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 parafoveal ring of mildly increased FAF. The midperiphery showed mildly decreased FAF. Patient C showed a parafoveal ring of moderately increased FAF. FAF was moderately decreased along the arcades and the midperiphery. The patient with RP showed a parafoveal 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),[10,13,15] 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*, *RPGRIP1*),[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 loss[35] 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-
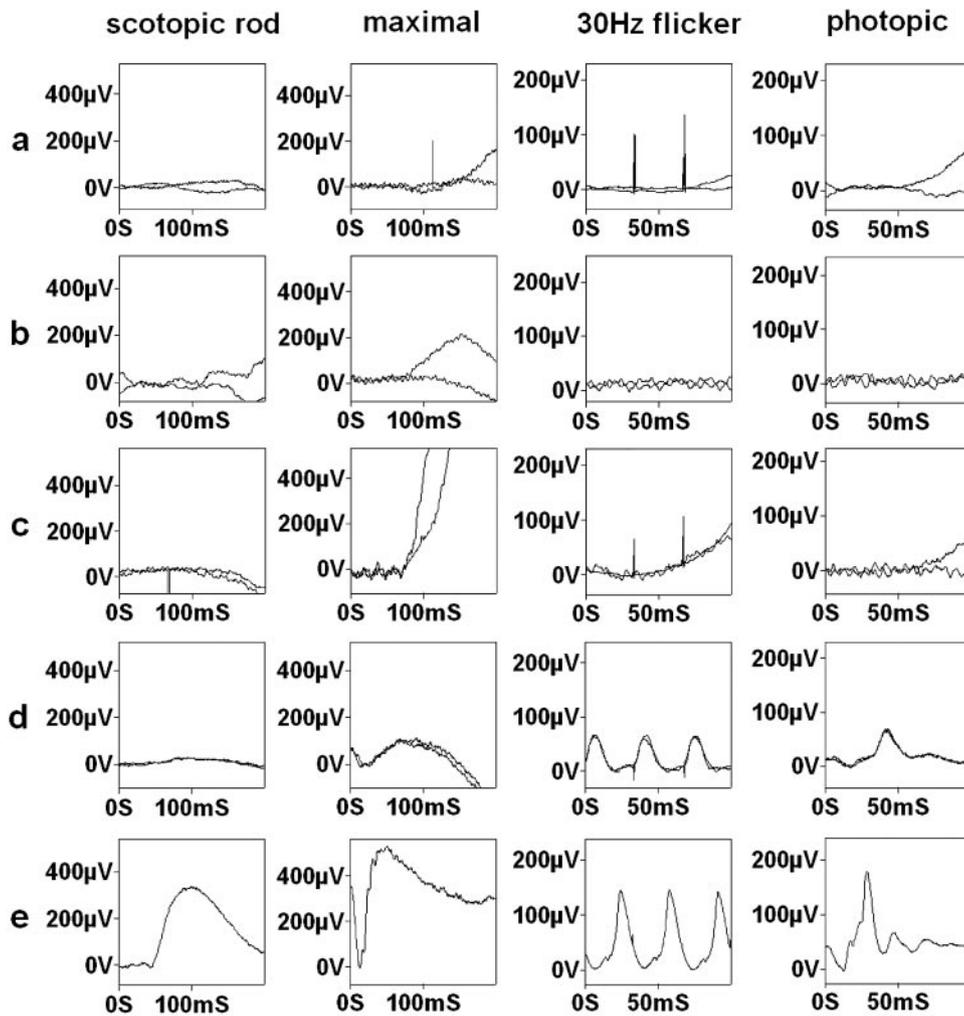
FIGURE 1. ISCEV-standard ERGs from the three patients with LCA (**a**, patient C; **b**, patient B; **c**, patient A), a patient with retinitis pigmentosa (**d**) and a normal example (**e**). Recordings in rows (**a**) to (**c**) show eye movement artifacts, inescapable in such patients.

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.[38] FAF was recorded with a confocal scanning laser ophthalmoscope (cSLO; Heidelberg Retina Angiograph, HRA; Heidelberg Engineering, Heidelberg, Germany), as previously described.[39,40] 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.[34] FAF images in normal eyes have been published previously.[34,41] Focally decreased or increased FAF was graded as mild, moderate, or severe, similar to grading in a previous report.[42] 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.[42]

