Quantitative Fundus Autofluorescence and Optical Coherence Tomography in PRPH2/RDS- and ABCA4-Associated Disease Exhibiting Phenotypic Overlap


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Purpose. To assess whether quantitative fundus autofluorescence (qAF), a measure of RPE lipofuscin, and spectral-domain optical coherence tomography (SD-OCT) can aid in the differentiation of patients with fundus features that could either be related to ABCA4 mutations or be part of the phenotypic spectrum of pattern dystrophies.

Methods. Autofluorescence images (30°, 488-nm excitation) from 39 patients (67 eyes) were acquired with a confocal scanning laser ophthalmoscope equipped with an internal fluorescent reference and were quantified as previously described. In addition, horizontal SD-OCT images through the fovea were obtained. Patients were screened for ABCA4 and PRPH2/RDS mutations.

Results. ABCA4 mutations were identified in 19 patients (mean age, 37 ± 12 years) and PRPH2/RDS mutations in 8 patients (mean age, 48 ± 13 years); no known ABCA4 or PRPH2/RDS mutations were found in 12 patients (mean age, 48 ± 9 years). Differentiation of the groups using phenotypic SD-OCT and AF features (e.g., peripapillary sparing, foveal sparing) was not reliable. However, patients with ABCA4 mutations could be discriminated reasonably well from other patients when qAF values were corrected for age and race. In general, ABCA4 patients had higher qAF values than PRPH2/RDS patients, while most patients without mutations in PRPH2/RDS or ABCA4 had qAF levels within the normal range.

Conclusions. The high qAF levels of ABCA4-positive patients are a hallmark of ABCA4-related disease. The reason for high qAF among many PRPH2/RDS-positive patients is not known; higher RPE lipofuscin accumulation may be a primary or secondary effect of the PRPH2/RDS mutation.

Keywords: ABCA4, lipofuscin, optical coherence tomography, pattern dystrophy, PRPH2/RDS, quantitative fundus autofluorescence, recessive Stargardt disease, retinal pigment epithelium, scanning laser ophthalmoscope

Recessive Stargardt disease (STGD1), caused by mutations in the gene ABCA4,1 follows an autosomal recessive mode of inheritance and usually manifests in the first two decades of life,2 although late-onset forms of STGD1 also exist.3 Typical phenotypic features of STGD1 include intensely fluorescent foci (flecks) that emerge with disease progression, mottling of the retinal pigment epithelium (RPE), and central atrophy.4,5 These fundus changes can be confined to the macula or extend beyond the vascular arcades. Another fundus feature that is considered pathognomonic for ABCA4-related disease is relative sparing of the peripapillary area from flecks and atrophy.6,7 However, peripapillary sparing does not necessarily persist with ABCA4-related disease progression.8 Foveal atrophy is common in STGD1 patients, even at young ages, although in some patients the fovea is spared.9 On fluorescein angiography, STGD1 patients also often, but not always, exhibit a "dark choroid."10

Despite the frequency with which STGD1 can be defined by these features, the phenotype is not necessarily exclusive to STGD1. For instance, peripapillary sparing can also be present in cases of pattern dystrophy (PD) without ABCA4 mutations,11 and both STGD1 and PD can present with pigmentary changes identified as flecks and mottling.12 Pattern dystrophy refers to a genetically heterogeneous group of retinal disorders, with mutations in PRPH2/RDS being the most common cause.4 Pattern dystrophy is usually inherited in an autosomal dominant manner and usually manifests in midlife with mild-to-moderate vision loss.13,14 In PD with an absence of ABCA4 mutations, foveal atrophy is generally found only in late stages of the disease.13

Histopathological studies, spectrophotometric analysis, and studies in mouse models have demonstrated over many years that the formation of RPE cell lipofuscin is augmented and pathogenic in ABCA4-associated disease.15-18 Retinal pigment epithelium lipofuscin, a mixture of fluorophores with spectral features that reflect its bisretinoid constituents,19 is the primary source of fundus AF, with a minor contribution from Bruch’s membrane.20 We recently demonstrated that the increase in
RPE lipofuscin in STGD1 can be measured indirectly by quantifying fundus autofluorescence intensities in images acquired by confocal scanning laser ophthalmoscope (cSLO).\textsuperscript{21,22} Quantitative fundus autofluorescence (qAF) was also shown to be a valuable tool for distinguishing \textit{ABCA4}-related from non-\textit{ABCA4}-related bull’s-eye maculopathy (BEM).\textsuperscript{23}

