Retinal Function in X-linked Ocular Albinism (OA1)

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ABSTRACT Purpose: To characterize retinal function in human recessive X-linked ocular albinism (OA1) across the normal lifespan. Methods: Retinal function was evaluated in 14 OA1 patients (ages 11 to 71 years) and five obligate carriers (ages 41 to 50 years) and compared to normal controls using full-field and multi-focal electroretinograms (ERG and mERG, respectively) and electro-oculography (EOG). Results: No consistent differences in ERG response parameters were observed when OA1 patients were compared as a group to normal controls. A trend in the direction of better correlations of response parameters with age was, however, observed in OA1. EOG Arden ratios were normal or hypernormal for all patients, but were uncorrelated with age. Central retinal function measured with the mERG suggested a flat response topography with depressed macular function compared to normal controls. Conclusions: Panretinal function in OA1 is within normal limits at all ages, consistent with previous reports in generalized albinism. The stronger correlations with age in OA1 may suggest a different rate of age-related change in OA1 compared to normal populations, but the precise nature of this change must await an appropriate prospective study. The topography of mERG amplitudes in OA1 is relatively flat across the central retina with a reduction in amplitude in the macular region consistent with anatomical studies demonstrating an underdeveloped macular region in albinism.

KEYWORDS albinism; electroretinogram; light damage; light toxicity; OA1; retinal function

INTRODUCTION

Albinism is a general term used to describe a group of hereditary disorders characterized by hypopigmentation of the skin, hair, and eyes. In recessive X-linked ocular albinism (OA1:OMIM; 300500), iris transillumination defects, hypopigmentation of the retinal pigment epithelium (RPE), and foveal hypoplasia are distinguishing features, whereas the skin and hair appear normal.1 Visual abnormalities associated with ocular albinism include reduced visual acuity, nystagmus, and hypersensitivity to light (photophobia). In addition, all albino mammals share a unique anomaly of the visual pathway in which nerve fibers originating from the temporal retina erroneously decussate at the optic chiasm,
disrupting retinotopic mapping throughout the visual pathway.\textsuperscript{2–5} Female carriers have also been shown to have iris transillumination defects and a variable pigmentary retinal appearance, although carriers are typically asymptomatic.\textsuperscript{6} X-linked recessive ocular albinism is caused by mutations in the RPE gene \textit{OAI} (approved gene symbol \textit{GPR143}) localized to chromosome Xp22.32.\textsuperscript{7} Examination of skin and/or RPE melanocytes have demonstrated that abnormal melanosomal biogenesis is associated with \textit{OAI} defects.\textsuperscript{1–4}

Mouse models of inherited ocular disease provide powerful tools for rapid genetic testing, structural and functional characterization, and gene identification in the study of human ocular disease.\textsuperscript{8,9} A mouse model of \textit{OAI} has recently been described.\textsuperscript{10} The \textit{OAI}\textsuperscript{−/−} mouse demonstrates characteristics typically observed in human albinism, including hypopigmentation of the fundus, fewer and larger melanosomes on microscopic analysis of the RPE, and misrouting of optic nerve fibers.\textsuperscript{10} However, the \textit{OAI}\textsuperscript{−/−} mouse also demonstrated significant electrophysiological abnormalities as measured with the electroretinogram (ERG), including significant reductions of both \textit{a}- and \textit{b}-wave amplitudes as well as delayed recovery of retinal function following exposure to intense bleaching lights.\textsuperscript{11} This is the first study to indicate that the lack of \textit{OAI} gene product can result in panretinal dysfunction of both rod- and cone-mediated vision. Interestingly, while an adeno-associated viral (AAV) vector-mediated \textit{OAI} gene transfer to the retina of the \textit{OAI}\textsuperscript{−/−} mouse increased melanosomes density in the RPE, the treatment only partially restored the normal ERG phenotype.\textsuperscript{11}

