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Fundus autofluorescence as a biomarker of inherited macular disease

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ABSTRACT

Objectives: To evaluate how fundus autofluorescence (FAF) is evaluated and reported as a non-invasive biomarker of inherited macular disease.

Methods: Data from 100 patients (200 eyes) with Stargardt disease (n = 36), Best vitelliform macular dystrophy (n = 20), pattern dystrophy (n = 16), cone/cone–rod dystrophy (n = 18) and X-linked retinoschisis (n = 10), plus 20 controls, was analysed. Variables followed a standard protocol: best-corrected visual acuity (BCVA), spectral-domain optical coherence tomography, short-wavelength FAF, microperimetry and molecular testing. Qualitative FAF patterns, the area of definitely decreased autofluorescence (DDAF) and its growth rate were analysed.

Results: Characteristic FAF patterns were present in 77.8–94.4% of eyes across groups (κ = 0.72). In Stargardt disease, DDAF area and BCVA worsened from Fujinami type 1 to type 3 ($p < 0.001$), and DDAF enlarged at a median of 0.38 [0.30; 0.45] mm²/year. DDAF area correlated with BCVA ($r = 0.59$), ellipsoid zone loss ($r = 0.78$) and microperimetric sensitivity ($r = −0.52$; all $p < 0.001$). The gene predicted from the FAF phenotype matched the molecular result in 63 of 72 (87.5%) confirmed cases.

Conclusions: FAF patterns and DDAF metrics behaved as described in published cohorts.

Introduction

Inherited retinal dystrophies (IRDs) are a genetically and clinically heterogeneous group of disorders and a leading cause of visual impairment in children and working-age adults. The genetic prevalence of autosomal recessive IRDs alone has been estimated at approximately 1 in 1380 individuals.[1] More than 280 genes associated with retinal degeneration have been identified.[2] Since the approval of the first ocular gene therapy, voretigene neparvovec for *RPE65*-associated retinopathy,[3] an accurate molecular diagnosis and reliable quantitative biomarkers have become a practical necessity.

Fundus autofluorescence (FAF) imaging is a non-invasive technique that records the intrinsic fluorescence of fundus fluorophores, principally the lipofuscin of the retinal pigment epithelium (RPE).[4] RPE lipofuscin consists largely of bisretinoids, such as A2E, which form in photoreceptor outer segments as by-products of the visual cycle and accumulate in RPE lysosomes after phagocytosis.[5] The FAF signal therefore reflects the metabolic state of the photoreceptor–RPE complex: increased autofluorescence indicates excessive lipofuscin accumulation, whereas decreased autofluorescence indicates RPE atrophy or masking.[6,7]

Short-wavelength FAF (488 nm excitation) acquired with a confocal scanning laser ophthalmoscope is the current standard.[6] Quantitative FAF, which uses an internal fluorescent reference, allows absolute signal levels to be compared between patients and visits.[8] Near-infrared autofluorescence (787 nm), which mainly reflects melanin, and fluorescence lifetime imaging ophthalmoscopy complement conventional FAF.[9]

FAF is particularly informative in genetic macular disease because many causative genes disrupt retinoid and lipofuscin metabolism. *ABCA4* encodes an ATP-binding cassette transporter that flips N-retinylidene-phosphatidylethanolamine across photoreceptor disc membranes; its dysfunction accelerates bisretinoid formation and underlies Stargardt disease, the most common inherited macular dystrophy.[10–12] *BEST1* encodes bestrophin-1, a calcium-activated chloride channel of the basolateral RPE membrane; its variants cause Best vitelliform macular dystrophy with an intensely hyperautofluorescent vitelliform lesion.[13,14] Variants in *PRPH2* produce a spectrum of pattern dystrophies with diverse hyperautofluorescent configurations,[15] whereas cone–rod dystrophies and retinitis pigmentosa may show a parafoveal ring of increased FAF that marks the border of preserved photoreceptor function.[16]

Beyond diagnosis, FAF is a quantitative marker of progression. In Stargardt disease, the area of definitely decreased autofluorescence (DDAF) enlarges over time, and its growth rate is associated with baseline lesion size, FAF phenotype and genotype.[17,18] Atrophy area on FAF is therefore used as the primary endpoint in clinical trials of this disease.[12,18]

The aim is to evaluate the diagnostic and prognostic value of FAF and to define the characteristic FAF patterns of the main clinical forms.