Here we present a patient cohort that, because they presented with clinical features of STGD1, were referred to our medical center for \textit{ABCA4} genetic screening. In all patients the clinical evaluation included qAF imaging and analysis. We report that some of these patients were ultimately found to carry mutations in \textit{PRPH2}/\textit{RDS}. The inheritance pattern of PD can often not be readily discerned because incomplete penetrance\textsuperscript{24} and variable expression can mask dominant inheritance. Whether \textit{PRPH2}/\textit{RDS} patients exhibit increased lipofuscin levels has been a matter of debate.\textsuperscript{4,25} Thus our objective was to consider whether qAF can assist in distinguishing \textit{ABCA4}-related disease from disease caused by mutations in \textit{PRPH2}/\textit{RDS} and other unknown genes. Given the phenotypic overlap between some cases of \textit{ABCA4} and \textit{PRPH2}/\textit{RDS-associated disease},\textsuperscript{4,5} we also assessed AF and spectral-domain optical coherence tomography (SD-OCT) images qualitatively to attempt to identify characteristic fundus features that may aid in the differentiation of these diseases. \textit{PRPH2}/\textit{RDS}-associated PD is generally considered to have a better prognosis than \textit{ABCA4}-associated disease; thus it is important to differentiate the two conditions clinically.

**METHODS**

**Patients and Genetic Testing**

The cohort consisted of 39 patients (67 eyes) from 35 families. All patients were prospectively recruited at Columbia University, were examined by a retina specialist (SHT), and had clear media except for some floaters. Cases included those for which STGD1 could be considered as a potential diagnosis, with the final diagnosis depending on the results of genetic testing. Recruitment to the cohort was based on the presence of central atrophy, mottling, and/or macular flecks in fundus AF (488-nm excitation) images. Patients included in this cohort were (1) those presenting with fundus features that could be observed in both confirmed \textit{ABCA4}-associated disease and in patients considered to carry the PD phenotype (central lesion with jagged border, butterfly-shaped lesion in the macula); (2) patients with a phenotype having some features that were atypical for \textit{ABCA4}-associated STGD1 (no peripapillary sparing; atypical size, shape, and distribution of flecks); and (3) patients in whom \textit{ABCA4} mutations were not detected despite \textit{ABCA4}-like fundus features but in whom additional genetic screening revealed \textit{PRPH2}/\textit{RDS} mutations. The study cohort was compared to 374 healthy eyes of 277 subjects with no family history of inherited retinal dystrophies\textsuperscript{26} and 36 of 42 previously reported patients\textsuperscript{27} (57 eyes) with confirmed \textit{ABCA4} mutations that did not have a PD-like phenotype. Visual acuity data, recorded as logMAR, was obtained using the recently obtained refractive correction and a Snellen chart.

Screening with the \textit{ABCA4} array was performed on most study subjects followed by direct Sanger sequencing to confirm identified changes, as previously described.\textsuperscript{28} Since recruitment proceeded over many years, versions of the \textit{ABCA4} chip included the least representative (~300 mutations) to the most recent version of the array (>600 variants). When array screening identified only one mutated \textit{ABCA4} allele or no \textit{ABCA4} mutations, next-generation sequencing (NGS) was carried out. In the latter case, the 50 exons and exon–intron boundaries of the \textit{ABCA4} gene were amplified (Illumina TruSeq Custom Amplicon protocol; Illumina, San Diego, CA, USA), and then submitted to NGS on the Illumina MiSeq platform with analysis using the variant discovery software NextGENe (SoftGenetics LLC, State College, PA, USA) and reference genome GRCh37/hg19. Variants were confirmed by Sanger sequencing and analyzed with Alamut software (http://www.interactive-biosoftware.com (in the public domain)). To assess cosegregation of the new variants with disease status, family members were studied if available.

