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Correlation of Ultra-widefield Fundus Autofluorescence Patterns with the Underlying Genotype in Retinal Dystrophies and Retinitis Pigmentosa

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Abstract

Purpose: Ultra-widefield fundus autofluorescence (UW-FAF) allows the characterization of the peripheral retinal features of vitreoretinal diseases. The purpose of this study was to examine possible genotypic/phenotypic correlations of UW-FAF patterns in patients with a variety of retinal dystrophies and retinitis pigmentosa (RP).

Methods: An IRB-approved retrospective consecutive case series study was performed of genetically characterized retinal dystrophy or RP patients who underwent UW-FAF imaging. UW-FAF was performed with the Optos 200Tx system. Clinical variables, genotypic analysis, and phenotypic characteristics were reviewed.

Results: Seventeen patients were identified who had identified mutations in retinal dystrophy or RP genes and who also had undergone UW-FAF. Three patients had X-linked RP with *RPGR* mutations. Six patients had autosomal dominant RP (four with *RHO* mutations and one with a *PRPF31* mutation, and one with *RDS/PRPH2* mutation). Four patients had autosomal recessive RP (four with *USH2A* mutations). Three patients had Leber Congenital Amaurosis (LCA) with mutations including *CRB1*, *CEP290*, and *RPGRIP1*. Macular hyperautofluorescence was noted in all patients. A ring of hyperautofluorescence was clear in patients with *RHO* and *USH2A* mutations, and patients with *USH2A* mutations demonstrated a second ring of hyperautofluorescence. In the periphery, patients with *RHO* or *RPGR* mutations exhibited hyperautofluorescence with patchy areas of hypoautofluorescence. Patients with *USH2A* mutations had a distinctive pattern of diffuse and homogeneous peripheral hypoautofluorescence.

Conclusion: UW-FAF may provide important information to facilitate diagnosis and further research is needed to better characterize this technology as an imaging biomarker for genotype association in retinal dystrophies and RP.

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Introduction

Retinal dystrophies and retinal degenerations are a heterogeneous group of disorders with widely varied phenotypes. Retinitis Pigmentosa (RP) affects 1/3,000–1/5,000 people.[1] It is characterized by symptoms of night blindness and loss of peripheral vision and slowly progressive decreased central vision. It has classic fundus findings that include bone spicule hyperpigmentation in the mid periphery, vascular attenuation and optic nerve waxy pallor. [2,3] RP can be inherited as autosomal dominant, autosomal recessive, X-linked disease or can be sporadic from spontaneous mutations. [4].

To date, mutations in more than 50 genes are known to cause RP. The most severe cases of infantile or early-onset childhood RP are termed Leber congenital amaurosis (LCA) whereas the less aggressive forms are usually considered juvenile Retinitis Pigmentosa. Usher syndrome is a condition characterized by hearing loss or deafness and progressive vision loss, due to retinitis pigmentosa. It accounts for up to 10–20% of RP cases [5–9]

Fundus autofluorescence (FAF) imaging is an emerging technology that allows the functional evaluation of photoreceptors and retinal pigment epithelium (RPE). [10] The green light (532nm) widefield autofluorescence imaging modality records signal mainly from RPE lipofuscin but also other fluorophores in the macula and periphery. Lipofuscin contains more than 10 different fluorophores with discrete emission in the green, yellow and red wavelengths.[11] Reduced FAF is thought to result from photoreceptor loss in early life and is absent with RPE loss. Increased recorded autofluorescence occurs with increased lipofuscin in the RPE or loss of rod outer segments.[12] Ultra-wide-field imaging (Optos 200 Tx) offers a 200-degree view of the retina on a single frame, which allows simultaneous visualization of the FAF levels in the macula and periphery. The topographical distribution of FAF has been described in normal eyes.[13] The optic nerve head typically appears dark due to absence of RPE. The retinal vessels are hypofluorescent because of light absorption by blood. In the macula the FAF signal is reduced due to increased concentration of lutein and zeaxanthin. *In vivo* recording of pigment epithelial autofluorescence is considered an index of lipofuscin accumulation that gives indirect information about the metabolic activity of the RPE.[14–16] Histopathologic studies in RP have shown that after the death of all photoreceptors, RPE cells may migrate to perivascular sites producing the typical bone spicule pigmentation.[17,18] On the other hand lipofuscinopathies including Stargardt disease, Best disease and adult vitelliform macular dystrophy have increased hyperautofluorescence with characteristic patterns of lipofuscin accumulation. Patients with age related macular degeneration (AMD) display RPE hyperautofluorescence preceding geographic atrophy and eventually RPE hypoautofluorescence.

