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## Ultra-widefield Fundus Autofluorescence Patterns in Retinitis Pigmentosa and Other Retinal Dystrophies

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### Abstract

Ultra-widefield fundus autofluorescence (UW-FAF) allows for 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). Seventeen patients were identified who had identified mutations in retinal dystrophy or RP genes and who also had undergone UW-FAF. Mutations with genes included *RPGR*, *RHO*, *PRPF31*, *RDS/PRPH2*, *USH2A*, *CRB1*, *CEP290*, and *RPGRIP1*. Variable UW-FAF patterns including ring hyperautofluorescence, double ring hyperautofluorescence, and peripheral hypoautofluorescence were identified. 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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Inherited retinal dystrophies comprise a heterogeneous group of disorders with widely varied phenotypes. Retinitis Pigmentosa (RP) is most common and affects 1/3,000–1/5,000 people[1–3], and can be inherited as autosomal dominant, recessive, or X-linked.[4] To date, mutations in more than 50 genes are known to cause RP. The most severe phenotype of infantile or early-onset childhood RP is termed Leber congenital amaurosis (LCA). Usher syndrome is characterized by hearing loss and progressive vision loss, due to retinitis pigmentosa. It accounts for up to 10–20% of RP cases.[5–9] Significant advances have been made in recent years in the molecular diagnosis of retinal dystrophies and there are a number of current trials of gene and other therapies for this group of disorders.

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 FAF imaging modality records signal mainly from RPE lipofuscin but also other fluorophores in the macula and periphery.[11] Reduced FAF is thought to result from photoreceptor loss in early life and is absent with RPE loss. Increased FAF occurs with increased lipofuscin in the RPE or loss of rod outer segments.[12] Ultra-

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widefield imaging (Optos 200 Tx) offers a 200-degree view of the retina on a single frame, which allows simultaneous FAF visualization in the macula and periphery.

We analyze ultra-widefield FAF (UW-FAF) in patients with retinal dystrophies and RP and attempt genotypic-phenotypic characterization of UW-FAF patterns.

We performed a retrospective, consecutive case series study 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 disorder. All patients underwent genetic counseling and were consented for molecular genetic testing. Molecular diagnostic testing was performed at CLIA approved laboratories.

All patients underwent visual acuity testing, slit lamp biomicroscopy and dilated fundus examination. All eyes underwent UW-FAF imaging was performed using the Optos 200 Tx. 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. UW-FAF characteristics were analyzed by two reviewers. The study included 34 eyes of 17 patients. The diagnoses and mutations (Table 1) included 3 patients with X-linked RP (*RPGR* mutations), 7 patients with dominant RP [*RHO* mutations (n = 4), *PRPF31* mutation (n = 1), *RDS/PRPH2* (n = 1), and *EYS* mutation (n = 1)], 3 patients with LCA [*CRB1* mutation (n = 1), *CEP290* mutation (n = 1), and *RPGRIP1* mutation (n = 1)], and 4 patients with autosomal recessive Usher syndrome (all with *USH2A* mutations).

Macular hyperautofluorescence was noted in all patients. Ring hyperautofluorescence was prominent in patients with *RHO*, *USH2A*, *CEP290*, *RPGRIP1* and *RPGR* mutations. (Figure 1A) Patients with *USH2A* mutations demonstrated a second macular ring of hyperautofluorescence. (Figure 1B) Peripheral UW-FAF patterns in patients with *RHO* or *RPGR* mutations consisted of hyperautofluorescence with patchy areas of hypoautofluorescence. (Figure 1C) 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. (Figures 1D and 1E) 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 1F)

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.[13,14] 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 and was frequently observed in this study.[15] One hypothesis is that this represents a circumscribed area of increased photoreceptor phagocytosis that results in accumulation of lipofuscin. In this study, all eyes exhibited hyperautofluorescence in the macula even in absence of a ring. However, we observed specific patterns of UW-FAF associated with select mutations. 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 including constriction of the ring thought to be related to disease progression.[16]

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 (*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.

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 is 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 genotypes of inherited retinal diseases.