Genetic analyses were originally performed according to the following scheme. Patients whose phenotypes were more likely compatible with \textit{ABCA4}-associated disease were first screened for variants in the \textit{ABCA4} gene. If two or more \textit{ABCA4} variants were identified that segregated with the disease, the case was considered an \textit{ABCA4}-associated disease. All patients in whom complete sequencing of the gene identified only one or no disease-associated \textit{ABCA4} alleles were sequenced for variants in the \textit{PRPH2}/\textit{RDS} gene. Conversely, some patients presenting with likely \textit{PRPH2}/\textit{RDS}-associated phenotypes were first sequenced for this gene, and if a causal mutation was identified, the case was considered genetically solved and \textit{ABCA4} was not sequenced. This scheme was followed because the statistical probability that an individual carries mutations in both the \textit{ABCA4} and \textit{PRPH2}/\textit{RDS} genes is very low. Since \textit{ABCA4} is located on chromosome 1 and \textit{PRPH2}/\textit{RDS} on chromosome 6, variants in the two genes are inherited independently. While the exact population frequency of \textit{PRPH2}/\textit{RDS}-associated disease is not known, it comprises between 10% to 20% of all cases of autosomal dominant PD.\textsuperscript{29,30} If we consider that the population frequency of autosomal dominant PD is 1:50,000 (which is likely an overestimate), then no more than 1 in 250,000 people are affected by autosomal dominant PD due to \textit{PRPH2}/\textit{RDS} mutations. The carrier frequency of disease-associated \textit{ABCA4} alleles is estimated to be 1:20; therefore only 1 in 5 million patients with \textit{PRPH2}/\textit{RDS}-associated disease would also carry an \textit{ABCA4} allele. Despite the extremely low probability of finding disease-causing mutations in both genes in one individual, we sequenced both genes in all patients and confirmed that no patients carried disease-causing mutations in both genes (Table).

All procedures adhered to the tenets of the Declaration of Helsinki, and written informed consent was obtained from all subjects after a full explanation of the study procedures had been provided. The study was approved by the Institutional Review Board of Columbia University and complied with the Health Insurance Portability and Accountability Act of 1996.

**Electrophysiology**

A Diagnosys Espion Electrophysiology System (Diagnosys LLC, Littleton, MA, USA) was used to perform electroretinography (ERG) according to the International Society for Clinical Electrophysiology of Vision standards.\textsuperscript{31} For all recordings, tropicamide (1%) and phenylephrine hydrochloride (2.5%) were used for mydriasis before full-field ERG recording, and a drop of proparacaine (0.5%) was used to anesthetize the corneas. Full-field ERGs were performed using silver-impregnated fiber electrodes (DTL; Diagnosys LLC) with a ground electrode on the forehead.

**Image Acquisition**

Autofluorescence images (30° × 30° field, 768 × 768 pixels; 488-nm excitation; barrier filter 500–680 nm; beam power < 260 μW) were obtained with a scanning laser ophthalmoscope (cSLO, Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) modified by incorporation of a fluorescent reference internal to the imaging device such that the
reference is displayed in the AF image. As discussed below, evaluating gray levels (GL) in the AF image relative to the GL of the reference accounted for variable laser power and detector gain.21 Previously published protocols were followed to ensure the acquisition of high-quality AF images that permit meaningful quantification.21,22,26,32 Briefly, pupils were dilated to at least 7 mm (1% tropicamide and 2.5% phenylephrine); rhodopsin was bleached in AF mode for 20 to 30 seconds 21; detector sensitivity was adjusted so that the GLs were within the linear range of the detector (GL < 175)21; and focus and alignment of the camera were adjusted to produce a uniform fundus signal of maximal intensity. Two or more images (each of 9 frames; video format) were recorded in high-speed mode (8.9 frames/s), followed by a second imaging session for estimating the reproducibility. All videos were reviewed, and frames without localized or generalized decreased AF signal21 were aligned, averaged, and saved in “nonnormalized” mode (two images per session). The data presented in this study were based on AF images of 67 eyes, with 44 of these eyes having a second AF imaging session; an additional 13 imaging sessions were excluded because of insufficient image quality. Autofluorescence images from all eyes included in this study are presented in the Supplementary Material.

