Fundus Autofluorescence in Patients with Leber Congenital Amaurosis

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PURPOSE. Fundus autofluorescence (FAF), as an index of lipofuscin accumulation in the retinal pigment epithelium (RPE), provides indirect information on the level of metabolic activity of the RPE and thus the integrity of the RPE/photoreceptor complex. To investigate whether the photoreceptor/RPE complex is still viable in patients with Leber congenital amaurosis (LCA), FAF imaging was performed.

METHODS. Three patients with LCA (patients A, B, and C; ages, 24, 15, and 37 years, respectively) were enrolled and one patient with RP with preserved visual acuity (age, 28 years) was included as a control. The diagnosis was based on history, visual function, and Ganzfeld electroretinography (International Society for Clinical Electrophysiology of Vision [ISCEV] standard). FAF was recorded with a confocal scanning laser ophthalmoscope (cSLO; Heidelberg Retina Angiograph; Heidelberg Engineering, Heidelberg, Germany).

RESULTS. All patients with LCA had vision reduced to perception of light and had undetectable ERGs. FAF was normal in patient A. In patient B, there was a paraffoveal ring of mildly increased FAF. The midperiphery showed mildly decreased FAF. Patient C showed a paraffoveal ring of moderately increased FAF. FAF was moderately decreased along the arcades and the midperiphery. The patient with RP showed a paraffoveal ring of moderately increased FAF and severely decreased FAF eccentric to the macula including the periphery.

CONCLUSIONS. The FAF findings in these patients with LCA suggest that there is continuous metabolic demand from the photoreceptors and that the RPE/photoreceptor complex is, at least in part, anatomically intact, but the photoreceptors have lost function. These findings may have implications for future treatment. It is notable that more than 20 years of severe visual impairment associated with LCA can be associated with normal FAF, indicating that photoreceptor function may be rescuable. (Invest Ophthalmol Vis Sci. 2004;45:2747–2752) DOI:10.1167/iovs.03-1208

Leber congenital amaurosis (LCA) accounts for 5% of all inherited retinal dystrophies and represents the earliest and most severe form of inherited retinal disease.1–4 LCA is clinically and genetically heterogeneous; most forms show autosomal recessive inheritance. Patients with nonsyndromic LCA typically have an onset of poor vision and nystagmus before 6 months of age and have an undetectable ERG.5 Visual acuity, when the children are old enough for formal assessment, is usually less than 20/400. The appearance of the fundus is highly variable.6–8 A normal-appearing fundus may be encountered in infancy,6,8,9 although later in childhood a variety of fundus abnormalities may be present.6,7 These include typical retinitis pigmentosa (RP),6,7,10 salt-and-pepper appearance of the fundus,10–15 increased granularity of the retinal pigment epithelium (RPE),10,15–17 white spots or fundus flecks,6,10,16 macular coloboma,6–8,10,17 marbled fundus,6,7,10,18,19 peripheral nummular pigmentation,6,10,20 attenuation of the retinal vessels,14,15 and optic atrophy.11 The clinical heterogeneity of the disease is reflected by the genetic heterogeneity. To date, six causative genes have been identified that together account for approximately half of all patients with LCA.21 These genes are expressed preferentially in the retina or the RPE. Their putative functions are diverse and include phototransduction (RetGC1/GUCY2D),22,23 vitamin A metabolism (RPE65),24,25 retinal embryonic development (CRX),26 protein trafficking (AIPL1, RPRG1),27–29 and photoreceptor cell structure (CRB1).30 These clinical and genetic studies suggest that there is a relatively uniform loss of retinal function in LCA, but that the underlying pathophysiological mechanisms and retinal morphologic changes may be extremely heterogeneous.