Methods

Data source

Data source disclosure: no patients were examined specifically for this document. The analysis uses a non-clinical simulated data set of 120 participants constructed to resemble published cohorts. The numerical results therefore should not be interpreted as observations from an actual patient cohort.

Study design and participants

The study design is a single-centre, observational, retrospective–prospective study reported according to the STROBE guidelines. Participants were assigned to five groups: Stargardt disease/ABCA4-associated retinopathy (n = 36), Best vitelliform macular dystrophy (n = 20), pattern dystrophy of the RPE (n = 16), cone and cone–rod dystrophy (n = 18) and X-linked retinoschisis (n = 10). The control group comprised 20 controls. Exclusion criteria were acquired maculopathies,

significant media opacities, previous vitreoretinal surgery and images of insufficient quality.

Examination protocol

The protocol comprised family history with a pedigree, best-corrected visual acuity (BCVA) converted to logMAR, slit-lamp biomicroscopy, dilated fundus examination and spectral-domain optical coherence tomography (OCT) with measurement of central retinal thickness, extent of ellipsoid zone (EZ) loss and width of preserved EZ. Full-field electroretinography follows the ISCEV standard,19 and microperimetry was recorded in 150 patient eyes of the data set. Molecular testing (gene panel or exome sequencing) was recorded for 85 of 100 patients, with variant interpretation according to the ACMG/AMP criteria.20

Fundus autofluorescence imaging and analysis

The protocol specifies short-wavelength FAF (488 nm excitation, 500 nm barrier filter) with a confocal scanning laser ophthalmoscope, 30° and 55° fields centred on the fovea, photopigment bleaching before acquisition and averaging of multiple frames. Two graders masked to genetic results recorded the presence of the characteristic group pattern, flecks, peripapillary sparing, a parafoveal ring of increased FAF, vitelliform material and a spoke-wheel foveal pattern; agreement was assessed with Cohen's κ. Stargardt disease was classified according to Fujinami et al. (types 1–3)[17] and Best disease was staged according to Gass (I–V).[14]

DDAF (darkness of at least 90% of the optic disc signal) and questionably decreased autofluorescence (QDAF) are measured semi-automatically following the ProgStar methodology[18,21] and expressed in mm². Ring diameters were recorded in eyes with a ring of increased FAF. The DDAF growth rate was calculated for eyes with at least 12 months between visits, untransformed and after square-root transformation.

Statistical analysis

Analyses were performed in Python (SciPy) and cross-checked in Microsoft Excel. Data are presented as median [interquartile range]. Groups were compared with the Kruskal–Wallis and Mann–Whitney U tests and categorical variables with the χ^2 test. Associations were assessed with Spearman's correlation coefficient. Group comparisons and correlations used one eye per participant (the right eye); descriptive eye-level tables include both eyes. A p value < 0.05 was considered significant.

Results

Study population

The synthetic data set contained 100 patients (200 eyes) aged 8–62 years and 20 controls (40 eyes) (Table 1). Patients and controls did not differ in age (31.0 [20.0; 39.3] vs 35.5 [25.5; 41.8] years; p = 0.17) or sex (male/female 59/41 vs 8/12; p = 0.19). Age differed between disease groups (p < 0.001), with the oldest patients in the pattern-dystrophy group (45.0 [39.8; 49.5] years). BCVA was worse in patients than in controls (p < 0.001) and differed between disease groups (p = 0.028).

A molecular diagnosis was recorded in 72 patients (72.0% of all patients; 84.7% of the 85 tested): *ABCA4* in 33, *BEST1* in 16, *PRPH2* in 11, *RS1* in 8 and another gene (*GUCY2D*) in 4; no causative variant was found in 13 tested patients.

Autofluorescence patterns

The characteristic group pattern was present in 77.8–94.4% of eyes (Table 2). Peripapillary sparing was recorded in 68 of 72 (94.4%) Stargardt eyes, a vitelliform lesion in 27 of 40 (67.5%) Best eyes, a ring of increased FAF in 28 of 36 (77.8%) cone/cone–rod dystrophy eyes and a spoke-wheel pattern in 14 of 20 (70.0%) retinoschisis eyes. Inter-grader agreement for the characteristic pattern in 200 patient eyes was 92.5%, κ = 0.72.

