Differences in Intraretinal Pigment Migration Across Inherited Retinal Dystrophies

JIN KYUN OH, SARAH R. LEVI, JOONPYO KIM, JOSE RONALDO LIMA DE CARVALHO JR, JOSEPH RYU, JANET R. SPARROW, AND STEPHEN H. TSANG

• PURPOSE: To determine whether there are differences in the prevalence of intraretinal pigment migration (IPM) across ages and genetic causes of inherited retinal dystrophies (IRDs).
• DESIGN: Retrospective cohort study.
• METHODS: Patients were evaluated at a single tertiary referral center. All patients with a clinical diagnosis of IRD and confirmatory genetic testing were included in these analyses. A total of 392 patients fit inclusion criteria, and 151 patients were excluded based on inconclusive genetic testing. Patients were placed into 3 groups, ciliary and ciliary-related photoreceptor, nonciliary photoreceptor, and retinal pigment epithelium (RPE), based on the cellular expression of the gene and the primary affected cell type. The presence of IPM was evaluated by using slit lamp biomicroscopy, indirect ophthalmoscopy, and wide-field color fundus photography.
• RESULTS: IPM was seen in 257 of 339 patients (75.8%) with mutations in photoreceptor-specific genes and in 18 of 53 patients (34.0%) with mutations in RPE-specific genes ($P < .0001$). Pairwise analysis following stratification by age and gene category suggested significant differences at all age groups between patients with mutations in photoreceptor-specific genes and patients with mutations in RPE-specific genes ($P < .05$). A fitted multivariate logistic regression model was produced and demonstrated that the incidence of IPM increases as a function of both age and gene category.
• CONCLUSIONS: IPM is a finding more commonly observed in IRDs caused by mutations in photoreceptor-specific genes than RPE-specific genes. The absence of IPM does not always rule out IRD and should raise suspicion for disease mutations in RPE-specific genes. (Am J Ophthalmol 2020;217: 252–260. © 2020 Elsevier Inc. All rights reserved.)

INHERITED RETINAL DISEASES (IRDS) REFER TO A GROUP of rare, heterogeneous disorders that affect an estimated 1 in 2,000 individuals worldwide.[1,2] These irreversible disorders are currently a leading cause of blindness, and although disease due to biallelic mutations in *RPE65* has been successfully treated and other causes of disease are currently being targeted for therapy, most of these IRDs are currently untreatable.[2,3] Typically, the genetic cause of these conditions can be traced either to mutations in genes that are expressed within the inner and outer segments of photoreceptors or the retinal pigment epithelium (RPE).[4] The phenotypic variability associated with these conditions reflects the manner in which photoreceptor and RPE cells respond to disease.[5–9] In IRDs involving mutations in genes expressed within the RPE, RPE degeneration may occur as a result of interruption of metabolic function (*CYP4V2*), accumulation of misfolded protein (*C1QTNF5*), or unclear mechanism (*RPE65*).[10–13] In contrast, IRDs caused by mutations within photoreceptor genes can lead to the degeneration of photoreceptors as a consequence of the accumulation of toxic substrates (*PDE6A* and *PDE6B*), stress-induced apoptosis (*RHO*), or unknown mechanisms (*USH2A*).[14–16]

A hallmark of inherited retinal dystrophies is the appearance of bone spicule pigmentation caused by the translocation of pigment-containing cells derived from the RPE to the surface of the retina.[4] Previously, this intraretinal pigment migration (IPM) was believed to occur after roughly 5 years in patients with a diagnosis of retinitis pigmentosa sine pigmento.[17] The identification of IPM is particularly important in order to rule out more severe disease such as cancer-associated retinopathy or melanoma-associated retinopathy, which typically have rapid symptomatic progression.[18] IPM was also shown to be relevant in the evaluation of disease severity in patients with recessive Stargardt disease. The appearance of IPM corresponded to a more severe phenotype.[19] As such, the appearance of IPM may serve as a critical diagnostic tool and biomarker for disease.

This study compared a large cohort of patients with IRDs caused by mutations in photoreceptor genes with those carrying mutations in RPE genes. Specifically, the authors

AJO.com Supplemental Material available at AJO.com.
Accepted for publication May 8, 2020.
From the Department of Ophthalmology (J.K.O., S.R.L., J.R.L.dC., J.R., J.R.S., S.H.T.), Columbia University Irving Medical Center, New York, New York, USA; College of Medicine, State University of New York at Downstate Medical Center (J.K.O.), Brooklyn, New York, USA; Department of Statistics (J.K.), Seoul National University, Seoul, South Korea; Department of Ophthalmology (J.R.L.dC.), Empresa Brasileira de Servicos Hospitalares, Hospital das Clinicas de Pernambuco, Federal University of Pernambuco, Recife, Pernambuco, Brazil; Department of Ophthalmology (J.R.L.dC.), Federal University of São Paulo, São Paulo, São Paulo, Brazil; Department of Pathology & Cell Biology, and Columbia Stem Cell Initiative, Columbia University Irving Medical Center, New York, NY, USA (J.R.S., S.H.T.).
Inquiries to: Stephen H. Tsang, Harkness Eye Institute, Columbia University Medical Center, 635 West 165th Street, Box 212, New York, New York 10032, USA; e-mail: sht2@cumc.columbia.edu