A horizontal 9-mm SD-OCT image through the fovea, registered to a simultaneously acquired AF or near-infrared reflectance (NIR-R) image, was recorded in high-resolution mode as an average of 100 individual images for each eye. The optical depth resolution in the Spectralis is currently ~7 lm. In cases in which the SD-OCT image had been registered to an NIR-R image, i2kRetina software (DualAlign LLC, Clifton Park, NY) was used for alignment. The summary of data is provided in Table 1.
AF Image Analysis

As formerly reported, AF images were analyzed under the control of an experienced operator with dedicated image analysis software written in IGOR (WaveMetrics, Lake Oswego, OR, USA) to determine qAF. Briefly, the software recorded the mean GLs of the internal reference and of eight circularly arranged segments positioned at an eccentricity of approximately 7° to 9° (Fig. 1) that were scaled to the horizontal distance between the fovea and the temporal edge of the optic disc. Quantitative AF was calculated from the mean GLs of each segment taking into account the Reference Calibration Factor, the internal reference GL, zero GL, magnification, and optical media density from normative data on lens transmission spectra. For each image, the average qAF from the eight segments was generated.

Statistical Analyses

Analyses were performed using Prism 5 (GraphPad Software, La Jolla, CA, USA) and Stata (version 12.1; College Station, TX, USA). Where appropriate, mixed-effects models were used to account for data from two eyes and familial and twin relationships. When comparing qAF between groups, age and race were included in the model. To evaluate between-session repeatability of the measurements and coefficient of agreement between right and left eyes, the method of Bland and Altman was utilized. For each qAF value, z-scores were also calculated and plotted as a measure of the numbers of standard deviations that describe each value relative (negative and positive) to the mean of healthy eyes.

Results

ABCA4 mutations were detected in 19 patients (31 eyes) from 18 families, including 11 cases (58%) with two disease-causing ABCA4 variants. Of those patients, 17 were white and 2 were Indian. Mutations in PRPH2/RDS were identified in 8 patients (16 eyes) from 5 families, all of whom were white. No mutations in ABCA4 or PRPH2/RDS were found in 12 patients (20 eyes). Of those patients, 11 were white and 1 was black. Demographic, clinical, and genetic information of the patients is presented in Table 1.

The group with ABCA4-associated disease (mean age, 37 years; range, 20–60 years) was younger than both the PRPH2/RDS-positive group (mean age, 48; range, 30–62 years; mixed-effects regression, \( P = 0.03 \)) and patients without mutations in ABCA4 or PRPH2/RDS (mean age, 48; range, 28–58 years; \( P = 0.01 \)). There was no difference in best-corrected visual acuity among the three groups of patients (mixed-effects regression, \( P > 0.19 \)).

Electroretinogram results (digital scans or clinical interpretations) were available for 30 out of 39 (30/39) patients: 20 patients (12 ABCA4 positive, 3 PRPH2/RDS positive, 5 ABCA4 and PRPH2/RDS negative) exhibited focal disease with a normal full-field ERG; 9 patients (4 ABCA4 positive, 2 PRPH2/RDS positive, 3 ABCA4 and PRPH2/RDS negative) had generalized cone ERG abnormalities, and 1 patient (1 ABCA4 positive) exhibited generalized cone and rod ERG abnormalities. Mean scotopic and photopic values and latency are summarized in Supplementary Table S1.

Representative AF images from patients with ABCA4-associated disease are shown in Figure 2. These patients either had a central lesion with a jagged border and no flecks in the periphery, a phenotype that can be found in both ABCA4-associated and non-ABCA4-associated disease, or had fundus features that may be considered atypical for STGD1, for instance, foveal sparing or peripapillary involvement.

Figure 3 illustrates the phenotypic range within AF images of PRPH2/RDS-positive PD patients. Prior to genetic screening these patients had been misdiagnosed as STGD1. The AF image of patient (P12) is shown qualitatively normal, while in the image of the other eye of P12 there is a remarkable phenotypic similarity to the ABCA4-positive cases presented in Figure 2.

Relative sparing of the peripapillary region in terms of flecks and atrophy is often regarded as a characteristic feature of STGD1 disease. However, as illustrated in Figure 6, within our cohort some ABCA4-positive cases (upper row) did not have complete peripapillary sparing, as these patients had flecks in close proximity to the optic nerve. Conversely, some PRPH2/RDS-positive patients (lower row) presented with “classic” peripapillary sparing. Foveal sparing, another fundus feature that could potentially help to discriminate patients with ABCA4-associated disease from patients presenting with PD due to mutations in other genes, was also observed in a subgroup of patients irrespective of whether they were PRPH2/RDS positive or ABCA4 positive or patients without mutations in either ABCA4 or PRPH2/RDS.

