Macular Pigment and Lutein Supplementation in *ABCA4*-Associated Retinal Degenerations

Tomas S. Aleman,[1] *Artur V. Cideciyan,*[1] *Elizabeth A. M. Windsor,*[1] *Sharon B. Schwartz,*[1] *Malgorzata Swider,*[1] *John D. Chico,*[1] *Alexander Sumaroka,*[1] *Alexander Y. Pantelyat,*[1] *Keith G. Duncan,*[2] *Leigh M. Gardner,*[1] *Jessica M. Emmons,*[1] *Janet D. Steinberg,*[1] *Edwin M. Stone,*[3] *and Samuel G. Jacobson*[1]

Purpose. To determine macular pigment (MP) optical density (OD) in patients with *ABCA4*-associated retinal degenerations (*ABCA4*-RD) and the response of MP and vision to supplementation with lutein.

Methods. Patients with Stargardt disease or cone–rod dystrophy and known or suspected disease-causing mutations in the *ABCA4* gene were included. All patients had foveal fixation. MPOD profiles were measured with heterochromatic flicker photometry. Serum carotenoids, visual acuity, foveal sensitivity, and retinal thickness were quantified. Changes in MPOD and central vision were determined in a subset of patients receiving oral supplementation with lutein for 6 months.

Results. MPOD in patients ranged from normal to markedly abnormal. As a group, patients with *ABCA4*-RD had reduced foveal MPOD, and there was a strong correlation with retinal thickness. Average foveal tissue concentration of MP, estimated by dividing MPOD by retinal thickness, was normal in patients, whereas serum concentration of lutein and zeaxanthin was significantly lower than normal. After oral lutein supplementation for 6 months, 91% of the patients showed significant increases in serum lutein, and 63% of the patients' eyes showed a significant augmentation in MPOD. The retinal responders tended to be female and to have lower serum lutein and zeaxanthin, lower MPOD, and greater retinal thickness at baseline. Responding eyes had significantly lower baseline MP concentration than did nonresponding eyes. Central vision was unchanged after the period of supplementation.

Conclusions. MP is strongly affected by the stage of *ABCA4* disease leading to abnormal foveal architecture. MP could be augmented by supplemental lutein in some patients. There was no change in central vision after 6 months of lutein supplementation. Long-term influences of this supplement on the natural history of these macular degenerations require further study. (*Invest Ophthalmol Vis Sci.* 2007;48:1319–1329) DOI:10.1167/iovs.06-0764

From the [1]Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania, Philadelphia, Pennsylvania; the [2]Department of Ophthalmology, University of California, San Francisco, California; and the [3]Department of Ophthalmology, University of Iowa Carver College of Medicine, Iowa City, Iowa.

Supported by National Eye Institute Grant EY13203; The Foundation Fighting Blindness, Inc.; the Macula Vision Research Foundation; The Macular Disease Foundation; The Chatlos Foundation, Inc.; Howard Hughes Medical Institute; the Ruth and Milton Steinbach Award, Alcon Research Institute; and Research to Prevent Blindness.

Submitted for publication July 6, 2006; revised September 19, 2006; accepted January 10, 2007.

Disclosure: **T.S. Aleman**, None; **A.V. Cideciyan**, None; **E.A.M. Windsor**, **S.B. Schwartz**, None; **M. Swider**, None; **J.D. Chico**, None; **A. Sumaroka**, None; **A.Y. Pantelyat**, None; **K.G. Duncan**, None; **L.M. Gardner**, None; **J.M. Emmons**, None; **J.D. Steinberg**, None; **E.M. Stone**, None; **S.G. Jacobson**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Tomas S. Aleman, Scheie Eye Institute, 51 North 39th Street, Philadelphia, PA 19104; aleman@mail.med.upenn.edu.

The *ABCA4* gene encodes the ABCR protein, which localizes to the rims of rod and cone outer segments[1,2] and accelerates removal of all-*trans*-retinal from light-exposed photoreceptors by transporting A2-PE, a retinoid adduct formed by all-*trans*-retinal and phosphatidylethanolamine.[3–5] Mutations in the *ABCA4* gene cause a major proportion of autosomal recessive retinal degenerations (RD) with macular involvement.[6–12] Pathophysiology of *ABCA4*-RD involves trapping of A2-PE[3,13,14] within disc membranes of the photoreceptor outer segments (POS). Phagocytosis of the shed POS by adjacent retinal pigment epithelial (RPE) cells in ABCA4-deficient retinas results in excessive intracellular accumulation of lipofuscin, an aggregate of lipids, proteins, and fluorescent retinoids, including cytotoxic *bis*-retinoid A2E derived from the trapped A2-PE.[3,15] In extramacular retinas of patients with known *ABCA4* mutations, we have provided evidence supporting an abnormal increase in lipofuscin autofluorescence in the RPE preceding dysfunction and degeneration of the overlying retina.[11] We have also shown that the parapapillary retina is relatively spared from retinal degeneration.[12] Details of the macular disease sequence remain to be studied.

Macular degenerations, including those caused by *ABCA4* mutations, commonly go through a counterintuitive stage during which foveal vision and structure are relatively preserved compared with the surrounding parafoveal region.[9–11,16–18] It has been hypothesized that macular pigment (MP), a yellowish carotenoid mainly composed of lutein and zeaxanthin concentrated at the fovea, contributes to relative preservation of this retinal region in macular degenerations.[19] The mechanism of MP protection may involve passive absorption of shorter wavelengths of light.[20–25] Exposure to light not only causes A2E accumulation but also increases the potential toxicity of the accumulated A2E via photooxidation.[26,27] Further, it has been proposed recently that lutein and zeaxanthin specifically protect A2-PE in photoreceptors and A2E in RPE cells from photooxidation, and thus MP may have a particularly important role in *ABCA4* disease.[28]

To understand better the preserved foveas in *ABCA4*-RD, we explored the relationship between MP and systemic, ocular, and retinal features. Seeking ways to prevent loss of this remaining foveal vision in *ABCA4*-RD, we also performed a short-term open-label pilot study asking whether retinal MP could be modified with oral lutein supplementation.

TABLE 1. Clinical and Molecular Characteristics of the Patients

Patient	Age (y)/Gender	*ABCA4* Mutation	Visual Acuity*		Refraction†		Kinetic Visual Field Extent (V-4e)‡		Lutein Trial Participant?
			RE	LE	RE	LE	RE	LE	
1	18/M	G863A/R943Q	20/32	20/32	−0.50	−0.50	109	105	Y
2	18/F	E1087K/G1961E	20/25	20/25	−1.00	−1.25	103	104	N
3	18/M	‖	20/20	20/125	−1.00	−1.00	126	105	N
4§	19/F	R1129L/L1940P	20/40	20/50	+0.25	+0.25	90	93	Y
5	21/M	P1511del1ccgC/R1705Q	20/25	20/25	−0.75	−0.25	103	107	Y
6	24/M	T1019M/G1961E	20/50	20/200	−1.25	−1.50	112	105	Y
7§	26/M	‖	20/40	20/32	+1.00	+0.75	86	88	Y
8	30/F	‖	20/50	20/40	+2.25	+1.75	105	110	Y
9	30/M	R1108C/R152Q	20/20	20/32	−2.25	−3.50	99	93	Y
10	32/F	V935A/IVS40+5G→A	20/32	20/40	−0.75	−1.25	103	92	N
11	34/F	R681X/R1300Q	20/20	20/20	−1.50	−1.75	110	96	N
12	37/M	C54Y/G1961E	20/32	20/25	−3.00	−2.00	99	105	Y
13¶	38/F	V256V/G1961E	20/25	20/25	−1.00	−1.25	106	101	Y
14¶	42/F	V256V/G1961E	20/25	20/32	−0.50	−0.75	107	94	Y
15	47/F	R1300Q/R2107H	20/32	20/20	+0.75	+0.25	108	103	N
16§	49/M	‖	20/32	20/32	−4.50	−4.50	84	79	Y
17	56/M	G1977S	20/25	20/25	−5.50	−5.50	99	109	N

* Best corrected visual acuity.
† Spherical equivalent.
‡ Expressed as a percentage of normal mean of V-4e target; 2 SD below normal is 90%.
§ Clinical diagnosis of cone-rod dystrophy; remaining patients had a clinical diagnosis of Stargardt disease.
‖ Mutation unknown.
¶ Patients are siblings.