Retinal function in human albinism has rarely been shown to be abnormal even when evaluated in older patients, and when abnormal has been attributed to causes other than retinal cell death.\textsuperscript{6,12–14} However, there are only a few isolated case descriptions of retinal function in human \textit{OAI}.\textsuperscript{6,12} and there have been no studies that have sampled retinal function across the lifespan. In light of the functional abnormalities observed in the \textit{OAI}\textsuperscript{−/−} mouse model, and the paucity of human \textit{OAI} data, we undertook a simultaneous study of retinal function in both the mouse and in \textit{OAI} patients in an attempt to reconcile the different observations in human and mouse \textit{OAI} and to establish the relevance of the \textit{OAI}\textsuperscript{−/−} mouse model to human disease. This article describes our findings in human \textit{OAI}. Our primary goal was to evaluate panretinal rod- and cone-mediated function at different ages, which we accomplished by recording full-field ERGs under standardized conditions across the lifespan. In addition, since abnormal melanosomal biogenesis in the RPE is associated with \textit{OAI} defects, the electro-oculogram (EOG) was used to evaluate the integrity of the photoreceptor-RPE interface. Finally, while foveal hypoplasia is characteristic of \textit{OAI} albinism, the status of macular function is largely unknown. In an attempt to gain some understanding of macular function in \textit{OAI}, multi-focal ERGs were also recorded from a subset of eyes.

## METHODS

### Subjects

Fourteen \textit{OAI} patients (ages 11 to 71 yr) and five obligate carriers (ages 41 to 50 yr) participated in the study. To be included in the study, all males were required to have a diagnosis of X-linked ocular albinism from a retinal specialist and to demonstrate classical clinical findings, including iris transillumination, blonde fundus, foveal aplasia identified by an indistinct foveal and foveolar reflex, and nystagmus (Table 1 and Fig. 1). Male patients younger than 10 years of age were excluded from participation. There were no other exclusion criteria. X-linked transmission was confirmed by pedigree analysis. Visually evoked cortical potentials (VECPs) were performed on some patients (data not shown) to confirm the misrouting of optic nerve fibers that is typical in albinism.\textsuperscript{5} All female carriers were asymptomatic but showed the typical variegated pigmented fundus. Patients were recruited nationwide using an ocular albinism registry maintained by the Vision of Children Foundation (San Diego, CA) and brought to the University of California, Los Angeles (UCLA) for evaluation. Age-matched normal controls obtained in our laboratory served as comparison. The study was carried out with approval of the UCLA Institutional Review Board (IRB), informed consent was obtained from all patients, and the study was conducted in accordance with regulations of the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

## Electrophysiology

Retinal function of each patient, female carrier and normal control, was assessed with the full-field electroretinogram (ERG), the multi-focal electroretinogram (mERG), and the electro-oculogram (EOG). All
testing protocols were performed in accordance with the standards set by the International Society for Clinical Electrophysiology of Vision (ISCEV).

Full-field ERGs were performed in standard fashion. ERGs were recorded from the corneal surface of both eyes after pupil dilation (0.5% cyclopentolate hydrochloride and 1% phenylephrine hydrochloride) using a Burian-Allen contact lens electrode (Hansen Ophthalmic Development, Coralville, IA, USA). A drop of methylcellulose (2.5%) was placed on the corneal surface to ensure electrical contact and to maintain corneal integrity. Responses were amplified (Grass CP511 AC amplifier, West Warwick, RI, USA × 10,000; 3 dB down at 2 and 10,000 Hz) and digitized using an I/O board (National Instruments, Austin, TX, USA, PCI-1200) in a personal computer. Signal processing was performed with custom software (National Instruments, LabWindows/CVI). Viewing position was orthogonal to an opening in a large Ganzfeld dome (LKC Technologies), the interior surface of which was painted with a highly reflective white matte paint (Eastman Kodak Corporation, Rochester, NY, USA, #6080). A flash head affixed to the outside of the dome at 90° to the viewing porthole illuminated the interior surfaces with brief flashes of light. A skin electrode on the surface of the forehead served as the ground.

Following 30 min of dark adaptation, rod-mediated ERGs were recorded to a dim achromatic flash (−1.87 log cd-s/m²), and a mixed rod-cone response was recorded to a bright flash (0.405 log cd-s/m²) to obtain the dark-adapted maximal retinal response. Flash presentation frequency was set to 1 Hz for the dimmest flashes and 0.2 Hz for the brightest flashes to avoid adaptation effects. Cone-mediated ERGs were recorded to achromatic flashes (0.405 log cd-s/m²) on a rod-saturating background (32 cd/m²) after 10 min of light adaptation with flash presentation rates of 1 and 30 Hz in accordance with the ISCEV standard protocol. Responses were computer averaged at all intensities with up to 20 records averaged for the weakest signals. A signal rejection window was used to eliminate electrical artifacts produced by blinking and eye movements.