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0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2020.05.010

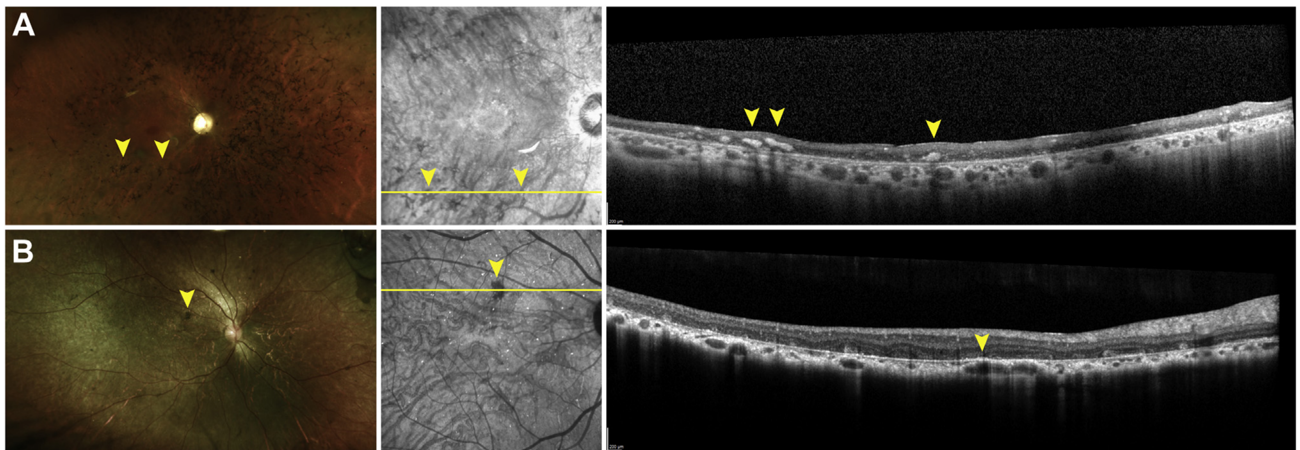

FIGURE 1. Intraretinal and subretinal pigment visualized on spectral-domain optical coherence tomography and color fundus photography. Spectral-domain optical coherence tomography images and wide-field color fundus photographs of 2 patients with mutations (A) in a ciliary gene (*MAK*) and (B) an RPE-specific gene (*CYP4V2*) are shown. (A) Intraretinal pigment has a bone spicule-like appearance in color fundus photographs and can be visualized on the near-infrared reflectance scan and within the neurosensory retina in the spectral-domain optical coherence tomography (yellow arrowheads). (B) In contrast, subretinal pigment appears nummular (coin-like) in color fundus photographs and is seen deposited beneath the outer retinal layers and above the retinal pigment epithelium (yellow arrowheads). In both cases, posterior shadowing beneath the pigment is seen on spectral-domain optical coherence tomography.

hypothesized that patients with mutations in RPE-specific genes would not have evidence of IPM as mutations in RPE-specific genes would lead to primary RPE degeneration as opposed to migration. In contrast, it was hypothesized that IPM would be prominent in patients with mutations in photoreceptor-specific genes due to photoreceptor degeneration leading to loss of the apposition between photoreceptors and RPE cells, allowing for RPE cells to access the neurosensory retina. Given that genes associated with ciliary disease have been linked to more rapid disease progression, an additional distinction was made between ciliary and ciliary-related (CR) genes and nonciliary photoreceptor (PR) genes in order to identify potential differences in the prevalence and timing of IPM.[20]

SUBJECTS AND METHODS

• **PATIENT SELECTION:** A retrospective chart review was performed of patients seen and evaluated at Columbia University Medical Center between January 2009 and December 2019 whose clinical diagnosis was IRD and had received confirmatory diagnostic genetic testing. Clinical diagnosis of these patients was performed using a combination of complete ophthalmic examination, imaging, and full-field electroretinography testing. A total of 392 patients were identified who fit inclusion criteria, and 151 patients were excluded based on inconclusive genetic testing. Of the 392 patients, a total of 343 patients had a diagnosis with a rod-first pattern of degeneration, including retinitis pigmentosa, late-onset retinal degeneration, Bietti crystalline dystrophy, and choroideremia. The remaining 49 patients had diagnoses of macular, cone, or cone-rod dystrophy. The study was conducted under Columbia University Institutional Review Board approval (protocol AAAR8743) and the need for informed consent was waived due to the retrospective nature of the study design and the minimal risk conferred to patients. All procedures were carried out in accordance with the tenets of the Declaration of Helsinki.