METHODS

Subjects

Stargardt disease or cone–rod dystrophy patients (n = 17), most of whom had known *ABCA4* gene mutations,[11,12,29] were included in the study (Table 1). All participants were in general good health. The subjects, all with central retinal disease, were selected for participation because of their relatively spared foveal function in at least one eye. All subjects had a routine ocular examination and best corrected visual acuity (VA) determined with the ETDRS (Early Treatment Diabetic Retinopathy Study) chart. Eyes included in the study had stable foveal fixation, as documented by the correspondence of the center of the anatomic fovea to fixation with optical coherence tomography (OCT). When two eyes were eligible, only the one with full MP profile and/or highest peak MP density was included in the analyses related to baseline parameters. This choice was made to be consistent with de facto inclusion of the "better" eye in patients with only one eye with foveal vision. Normal data from a group of subjects (n = 29) without ocular disease who participated in our previous study[30] were reanalyzed according to the current methods. A subset of the subjects (11 patients and 8 control subjects) underwent a pilot trial of supplementation with oral lutein for 6 months. In patients with interocular differences in disease severity, we anticipated possible interocular differences in response to lutein supplementation. Therefore, data from individual eyes are presented for the lutein supplementation section of the article. Informed consent was given by all subjects in compliance with the Declaration of Helsinki, and institutional review board approval was obtained.

Evaluation of the Macula: Macular Pigment and Central Retinal Function and Structure

Macular pigment optical density (MPOD) was measured by heterochromatic flicker photometry (HFP) using an LED-based MP densitometer (Macular Metrics Corp., Rehoboth, MA). This psychophysical technique compares flicker photometric sensitivity measured at and near the fovea with that obtained at a more peripheral retinal location.[22,31] Sensitivity is determined by alternating a short wavelength test light that is maximally absorbed by MP in counterphase with a longer wavelength reference light that is not absorbed by MP. The intensity of the test light is adjusted until the perception of flicker is minimized or eliminated, at which point the two lights are equated in apparent brightness. The peripheral-to-foveal sensitivity ratio is used to determine the peak density of MP. Details of the methodology in patients with hereditary retinal degenerations have been provided.[30,32] In brief, flickering stimuli (460 nm, test; 570 nm, reference, 1.7 log td) were centered on a 6° diameter background field (1.5 log td, 470 nm) while the patients fixated centrally on a 5-minarc spot. Four different stimuli were used that consisted of two discs (0.34° and 1° diameter) and two annuli (2° and 4° diameter, 0.4° wide). We will assume that flicker perception is dominated by the edges,[33] although other work has suggested that flicker may not be detected at the edge but is perceived by more central retinal eccentricities when using discoid stimuli.[34] Using the former assumption, these stimuli represent eccentricities of 0.17° and 0.5° (henceforth referred to as "foveal") and 1° and 2° (henceforth referred to as "parafoveal"); 0.17° eccentric stimulus will be referred to as 0.2°. Peripheral sensitivities were determined with a 2° diameter disc centered on the background while subjects fixated on a small red LED situated 7° to the nasal side of the background field; the radiance setting of the 460-nm test light needed for a flicker null at this eccentricity was not significantly different between patients and normal subjects (171 ± 61 vs. 157 ± 18 counts; P = 0.17).

Foveal visual function was measured using a modified[35] automated perimeter (Humphrey Instruments; Carl Zeiss Meditec, Inc., Dublin, CA) and a red (650 nm) target (1.7° diameter, 200 ms duration) in the dark-adapted state. Macular structure was quantified by OCT (Carl Zeiss Meditec, Inc.). The principles of OCT[36] and our methodology[11,37] have been published. Horizontal scans crossing the anatomic fovea were obtained in all subjects. Retinal thickness at the center of the fovea and at 0.5° of eccentricity was measured.[37] Serum carotenoids (lutein, zeaxanthin, and β-carotene) were measured using high-performance liquid chromatography (HPLC; Craft Technologies, Inc., Wilson, NC). Dietary information was obtained with the Health Habits and History questionnaire (HHHQ) developed by the National Cancer Institute[38]; data were analyzed by using the HHHQ Diet System Analysis Software.[39]

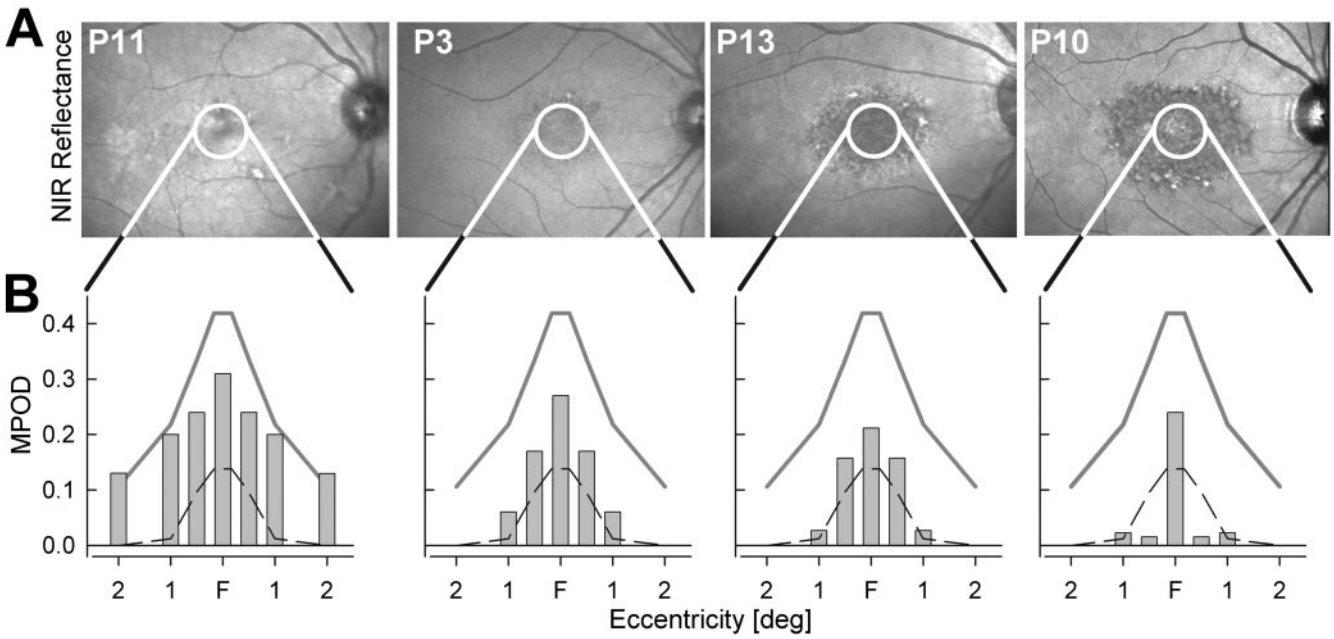

FIGURE 1. Patients representing the spectrum of fundus appearance and MPOD. (**A**) Near-infrared (NIR) reflectance images of the central retina in four patients. *Overlaid circles* delimit the central 5° diameter region within which MPOD measurements were performed. (**B**) MPOD in patients shown in (**A**). The foveal center (F) corresponds to MPOD determined with a 0.2° radius stimulus; MPOD determined with circular stimuli of 0.5°, 1°, and 2° radius are plotted with symmetric duplication from F. *Gray lines*: mean normal MPOD; *dashed lines*: lower limit (mean − 2 SD) of normal.