Fundus autofluorescence (FAF) imaging with a confocal scanning laser ophthalmoscope (CSLO) is a recent imaging technique that visualizes the RPE, taking advantage of its autofluorescence derived from lipofuscin.31–34 In vivo recording of RPE autofluorescence as an index of lipofuscin accumulation provides indirect information on the level of metabolic activity of the RPE, largely determined by the rate of turnover of photoreceptor outer segments. Progressive loss of lipofuscin occurs when there is reduced metabolic demand due to photoreceptor cell death. This is consistent with studies of RP that have shown correspondence between areas of decreased FAF and areas of photoreceptor cell loss35 and suggests that decreased FAF may be a good marker for the integrity of the RPE/photoreceptor complex and that FAF may be useful for investigating whether it is possible to restore retinal function with treatment.

Histopathologic studies in RP have shown that after the death of all photoreceptors in a given retinal area, RPE cells may detach from Bruch’s membrane and migrate to perivascular sites in the inner retina, producing the typical intraretinal pigmentation.36 The stimulus for RPE migration is unknown, but it has been speculated that it is due to the loss of metabolic byproducts derived normally from the photoreceptors.37 Thus, intraretinal pigmentation represents a late sign of photoreceptor cell death. In LCA with near normal fundi (i.e., without such pigmentation), it is questionable whether the photore-
ceptor/RPE complex is still viable. To address this question we performed FAF imaging in selected patients with LCA.

METHODS

Three patients with LCA were studied, and one typical patient with RP with good visual acuity was also studied as a control. The diagnosis was based on history of visual loss from early infancy, the findings of severe bilateral visual impairment, nystagmus, and undetectable flash ERGs. The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Moorfields Hospital Ethics Committee. For electroretinography, the protocol recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV) was used. FAF was recorded with a confocal scanning laser ophthalmoscope (cSLO; Heidelberg Retina Angiograph, HRA; Heidelberg Engineering, Heidelberg, Germany), as previously described. Because of nystagmus, single images were obtained that resulted in a poorer image quality compared with averaged images (for a comparison of image quality between such single and averaged images, see Fig. 1 in Ref. 40). Abnormal FAF was defined as either an increased or decreased FAF signal, compared with normal. FAF images in normal eyes have been published previously. It has been shown that FAF is dependent on age (for a range of normal FAF images in normal subjects of different age, see Fig. 1 in Ref. 42) with significantly increasing FAF intensity with age (see Fig. 2 in Ref. 42). However, interindividual variability in FAF intensity has been shown to be small compared with pathologic changes in retinal diseases.

RESULTS

All three patients with LCA had a lifelong visual function of perception of light in each eye, nystagmus, and undetectable flash ERGs. For comparison, data of a patient with typical RP and good visual acuity are presented, to illustrate the difference in FAF imaging between typical RP and this subset of patients with LCA.

RP Patient

A 28-year-old female patient with a family history of autosomal recessive RP had an 18-year history of nyctalopia. Visual acuity was 6/9 bilaterally. Fundus examination showed typical intraretinal bone spicula pigment deposition and attenuated vessels. Visual fields were bilaterally constricted to 10°. ERGs showed generalized retinal dysfunction affecting both the rod and the cone system, with the rod system being more affected (Fig. 1d).

FAF imaging revealed a parafoveal ring of moderately increased FAF and severely decreased FAF eccentric to the macula (Fig. 2), including the periphery.

LCA Patient A

This 24-year-old woman had a history of poor vision and nystagmus first noted at 6 weeks of age. There was no relevant
family history. Her visual function was perception of light in each eye. She had pendular nystagmus and normal anterior segments. The fundus examination showed mild attenuation of the retinal vessels but was otherwise normal. ERG testing showed undetectable responses under both photopic and scotopic conditions (Fig. 1c).

Several single FAF images including both the posterior pole and the periphery from both eyes could be obtained, despite the pendular nystagmus. They were all indistinguishable from normal when movements and optical artifacts were disregarded. Figure 3 shows one FAF image obtained from the posterior pole of the left eye. It shows a relatively even distribution of FAF, with a typical decrease in intensity in the macular area and lower FAF signals along large retinal vessels and at the optic disc. The slightly darker area in the superior/temporal area of Figure 3 is an illumination artifact. Analysis of other FAF images in this patient (not shown) showed that this darker area indeed represented the central macular area. Such decreased FAF at the macular area is at least partly due to absorption of the incident short wavelength light by the macular pigment. The optic disc showed some distortion in the vertical direction, and the corners of the FAF image showed some clouding, both of which represent artifacts due to eye movements.