• **GENE CLASSIFICATION:** Patients were divided into 1 of 3 categories (CR, PR, and RPE) based primarily on the subcellular localization of the gene product and, if known, the function of the gene implicated in their condition as described in medical literature. Genes with protein products localizing to the connecting cilium, including the basal bodies and the ciliary axoneme, the periciliary membrane complex, or the calyceal processes of photoreceptors were labeled CR genes. All other photoreceptor specific genes were labeled PR genes. Finally, genes specific to the RPE were labeled RPE genes. Patients with choroideremia due to mutations in *CHM* were placed within the RPE cohort, as studies have suggested that, although choroideremia affects photoreceptors and the RPE independently, choroideremia predominantly affects the RPE.[21–23]

• **INTRARETINAL PIGMENT MIGRATION EVALUATION:** Each patient underwent slit lamp biomicroscopy and indirect ophthalmoscopy of the posterior pole and peripheral

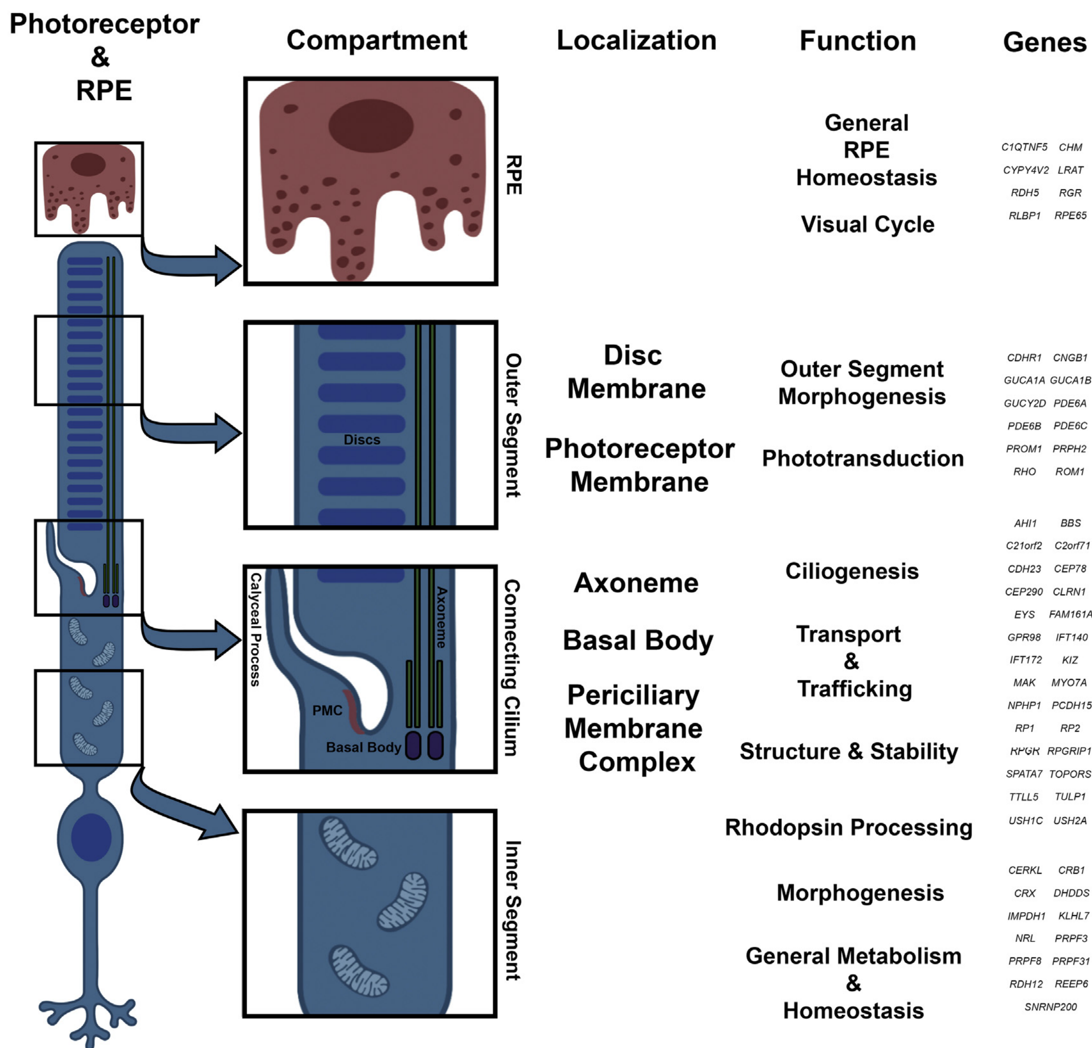

FIGURE 2. Cellular localization of genes associated with inherited retinal degenerations. A diagram of photoreceptors and retinal pigment epithelium (RPE) illustrates the various subcellular compartments of the photoreceptors, including the inner and outer segment as well as the connecting cilium and surrounding periciliary areas, which includes the periciliary membrane complex (PMC) and the calyceal processes. The functions of the inner and outer segment were categorized into photoreceptor morphogenesis, rhodopsin processing, phototransduction and general metabolism and homeostasis. The functions of the connecting cilium were simplified as ciliogenesis, ciliary transport and trafficking, and ciliary structure and stability. Finally, the functions of the RPE were classified as either general homeostasis or associated with the visual cycle.

retina following pupillary dilation with topical phenylephrine (2.5%) and tropicamide (1%). Afterward, each patient underwent a series of imaging tests including spectral domain-optical coherence tomography (SD-OCT) and digital fundus photography or wide-angle color fundus photography using an Optos 200 Tx unit (Optos; PLC, Dunfermline, United Kingdom). Pigment migration was defined as the visible presence of bone spicule pigmentation in the inner retina. Determination of the presence of IPM was made by slit lamp biomicroscopy and indirect ophthalmoscopy and corroborated by fundus images and SD-OCT. Fundus photos were evaluated at the initial and most recent visits for evidence of IPM. Cases with nummular (coin-like) subretinal pigment deposition were excluded based on evaluation of pigment seen on SD-OCT (Figure 1).