Supplementation with Lutein

A subset of patients ($n = 11$) participated in an open-label, 6-month pilot trial of oral lutein supplementation (Table 1). There was no placebo control group. After two baseline visits (separated by no more than 1 month; except patient 4, who had a single baseline visit), subjects supplemented their diets with a commercially available form of lutein at 20 mg per day (Twin Laboratories Inc., Hauppauge, NY). Subjects were instructed to take the lutein supplement with a meal with the most fat of the day, presuming this would enhance absorption of the supplement.[40] A further visit occurred 6 months after supplementation began. Baseline and follow-up visits included a clinical examination, fasting (overnight) venous blood sample for serum carotenoids, and measurements of MPOD and absolute dark-adapted sensitivity at the fovea with a 650-nm target.[30,32]

Data Analysis

Statistical software (SAS, ver. 9.1; SAS, Cary, NC) was used to analyze data. Mean data from the two baseline visits were used to describe the study groups and calculate the change after lutein supplementation. *t*-Tests were performed to compare means and significance levels for correlation coefficients. Intersession variability was assessed with signed and absolute differences of measurements between the first and second baseline visits. Means of intersession differences and person-specific variables were compared with independent *t*-tests. Proportions were compared using χ^2 tests with exact computation of the probabilities.

RESULTS

Patients had a clinical diagnosis of either Stargardt disease ($n = 14$) or cone–rod dystrophy ($n = 3$) and all but four had known mutations in the *ABCA4* gene (Table 1). All patients had macular disease with foveal sparing in at least one eye. The macular appearance on infrared reflectance imaging ranged from a mottled pattern with scattered dark and light lesions, to dark areas surrounding a lighter foveal center and bordered by a granular-appearing annulus (Fig. 1A). Kinetic visual fields were full in peripheral extent in all but two patients (Table 1); small scotomas around fixation were frequently detected. The four examples of MPOD at different eccentricities displayed below the fundus images (Fig. 1B) indicate that diseased eyes could have results that are within normal limits or show abnormalities.

Low Macular Pigment in *ABCA4*-RD

Eyes of patients had significantly lower MPOD than did those of normal subjects (Table 2). The distribution of MPOD in patients' eyes was shifted toward lower values than in normal eyes (Fig. 2A). At 0.5° eccentricity, 76% of the patients showed MPOD below 0.2, whereas only 7% of normal subjects had such low values.

MPOD normally peaks near the center of the fovea and declines with eccentricity.[41,42] The spatial distribution of MP was studied in a subset of 11 patients' eyes in which MPOD was measurable at all four eccentricities (Fig. 2B). On average, MPOD in these eyes was lower than normal at each eccentricity (Table 2, MPOD Profile). The distribution of MPOD profiles as estimated from the half-width at half-peak was narrower in these 11 patients than in the normal subjects (Table 2). The remaining six patients could not perform HFP at the parafoveal locations due to a lack of perception of the flicker. These eyes with an indeterminate spatial MPOD distribution corresponded to some of the lowest foveal MPOD (Fig. 2B, stars).

MP levels have been related to dietary,[22,43–46] demographic,[42,46–48] lifestyle,[49] systemic,[45,50–53] and ocular[54] characteristics in studies of normal populations. Examination of some of these factors (Table 2) showed that the patients and normal subjects included in this study were well matched in age, body mass index (BMI), gender, smoking status, race, and color of irides. Mean dietary fat intake was higher in the patients than the normal subjects but the groups were not significantly different and the patients' levels were similar to those reported in other studies in

TABLE 2. Baseline Group Summary Statistics*

	Patients (n = 17)	Normal (n = 29)	P†
General			
Age (y)	32 ± 12	30 ± 12	NS
BMI (kg/m^2)	24 ± 5	23 ± 3	NS
Female gender (%)	47	55	NS
Smoker (%)	18	14	NS
White (%)	82	90	NS
Light irides (%)	44	48	NS
Diet			
Lutein (mg/day)	2.6 ± 1.7	2.8 ± 2.1	NS
Fat (g/day)	89 ± 51	66 ± 41	NS
Serum			
Lutein (μmol/L)	0.20 ± 0.10	0.31 ± 0.14	0.003
Zeaxanthin (μmol/L)	0.07 ± 0.04	0.13 ± 0.06	0.002
β-Carotene (μmol/L)	0.47 ± 0.61	0.59 ± 0.39	NS
MPOD			
0.2°	0.22 ± 0.12	0.42 ± 0.14	<0.001
0.5°	0.15 ± 0.13	0.33 ± 0.12	<0.001
Retinal thickness			
0° (μm)	103 ± 50	198 ± 14	<0.001
0.5° (μm)	116 ± 53	205 ± 14	<0.001
MP concentration			
0.2° (μm^{-1})	0.23 ± 0.12	0.22 ± 0.07	NS
0.5° (μm^{-1})	0.12 ± 0.09	0.16 ± 0.06	0.030
MPOD profile			
0.2°	0.26 ± 0.13‡	0.42 ± 0.14	0.002
0.5°	0.20 ± 0.14‡	0.33 ± 0.11	0.004
1°	0.12 ± 0.11‡	0.22 ± 0.10	0.012
2°	0.07 ± 0.09‡	0.11 ± 0.06	NS
Half-width at half-peak (deg)	0.98 ± 0.63‡	1.28 ± 0.33	0.045

* Noncategorical variables are specified as mean ± SD
† Not significant (NS) values correspond to $P > 0.05$.
‡ Determined in subset of nine patients with full spatial profiles.

normal subjects.[45,55,56] Dietary lutein was similar in patients and normal subjects. The effect of factors contributing to low MPOD were further explored in patients by comparing subgroups in the first (MPOD ≤ 0.08) and fourth (MPOD ≥ 0.19) quartiles of the distribution of MP data in response to the conventional 0.5° eccentric stimulus. Females (60% vs. 40%, $P = 0.36$) and subjects with light-colored irides (60% vs. 20%, $P = 0.52$) were more commonly observed in the low MPOD compared with the high MPOD group of patients; the only smoker in these two subsets was in the low MPOD group. These results are in agreement with our previous observations in patients with other hereditary retinal degenerations[30,32] and in reports of normal subjects.[43,46,49,54]