Multi-focal ERG: Multi-focal ERGs (VERIS System, EDI Inc., Redwood City, CA, USA, Version 5.0) were recorded to map the topography of retinal function in the central retina. The stimulus array consisted of 103 hexagons that were modulated between black and white in a pseudo-random sequence called an $m$-sequence. Local responses were mathematically extracted by cross-correlating the stimulus $m$-sequence with the recorded signal from the eye as previously described. The $m$-sequence (with an exponent of 15) was divided into at least 16 equal segments, each requiring 34.5 sec to complete. Shorter segments were used in patients who had difficulty maintaining direction of gaze or who had pronounced nystagmus.
As with most patients with poor central vision, steady fixation was difficult for OA1 patients. The direction of gaze was monitored continuously with a camera imaging the corneal surface. Most patients had little difficulty maintaining direction of gaze, despite the presence of nystagmus, when a fixation circle, concentric with the macula, was used. With gaze directed to the center of the circle, the angular diameter of the fixation circle was gradually decreased until the patient no longer reported a continuous and unbroken circle. The presence of a continuous circle was required for a successful trial, and patients were instructed to report the trials for which the circle perimeter was broken. These trials were then replicated. In addition, those trials that were deemed noisy due to loss of fixation gaze, as observed with the camera imaging the corneal surface, were also replicated. Finally, after each segment was recorded, the segment was analyzed using an algorithm provided by the manufacturer to show the distribution of sampled values for the entire segment, ordered by height and sorted from negative to positive. If the distribution of sampled values deviated significantly for the average reference value, or demonstrated evidence of signal saturation, the entire segment was replicated. These procedures minimized the inclusion of “noisy” trials due to loss of fixation, and more importantly, established a level of confidence in the interpretation of our results. The amplitude and frequency of the superimposed nystagmus was not measured. Subjectively, the magnitude of the nystagmus was greatest...
for the younger patients and appeared to diminish with age (see below for further discussion).

Electro-oculogram (EOG): The EOG measures a standing electrical potential that exists between the cornea and retina. There are two components to the EOG: one that is light sensitive and the other that is light insensitive. Both the light-sensitive and insensitive components of the EOG are dependent on the integrity of the retinal pigment epithelium (RPE) and photoreceptor interface. In the clinical EOG, eye movements are used to measure the corneal-fundal potential on the assumption that eye movements do not affect this potential. The potential was measured for 15 sec each minute, first under light-adapted conditions for 5 min, then in the dark for 15 min, and finally under light-adapted conditions again for 10 min. The ratio (called the Arden Ratio) of the amplitude of the dark trough (the lowest amplitude during dark adaptation) to the asymptotic amplitude during final light adaptation, was calculated and used as a measure of the integrity of the RPE/photoreceptor interface.

Some patients can have difficulty performing the saccadic eye movements required of the EOG if central vision is not good or when there is nystagmus. To ensure compliance with the task, we used a relatively bright focal target presented at a lower temporal frequency (0.5 Hz) to enhance target detection. Real-time viewing of the EOG potentials occurred continuously throughout testing and was used to evaluate the quality of the recordings. The patients could clearly perform the saccadic eye movements, although in most records, a small overshoot of the target location was observed. The small overshoot was present in both the light and dark segments of the recording session, and therefore added a constant level of noise to the recordings. However, because the temporal frequency of the stimulus modulation was slower than commonly used in EOG recordings, the response amplitude for a particular direction of gaze was averaged over a longer time period (approximately 2 sec), thereby minimizing the effects of the overshoot. In addition, some OA1 patients had difficulty with the transition from the dark- to the light-adapted portions of the EOG because of photophobia. These effects were minimized by having the patient close their eyes for the first minute of the transition from dark to light to give them an opportunity to adjust to the light. By the end of the 10 min of light adaptation, patients had no difficulty performing the saccadic eye movements and could easily maintain open eyes.

Optical Coherence Tomography (OCT): The present study used Zeiss Stratus OCT 3 (Carl Zeiss Meditec, Dublin, CA, USA) imaging to scan the central retina of each patient. The testing protocol was similar to that previously described.\textsuperscript{18} The OCT images were recorded after dilation and were obtained by an experienced photographer using a fast macular thickness protocol. The fast macular scan was used to minimize the effects of unsteady fixation due to nystagmus. Fixation was stabilized using either an internal fixation target presented to the tested eye or to an external target presented to the opposite eye. On occasions when the fixation target could not be located, the patient was instructed to shift their gaze until the scan line, which was under continuous view of the photographer, bisected the macular region. Numerous scans were recorded if the images were blurred or when the retinal laminae were broken due to eye movements.