Statistical analysis was performed using R statistical software version 3.6.1 (R Foundation, Vienna, Austria). A Fisher exact test was performed to determine whether the prevalence of IPM was independent of the category of genes. Analysis was then repeated to determine whether the prevalence of IPM by category of genes differed following stratification by age. A multivariate logistic regression model using gene category and age category as independent variables was fitted to predict the prevalence of IPM based on gene category and age.

TABLE 1. Demographics and Diagnoses of 392 Patients with Inherited Retinal Dystrophy

	Sex		Diagnosis								
			Rod-First Degeneration						Cone-First Degeneration		
	Males	Females	ARRP	ADRP	XLRP	BCD	LORD	CHM	Cone-Rod Dystrophy	Cone Dystrophy	Macular Dystrophy
PR	70	70	52	54	0	0	0	0	4	10	20
CR	130	69	144	8	32	0	0	0	5	2	8
RPE	35	18	14	0	0	13	2	24	0	0	0

ADRP = autosomal dominant retinitis pigmentosa; ARRP = autosomal recessive retinitis pigmentosa; BCD = Bietti crystalline dystrophy; CHM = choroideremia; CR = patients with mutations in ciliary and ciliary-related photoreceptor-specific genes; LORD = late onset retinal degeneration; PR = patients with mutations in nonciliary photoreceptor-specific genes; RPE = patients with mutations in retinal pigment epithelium-specific genes; XLRP = X-linked retinitis pigmentosa.

RESULTS

• **GENE CLASSIFICATION:** Identified genetic causes of disease in the present cohort were mutations in the following PR genes *CDHR1*, *CERKL*, *CNGB1*, *CRB1*, *CRX*, *DHDDS*, *GUCA1A*, *GUCA1B*, *GUCY2D*, *IMPDH1*, *KLHL7*, *NRL*, *PDE6A*, *PDE6B*, *PDE6C*, *PROM1*, *PRPF3*, *PRPF8*, *PRPF31*, *PRPH2*, *RDH12*, *REEP6*, *RHO*, *ROM1*, and *SNRNP200*; in the following CR genes: *AHI1*, *BBS1*, *BBS10*, *C21orf2*, *C2orf71*, *CDH23*, *CEP290*, *CEP78*, *CLRN1*, *EYS*, *FAM161A*, *GPR98*, *IFT140*, *IFT172*, *KIZ*, *MAK*, *MYO7A*, *NPHP1*, *PCDH15*, *RP1*, *RP2*, *RPGR*, *RPGRIP1*, *SPATA7*, *TTLL5*, *TOPORS*, *TULP1*, *USH1C*, and *USH2A*; and in the following RPE genes: *C1QTNF5*, *CHM*, *CYP4V2*, *LRAT*, *RDH5*, *RDH11*, *RGR*, *RLBP1*, and *RPE65*. Gene functions and localizations are summarized in Figure 2 and Supplemental Table 1.