Low Serum Lutein in *ABCA4*-RD

Serum levels of lutein and zeaxanthin were significantly lower in the patients compared with those in the group of normal subjects (Table 2). Serum levels of xanthophylls in most patients also fell within the lower end of normal values reported by other investigators in large population-based studies.[55–58] When the subset of subjects with low serum lutein (≤0.19 μmol/L, lowest quartile of our normal population) was considered, the patients had significantly lower zeaxanthin levels than did the normal subjects but could not be otherwise distinguished from them by other variables. Low serum xanthophyll levels were not associated with any one category of patients. For example, there were no significant differences

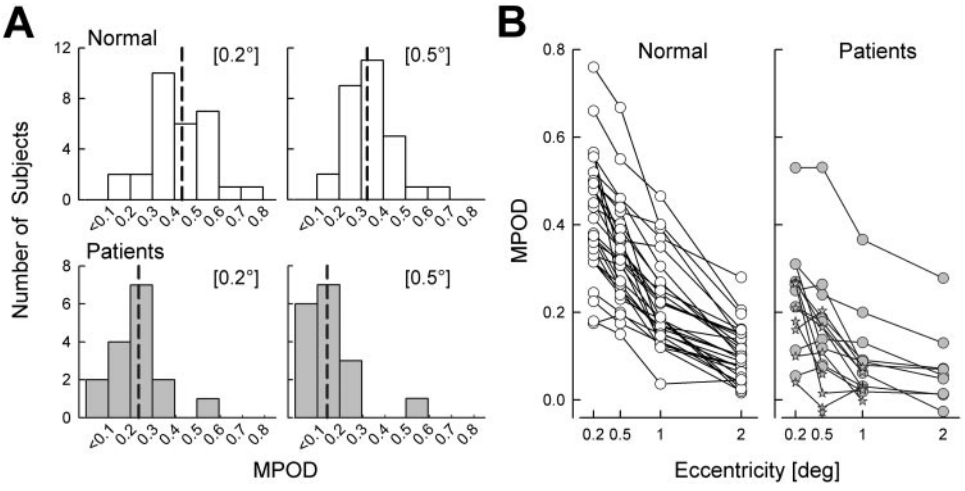

FIGURE 2. MPOD in patients compared with normal subjects. (**A**) Frequency-distribution histograms of MPOD measured at foveal locations (0.2° and 0.5°). *Vertical dashed lines*: median value of each distribution. (**B**) All individual MPOD values measured in patients and normal subjects. *Circles*: full profiles; *stars*: patient eyes in which MPOD could only be determined in a subset of the four eccentricities.

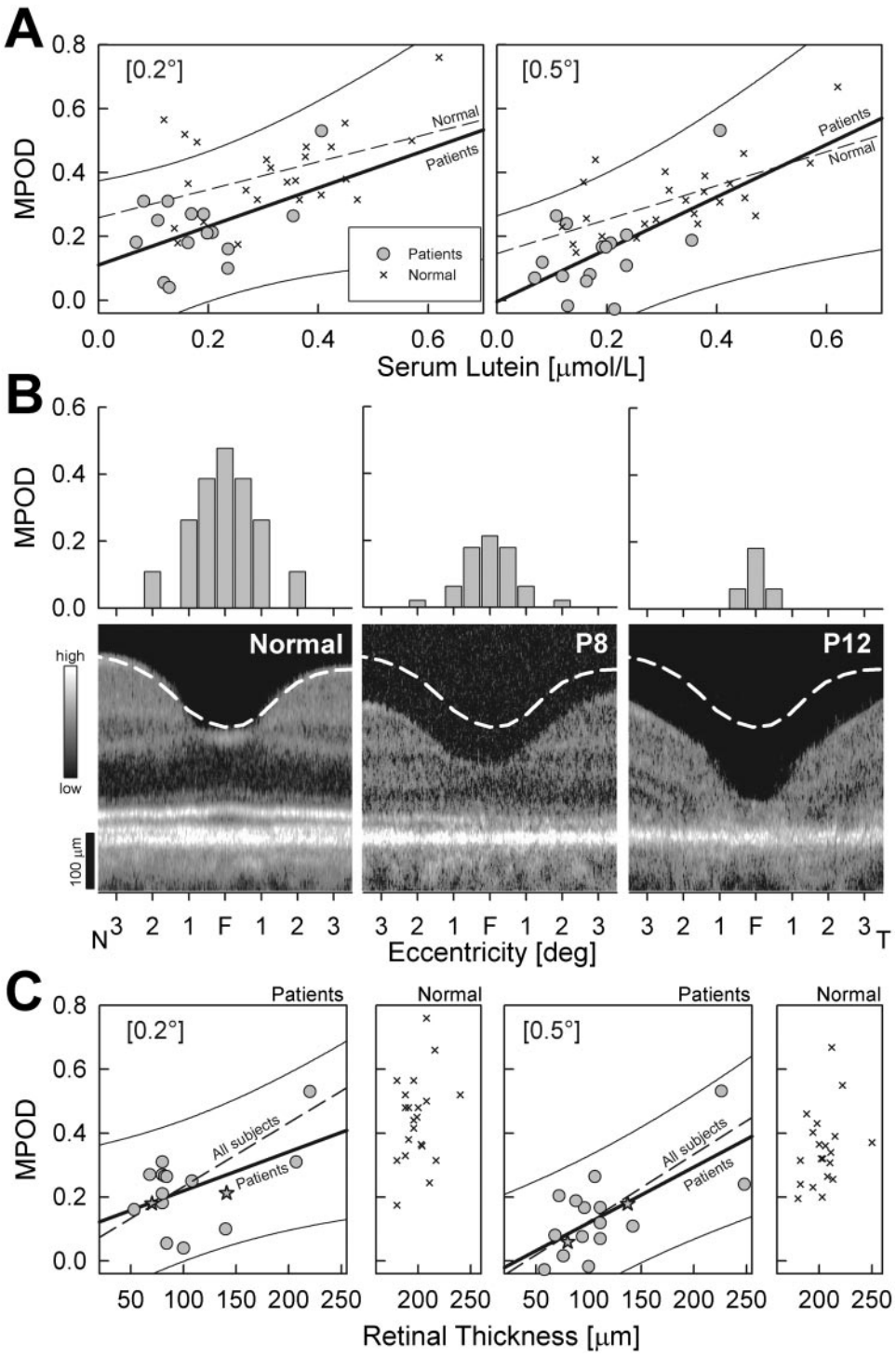

FIGURE 3. Systemic and ocular factors in relationship to MPOD. (**A**) Foveal MPOD (0.2° and 0.5°) as a function of serum lutein concentrations in patients compared with normal subjects. Linear regressions for patients (*thick line*) and normal subjects (*dashed line*) are shown; for clarity the 95% prediction interval (*thin lines*) is shown for the patient data only. (**B**) MPOD as a function of eccentricity (*top*; plotted as in Fig. 1B) and corresponding foveal microstructure by OCT (*bottom*) in two patients (P8 and P12) compared to a normal subject. OCT scans are crossing the anatomic foveal center (F) horizontally, from 3.5° temporally (T) to 3.5° nasally (N). Images are displayed with the logarithm of reflectivity mapped to a gray scale (*left*). *Dashed white lines*: represent mean normal location of the vitreoretinal boundary. (**C**) Foveal MPOD (0.2° and 0.5°) as a function of retinal thickness in patients compared with normal subjects. Linear regressions for patients (*thick line*) and for all subjects (patients + normal subjects; *dashed lines*) are shown; for clarity the 95% prediction interval (*thin lines*) are shown for the patient data only. *Stars*: patients P8 and P12 shown in (**B**).

between serum xanthophyll levels in patients based on gender, smoking status, and color of irides (data not shown). Examination of concurrent use of dietary supplements revealed that 8 of 17 patients were using multivitamins containing low doses of carotenoids before admission to the study. There were no differences, however, in any of the variables measured between those who had used supplements and the rest of the patients (data not shown).