**LCA Patient B**

This 15-year-old daughter of patient C had been severely visually impaired from birth. Both her father and her mother had LCA. She was otherwise healthy. On examination, visual function was perception of light in each eye. Both fundi showed only a mild pallor of the optic disc; mild attenuation of the retinal vessels; and, in the midperiphery, a subtle salt-and-pepper appearance. Flash ERG responses were undetectable under both photopic and scotopic conditions (Fig. 1b).

Figure 4 shows FAF images obtained from the lower half of the posterior pole of the right eye (Fig. 4a) and from the nasal midperiphery of the left eye (b), respectively. FAF at the posterior pole showed a parafocal ring of mildly increased FAF, the radius of which measured between about 5° and 7° eccentricity (cut in half by the top edge of the image) and a few tiny spots of increased FAF. The midperiphery showed mildly decreased FAF of a mottled appearance corresponding in retinal location to the salt-and-pepper appearance on funduscopy.

**LCA Patient C**

This 37-year-old female patient had been severely visually impaired from birth. She was otherwise healthy. She was the mother of patient B. On examination, visual function was perception of light in each eye. Both fundi were very symmetrically affected and showed pale optic discs; normal macular appearance, attenuated retinal vessels, and both mild hypopigmentation at the level of the RPE and intraretinal pigment in the midperiphery. Standard ERG responses were undetectable under both scotopic and photopic conditions (Fig. 1a).
lipofuscin concentration of the opposing RPE cells. More- 
over, it has been demonstrated that lipofuscin deposition de-
creased significantly in retinal degenerate rats only after pho-
toreceptors were lost.\\58 The residual bodies containing 
autofluorescent material have a finite half-life, because there 
is a constant degradation of residual bodies in the RPE.\\59–\\60

Impaired outer segment phagocytosis or a disrupted reti-
noid cycle may also contribute to reduced FAF. Mutation of a 
receptor tyrosine kinase gene (MERTK), in the Royal College 
of Surgeons (RCS) rat results in defective phagocytosis of pho-
toreceptor outer segments by the RPE and retinal degeneration. 
Mutations in MERTK cause RP in humans.\\51 Despite the RPE-

specific nature of the defect, photoreceptors are affected first, 
through the process of apoptosis\\52 and the time course of 
photoreceptor degeneration is rapid. Vitamin A (retinoids) 
plays a key role in formation of lipofuscin in the RPE. It has 
been shown that formation of RPE lipofuscin fluorophores is 
almost completely dependent on a normal visual cycle, be-
cause absence of retinal (both all-trans and 11-cis) in RPE65

knockout mice drastically reduces the formation of lipofuscin 
fluorophores in such animals.\\53 Thus, normal FAF is able to 
reflect the anatomic integrity of both the RPE and the photo-
toreceptors, normal outer segment turnover, and normal vitamin 
A metabolism.

Photoreceptor cell loss is a histologic hallmark of typical 
RP.\\57 von Rückmann et al.\\55 found profoundly abnormal (both 
increased and decreased) FAF at the posterior pole in most 
patients with RP and (exclusively) decreased FAF in the pe-

riphery in 100% of cases. Our patient with autosomal recessive 
RP showed similar findings.\\54 The FAF recordings in our 
patients with LCA differed markedly from those in RP. The nor-
mal FAF in patient A and the mild increase/decrease in FAF in 
patient B suggests continuous metabolic demand from the 
photoreceptors, with normal outer segment turnover and reti-
noid recycling. Thus, the RPE/photoreceptor complex in 
these patients may be, at least in part, anatomically intact and 
may have normal vitamin A metabolism and normal outer 
segment turnover, but the photoreceptors do not subserve 
visual function.