• **PATIENT SUMMARY:** A total of 392 patients were evaluated. Within this cohort were 140 patients in the PR group, 199 patients in the CR group, and 53 patients in the RPE group. Within these 3 groups, 106 patients in the PR group, 184 patients in the CR group, and 53 patients in the RPE group had diagnoses with a type of rod-first degeneration. Mean and median ages at initial evaluation were 40 and 37.5 for the PR cohort, 41.3 and 43 for the CR cohort, and 37 and 34 for the RPE cohort, respectively. Mean follow-up time for all patients who were seen at more than 1 visit was 4.09, 3.77, and 2.8 years in these 3 groups, respectively. Demographic and diagnostic information are summarized in Table 1.

• **INTRARETINAL PIGMENT MIGRATION:** On evaluation of the PR cohort, a total of 99 of 140 patients (71%) had evidence of IPM at the most recent visit. A total of 95 of the 106 patients (90%) with a diagnosis of rod-first degeneration and 4 of the 34 patients (12%) with a diagnosis of cone-first degeneration were found to have IPM in the PR cohort. A total of 158 of 199 patients (79%) had signs of IPM on examination of the CR cohort. Prevalence of IPM in patients with rod-first degeneration was 154 of 184 (84%) compared to 4 of 15 patients (27%) with cone-first degeneration within this cohort. In the RPE cohort, the prevalence of IPM was 18 of 53 patients (34%). Two of 14 patients (14%) with RP and 16 of 24 patients (67%) with choroideremia had IPM, whereas no patients with Bietti crystalline dystrophy or late-onset retinal degeneration showed any signs of IPM. Comparison of the 3 cohorts following stratification by age was performed among the patients with a diagnosis of rod-first degeneration. Patients in each group were divided into 5 age categories (0-15, 16-30, 31-45, 46-60, and 61+ years old). The prevalence of IPM by age can be seen in Table 2.

The Fisher exact test was applied to determine the homogeneity of prevalence of IPM among the 3 gene categories, yielding a P value of 1×10^{-13}, suggesting a significant difference. Following further stratification of prevalence rates by age, Fisher exact test results demonstrated significant differences among the prevalence rates of IPM in each age group ($P < .02$). Pairwise comparison of gene categories revealed no significant differences between the PR and CR group at any age group; however, significant differences were seen between the PR and RPE cohorts at all age groups ($P < .05$) and between the CR and RPE cohorts at all age groups ($P < .05$), except for those who were 0-15 and 16-30 years old (Supplemental Data 1).

A multivariate logistic regression model was first produced using only the gene category as the explanatory variable. The model (Supplemental Data 2 and 3, Supplemental Tables 2 through 5) similarly suggested statistically significant differences in rates of IPM between the CR and RPE gene categories and the PR and RPE gene categories ($P < 1 \times 10^{-10}$). Prevalence rates predicted for IPM were 0.90, 0.84, and 0.34 among the PR, CR, and RPE gene categories, respectively. The model was then refitted to include age as an additional explanatory variable and revealed persistent statistical significance in the prevalence of IPM among the gene categories.

TABLE 2. Prevalence of Intraretinal Pigment Migration as Stratified by Age and Gene Category In Patients with Rod-First Degeneration

Age, y	PR Incidence	CR Incidence	RPE Incidence	Fisher Exact Test P Values
0-15 n/N (%)[a]	10/14 (71%)	9/23 (39%)	0/7 (0%)	.002
16-30 n/N (%)[a]	20/24 (83%)	26/36 (72%)	6/15 (40%)	.017
31-45 n/N (%)[a]	28/30 (93%)	45/46 (98%)	4/13 (31%)	1.22×10^{-7}
46-60 n/N (%)[a]	17/18 (94%)	47/50 (94%)	4/11 (36%)	5.20×10^{-5}
61+ n/N (%)[a]	20/20 (100%)	27/29 (93%)	4/7 (57%)	.006
Cumulative n/N (%)[a]	95/106 (90%)	154/184 (84%)	18/53 (34%)	5.97×10^{-14}

CR = ciliary and ciliary-related gene; PR = photoreceptor gene; RPE = retinal pigment epithelium gene.
[a]Statistically significant at $P < .05$.

TABLE 3. Predicted Incidence of Pigment Migration in Patients with Rod-First Degeneration by Age and Gene Category, Using a Fitted Regression Model

Age, y	PR Incidence (95% CI)	CR Incidence (95% CI)	RPE Incidence (95% CI)
0-15	0.61 (0.41-0.78)	0.44 (0.28-0.61)	0.05 (0.02-0.13)
16-30	0.87 (0.75-0.94)	0.78 (0.65-0.87)	0.20 (0.10-0.36)
31-45	0.96 (0.90-0.98)	0.92 (0.84-0.96)	0.45 (0.26-0.65)
46-60	0.96 (0.89-0.98)	0.92 (0.83-0.96)	0.44 (0.25-0.65)
61+	0.97 (0.92-0.99)	0.95 (0.86-0.98)	0.57 (0.30-0.80)

CI = confidence interval; CR = ciliary and ciliary related gene; PR = photoreceptor gene; RPE = retinal pigment epithelium gene.