Serum lutein was related to MPOD in patients and in normal subjects (Fig. 3A). Linear correlation coefficients between these variables were similar in patients ($r = 0.46$; $P = 0.03$) and normal subjects ($r = 0.44$; $P=0.002$) at 0.2° eccentricity. This relationship improved notably for the more eccentric 0.5° location in normal subjects ($r = 0.63$; $P < 0.001$), but remained unchanged in patients ($r = 0.47$; $P = 0.02$).

Relationship of Foveal Structure to MPOD

Cross-sectional images through the fovea in two patients (Fig. 3B, bottom) illustrate the types of abnormalities encountered. Patient 8 showed some photoreceptor layer thinning and localized disruption of the signal originating from the photoreceptor inner–outer segment interface.[11,37] Patient 12, with more advanced disease, showed severe central retinal thinning and an adjacent (~1°–3° eccentric) region with loss of the photoreceptor layer. MPOD in patient 8 was reduced but

TABLE 3. Supplemented Group Summary Statistics*

	Patients (n = 11)	Normal (n = 8)	P†
General			
Age (y)	30 ± 11	27 ± 8	NS
BMI (kg/m^2)	23 ± 4	24 ± 4	NS
Female gender (%)	36	50	NS
Smoker (%)	27	13	0.042
White (%)	82	75	NS
Light irides (%)	55	38	NS
Serum (before supplementation)			
Lutein (μmol/L)	0.18 ± 0.08	0.34 ± 0.11	0.002
Zeaxanthin (μmol/L)	0.07 ± 0.03	0.14 ± 0.05	0.001
β-Carotene (μmol/L)	0.40 ± 0.51	0.44 ± 0.24	NS
Serum (after supplementation)			
Lutein (μmol/L)	0.84 ± 0.50	1.06 ± 0.41	NS
Zeaxanthin (μmol/L)	0.12 ± 0.04	0.21 ± 0.07	0.001
β-Carotene (μmol/L)	0.40 ± 0.48	0.44 ± 0.18	NS

* Noncategorical variables are specified as mean ± SD.
† Not significant (NS) values correspond to $P > 0.05$.

measurable at all eccentricities, but in patient, 12 MPOD was measurable only at the foveal locations (Fig 3B, top).

Average foveal thickness in patients was reduced to approximately half of normal—a significant difference (Table 2). The relationship between foveal MPOD and retinal thickness was examined in the patients compared with normal subjects (Fig. 3C). Patients showed a positive correlation between MPOD at 0.2° and retinal thickness at the foveal center (Fig. 3C, r = 0.43, P = 0.03) and a robust relationship (r = 0.66, $P < 0.001$) at the 0.5° locus, consistent with previous observations.[30,32,59] The relationship between retinal thickness and MPOD was much stronger when the patients and normal subjects were considered as a single group, under the assumption that patients' foveas had normal thickness before the onset of retinal degeneration. The regression lines considering all subjects (Fig. 3C) had robust correlation coefficients (0.2° = 0.69 and 0.5° = 0.76, $P < 0.0001$); the intercepts were not significantly different from zero (0.2° = 0.10, P = 0.58; 0.5° = −0.06, P = 0.10).

To a first approximation, retinal tissue concentration of MP can be estimated by dividing MPOD by retinal thickness. Foveal MP concentration in the patients was not different from that in normal subjects at 0.2° but it was lower at 0.5° (Table 2). Serum lutein concentration in the patients did not correlate significantly with MP concentrations at 0.2° (r = 0.26, P = 0.44) and 0.5° (r = 0.36, P = 0.12) unlike the stronger relationship observed in the normal subjects (r = 0.28, P = 0.03 and r = 0.65, $P < 0.001$, for 0.2° and 0.5°, respectively).

The relationship between central visual function and MPOD was probed with visual acuity and dark-adapted sensitivity to a 650 nm stimulus. MPOD at 0.5° did not correlate significantly with visual acuity (r = 0.36, P=0.07) and dark-adapted foveal sensitivity (r = 0.33, P = 0.11).

Effects of Lutein Supplementation

The subset of patients (n = 11) and normal subjects (n = 8) who took part in the 6-month pilot trial of lutein supplementation (Table 1) were well matched in age, BMI, gender, smoking status, race, and color of irides (Table 3). All but one of the 11 patients responded with an increase in serum lutein. In the entire group of patients, the change in serum lutein with supplementation (postsupplementation level minus the mean of the two baseline values) was significantly greater than the mean absolute difference between the two baseline values (0.74 ± 0.44 vs. −0.01 ± 0.03 μmol/L; $P < 0.001$). After supplementation, there was no significant difference in serum lutein remaining between patients and normal subjects (Table 3). Serum zeaxanthin levels also showed an increase in both groups with supplementation, as expected from the small amounts of zeaxanthin contained in the marigold extracts, the main component of the supplementation capsule.[52] However, serum zeaxanthin in the patients remained significantly lower than that in the normal subjects after supplementation (Table 3). There were no changes measured in serum β-carotene levels in either group (Table 3).

MPOD measurements before and after oral lutein supplementation are illustrated by using the data from two patients (Fig. 4A). Both patients had increases in serum lutein (patient 8: from 0.21 to 0.51 μmol/L; patient 6: from 0.08 to 0.64 μmol/L) after supplementation, but showed different retinal responses by MPOD. Patient 8 had increases in MPOD at each eccentricity when compared with the baseline levels; in contrast, the postsupplementation MPOD profile of patient 6 was very similar to the baseline profiles. The apparent differences in response to supplementation were not due to a lack of reliability in MPOD estimates. Intervisit reproducibility of the MPOD profiles was measured in two visits in 11 patients (13 eyes). Absolute differences between the MPODs obtained at each visit in the patients were not significantly different from those in the normal subjects (Fig. 4B). Previously published intervisit density differences[21,41,47,60–63] showed similar ranges, suggesting that MPOD can be reproducibly obtained with HFP in eyes with maculopathy and foveal fixation.

Sixteen eyes of 10 patients (six bilateral, four unilateral measurements) were used for summary statistics of the MPOD response to lutein supplementation. Patient 4 showed no serum response to lutein supplementation and was not included in this analysis (she had no MPOD change). Mean foveal MPOD increased with supplementation: at 0.2°, from 0.17 ± 0.09 to 0.28 ± 0.14 (paired t-test, $P < 0.001$); at 0.5°, from 0.11 ± 0.06 to 0.18 ± 0.10 ($P < 0.001$). Parafoveal increases were not significant. Magnitude of MPOD changes with supplementation were larger in patients compared to normal subjects: at 0.2°, 0.12 ± 0.12 vs. 0.07 ± 0.06 (P = 0.28); at 0.5°, 0.06 ± 0.07 vs. 0.01 ± 0.04 (P = 0.02). These two foveal locations were used to assess changes in MP concentration with supplementation. In normal subjects, oral lutein led to significant increases in MP concentration at the foveal center but not at 0.5°. In patients, the retinal MP concentration increased at both foveal locations (Fig. 4C).