Seo et al.\textsuperscript{19} proposed an OCT classification of albinism based on 13 patients and correlated the macular OCT findings with iris transillumination and foveal hypoplasia severity. The OCT grading scheme assessed choroidal transillumination and reflectivity and foveal depression. OCT images were assessed for the above criteria and, in addition, foveal thickness and perifoveal thickness values were measured.

**Statistical Analysis**

Data from both eyes were included in all analyses, and a repeated measures linear regression model with a compound symmetry covariance structure was performed to account for correlations between the two eyes of each subject in all analyses. These types of models have been developed for the analysis of correlated data and are widely used in many applications.\textsuperscript{20} Summary descriptive statistics shown in Table 2 are for both eyes combined. Due to the skewed distribution in amplitude and timing, a natural logarithmic transformation of the amplitude and timing was used in all analyses. The differences in amplitude and timing between OA1 and normal groups were compared using repeated measures linear regression models with interaction terms of group and response. The difference between normal and OA1 for each response was evaluated by a contrast from the model estimate, instead of separate \textit{t}-tests. To account for multiple comparisons, a Bonferroni correction was applied. The relationship between age and amplitude or timing was estimated using a repeated measures linear
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S¹ = significant after Bonferroni correction for multiple comparisons.
ns = not significant.
*= significant at p < 0.05.
regression model with an interaction term of group and age for each response separately. All statistical analyses were performed using statistical software SAS Version 9.1 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Full-Field ERG

The summary statistics for each response are presented separately for OA1 and normal groups in Table 2. Response amplitude and implicit times derived from standard protocol stimuli are summarized graphically in Figures 2 and 3, respectively.

Difference in Amplitude between OA1 and Normal

When comparing OA1 to normal, there were no statistically significant differences in amplitude for the b-wave of the maximal response (0.083 ± 0.076 ln μV; p = 0.279), the cone b-wave (0.087 ± 0.086 ln μV; p = 0.313), and cone 30-Hz flicker response (0.16 ± 0.096 ln μV; p = 0.096), but OA1 patients appeared to have significantly larger amplitude signals for the rod response (0.18 ± 0.076 ln μV; p = 0.020), for the a-wave of the maximal response (0.21 ± 0.098 ln μV; p = 0.032), and the cone a-wave (0.28 ± 0.098 ln μV; p = 0.0047). However, after applying the Bonferroni correction, only cone a-wave showed a borderline significant difference.

Difference in Implicit Timing of Responses between OA1 and Normal

There were no statistically significant differences in the implicit times for the a-wave of the maximal response (−0.024 ± 0.026 msec; p = 0.354), the b-wave of the maximal response (−0.030 ± 0.045 msec; p = 0.503), and the cone 30-Hz flicker response (−0.058 ± 0.037 msec; p = 0.122). However, OA1 patients appeared to have shorter implicit times compared to normal for the rod response (−0.14 ± 0.032 msec; p < 0.0001), for the cone a-wave (−0.058 ± 0.031 msec; p = 0.061), and the cone b-wave (−0.079 ± 0.038 msec; p = 0.039). Only the rod b-wave implicit time remained statistically significant after applying the Bonferroni correction.

Relationship between Amplitude and Age

Amplitude was not correlated with age for the a-wave of the maximal response for both the OA1 (slope = −0.0048 ± 0.0038; p = 0.205) and normal (slope = −0.0021 ± 0.0029; p = 0.495) groups for the amplitude of the cone a-wave (OA1: slope = −0.0017 ± 0.0039; p = 0.666; normal group: slope = −0.0042 ± 0.0031; p = 0.174), cone b-wave (OA1: slope = −0.0060 ± 0.0033; p = 0.074; normal group: slope = −0.0008 ± 0.0027; p = 0.768), and the cone 30-Hz flicker response (OA1: slope = −0.0015 ± 0.0037; p = 0.69; normal group: slope = −0.0038 ± 0.0032; p = 0.242).

Amplitude for the dark-adapted rod-isolated response was not related to age in the normal control group (slope = −0.0016 ± 0.0021; p = 0.450), but showed evidence of decreasing values with increasing age in the group of OA1 patients (slope = −0.0096 ± 0.0026; p = 0.0007). A similar relationship was observed for the b-wave of the maximal response (OA1: slope = −0.0088 ± 0.0027; p = 0.002; normal group: slope = −0.0017 ± 0.0022; p = 0.443).