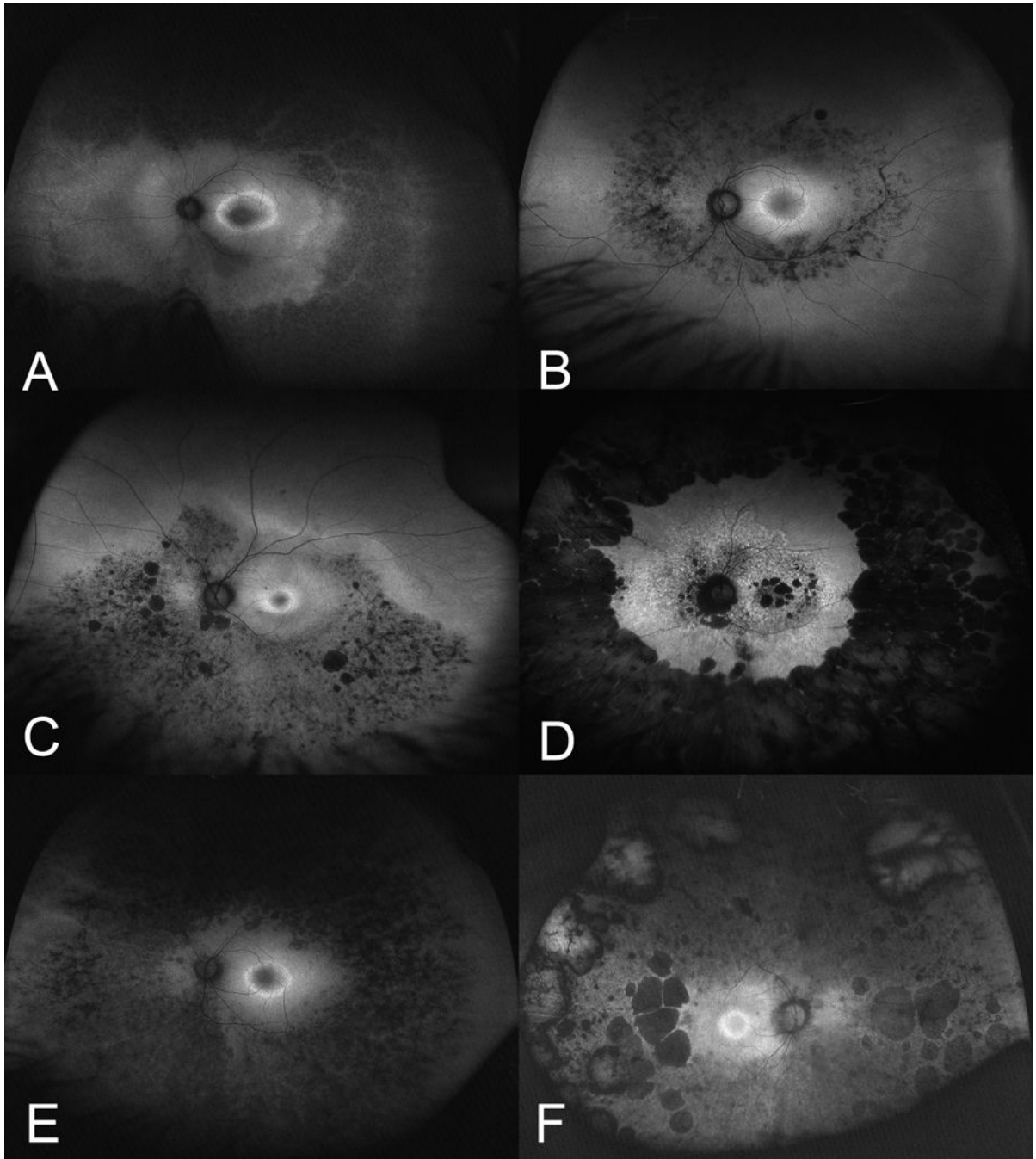
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**Figure 1. Ultra-widefield Fundus Autofluorescence Patterns in Retinitis Pigmentosa and Other Retinal Dystrophies**

A. Macular ring hyperautofluorescence with as seen in patients with *RHO*, *USH2A*, *CEP290*, *RPGRIP1* and *RPGR* mutations. B. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with *USH2A* mutation demonstrates diffuse macular hyperautofluorescence and a ring of hyperautofluorescence. C. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with *RHO* or *RPGR* with hyperautofluorescence with patchy areas of hypoautofluorescence. D. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with *RDS* mutations had a distinct pattern of diffuse peripheral

hypoautofluorescence. E. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with *USH2A* mutation had diffuse peripheral hypoautofluorescence. F. Ultra-widefield fundus autofluorescence in retinitis pigmentosa with *PRPF31* or *RHO* mutations with associated optic nerve pallor appeared to have optic nerve hyperautofluorescence.

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**Table 1**

Clinical Characteristics.

Patient	Age	Gender	Diagnosis	Mutation	Best Corrected Visual Acuity Right - Left
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