Retinal MPOD responders to lutein supplementation were then compared to nonresponders to seek explanations for their

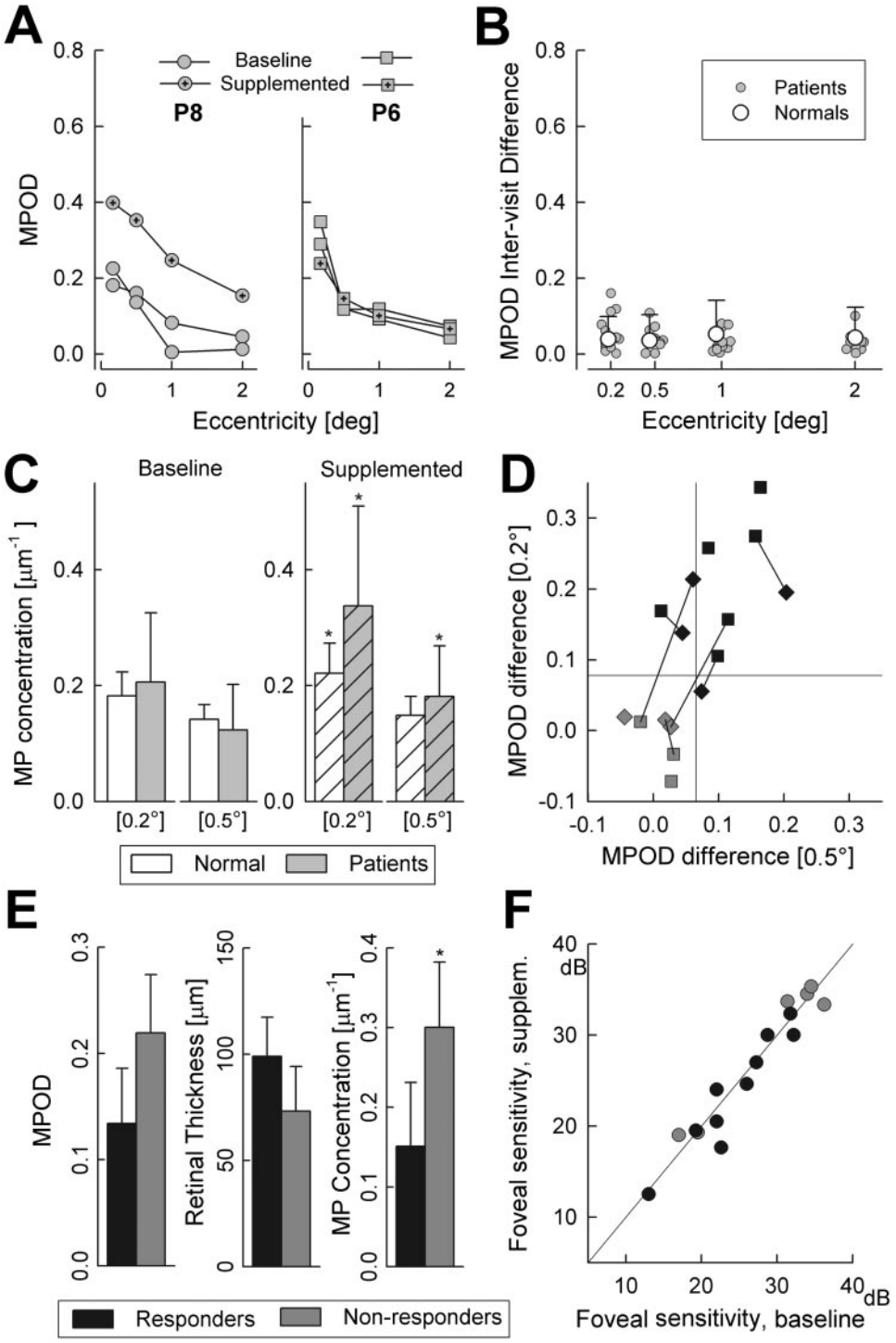

FIGURE 4. Effects of lutein supplementation. (**A**) MPOD profiles at two baseline visits and after supplementation in two patients. (**B**) Intervisit absolute MPOD differences for each eccentricity in individual patients compared to normal mean + 2 SD. Some of the patients' symbols are laterally shifted for better visibility. (**C**) MP concentrations (MPOD divided by retinal thickness) for foveal locations (0.2° and 0.5°) in patients (*gray bars*) compared with normal subjects (*unfilled bars*) at baseline and at 6 month after supplementation (*hatched bars*). Error bars, 1 SD from mean; *significant ($P < 0.05$) change compared with baseline. (**D**) Change in MPOD after supplementation at 0.2° plotted against 0.5° eccentricity. Baseline intersession variability (95% confidence limits) is defined by the *horizontal* and *vertical lines*. *Black symbols*: retinal responders; *gray symbols*: retinal nonresponders; *squares*: right eyes; *diamonds*: left eyes. Lines connect the symbols for the two eyes of the same patient. (**E**) Baseline variables at 0.2° eccentricity in retinal responders (black circles) and nonresponders (gray circles); *statistically significant difference. (**F**) Foveal sensitivity (650 nm, 1.7° diameter, dark-adapted) in patients at baseline and after 6 months of lutein supplementation. *Diagonal line* represents no change.

differences. For this analysis, responding was defined by the 95th percentile for differences between the two baseline MPODs (Fig. 4D). Over half of the eyes responded with a significant increase in MPOD (Fig. 4D, black squares). In most, the response occurred at both eccentricities, but in some eyes, the response was limited to one retinal location. Baseline serum levels of lutein and zeaxanthin in the responders were lower on average than those in the nonresponders, suggesting that changes in the retina are related to initial levels of serum xanthophylls. Although there was a small sample of subjects, we asked whether there was an association between the MPOD response and some of the general characteristics examined earlier. Of interest, the three female patients (5/5 eyes) who took supplements responded with an increase in MPOD, whereas responses in the male participants were less frequent (4/7 male patients; 5/11 eyes). Retinal responders and nonresponders did not differ significantly in age (36 ± 7 vs. 27 ± 12 years), frequency of lighter irides (60% vs. 50%), or smoking (both groups, 20%).

Were there eye-specific variables that could be related to the MPOD response? The responders tended to have lower MPOD at 0.2° and thicker foveas, but these differences did not reach statistical significance at this central foveal location (Fig. 4E) or at 0.5°. Baseline mean MP concentration on the other hand, was significantly lower in the responders than in the nonresponders (Fig. 4E), suggesting that MPOD changed in

those with the lowest initial MP concentrations. In terms of central visual function, the responders and nonresponders showed no differences in baseline visual acuity (0.19 ± 0.12 vs. 0.21 ± 0.10 logMAR) and foveal sensitivity (25.5 ± 4.5 vs. 28.8 ± 8.3 dB; Fig. 4E) group. An estimate of interocular differences in MPOD response to lutein supplementation was obtained in six patients with bilateral measurements. Three patients responded bilaterally, whereas one did not respond in either eye (Fig. 4D). Two patients showed unilateral responding; in both cases, eyes that responded had thicker retinas than did the eyes that did not respond.