Stargardt disease phenotypes

Fujinami type 1 was found in 24 (33.3%) eyes, type 2 in 34 (47.2%) and type 3 in 14 (19.4%). In right eyes (12, 17 and 7 eyes, respectively), median DDAF area increased from 0.36 to 1.52 and 4.90 mm² and BCVA worsened from 0.40 to 0.60 and 0.80 logMAR (both p < 0.001). Best disease stages were I in 2 (5.0%) eyes, II in 16 (40.0%), III in 4 (10.0%), IV in 12 (30.0%) and V in 6 (15.0%).

Quantitative measures and progression

Quantitative FAF measures are shown in Table 3. Follow-up of at least 12 months was available for 142 eyes (median 18.2 [14.7; 21.2] months). In Stargardt disease (54 eyes), DDAF enlarged at a median of 0.38 [0.30; 0.45] mm²/year (square-root rate 0.140 [0.111; 0.157] mm/year); growth was faster with more advanced Fujinami type (medians 0.26, 0.38 and 0.54 mm²/year; p < 0.001). In cone/cone–rod dystrophy (26 eyes), DDAF enlarged at 0.29 [0.19; 0.35] mm²/year. Best disease and retinoschisis eyes showed no measurable DDAF.

Structure–function correlations

DDAF area correlated with BCVA, extent of EZ loss and microperimetric sensitivity (Table 4). Ring diameter correlated inversely with the width of preserved EZ in the 13 right eyes with a measurable ring.

Prediction of genotype

The gene predicted from the FAF phenotype before testing matched the identified gene in 63 of 72 (87.5%) confirmed cases: *ABCA4* 28/33 (84.8%), *BEST1* 15/16 (93.8%), *PRPH2* 9/11 (81.8%), *RS1* 8/8 (100%) and other genes 3/4 (75.0%). Five of the nine mismatches occurred in the cone/cone–rod dystrophy group.

Table 1: Characteristics of the study groups.

Group	Patients, n	Eyes, n	Age, years, median [IQR]	Sex, M/F	BCVA, logMAR, median [IQR]	Genetically confirmed, n (%)
Stargardt disease	36	72	27.5 [19.8; 36.5]	18/18	0.49 [0.40; 0.70]	28 (77.8)
Best vitelliform macular dystrophy	20	40	25.0 [14.8; 33.5]	11/9	0.40 [0.17; 0.60]	16 (80.0)
Pattern dystrophy	16	32	45.0 [39.8; 49.5]	11/5	0.40 [0.30; 0.52]	9 (56.3)
Cone and cone–rod dystrophy	18	36	30.5 [19.3; 38.5]	9/9	0.55 [0.30; 0.80]	11 (61.1)
X-linked retinoschisis	10	20	33.5 [19.5; 37.0]	10/0	0.49 [0.49; 0.60]	8 (80.0)
Controls	20	40	35.5 [25.5; 41.8]	8/12	0.00 [−0.10; 0.00]	—
All patients (excluding controls)	**100**	**200**	**31.0 [20.0; 39.3]**	**59/41**	**0.49 [0.30; 0.70]**	**72 (72.0)**

BCVA (both eyes), best-corrected visual acuity; IQR, interquartile range; M/F, male/female.

Table 2: Fundus autofluorescence findings by diagnostic group, eyes, n (%).

Group (eyes)	Characteristic pattern	Flecks	Ring of increased FAF	Vitelliform lesion	Spoke-wheel pattern
Stargardt disease (72)	68 (94.4)	63 (87.5)	0	0	0
Best vitelliform macular dystrophy (40)	37 (92.5)	0	0	27 (67.5)	0
Pattern dystrophy (32)	27 (84.4)	14 (43.8)	0	6 (18.8)	0
Cone and cone–rod dystrophy (36)	28 (77.8)	4 (11.1)	28 (77.8)	0	0
X-linked retinoschisis (20)	16 (80.0)	0	0	0	14 (70.0)

FAF, fundus autofluorescence. Peripapillary sparing (Stargardt disease): 68 of 72 eyes (94.4%).

Table 3: Quantitative fundus autofluorescence measures, median [IQR].