ABCA4 mutations in PRPH2/RDS- and ABCA4-Associated Disease

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Quantitative Fundus AF

For qAF of right and left eyes (n = 28), the Bland-Altman coefficient of agreement was 16.4%. The between-session Bland-Altman coefficient of repeatability was 9.3% (44 eyes of 26 patients).

As shown in Figure 7A, the group of patients with ABCA4-associated disease had higher qAF than those with healthy eyes (mixed-effects regression, P < 0.001), with 16/19 patients (26/31 eyes) having higher qAF than the 95% confidence interval for the 374 healthy eyes (adjusted for age and race). As shown in Figure 7B, the group of patients with ABCA4-
associated disease had, however, lower qAF than other previously published \(27\) \(ABCA4\)-positive patients with non-PD-like phenotypes (\(P = 0.002\)).

As shown in Figure 7A, the \(PRPH2/RDS\)-positive PD group had higher qAF than those with healthy eyes (\(P < 0.001\)), with 4/8 patients (9/16 eyes) having higher qAF than the 95% confidence interval for the healthy eyes. The increases in RPE autofluorescence measured as elevated qAF in some \(PRPH2/RDS\)-positive patients are unlikely to be due to a "window defect"\(^36\) resulting from photoreceptor cell degeneration since visual pigment was bleached before imaging. The \(PRPH2/RDS\)-positive PD group had lower qAF than the group of patients with \(ABCA4\)-associated disease (\(P = 0.016\)). The group of PD patients without mutations in \(ABCA4\) or \(PRPH2/RDS\) had qAF that was not different from the normal range for age (\(P = 0.69\)), with 3/12 patients (5/20 eyes) having high qAF and 3/20 eyes having qAF below the 95% confidence interval for healthy eyes (Fig. 7A).

These differences in qAF levels between patients are further demonstrated in Figure 8. In the upper row, AF images from a healthy subject and three age-similar patients (age: ~50 years) with different genotypes are shown. In the lower row, the corresponding color-coded qAF maps are presented. While the healthy subject and the PD patient without mutations in \(ABCA4\) or \(PRPH2/RDS\) (P21) have similar qAF levels, qAF levels are clearly elevated in the \(PRPH2/RDS\)-positive patient (P12) and most dramatically increased in the \(ABCA4\)-positive patient (P26).

Of interest is whether it is possible to use qAF to discriminate patients with \(ABCA4\)-associated disease from patients presenting with PD due to mutations in other genes. Initially, we approached this question using raw qAF values, but the result gave poor discrimination \(d' = 1.09\), sensitivity = 0.94, specificity = 0.33 at a criterion of qAF \(= 355\)). Next, we investigated the ability to discriminate patients with \(ABCA4\)-associated disease using qAF values that were adjusted for age and race. To do that, we determined the z-score based on the fit of the healthy eye data (information provided in Table 2 of Greenberg et al.\(^26\)). The z-score of a given value indicates how many standard deviations above or below the mean that value is.\(^37\) The qAF z-score provided better discrimination \(d' = 1.52\), sensitivity = 0.71, specificity = 0.83 at a criterion of qAF = 2.5 standard deviations) than the raw qAF (Fig. 9). Note that since \(d'\) combines sensitivity and specificity into a single metric that describes the ability to distinguish the signal (i.e., presence of \(ABCA4\) mutation) from noise (absence of \(ABCA4\) mutation), a z-score criterion of 2.5 indicates the value above which the qAF z-score is likely to be indicative of \(ABCA4\)-associated disease.

![Figure 4](image4.jpg)

**Figure 4.** Montage from multiple fundus AF images of P12, illustrating the distribution of AF changes across the fundus.