Relationship between Timing and Age

Implicit time was not related to age in both OA1 and normal groups for the rod b-wave (OA1: slope = 0.0017 ± 0.0012; p = 0.176; normal: slope = 0.00097 ± 0.00098; p = 0.328), for b-wave of the maximal response (OA1: slope = 0.0012 ± 0.0017; p = 0.470; normal: slope = 0.0019 ± 0.0014; p = 0.178), and for the cone 30-Hz flicker response (OA1: slope = 0.0016 ± 0.0014; p = 0.270; normal: slope = 0.0004 ± 0.0012; p = 0.753). The implicit time for the a-wave of the maximal response was not related to age in the normal group (slope = 0.0011 ± 0.0008; p = 0.159), but showed evidence of increasing values with increasing age in OA1 (slope = 0.0022 ± 0.0009; p = 0.023). Implicit time for the cone a-wave was not related to age in the OA1 (slope = 0.0011 ± 0.0011; p = 0.328), but showed evidence of progressively longer implicit times with increasing age in the normal group (slope = 0.0024 ± 0.0009; p = 0.012). A similar relationship was observed for the implicit time of the cone b-wave (OA1: slope = 0.0012 ± 0.0014; p = 0.391; normal: slope = 0.0024 ± 0.0011; p = 0.043).

Multi-Focal ERG (mERG)

The mERG was used to assess local function in the central retina, including the region of foveal aplasia. Representative first-order mERG responses are shown in the right column of Figure 1 for different ages. Response density plots are not shown because the
FIGURE 2  Full-field ERG parameters obtained from standard ISCEV protocol responses. In each panel, parameters for OA1 patients are shown as open and filled circles for the right and left eyes, respectively, and for OA1 carriers (triangles). The relationship between age and amplitude was estimated using a repeated measures linear regression model as described in the Methods section, and the fits are shown as the heavy solid line for OA1 and the thin solid line for normal controls. Dashed lines are the 95% confidence limits for the normal controls. Panel A: rod-isolated response amplitude. Panels B and C: a- and b-wave amplitudes of the maximal response, respectively. Panels D and E: a- and b-wave amplitudes of the cone single flash response, respectively. Panel F: 30-Hz cone flicker amplitudes.
“effective” area of retinal stimulation was unknown due to the presence of varying degrees of nystagmus in OA1 patients. The nystagmus was not measured. Because the retinal stimulus is smeared as a result of the nystagmus, our approach to characterizing central retinal function in OA1 was to average “local” responses over larger areas and across patients in order to minimize the effects of the nystagmus-produced image blur. The mERG data was analyzed by sub-dividing the response array into six rings concentric with the macula. Ring 1 was the most central region corresponding to the putative macula, and ring 6 was the most eccentric at about 15° eccentricity. Amplitude and timing of the first positive peak (P1) was determined for each of the six rings. These parameters, used in all subsequent statistical analyses, are summarized in Figure 4.

**Difference in Amplitude between OA1 and Normal**

When comparing OA1 to the normal, there was no statistically significant difference in amplitude for ring 2 (0.084 ± 0.110 ln µV; p = 0.445), but OA1 appeared to have smaller amplitudes for ring 1 (−0.488 ± 0.116 ln µV; p < 0.0001) and larger amplitudes for ring 3 (0.314 ± 0.112 ln µV; p = 0.0052), ring 4 (0.456 ± 0.116 ln µV; p = 0.0001), ring 5 (0.548 ± 0.121 ln µV; p < 0.0001), and ring 6 (0.594 ± 0.124 ln µV; p <
FIGURE 4  Analysis of mERG responses. Mean amplitude (Panel A) and implicit times (Panel B) for six concentric rings centered on the macula in OA1 (open circles) and normal controls (filled circles). Error bars are standard errors. Panels C and D: Age-related changes in central retinal function for ring 1 (macula) and ring 3 (parafoveal) regions.

0.0001). After applying the Bonferroni correction, the difference between normal and OA1 for rings 1, 4, 5, and 6 remained statistically significant.

**Difference in Timing between OA1 and Normal**

Compared to normal, OA1 appeared to have shorter P1 implicit times for all rings. The difference (in logarithmic units) in each ring was: ring 1 (−0.077 ± 0.016 msec; p < 0.0001), ring 2 (−0.050 ± 0.013 msec; p = 0.0001), ring 3 (−0.037 ± 0.019 msec; p = 0.054), ring 4 (−0.047 ± 0.020 msec; p = 0.020), ring 5 (−0.032 ± 0.016 msec; p = 0.046), and ring 6 (−0.024 ± 0.019 msec; p = 0.208). However, after applying the Bonferroni correction, only the difference for rings 1 and 2 remained statistically significant.