After supplementation, foveal absolute sensitivity as a measure of central visual function was little changed from the mean baseline of 26.2 ± 6.3 to 26.0 ± 6.7 dB. Pre- and postsupplementation results correlated highly ($r = 0.94$, $P < 0.001$; Fig. 4F). The mean change in foveal sensitivity (−1.20 ± 2.5 dB) in eyes that responded with an increase in MPOD (Fig. 4F, black symbols) was not different from the mean change in nonresponding eyes (1.21 ± 2.7 dB; $P > 0.05$; Fig. 4F, gray symbols). Similarly, the mean change in logMAR acuity in responding eyes (−0.02 ± 0.03) was not different from the mean change in nonresponding eyes (−0.02 ± 0.06; $P > 0.05$).

Discussion

The macular pigments, lutein and zeaxanthin, are highly concentrated at the fovea and are hypothesized to improve normal vision and protect photoreceptors and the RPE from oxidative damage.[64] MPs originate from dietary consumption of lutein, and lutein-free diets in nonhuman primates can result in abnormalities of foveal photoreceptors and RPE.[65–67] Epidemiologic studies have shown an association between lower dietary and serum levels of lutein and higher risk of age-related maculopathy,[68,69] but questions about causality remain[64] and await experimental clarification. Recently, one such specific antioxidant mechanism has been proposed. Lutein and zeaxanthin appear to protect visual cycle byproducts A2-PE and A2E from photooxidation.[28] To extend the understanding of this new mechanism, we evaluated a cohort of patients with a shared prototypical lipofuscinopathy due to *ABCA4* mutations and at a similar disease stage with relative preservation of the fovea compared with the surrounding parafoveal retina. Pathogenesis of human *ABCA4* disease involves a dramatic increase in A2-PE and A2E.[3,13,14] Consistent with earlier reports,[70,71] we found foveal MPOD to be significantly lower than normal in these patients. Unexpectedly, we found MP concentration to be normal at the preserved foveal center in these patients reemphasizing the importance of measuring foveal structure when interpreting MPOD abnormalities.[30,32] Our results lend support to the notion that MP has a role in *ABCA4*-RD and do not contradict the long-held hypothesis that protection afforded by MP contributes to foveal sparing.[19] Future longitudinal studies could directly test whether the rate of foveal disease progression is related to MPOD and/or MP concentration.

Microdensitometry studies in primate retinas have shown that MP concentration is not uniform across the foveal depth; there is a major peak at the Henle fiber layer and relatively uniform lower concentration along photoreceptor nuclei and inner and outer segments.[72] Our estimate of MP concentration derived by dividing the total MPOD by the total retinal thickness would thus not represent the true MP concentration in any given retinal layer. The estimate, however, may be a useful approximation of the average foveal MP concentration, under the assumption that cone outer segments and nuclei as well as cone axons in the Henle fiber layer thin proportionally in retinal degenerative disease. Future studies combining polarization-sensitive, OCT-based[73] delineation of Henle fiber layer thickness and MP imaging[42] may allow a better estimate of the maximum tissue concentration of MP and its relationship to foveal sparing in disease.

The psychophysical HFP technique used in the current work to estimate MPOD is the most common method[74]; alternatives include retinal reflectance,[70,75] lipofuscin fluorescence,[76] Raman spectroscopy,[77] and other psychophysical methods.[78,79] All psychophysical methods, including HFP, require stable foveal fixation, and patients' eyes were selected accordingly. HFP-based MPOD values in our patients were highly repeatable, with an intersession variability that was comparable to that in normal subjects in this study and other published work.[21,41,47,60–63] Repeatability does not necessarily imply validity, and assumptions implicit in the use of the HFP technique to estimate MPOD were not explicitly proven in our study. It is assumed, for example, that the difference in L/M-cone mediated sensitivity to the blue and green stimuli is invariant across the measured central retinal locations.[22,80] Theoretically, outer retinal degeneration could affect the relative abundance and/or photopigment density of L and M cones differentially across the regions tested. Our use of a molecularly homogeneous group of patients with *ABCA4* lipofuscinopathy would be expected to minimize the potential for spatially variant degeneration of L and M cones. Further, reduced L/M-cone photopigment density previously reported in patients with retinal degenerations and/or maculopathies[80–84] would be expected to diminish the spatial differences in cone pigment optical density and reduce the extent of MP density measurement error.

Serum lutein and zeaxanthin levels of the patients at baseline as a group were about half of that observed in our normal subjects even though estimates of dietary lutein intake were not different between the two groups. Serum carotenoids in our patients were also at the low end of the distribution of values from large population-based studies.[55–58] Patients with the lowest serum levels of lutein were not different from the rest of the patients or normal subjects in variables such as age, gender, diet, or BMI, although some showed concomitant low levels of zeaxanthin, possibly reflecting an overall low carotenoid intake or uptake not revealed by the dietary questionnaire. Analysis of patients with and without a previous history of multivitamin supplementation disclosed no differences in serum lutein and zeaxanthin concentrations, arguing against possible interactions with other carotenoids present in those preparations.[85] It is tempting to consider a causal relationship between the serum lutein concentration and maculopathy. One possibility is that lower serum lutein levels predisposed this cohort of patients to more severe maculopathy, as has been hypothesized in age-related macular degeneration.[68] Support for such a hypothesis is lacking, however, since the patients included in this study had relatively mild disease within the severity spectrum of *ABCA4* lipofuscinopathy.[11,12] An alternative hypothesis would be to consider the involvement of a systemic regulatory mechanism for lowering serum xanthophyll in response to reduced demand from degeneration of photoreceptors and RPE. Systemic signaling from the retina has been proposed to explain reduced blood levels of docosahexaenoic acid observed in many hereditary retinal degenerations.[86]

Augmentation of retinal MP concentration has been proposed not only to prevent age-related multifactorial degenerative diseases but also to prevent or delay retinal degeneration in Mendelian hereditary conditions.[87,88] We supplemented the diets of our *ABCA4* patients with lutein for 6 months to evaluate short-term effects as a prelude to longer-term studies. Serum lutein increased significantly in patients with supplemented diets, consistent with previous observations.[30,32,63,89,90] Retinal MPOD and MP concentration also increased significantly in more than half of the eyes of patients, consistent with previous studies.[30,32,63,89] Responders had a

tendency to be female and, at baseline, have lower serum lutein, greater retinal thickness and lower retinal MP concentrations. This suggests that low levels of baseline xanthophylls in serum and retina may help predict an increase of MPOD after supplementation. Whether other techniques of measuring MPOD would detect higher percentages of responders to supplementation or would be capable of detecting accumulation at cellular compartments such as the RPE[91] not probed by the HFP method awaits further study.

Increasing knowledge about the molecular basis of genetic retinal degenerations has provided an opportunity to consider gene- or mechanism-specific therapeutic interventions in otherwise incurable diseases such as *ABCA4*-RD. In the present study we built on available knowledge and used clinically feasible techniques to evaluate molecularly identified patients at a specific disease stage. In this disease subset, we tried to understand whether there is vulnerability to a recently described antioxidant mechanism.[28] The data were then used to perform a pilot trial of nutrient supplementation that could decrease vulnerability. Such strategic approaches in molecularly clarified retinopathies with specific consideration of baseline parameters that have high predictive value could reduce the variability of results and the length of clinical trials of these slowly progressive degenerative disorders.

Acknowledgments

The authors thank Andy Cheung, Michelle Doobrajh, Elaine Smilko, Alejandro Román, and Marisa Román for their critical help.