![Figure 5](image5.jpg)

**Figure 5.** Fundus AF images of patients without \(ABCA4\) or \(PRPH2/RDS\) mutations: P33 (A), P6 (B), P5 (C), P29 (D), P7 (E), P22 (F), P37 (G), and P17 (H).
As illustrated in Figure 10, there was a wide range of changes observed by SD-OCT within the group of PRPH2/RDS-positive patients (nomenclature of anatomic landmarks in SD-OCT images was based on the proposed lexicon by Straurenghi et al.33). Patient 35 did not have any obvious fundus changes. In P20, flecks exhibiting a high AF signal were present in the macula. These flecks traversed the photoreceptor ellipsoid zone (EZ) and external limiting membrane (ELM), and one...
fleck even extended into the outer nuclear layer (ONL). The choroidal signal under the flecks was attenuated. Patient 13 and P8 both had significant outer retinal loss temporally, while outer retinal damage was less pronounced nasally and at the fovea. Increased choroidal reflectivity, a feature that is generally attributed to RPE atrophy, was also more distinct temporally than nasally in P13 and P8. Patient 8 had parfoveal cystic-like spaces in the inner retina. Patient 11 had the most pronounced fundus changes. Except for a spared foveal island, the choroid was severely thinned and RPE and outer retina were atrophic. The inner retina seemed mostly intact and had prolapsed into areas of outer retina and RPE loss, leaving the impression of optical empty spaces bordering areas of preserved outer retina and RPE.

In some patients, differentiating ABCA4-associated disease from retinal phenotypes stemming from mutations in other genes such as PRPH2/RDS can be challenging. This difficulty is related to phenotypic similarities on funduscopy and the lack of diagnostic tests that can be used for better differentiation. For example, the full-field ERG in PRPH2/RDS-associated disease is often normal, but can also show profound variability even within families carrying the same mutation.

**DISCUSSION**

In some patients, differentiating ABCA4-associated disease from retinal phenotypes stemming from mutations in other
In ABCA4-associated disease, scotopic and photopic responses can also be normal.\textsuperscript{42,43} In the present study, we assessed whether qAF, an indirect in vivo measure of RPE lipofuscin, could aid in differentiating these patients. Our data showed, as expected, that ABCA4-positive patients had the highest qAF\textsubscript{8} levels within the cohort. However, it is notable that PRPH2/RDS-positive patients also had significantly increased values. Patients without mutations in ABCA4 and PRPH2/RDS had the lowest qAF\textsubscript{8} values within the cohort, and in most cases qAF\textsubscript{8} levels were either within or below the normal range for age. It was possible to discriminate patients with ABCA4-related disease from PD patients without ABCA4 mutations with an accuracy of 78% in our sample of 67 eyes using the age- and race-adjusted qAF z-score.

In STGD1, the mechanism for accentuated RPE lipofuscin formation is known. Insufficient ABCA4 protein activity leads to a buildup of bisretinoids in the outer segment disc membranes; this material includes an adduct of glycerophosphoethanolamine and two vitamin A molecules (A2-GPE), all-trans-retinal dimer and A2E as just some of the components.\textsuperscript{19} These bisretinoids are transferred to the RPE by phagocytosis where they form the lipofuscin of these cells. It is likely that the process of outer segment membrane shedding and phagocytosis by RPE serves to protect photoreceptor cells. If allowed to accumulate in outer segments, these bisretinoid fluorophores would trap incoming photons and compete with visual pigment photoisomerization. Importantly, bisretinoids are also photosensitizers that initiate photooxidation reactions.\textsuperscript{19} Yet because photoreceptor cells are specialized for phototransduction, the outer segments are not equipped with the levels of reducing equivalents and antioxidant defense systems expressed by RPE.\textsuperscript{44–46}

But what is the basis for an increase of RPE lipofuscin in the presence of PRPH2/RDS mutations? PRPH2/RDS is a photoreceptor-specific glycoprotein that is essential for the construction and preservation of the outer segment (OS) rim region of rods and cones. PRPH2/RDS functions by assembling into protein complexes that include rod OS membrane protein-1 (ROM-1). In the inner segment, PRPH2/RDS and ROM-1 form tetramers that are held together by noncovalent interactions between the second intradiscal (D2) loop of the two proteins.\textsuperscript{47–49} These complexes are then trafficked to the OS where they form higher-order oligomERIC structures, both hetero- and homologomers\textsuperscript{50} that interact by means of disulfide