Moreover, it also suggested that prevalence of IPM increases with age ($P < 1 \times 10^{-14}$). A summary of the predicted prevalence rates by gene category and age grouping are shown in Table 3, and their respective odds ratios are illustrated in the Supplemental Figure.

DISCUSSION

IPM IS A DEFINING FEATURE OF IRDS. THESE CLUMPS OF pigment are known to be derived from RPE cells that detach from Bruch's membrane and migrate toward the inner retina following the degeneration of photoreceptors and loss of connection between the RPE and photoreceptors.[4,24,25] Despite detailed histologic understanding of what constitutes this classic pathologic finding, the mechanism and pathophysiology of IPM is poorly understood.[4] Prior studies in RPE models have suggested the role of vascular affinity of RPE cells, whereas others have implicated the inactivation of phosphatase and tensin homolog (PTEN) in the phosphatidylinositol 3-kinase pathway (PI3K) pathway.[4,24,26] Studies of the migration of RPE cells into the vitreous cavity in proliferative vitreoretinopathy have suggested that the process is dependent on a number of factors including tumor necrosis factor-alpha (TNF-alpha), epithelial growth factor receptor (EGFR), PI3K, and protein kinase B (AKT) signaling.[27–29]

To date, the IRDs and their phenotypes have been typically classified according to an inheritance pattern.[3,30,31] Fishman[30] first correlated prognosis with pattern of inheritance, showing that autosomal dominant RP had the mildest manifestations, followed by autosomal recessive RP, and most severely X-linked RP.[3,31] The gene-specific treatment of IRDs is also closely tied to the inheritance pattern, as both recessive and X-linked conditions require the introduction of 1 functional allele, whereas dominant mutations, such as those that behave in a dominant negative fashion, impair the function of the normal allele and necessitate a more complex intervention.[32–34] Despite these factors, there is an argument for a greater emphasis on the functional grouping of IRD causes, as both disease pathophysiology and mechanism are dependent on the causative gene.[35,36] This has been previously suggested in the medical literature, and prior studies of ciliopathies, compared to nonciliopathies involving the retina, have shown a more rapid phenotypic progression in ciliopathies.[19,35,36] In the present study, differences in IPM were examined across functional categories and suggested a role for IPM as a biomarker in candidate gene testing and the characterization of disease progression.