**Difference in Amplitude with Eccentricity**

A trend in the direction of decreasing amplitude with eccentricity was observed in OA1. When compared to the amplitude of ring 1 (the putative macula), the difference in amplitude was −0.030 ± 0.148 ln µV for ring 2 (p = 0.838), −0.285 ± 0.149 ln µV for ring 3 (p = 0.057), −0.448 ± 0.152 ln µV for ring 4 (p = 0.0035), −0.549 ± 0.155 ln µV for ring 5 (p = 0.0005), and −0.670 ± 0.158 ln µV for ring 6 (p < 0.0001). However, after applying the Bonferroni correction, only the
decrease in amplitude for rings 4, 5, and 6 remained statistically significant (Fig. 4A).

Amplitude also decreased with eccentricity in the normal group, but the amount of change was greater than that observed for OA1. When compared to ring 1, the amplitude difference was $-0.602 \pm 0.059 \ln \mu V$ for ring 2 ($p < 0.0001$), $-1.087 \pm 0.059 \ln \mu V$ for ring 3 ($p < 0.0001$), $-1.391 \pm 0.059 \ln \mu V$ for ring 4 ($p < 0.0001$), $-1.584 \pm 0.060 \ln \mu V$ for ring 5 ($p < 0.0001$), and $-1.752 \pm 0.061 \ln \mu V$ for ring 6 ($p < 0.0001$). All comparison within the normal group remained statistically significant after applying the Bonferroni correction, indicating a much sharper decline in amplitude with eccentricity in the normal group compared to the OA1 group.

**Difference in Timing with Eccentricity**

The implicit time of P1 appeared to increase with eccentricity in the OA1 group, but the differences (in logarithmic units) were small. When compared to Ring 1, the difference in implicit time of P1 was $0.0035 \pm 0.019$ msec for ring 2 ($p = 0.855$), $0.0040 \pm 0.023$ msec for ring 3 ($p = 0.863$), $0.028 \pm 0.024$ msec for ring 4 ($p = 0.246$), $0.064 \pm 0.021$ msec for ring 5 ($p = 0.0024$), and $0.073 \pm 0.023$ msec for ring 6 ($p = 0.0018$), and the difference in rings 5 to 6 remained statistically significant after applying the Bonferroni correction (Fig. 4B).

There was some evidence of J-shaped trend in timing with eccentricity in the normal group since the timing in rings 2 and 3 was shorter than that for ring 1, while the timing in rings 5 and 6 was greater. When compared to ring 1, the difference in implicit time was $-0.024 \pm 0.0082$ msec for ring 2 ($p = 0.0041$), $-0.044 \pm 0.0097$ msec for ring 3 ($p < 0.0001$), $-0.0023 \pm 0.010$ msec for ring 4 ($p = 0.822$), $0.019 \pm 0.0089$ msec for ring 5 ($p = 0.036$), and $0.021 \pm 0.0098$ msec for ring 6 ($p = 0.035$). The difference in rings 2 to 3 remained statistically significant after applying the Bonferroni correction.

**Relationship between Amplitude and Age**

The relationship between response amplitude and age was determined in the macular region (ring 1; Fig. 4C) and for ring 3 covering the paramacular region (Fig. 4D), the first ring where OA1 amplitudes are not significantly different from normal. Amplitude was not related to age in ring 1 for both OA1 and normal groups (OA1: slope = $-0.0080 \pm 0.0050$; $p = 0.113$; normal; slope = $-0.0031 \pm 0.0028$; $p = 0.255$), or ring 3 (OA1: slope = $-0.0029 \pm 0.0049$; $p = 0.553$; normal; slope = $-0.0029 \pm 0.0026$; $p = 0.282$).

**Electro-Oculogram (EOG)**

EOG Arden ratios are plotted in Figure 5. The normal range for the EOG Arden ratios (mean + 2 SD) are shown as the cross-hatched region. With the exception of only two data points, which fell just outside the lower limits of normal, all other data points are either within normal limits or are better than normal. These results suggest normal function of the retinal pigment epithelium/photoreceptor interface. No age-related trends were apparent.

**Optical Coherence Tomography (OCT)**

OCT thickness measurements are shown in Figure 1 together with topographical maps of the posterior pole. The topographical maps are included to show that there is no evidence of a foveal depression outside the center of the topographical map that may have occurred as a result of eccentric fixation and/or nystagmus. The topographical maps were used to ensure that our thickness measurements were made through the putative foveal region.