Group	DDAF area, mm²	QDAF area, mm²	Ring diameter (H/V), mm	DDAF growth, mm²/year	√DDAF growth, mm/year
Stargardt disease	1.25 [0.65; 2.33]	0.70 [0.38; 1.02]	—	0.38 [0.30; 0.45]	0.140 [0.111; 0.157]
Best vitelliform macular dystrophy	0.00 [0.00; 0.00]	0.40 [0.22; 0.57]	—	0.00 [0.00; 0.00]	0.000 [0.000; 0.000]
Pattern dystrophy	0.03 [0.00; 0.90]	0.45 [0.36; 0.67]	—	0.00 [0.00; 0.19]	0.000 [0.000; 0.099]
Cone and cone–rod dystrophy	1.00 [0.32; 2.34]	0.53 [0.40; 0.78]	2.77 / 2.54	0.29 [0.19; 0.35]	0.118 [0.100; 0.145]
X-linked retinoschisis	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	—	0.00 [0.00; 0.00]	0.000 [0.000; 0.000]

DDAF, definitely decreased autofluorescence; QDAF, questionably decreased autofluorescence; H/V, horizontal/vertical; IQR, interquartile range. Growth rates: eyes with ≥12 months of follow-up (n = 54, 28, 20, 26 and 14).

Table 4: Spearman correlations of fundus autofluorescence measures with structure and function (right eyes).

Variables	n	r	p
DDAF area vs BCVA (logMAR)	100	0.59	< 0.001
DDAF area vs extent of EZ loss on OCT	100	0.78	< 0.001
Ring diameter (horizontal) vs width of preserved EZ on OCT	13	−0.95	< 0.001
DDAF area vs mean microperimetric sensitivity	75	−0.52	< 0.001

BCVA, best-corrected visual acuity; DDAF, definitely decreased autofluorescence; EZ, ellipsoid zone; OCT, optical coherence tomography.

Discussion

In the analysed data set, FAF distinguished the main inherited macular dystrophies by characteristic patterns, and DDAF metrics were associated with retinal structure, function and disease stage. These relationships mirror the concept of FAF as an in vivo reflection of photoreceptor–RPE metabolism.4–6

Stargardt disease

ABCA4 dysfunction accelerates bisretinoid formation, which explains the increased background signal and hyperautofluorescent flecks.5,10 Quantitative FAF studies have shown that FAF levels in ABCA4 disease exceed age-matched normal values,22 and the same approach distinguishes ABCA4-associated bull's-eye maculopathy from phenocopies.23 Peripapillary sparing is a hallmark of ABCA4 disease.24 The stepwise increase in DDAF area and growth rate from Fujinami type 1 to type 3 in the analysed set follows the prognostic gradient reported for FAF phenotypes and electrophysiological groups.17,25

Best disease and pattern dystrophy

FAF reflects the stages of Best disease: vitelliform material is intensely autofluorescent, whereas the signal decreases at the atrophic stage.[14] FAF may be normal at the subclinical stage, so electro-oculography remains the key functional test. The lower rate of molecular confirmation in the pattern-dystrophy

group is consistent with the phenotypic overlap between *PRPH2*-associated and other adult-onset forms.[15]

Ring of increased autofluorescence

In retinitis pigmentosa the ring encloses preserved central photoreceptors and constricts over time, whereas in cone dystrophies it surrounds the central lesion and expands.16,26 The inverse correlation between ring diameter and preserved EZ width in the cone/cone–rod group is therefore the expected direction: a larger ring means a larger central lesion and less preserved EZ within the scan.

FAF as an endpoint and in genetic triage

DDAF area can be measured reproducibly with semi-automated methods21 and is the primary endpoint in natural history studies and clinical trials in Stargardt disease.18 Where access to sequencing is limited, the FAF phenotype narrows the list of candidate genes; most mismatches occurred in cone–rod dystrophy, the most genetically heterogeneous group.

Limitations

The principal limitation is that the analysed records are simulated rather than observations from an actual clinical cohort. Consequently, the numerical estimates are not clinical evidence and may underestimate missing data, measurement error and inter-device variability.

Conclusions

FAF was evaluated as a biomarker of inherited macular disease using characteristic patterns for each dystrophy, DDAF area linked to structure and function, DDAF growth as a progression measure and the FAF phenotype as a guide to targeted genetic testing.

Source of funding

None.

Conflict of interest

None.

Ethical approval

Not applicable: no human participants or real patient data were involved.

Declaration of generative AI and AI-assisted technologies

The text and statistical analysis were prepared with Claude (Anthropic).

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