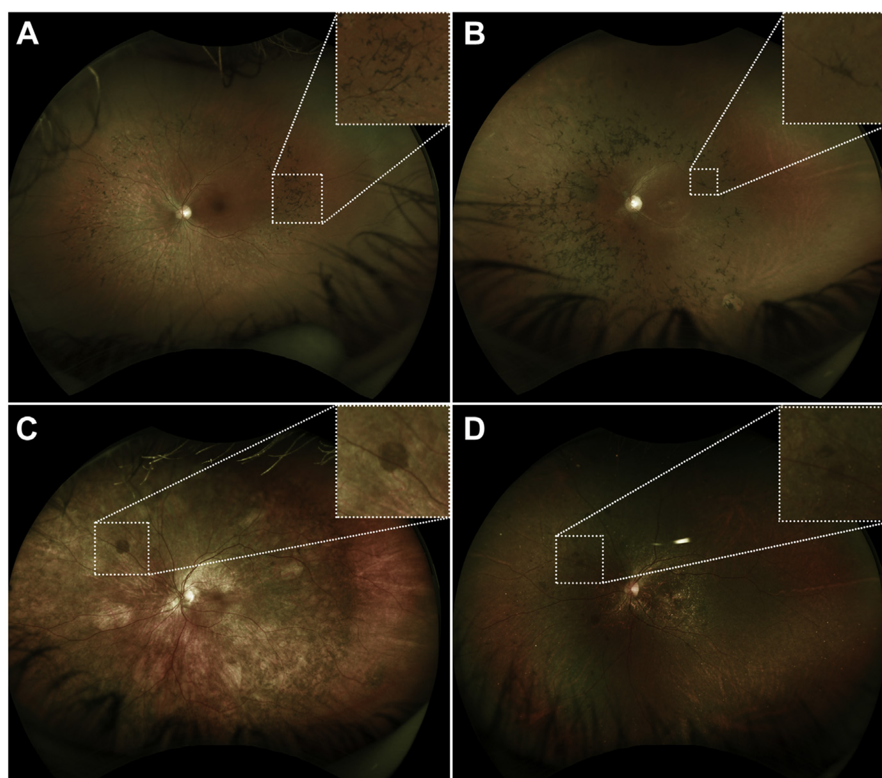

FIGURE 3. Intraretinal pigment migration in comparison with subretinal pigment migration near the branch vessels on color fundus photographs. Wide-field color fundus photos of patients with (A) a mutation in a phototransduction gene (*RHO*) and (B) mutations in a ciliary gene (*USH2A*) demonstrated the presence of intraretinal pigment overlying the ophthalmic veins, confirming that the pigment is anterior to the neurosensory retina. In contrast, fundus photos of patients with (C and D) mutations in the retinal pigment epithelium-specific genes *RGR* and *CYP4V2* respectively, demonstrated the presence of pigment below the vessels, suggestive of subretinal pigment.

Statistically significant differences in prevalence of IPM were seen among the 3 categories ($P < 1 \times 10^{-13}$) with the lowest prevalence of IPM found in patients with mutations in RPE genes. The absence of IPM in the RPE group was consistent with the hypothesis that, in retinal diseases caused by RPE-specific genes, RPE cells may undergo degeneration prior to secondary photoreceptor loss. Consequently, because the RPE degenerates while it remains apposed to intact photoreceptors, it is likely that they are not able to physically penetrate the neurosensory retina due to contact inhibition by the intact photoreceptor cell-RPE complex.[4,20,37] By the time secondary photoreceptor loss occurs, the RPE cells have already degenerated to a point where they are unable to undergo epithelial-mesenchymal transition and respond to PTEN inactivation and other stimuli that draw them toward the inner retina.[26,38] Notably, several patients in the RPE cohort, in particular those diagnosed with Bietti crystalline dystrophy and *RGR*-mediated retinal dystrophy, were found to have evidence of the nummular subretinal pigment clumping seen as deposits underneath the branch retinal veins and arteries on color fundus photographs (Figure 3). SD-OCT images through such pigment in several patients with Bietti crystalline dystrophy confirmed that these pigment were subretinal in all observed cases (Figure 4). Limitations of this analysis include the possibility that the presence of intraretinal pigment was not identified through the combination of dilated fundus examination and wide-field color fundus photography, especially in the periphery of severely progressive disorders such as Bietti crystalline dystrophy and late-onset retinal degeneration. Further studies using SD-OCT imaging in the far periphery will help confirm that these pigment clumps are subretinal even in the periphery.

Of the 18 patients in the RPE cohort who were found to have IPM, 16 patients had a clinical diagnosis of choroideremia. The relatively high prevalence of IPM in choroideremia compared to the other rod-cone degenerations may be explained by the unique pathophysiology of choroideremia. Choroideremia is found to be expressed in both photoreceptors and RPE cells, and while it is currently believed that the disease predominantly affects the RPE prior to photoreceptors, prior studies have suggested that the degeneration of the 2 cells occurs, to some degree, independently of one another.[21–23] Therefore, these authors hypothesize that the intraretinal pigment seen in patients