In this study, we analyze ultra-widefield FAF (UW-FAF) in patients with retinal dystrophies and RP and attempt genotypic-phenotypic characterization of UW-FAF patterns.

Methods

This retrospective, consecutive case series study was performed after Institutional Review Board approval was obtained at the Cleveland Clinic, OH, USA. All procedures conformed

to the tenets of the Declaration of Helsinki. Medical records of patients with RP and other retinal dystrophies at Cleveland Clinic were reviewed. Patients were included if they had UW-FAF and genotypic characterization of their retinal dystrophy.

UW-FAF imaging was performed using the Optos 200 Tx wide-field imaging after pupil dilation with topical 0.5% tropicamide and 2.5% phenylephrine drops. In AF mode the Optos 200 Tx uses a green (532nm) laser wavelengths to produce a digital, high-resolution image that is displayed on a monitor screen. In this mode, polarization discrimination optics are moved out of the return path (there is no reflection from the cornea) to optimize the detector collection of the AF component. Detection of the emitted signal occurs using a raster scan and detector for light in the wavelengths from 570 to 780nm (yellow-orange-red range). The ultra-widefield camera allows a 200° view of the retina with a single frame.

All patients underwent visual acuity testing, slit lamp biomicroscopy and dilated fundus examination. All patients had clinical findings consistent with RP. UW-FAF characteristics were analyzed qualitatively by two reviewers. The study included 34 eyes of 17 patients. All patients were identified and diagnosed clinically by an inherited retinal disease specialist (EIT) and the diagnosis was confirmed by genetic testing.

All patients underwent genetic counseling and were consented for molecular genetic testing. Molecular diagnostic testing was performed at CLIA approved laboratories.

Results

Genetics

Seventeen patients with retinal dystrophies and RP were identified. Three patients with X-linked RP had *RPGR* mutations. Seven had dominant RP with four having *RHO* mutations, one a *PRPF31* mutation, one *RDS/PRPH2* and one an *EYS* mutation. Patients with Leber Congenital Amaurosis (LCA) had *CRB1*, *CEP290* or *RPGRIP1* mutation. Four patients had autosomal recessive Usher syndrome with *USH2A* mutations. The genetics, demographics and clinical exam findings of the patients are summarized in Table 1.

Fundus Autofluorescence

Macular hyperautofluorescence was noted in all patients. Ring hyperautofluorescence was prominent in patients with *RHO*, *USH2A*, *CEP290*, *RPGRIP1* and *RPGR* mutations. (Figure 1) Patients with *USH2A* mutations demonstrated a second macular ring of hyperautofluorescence. (Figure 2) Peripheral UW-FAF patterns in patients with *RHO* mutations or *RPGR* consisted of hyperautofluorescence with patchy areas of hypoautofluorescence. (Figure 3) Patients with *USH2A* or *RDS/PRPH2* mutations had a distinct pattern of diffuse peripheral hypoautofluorescence and a characteristic dark appearance compared to the rest of the cohort. (Figure 4,5)

The optic nerve appeared dark in most patients. However, patients with *PRPF31* mutation or *RHO* mutations and optic nerve pallor appeared to have optic nerve hyperautofluorescence without optic nerve drusen. (Figure 6) One patient with optic nerve drusen had hyperautofluorescence that corresponded to the area of drusen. (Figure 7)

Discussion

FAF imaging is considered an indirect indicator of retinal function as it mainly results from lipofuscin and lipofuscin accumulation and is suggestive of oxidative stress and increased metabolic activity.[19,20] A common finding in patients with RP is hyperautofluorescence in the macula that indicates RPE stress. A hyperautofluorescent ring in the macula is commonly found in RP patients in this study and has been previously described.[21] One hypothesis is that this represents an circumscribed area of increased rate of photoreceptor phagocytosis that causes accumulation of lipofuscin. In this study, all eyes exhibited hyperautofluorescence in the macula even in absence of a ring. However, there appeared to be some genetic mutations that were associated with a specific pattern of FAF. It is not clear what the second ring of hyperautofluorescence represents but it was more evident in patients with *USH2A* mutations. Longitudinal changes in the hyperautofluorescent ring have been described in RP.[22] Lima et al demonstrated the constriction of the hyperautofluorescence ring in the macula and hypothesized that it is a sign of disease progression.