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 spicular 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
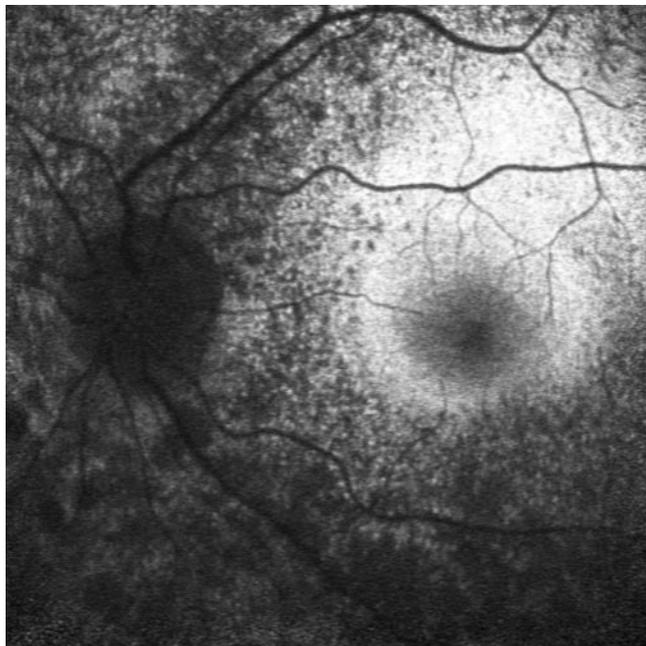
FIGURE 2. Fundus autofluorescence image of the patient with RP. The fovea was surrounded by a parafoveal ring of moderately increased FAF. There was severely decreased FAF eccentric to the macula.
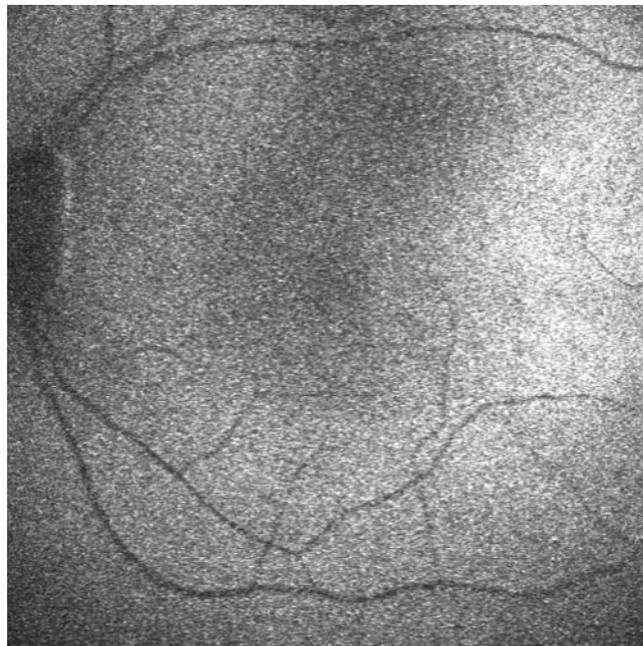

FIGURE 3. Fundus autofluorescence image obtained from the posterior pole in LCA patient A (left eye). FAF intensity and distribution were normal, apart from artifacts.

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.[34,41] 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 scotopic and photopic 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 (Fig. 4b), respectively. FAF at the posterior pole showed a parafoveal 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, a 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).

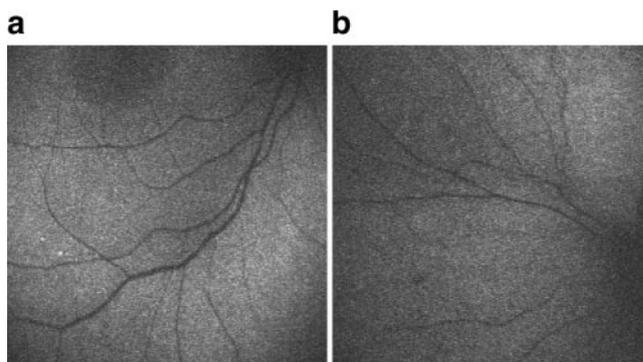**FIGURE 4.** Fundus autofluorescence images in patient B obtained from the lower posterior pole of the right eye (**a**) and from the nasal midperiphery of the left eye (**b**), respectively. FAF at the posterior pole showed a parafoveal ring of mildly increased FAF at between ~5° and 7° eccentricity and a few tiny spots of increased FAF (**a**). The midperiphery (**b**) showed mildly decreased FAF of a mottled appearance.
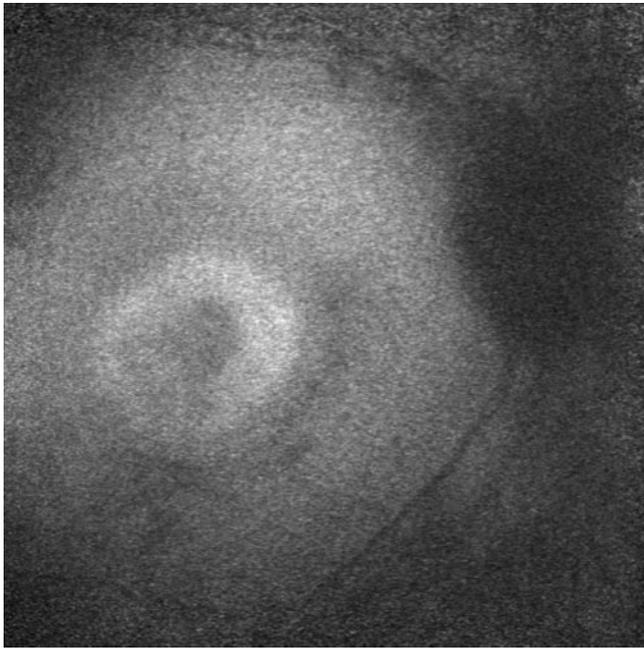
FIGURE 5. Fundus autofluorescence image obtained from the posterior pole in LCA patient C (right eye). The normal FAF signal from the center of the macula was surrounded by a ring of moderately increased FAF at between ~2° and 4° eccentricity. Along the arcades and the midperiphery, the FAF was moderately decreased, with a spotted appearance.