![FIGURE 5 EOG Arden ratios obtained from both eyes of OA1 patients (circles) and female carriers (squares). The shaded area defines the region of normal Arden ratios.](image)
OCT imaging revealed that 24 of 28 eyes failed to demonstrate a foveal depression, while the remaining 4 eyes had normal foveal anatomy with OCT imaging. Six of 28 eyes demonstrated increased choroidal reflectivity, and 4 of 28 eyes showed the more severe tram track sign (i.e., a double-contour image of a hyporeflective retina and choroid). Average central foveal thickness values were 235 ± 20.1 µm, with a range of 190 to 256 µm. The mean foveal thickness measurements are in agreement with prior measurements in albinism. Perifoveal thickness values were also measured in rings concentric with the putative macula at 3.0 mm and 6.0 mm eccentricity. Thickness values were 232.8 ± 19.2 µm for the 3-mm ring and 219.0 ± 20.2 µm for the 6.0-mm ring. There was a significant overall location effect. Except for a nonsignificant difference between the thickness of the macula and at 3 mm eccentrically (difference + SE = 2.33 ± 2.26 µm; p = 0.313), both the macula and 3-mm ring were significantly thicker than at 6 mm eccentricity (difference + SE = 16.11 ± 3.13 µm, p < 0.0001 and 13.78 ± 2.26 µm, p < 0.0001, respectively). Age was not related to OCT thickness for any location or all locations combined.

Representative OCT images from patients at three ages and from a female carrier are shown in Figure 1.

DISCUSSION

Traditionally, electrophysiological tests of visual function in albinism have been limited to recording the visually evoked response from the visual cortex (VECP). All albino mammals share a unique anomaly of the visual pathway in that nerve fibers originating in the temporal retina erroneously decussate at the optic chiasm, disrupting retinotopic mapping throughout the visual pathway. This disruption in the visual pathway is apparent in functional recordings from the visual cortex using the visually evoked cortical potential (VECP) and is a key clinical diagnostic indicator for confirmation of an ocular albinism diagnosis. The main goal of this study, however, was to evaluate retinal function in OA1 patients sampled across the normal lifespan. The study was motivated, in part, by the observation in a mouse model of OA1 of relatively severe panretinal dysfunction as measured with the ERG, including significant reductions of both a- and b-wave amplitudes as well as delayed recovery of retinal function following exposure to intense bleaching lights. Surace et al. offered no explanation as to the underlying mechanism to account for the observed ERG changes and, in particular, offered no evidence that the OA1−/− mice suffer from a degenerative retinal disease. Interestingly, an AAV-mediated OA1 gene transfer to the retina of the OA1−/− mice only partially rescued the normal ERG phenotype. The b-wave of the ERG, a post-receptoral response, remained significantly reduced from normal controls, but rescue was better for the a-wave, a signal that is generally assumed to originate within the photoreceptor. In light of the observed retinal abnormalities observed in the OA1−/− mouse model, we felt it necessary to re-examine retinal function in human OA1 to determine whether such changes could also be found in human albinism. The presence of retinal functional abnormalities in OA1 would have significant impact on the management of albino patients in a clinical environment.

Studies of retinal function in ocular albinism have been few. The limited studies that are available for albino patients of all specified types (OA and OCA) suggest that retinal function is either normal or mildly subnormal, or hypernormal (that is, better than normal). Studies of retinal function in OA1 have been few. As a remedy, we sampled retinal function across the lifespan in a small group of patients with a diagnosis of OA1 that was confirmed by pedigree analysis, fundus appearance, and VEP recordings. In this study, panretinal function in OA1, as measured with the full-field ERG, is consistent with prior reports of retinal function in generalized albinism. In general, both response amplitudes and implicit times for both rod- and cone-mediated function were not significantly different from normal. However, observed consistently was a trend in the direction of higher response amplitudes in OA1, particularly at the younger ages, which in some instances were also accompanied by shorter (faster) implicit timing of peak components of the ERG. While many of these differences were relatively small and did not reach a level of statistical confidence, these trends are in the opposite direction and are clearly not consistent with the profound retinal abnormalities observed in the mouse model of OA1 previously reported. Interestingly, recent ERG studies on this same mouse model in our own laboratory did not reveal any differences in retinal function between the OA1 mouse model and normal controls. The reason for this discrepancy is not yet understood but may have
occurred as a result of differences in ERG methodology and/or light exposure history for the $OAI^{-/-}$ mice used in the prior study.