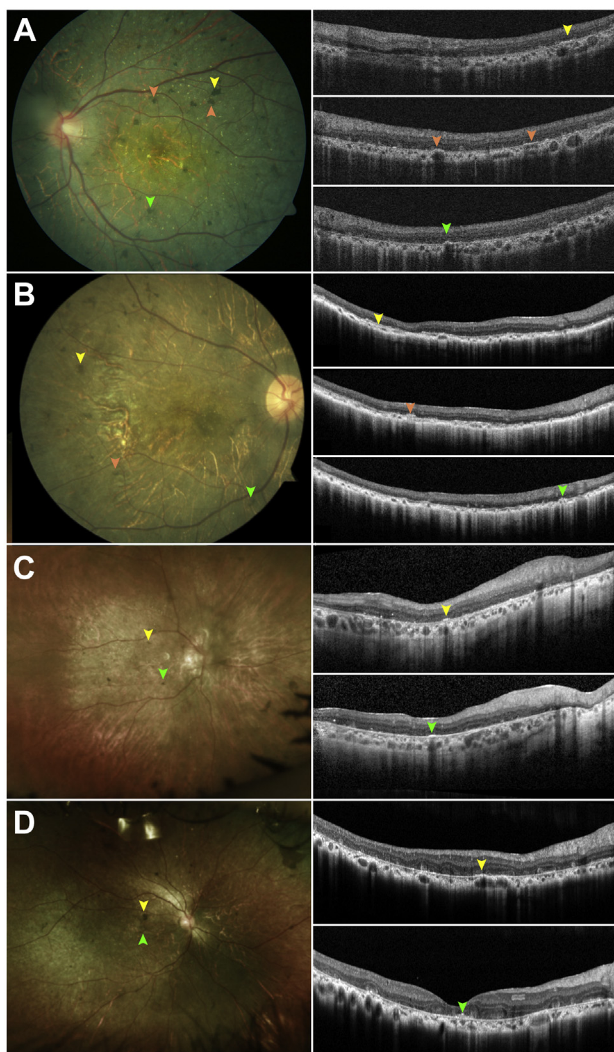

FIGURE 4. Subretinal pigment migration as seen on spectral-domain optical coherence tomography in patients with Bietti crystalline dystrophy. Color fundus photographs and optical coherence tomography of 4 patients with Bietti crystalline dystrophy (A through D) demonstrate that the foci of pigment on color photographs (arrowheads, yellow, orange, green) spatially correlate with subretinal deposits on optical coherence tomography (arrowheads, yellow, orange, green) as opposed to intraretinal deposits.

with choroideremia may occur at foci of the retina where photoreceptors degenerate concurrently or prior to RPE degeneration. This is in contrast to many other forms of IRDs, where degeneration of one cell type typically precedes the other.

No statistically significant differences were seen between the PR and CR groups overall. However, a relatively high prevalence of IPM was seen in the PR group (71%) compared to that in the CR group (39%) at 0-15 year of age. Prior studies have shown that ciliary dysfunction causes defects in RPE maturation that predate photoreceptor death, which suggests a potential mechanism for why IPM may develop more slowly and later on in the CR group compared to the PR group.[39] Limitations of this analysis include the small number of patients in each age group following stratification, and future evaluation of a larger cohort of patients may help elucidate significant differences between these 2 groups.

Finally, a multivariate logistic regression model was produced to predict prevalence of IPM, taking into account both age and gene categories. This model corroborated the fact that the prevalence of IPM was significantly higher in the CR and PR groups than in the RPE group and that the prevalence of IPM increases with age. Notably, deviance testing of the interaction between age and gene on IPM was found to be insignificant; however, adding age to the model resulted in a profound shift in the predicted prevalence of IPM based on gene category, suggesting that patient age may have an underlying effect on gene function as it relates to the development of IPM. Further mechanistic studies of the effects of age on various gene functions will be valuable in refining this model. Limitations of this predictive model include its fitting based on this specific cohort, and analysis of a larger cohort of patients will help validate the accuracy of the model. Additionally, the model does not take into consideration additional confounding factors which may influence the prevalence of pigment migration and the interactions between such factors.

IPM is a defining feature in several inherited retinal dystrophies, and the prevalence of pigment development was shown in this study to be a function of both age and gene category. In patients with PR and CR gene mutations, the fitted logistic regression model may serve as a predictor for the timeline and development of pigment in patients, supporting the use of IPM as a possible biomarker for monitoring disease progression and as a potential outcome measurement in future treatments that involve early intervention before appreciable pigmentary changes. Similarly, the lack of IPM seen in patients with RPE-specific gene mutations as compared to those with PR or CR gene mutations may be helpful to guide candidate gene testing.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

JIN KYUN OH: METHODOLOGY, INVESTIGATION, FORMAL analysis, Writing - original draft. **Sarah R. Levi:** Methodology, Investigation, Writing - original draft. **Joonpyo Kim:** Formal analysis, Methodology, Writing - review & editing. **Jose Ronaldo Lima de Carvalho:** Conceptualization, Methodology, Writing - review & editing. **Joseph Ryu:** Writing - review & editing, Visualization, Validation. **Janet R. Sparrow:** Writing - review & editing, Conceptualization, Supervision. **Stephen H. Tsang:** Writing - review & editing, Conceptualization, Project administration.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST and none were reported.

Funding/Support: Funding for this research was supported by the Jonas Children's Vision Care and Bernard & Shirlee Brown Glaucoma Laboratory are supported by the National Institutes of Health 5P30CA013696, U01 EY030580, U54OD020351, R24EY028758, R24EY027285, 5P30EY019007, R01EY018213, R01EY024698, R01EY024091, R01EY026682, R21AG050437, the Schneeweiss Stem Cell Fund, New York State [SDHDOH01-C32590GG-3450000], the Foundation Fighting Blindness New York Regional Research Center Grant [PPA-1218-0751-COLU], Nancy and Kobi Karp, the Crowley Family Funds, The Rosenbaum Family Foundation, Alcon Research Institute, the Gebroe Family Foundation, the Research to Prevent Blindness (RPB) Physician-Scientist Award, unrestricted funds from RPB, New York, NY, USA. The sponsor or funding organization had no role in the design or conduct of this research.

Financial disclosures: The authors have no financial disclosures.

The authors thank Jimmy K. Duong, consulting ophthalmic biostatistician, Department of Biostatistics, Columbia University, for validating the proposed model.

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