/−</sup> mice compared to fH<sup>m/m</sup> mice,50,56 suggesting that fP-deficiency resulted in more C3 and C5 available to damage the kidney and eye in an fP-independent manner.62 With impaired fH, excess C3b accumulated in the PR outer segments and sub-RPE/BuM regions (Fig 7B). Furthermore, the efficacy of anti-C5 antibody in reducing hypopigmented spots on fundus imaging and improving ERG amplitudes suggested that terminal complement was excessively activated and contributed to the eye phenotype of fH<sup>m/m</sup>/fP<sup>−/−</sup> mice.

An important question concerning complement-mediated retinopathy is the relative contributions of local versus systemic complement. Toomey et al. reported that RPE degeneration was lost in mice induced by a high fat diet only in 2-year-old fH<sup>−/−</sup> mice, not in fH<sup>−/−</sup> mice because of retention of serum C3 in fH<sup>−/−</sup>/mice versus complete serum C3 consumption in fH<sup>−/−</sup> animals.60 This is consistent with our results showing that higher C3 and C5 levels in fH<sup>m/m</sup>/fP<sup>−/−</sup> mice relative to fH<sup>m/m</sup> mice led to more C3 and C5 activation in the kidney and eye, with consequent degeneration of those tissues. Our finding that administration via intraperitoneal injection of anti-C5 mAb ameliorated local retinopathy in fH<sup>m/m</sup>/fP<sup>−/−</sup> mice further supports the role of systemic complement in contributing to retinal injury in the setting of fH dysfunction. Interestingly, male fH<sup>m/m</sup>/fP<sup>−/−</sup> mice exhibited more severe retinal pathology than females. A potential reason for this difference is that male mice have higher baseline serum C5 levels, which has been attributed to differences in sex hormones, such as testosterone.34,55 In support of this hypothesis, we found that intact C5 levels were significantly lower in female fH<sup>m/m</sup>/fP<sup>−/−</sup> compared to WT mice, whereas there was no difference between male fH<sup>m/m</sup>/fP<sup>−/−</sup> and WT mice. In addition, there was significant variability in phenotype severity among male fH<sup>m/m</sup>/fP<sup>−/−</sup> mice, including two mice with no hypopigmented spots on fundus photographs. This indicated incomplete penetrance of the fH<sup>m/m</sup>/fP<sup>−/−</sup> mutations, similar to the penetrance of defects induced by fH mutations in other organs, such as the kidney.

Our finding that anti-C5 mAb therapy ameliorated retinopathy in fH<sup>m/m</sup>/fP<sup>−/−</sup> mice suggested that C5 activation participated in the eye pathophysiology of this DDD mouse model. This result is consistent with our recent study showing that blocking C5 also prevented lethal glomerulonephritis in fH<sup>m/m</sup>/fP<sup>−/−</sup> mice.56 A recent study testing anti-C5a therapy in a fH<sup>−/−</sup> mouse model on a high fat diet showed that the treatment was ineffective.57 Perhaps an anti-C5 therapy that blocks C5a and membrane attack complex (MAC) formation would be more effective, as in our study. Furthermore, our results suggested a potential therapeutic role for anti-C5 therapy to treat retinopathy associated with DDD. Current guidelines recommend an ophthalmic exam on presentation and annual screenings beginning 10 years after diagnosis, with prompt treatment for neovascular membranes.42 Investigations of new treatment strategies for DDD-associated retinopathy are especially useful given that increasing survival after renal transplantation will likely increase its prevalence.6

In summary, we characterized the fH<sup>m/m</sup>/fP<sup>−/−</sup> mouse as a novel model of DDD-related retinopathy, with notable BLamD formation. The efficacy of systemic anti-C5 antibody treatment suggested that serum complement contributes to the observed pathology. This unique mouse represents a new tool to study complement-mediated rapid-onset retinal disease and RPE stress, and is a useful model to study BLamD in the absence of age-related confounding variables. Future directions for research include using electroretinography to assess RPE function in this model, dissecting how complement dysfunction may be associated with melanosome abnormalities, and determining which complement components are necessary for BLamD formation and for RPE/PR degeneration.

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