Age-related trends in ERG amplitudes also suggested stronger correlations with age in $OAI$ than in the normal control population. However, in most instances, amplitude was not significantly correlated with age for either the $OAI$ or control groups. The exceptions were the amplitude for the dark-adapted rod-isolated response, which was not related to age in the normal control group, but showed evidence of decreasing values with increasing age in the group of $OAI$ patients, and the b-wave of the maximal response, which showed a similar relation. In addition, there was a suggestion of different age-related trends in the implicit timing of peak components of the ERG. For example, the implicit time for the a-wave of the maximal response was not related to age in the normal group, but showed evidence of prolonged timing with increasing age in $OAI$. In general, the trend in prolonged timing was correlated with decreasing amplitudes. Whether these findings imply different rates of change of retinal function with age in $OAI$ cannot be determined precisely from this cross-sectional study. Plotting amplitude or timing parameters against age in a cross-sectional study cannot in itself be construed as informative with respect to the natural history of retinal function in patients with $OAI$. An appropriate prospective study will be required to determine whether the rate of change in $OAI$ is different from normal.

It is well known that the albino retina is more susceptible to light damage than is a normally pigmented retina. Factors affecting the susceptibility of photoreceptors to light damage include diet, circadian factors, ocular pigmentation, light exposure history, and genetic factors. While ERG responses observed in this study are robust and within normal limits at all ages, the consistent trend in the direction of more rapid change (decrease in amplitude and prolonged timing) with age in $OAI$ could be interpreted as consistent with an accelerated age-related loss of retinal function that might be attributed to phototoxic damage. Although equivocal, ocular melanin may play a role in protecting the retina from phototoxic damage, either by absorbing excess light, or by absorbing or retaining photoreactive products that might be toxic to the retina. Whether such changes occur in human $OAI$ will require a study of morphological data from human retinal tissue that is currently lacking.

Since the $OAI$ gene product is expressed in the RPE, we asked whether standard measures of RPE function in $OAI$ patients were also normal. Evaluation of RPE function was accomplished with the electro-oculogram (EOG). There have been few reports using the EOG to evaluate RPE function in human albinism. In one study, two patients with “generalized albinism” (1 male, 1 female) and two patients with “ocular albinism” (2 males) were studied, and the EOG was normal in two patients and hypernormal (better than normal) in two other patients (one from each type of albinism). Gouras and Gunkel reported a normal EOG for one patient with ocular albinism of unspecified type. Finally, EOGs were recorded from six patients with “complete” albinism and the EOGs were normal. In the current study, EOG parameters were variable at all ages, ranging from borderline normal to hypernormal, but few would be considered abnormal. These results, combined with the ERG results, suggest that the abnormal melanosomal biogenesis in the RPE is functionally silent in human $OAI$.

Foveal hypoplasia is characteristic of human OA1 and albinism generally. However, despite the hypoplastic foveal region, the status of macular cone function in $OAI$ is largely unknown. In this study, we measured central retinal cone function with the multi-focal ERG (mERG). However, while mERGs can be recorded in this patient population, the results must be interpreted cautiously because of the presence of nystagmus. Eye movements of any kind introduce a level of blur in the retinal image that will be variable and dependent on the extent of the eye movement. While we took special precautions (see Methods section) to ensure central fixation of the stimulus array as described in the Methods section, there are currently no available methods to ensure stabilization of the mERG stimulus on the retina, or to modify local measurements by the severity of the nystagmus to increase the accuracy of the mERG recordings. Nevertheless, while the nystagmus certainly increases the level of “noise” in the recordings, we felt that they could be generally informative as to the topography of central retinal function. The alternative was not to perform the testing.

Local responses were robust and well defined at all retinal locations. However, local response amplitudes in the putative macular region in $OAI$ patients were significantly lower than normal, whereas amplitudes derived from parafoveal and more peripheral locations were higher (Fig. 1, right panel, and Fig. 4A). Similar
topographies were observed recently in oculocutaneous albinism (OCA), although parafoveal and peripheral responses were better in this study than previously observed. In humans, the darker pigmentation in the macular region has been attributed to a greater number of melanin granules in this region. Topographically, melanin density shows a slight decrease from the peripheral retina to the posterior pole but with an increase in the macular region. Since a reduction in melanin content increases the probability of photon absorption, the higher amplitudes in the parafoveal retina may be attributable to the fact that more light is reaching these photoreceptors. Overall, the topography of mERG amplitudes in OA1 is relatively flat across the central retina, with a reduction in amplitude in the macular region consistent with anatomical studies demonstrating an underdeveloped macular region in albinism. However, the precise nature of macular function in OA1, and in albinism generally, will need to wait until better methodologies are developed to “correct” the image blur as a result of nystagmus, or of eye movements generally.

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