FAF was normal at the fovea, but there was a parafoveal ring of moderately increased FAF, the radius of which was between ~2° and 4° eccentricity (Fig. 5). Along the arcades and the midperiphery, the FAF was moderately decreased with a spotted appearance that showed some topographic correspondence to the RPE changes and intraretinal pigment observed on funduscopy. Peripheral to this area of decreased FAF in the midperiphery, FAF was moderately decreased in both eyes (not shown).

DISCUSSION

This is the first report of FAF recordings in patients with LCA and demonstrates that FAF may be preserved in the presence of severe photoreceptor dysfunction, as shown by undetectable full-field ERGs. Preservation of FAF indicates the presence of structurally intact photoreceptors and the integrity of the photoreceptor/RPE complex (discussed later). The distinction between photoreceptor cell death and cell dysfunction is likely to prove an important factor in the context of future therapies.

Physiologically reduced FAF is seen in the absence of RPE cells (e.g., at the optic disc) or may be due to absorption of the incident short wavelength light by melanin, macular pigment, and the retinal vessels.[41] Reduced FAF in retinal diseases may be due to a number of factors, including photoreceptor and/or RPE cell loss, disrupted phagocytosis or disruption of the retinoid cycle. In areas of geographic atrophy of the RPE, a late form of age-related macular disease (AMD), FAF is intensely reduced.[40,42,43] Very recently, it has been shown that both rod- and cone-driven sensitivity is reduced over such areas of decreased FAF in AMD, including geographic atrophy.[44] Loss of photoreceptors may also lead to reduced FAF, which may be expected because of several reasons. Progressive lipofuscin accumulation is mainly a byproduct of the constant phagocytosis of shed photoreceptor outer segment discs.[45,46] In humans, photoreceptor density was found to correlate with the lipofuscin concentration of the opposing RPE cells.[47] Moreover, it has been demonstrated that lipofuscin deposition decreased significantly in retinal degenerate rats only after photoreceptors were lost.[48] The residual bodies containing autofluorescent material have a finite half-life, because there is a constant degradation of residual bodies in the RPE.[49,50]

Impaired outer segment phagocytosis or a disrupted retinoid 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 photoreceptor 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, because 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 likely to reflect the anatomic integrity of both the RPE and the photoreceptors, normal outer segment turnover, and normal vitamin A metabolism.

Photoreceptor cell loss is a histologic hallmark of typical RP.[37] von Rückmann et al.[35] found profoundly abnormal (both increased and decreased) FAF at the posterior pole in most patients with RP and (exclusively) decreased FAF in the periphery 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 normal 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 retinoid 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 histopathologic 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.[55] However, another study in a patient with a mutated *AIPL1* gene has shown extensive photoreceptor cell death.[56] 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 images, the single images obtained with the HRA allowed reasonable 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, clinical examination, and electrophysiological findings and there have been very few published studies of genotype-phenotype correlation in LCA.[15,28,57–59] 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 (Wabbels 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,[34,60,61] disrupted phagocytosis,[62,63] or an inability of the RPE to recycle metabolites.[64,65] The high-density ring of FAF in the patient with RP (Fig. 2) resembled that in many other patients who manifest classic clinical signs of RP with preserved visual acuity.[34,54,66] 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.[54] Psychophysical studies based on high spatial resolution perimetry (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.[66] Age-related differences in ring size have been described in an autosomal dominant pedigree of RP in patients with preserved central macular function,[54] and it has been suggested that the ring may demarcate the internal edge of peripheral field constriction as it encroaches on central macular areas,[54] consistent with progressive visual field loss in RP.[67-69] 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.

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