The benefit of UW-FAF is that it allows for panretinal assessment of disease characteristics. Most of the RP patients in this study demonstrate variable hyperautofluorescence and hypoautofluorescence in the periphery. However patients with autosomal recessive Usher syndrome and *USH2A* mutations had distinctly dark UW-FAF in the periphery. The prominence of this finding suggests RPE death or atrophy in these areas. Typically, the optic nerve has a dark appearance on FAF imaging in healthy patients. Most of the patients with RP have also unremarkable FAF features to the optic nerve. However, patients with significant waxy pallor exhibited hyperautofluorescence at the disc. Various hypotheses can be considered for this finding. One explanation is that there is hyperautofluorescent material in the disc. Another is that the lamina cribrosa and the sclera hyperautofluorescence is better visualized because of the overlying optic nerve atrophy. The appearance of the hyperautofluorescence in these patients is different from that of those with optic nerve drusen: It is more diffuse and less intense. Conversely, optic nerve drusen have distinct borders and have intense autofluorescence signal.

The present study has several limitations. It is retrospective and does not provide data on time-dependent longitudinal changes. The number of patients with mutations in the same gene limits quantitative and statistical analysis and further characterization of genotype-phenotype correlation.

Prospective large-scale assessment of longitudinal changes on UW-FAF are needed to better characterize UW-FAF as a potential imaging biomarker for inherited retinal diseases with different underlying genetic alterations. This study suggests the UW-FAF findings in patients with retinal dystrophies is heterogeneous and may have distinctive features in different genetic types of inherited retinal diseases.

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Figure 1.
Ring hyperautofluorescence with ultra-widefield fundus autofluorescence in patients with RHO, USH2A, CEP290, RPGRIP1 and RPGR mutations.

Figure 2. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with USH2A mutations demonstrates diffuse hyperautofluorescence at the macula and a second ring of hyperautofluorescence.

Figure 3. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with RHO mutations or RPGR had hyperautofluorescence with patchy areas of hypoautofluorescence.

Figure 4. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with RDS mutations had a distinct pattern of diffuse peripheral hypoautofluorescence and characteristic dark appearance compared to the rest of the patients.

Figure 5. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with USH2A mutations had diffuse peripheral hypoautofluorescence.

Figure 6.

A. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with PRPF31 mutation or RHO and optic nerve pallor appeared to have optic nerve hyperautofluorescence on fundus autofluorescence. B. Color fundus image of the same patient with optic nerve pallor.

Figure 7.
A. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with optic nerve drusen demonstrated hyperautofluorescence that corresponded to the area of drusen B. color fundus image of the same patient with optic nerve drusen.

Table 1.

Clinical Characteristics of patients included in the study. (BCVA: Best corrected visual acuity)

Patient	Age	Gender	Diagnosis	Mutation	BCVA OD-OS
P1	49	Male	X-linked Retinitis Pigmentosa	RPGR	20/125 – 20/150
P2	19	Male	X-linked Retinitis Pigmentosa	RPGR	20/30 – 20/30
P3	29	Male	X-linked Retinitis Pigmentosa	RPGR	20/30 – 20/30–1
P4	50	Female	Autosomal Dominant RP	RHO	20/25 – 20/20
P5	15	Female	Autosomal Dominant RP	RHO	20/125 – 20/100
P6	43	Female	Autosomal Dominant RP	RHO	20/60 – 20/40
P7	60	Female	Autosomal Dominant RP	RHO	20/30 – 20/30
P8	47	Female	Autosomal Recessive RP	USH2A	20/20 – 20/40
P9	37	Female	Autosomal Recessive RP	USH2A	20/25 – 20/25
P10	46	Male	Autosomal Recessive RP	USH2A	20/20 – 20/20
P11	28	Male	Autosomal Recessive RP	USH2A	20/20 – 20/15
P12	65	Female	RP/Usher Syndrome	PRPF31	20/600 – 20/400
P13	72	Male	Autosomal Recessive RP	RDS/PRPH2	20/30 – 20/100
P14	24	Male	Autosomal Recessive RP	EYS	20/20 – 20/15
P15	8	Male	Leber Congenital Amaurosis	RPGRIP1	20/50 – 20/50
P16	31	Female	Leber Congenital Amaurosis	CRB1	20/200 – 20/200
P17	15	Male	Leber Congenital Amaurosis	CEP 290	20/40 – 20/50