These findings are in accordance with a recent histopatho-
logic report in LCA caused by mutant GUCY2D, in which the 
retina retained a substantial number of cones and rods in the 
macula and far periphery.\\59 However, another study in a pa-
tient with a mutated APLI gene has shown extensive photo-
toreceptor cell death.\\60 These histopathologic reports reflect 
the heterogeneity of LCA that is known from clinical and genetic 
studies. The variability of histologic findings highlights the 
importance of in vivo techniques in investigating the viability 
of the photoreceptor/RPE complex in individual patients. We 
conclude that FAF imaging is such a technique and may serve 
to distinguish between photoreceptor cell death and loss of 
function in LCA.

To date, there has been no report of FAF recordings in 
patients with LCA. This lack of FAF data in LCA may be 
explained by the difficulty in recording FAF in patients with 
nystagmus. Although nystagmus precludes averaging of im-
ages, the single images obtained with the HRA allowed reason-
able FAF imaging with only few artifacts (cf. Fig. 2 and Fig. 3–5; 
see also Fig. 1 in Ref. 40).

Molecular genetic data were not available in the patients 
with LCA studied. LCA is currently diagnosed by history, clini-
cal examination, and electrophysiological findings and there 
have been very few published studies of genotype-phenotype 
correlation in LCA.\\15,\\28,\\29,\\57–\\59 The FAF findings in this sample of 
patients with LCA suggest that they do not carry mutations, at 
least in MERTK or RPE65, because such genetic defects should 
cause extremely reduced FAF. There has been a preliminary 
report that patients with RPE65 mutations show absent or
minimal FAF (Wabbel-BK, et al. IOVS 2002;43;ARVO E-Abstract 520). Given the differences in FAF between our subset of patients with LCA and patients with typical RP, we suggest that FAF recording may be a valuable additional tool for phenotyping retinal degenerations and may identify patients who would benefit from attempts at photoreceptor cell rescue.

High-density FAF presumably results from abnormally high turnover of photoreceptor outer segments, disrupted phagocytosis, or an inability of the RPE to recycle metabolites. Pattern ERG studies in these patients demonstrate a high correlation between the size of the abnormal ring and the degree of macular dysfunction, suggesting that lipofuscin accumulates at the boundary between normal and severely abnormal retinal function. Psychophysical studies based on high spatial resolution perimeter (fine matrix mapping) also suggest dramatically changing sensitivity across the width of the high-density ring and a profound reduction in sensitivity in the surrounding retinal areas. Age-related differences in ring size have been described in an autosomal dominant pedigree of RP in patients with preserved central macular function, and it has been suggested that the ring may demarcate the internal edge of peripheral field constriction as it encroaches on central macular areas, consistent with progressive visual field loss in RP. The functional significance of the high-density ring in patients with abnormal visual acuity is not known, but it is notable that the ring in patient C (Fig. 5) is approximately half the radius of that in her 15-year-old daughter (patient B; Fig. 4a). It is tempting to speculate that, in these cases, the ring of high density may constrict with time, possibly indicating progressive or slowly changing metabolic disturbance that may occur without degeneration and in the presence of severe central visual impairment. Longitudinal studies are needed to investigate this proposal.

Given that normal or near-normal FAF may reflect the presence of structurally intact photoreceptors and the integrity of the photoreceptor/RPE complex, our findings may have implications for future therapies designed to restore vision at the photoreceptor level. Our finding of normal FAF in LCA patient A, who had had this retinal dystrophy more than 20 years, is notable. Furthermore, the findings in patient B and C (daughter and mother, respectively) suggest that the time course of progressive photoreceptor cell death may be slow in a subset of LCA families and patients. It is clearly important to distinguish between cell death and cell dysfunction when considering therapy. If the photoreceptor cells are viable but dysfunctional, gene therapy may allow recovery of photoreceptor function, but cell transplantation would be inappropriate. By contrast, if loss of vision is due to photoreceptor cell death, gene therapy would serve to delay the disease progress.

References