\textbf{FIGURE 10.} Spectral-domain optical coherence tomography (SD-OCT) images of PRPH2/RDS-positive patients. AF images (left) and corresponding SD-OCT images (right) of P35 (A), P20 (B), P13 (C), P8 (D), and P11 (E). Horizontal axis and extent of SD-OCT image indicated by white arrow in AF image. Outer nuclear layer (ONL), external limiting membrane (ELM), photoreceptor ellipsoid zone (EZ), interdigitation zone (IZ), and retinal pigment epithelium/Bruch’s membrane (RPE).
Mouse models have revealed that rods and cones have different constraints with respect to PRPH2/RDS. In the absence of PRPH2/RDS, rods fail to form OS while cones form open OS that lack rims and flattened membranous lamellae but are still capable of mediating function. Nonetheless, some PRPH2/RDS mutations result in rod-dominant retinal disease (such as autosomal dominant RP) while others, such as amino acid substitutions at position 172 in the D2 loop (R172W, R172Q), are associated with a cone-associated phenotype (autosomal dominant macular dystrophy and cone–rod dystrophy) has so far eluded explanation. One issue is that the architecture of OS in cones and rods is different: In rods the stack of OS discs is enclosed and separated from the plasma membrane, while in cones the membrane evaginations that form the OS do not become surrounded by plasma membrane and instead are exposed to extracellular space. Some investigations indicate that haploinsufficiency may underlie rod-dominant disease while gain-of-function defects lead to cone-related disease. Even then, however, it is not understood how photoreceptor defects associated with some PRPH2/RDS mutations translate into secondary disease features such as RPE atrophy. Whether loss of PRPH2/RDS could affect retinoid handling in OS as part of the primary disease process, or whether retinaldehyde adducts form secondarily due to photoreceptor cell dysfunctioning and degeneration, remains to be investigated. Lipofuscin formation as secondary effect could explain why PRPH2/RDS-positive patients exhibit fundus changes similar to STGD1 patients (high qAF and geographic atrophy) but later in life than STGD1 patients. The damaging effects of RPE lipofuscin would be expected to be the same, but the increase in lipofuscin could generally occur less rapidly.

We observed other phenotypic features in PRPH2/RDS-positive patients that deserve further discussion. It was our impression that flecks in PRPH2/RDS-positive patients appeared to be smaller and nonconfluent (e.g., P34; Fig. 3) while those in STGD1 often exhibited irregular profiles with adjacent flecks appearing to become contiguous. This may be an observation deserving further study. Additionally, at least in some PRPH2/RDS-positive patients, topographical differences in the disease were evident. In the SD-OCT images of P13 and P8 (Fig. 9), there were more disease-related changes present temporally than nasally. Also, in P12, P4, and P13 (Fig. 3) there were more fundus changes in the periphery and relatively less.
disease-related changes in the macular area, including the peripapillary area. It is interesting to consider that some PRPH2/RDS mutations are more specific to rods versus cones.

We observed that ABCA4-positive patients for whom PD may be considered as a differential diagnosis tended to have lower qAF than previously reported patients with non-PD-like STGD1 phenotypes and also tended to be more represented at older ages (Fig. 7B). ABCA4-positive patients exhibiting a BEM phenotype were also reported to have lower qAF than ABCA4-positive patients with other phenotypes, including those with extramacular disease. In the case of ABCA4-positive BEM, many of the patients carried the G1961E mutation, which was also the case for 9/19 of the ABCA4-positive patients in this study. In future studies we will examine for further genotype–phenotype correlations.

Color-coded qAF maps allow a rapid assessment of qAF levels and may therefore be valuable in the clinical assessment and follow-up of patients. However, since qAF also increases with age in healthy subjects, current comparisons of qAF maps can be made only between age-similar subjects. To compare patients regardless of age, it may be useful to develop color-coded qAF maps based on fold increases relative to healthy subjects of the same race and age. This could be done using the z-score approach that we used to discriminate patients with ABCA4-associated disease from patients presenting with PD due to mutations in other genes. The z-score approach furthermore allows for a comparison of qAF levels among patients regardless of their race.

As with any novel imaging technique, the clinical utility of qAF awaits further study. Nevertheless, the data presented here provide evidence that qAF can be used to establish genotype–phenotype correlations and can help to better understand the underlying pathomechanisms of retinal dystrophies. Quantitative AF can expose phenotypic variation among patients even under pathomechanisms of retinal dystrophies. Phenotypic variability and long-term follow-up of patients with known and novel PRPH2/RDS gene mutations. Am J Ophthalmol. 2009;147:518–530.