References

1. Papermaster DS, Reilly P, Schneider BG. Cone lamellae and red and green rod outer segment disks contain a large intrinsic membrane protein on their margins: an ultrastructural immunocytochemical study of frog retinas. *Vision Res*. 1982;22:1417–1428.
2. Molday LL, Rabin AR, Molday RS. ABCR expression in foveal cone photoreceptors and its role in Stargardt macular dystrophy. *Nat Genet*. 2000;25:257–258.
3. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in ABCR knockout mice. *Cell*. 1999;98:13–23.
4. Sun H, Molday RS, Nathans J. Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. *J Biol Chem*. 1999;274:8269–8281.
5. Beharry S, Zhong M, Molday RS. N-retinylidene-phosphatidylethanolamine is the preferred retinoid substrate for the photoreceptor-specific ABC transporter ABCA4 (ABCR). *J Biol Chem*. 2004; 279:53972–53979.
6. Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet*. 1997;15:236–246.
7. Martinez-Mir A, Paloma E, Allikmets R, et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. *Nat Genet*. 1998;18:11–12.
8. Cremers FPM, van de Pol DJR, van Driel M, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. *Hum Mol Genet*. 1998;7:355–362.
9. Fishman GA, Stone EM, Grover S, Derlacki DJ, Haines HL, Hockney RR. Variation of clinical expression in patients with Stargardt dystrophy and sequence variations in the ABCR gene. *Arch Ophthalmol*. 1999;117:504–510.
10. Fishman GA, Stone EM, Eliason DA, Taylor CM, Lindeman M, Derlacki DJ. ABCA4 gene sequence variations in patients with autosomal recessive cone-rod dystrophy. *Arch Ophthalmol*. 2003; 121:851–855.
11. Cideciyan AV, Aleman TS, Swider M, et al. Mutations in ABCA4 result in accumulation of lipofuscin before slowing of the retinoid cycle: a reappraisal of the human disease sequence. *Hum Mol Genet*. 2004;13:525–534.
12. Cideciyan AV, Swider M, Aleman TS, et al. ABCA4-associated retinal degenerations spare structure and function of the human parapapillary retina. *Invest Ophthalmol Vis Sci*. 2005;46:4739–4746.
13. Sparrow JR, Fishkin N, Zhou J, et al. A2E, a byproduct of the visual cycle. *Vision Res*. 2003;43:2983–2990.
14. Bergmann M, Schutt F, Holz FG, Kopitz J. Inhibition of the ATP-driven proton pump in RPE lysosomes by the major lipofuscin fluorophore A2-E may contribute to the pathogenesis of age-related macular degeneration. *FASEB J*. 2004;18:562–564.
15. Mata NL, Weng J, Travis GH. Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. *Proc Natl Acad Sci USA*. 2000;97:7154–7259.
16. Pinckers A, Cruysberg JR, aan de Kerk AL. Main types of bull's eye maculopathy. Functional classification. *Doc Ophthalmol*. 1984;58: 257–267.
17. Wajima R, Katsumi O, Mehta MC, Itabashi R, Hirose T. Pattern-reversal visual-evoked response in bull's eye maculopathy associated with Stargardt's disease. *Ophthalmic Res*. 1995;27:234–242.
18. Kurz-Levin MM, Halfyard AS, Bunce C, Bird AC, Holder GE. Clinical variations in assessment of bull's-eye maculopathy. *Arch Ophthalmol*. 2002;120:567–575.
19. Weiter JJ, Delori F, Dorey CK. Central sparing in annular macular degeneration. *Am J Ophthalmol*. 1988;106:286–292.
20. Landrum JT, Bone RA, Kilburn MD. The macular pigment: a possible role in protection from age-related macular degeneration. *Adv Pharmacol*. 1997;38:537–556.
21. Hammond BR Jr, Wooten BR, Snodderly DM. Preservation of visual sensitivity of older subjects: association with macular pigment density. *Invest Ophthalmol Vis Sci*. 1998;39:397–406.
22. Snodderly DM, Hammond BR Jr. In vivo psychophysical assessment of nutritional and environmental influences on human ocular tissues: lens and macular pigment. In: Taylor A, ed. *Nutritional and Environmental Influences on the Eye.* Boca Raton, FL: CRC Press; 1999:251–273.
23. Junghans A, Sies H, Stahl W. Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. *Arch Biochem Biophys*. 2001;391:160–164.
24. Thomson LR, Toyoda Y, Langner A, et al. Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. *Invest Ophthalmol Vis Sci*. 2002;43:3538–3549.
25. Krinsky NI, Landrum JT, Bone RA. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu Rev Nutr*. 2003;23:171–201.
26. Sparrow JR, Boulton M. RPE lipofuscin and its role in retinal pathobiology. *Exp Eye Res*. 2005;80:595–606.
27. Roberts JE, Kukielczak BM, Hu DN, et al. The role of A2E in prevention or enhancement of light damage in human retinal pigment epithelial cells. *Photochem. Photobiol*. 2002;75:184–190.
28. Kim SR, Nakanishi K, Itagaki Y, Sparrow JR. Photooxidation of A2-PE, a photoreceptor outer segment fluorophore, and protection by lutein and zeaxanthin. *Exp Eye Res*. 2006;82:828–839.
29. Webster AR, Heon E, Lotery AJ, et al. An analysis of allelic variation in the ABCA4 gene. *Invest Ophthalmol Vis Sci*. 2001;42:1179–1189.
30. Aleman TS, Duncan JL, Bieber ML, et al. Macular pigment and lutein supplementation in retinitis pigmentosa and Usher syndrome. *Invest Ophthalmol Vis Sci*. 2001;42:1873–1881.
31. Wooten BR, Hammond BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Invest Ophthalmol Vis Sci*. 1999;40:2481–2489.
32. Duncan JL, Aleman TS, Gardner LM, et al. Macular pigment and lutein supplementation in choroideremia. *Exp Eye Res*. 2002;74: 371–381.
33. Werner JS, Donnelly SK, Kliegl R. Aging and human macular pigment density: appended with translations from the work of Max Schultz and Ewald Hering. *Vision Res*. 1987;27:257–268.
34. Bone RA, Landrum JT, Gilbert J. Macular pigment and the edge hypothesis of flicker photometry. *Vision Res*. 2004;44:3045–3051.
35. Jacobson SG, Voigt WJ, Parel JM, et al. Automated light- and dark-adapted perimetry for evaluating retinitis pigmentosa. *Ophthalmology*. 1986;93:1604–1611.

36. Hee MR, Izatt JA, Swanson EA, et al. Optical coherence tomography of the human retina. *Arch Ophthalmol*. 1995;113:325–332.
37. Jacobson SG, Aleman TS, Cideciyan AV, et al. Identifying photoreceptors in blind eyes caused by RPE65 mutations: prerequisite for human gene therapy success. *Proc Natl Acad Sci USA*. 2005;102:6177–6182.
38. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. *Am J Epidemiol*. 1986;124:453–469.
39. Block G, Coyle LM, Hartman AM, Scoppa SM. Revision of dietary analysis software for the Health Habits and History Questionnaire. *Am J Epidemiol*. 1994;139:1190–1196.
40. Khachik F, Beecher GR, Smith JC Jr. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *J Cell Biochem*. 1995;22:236–246.
41. Hammond BR Jr, Wooten BR, Snodderly DM. Individual variations in the spatial profile of human macular pigment. *J Opt Soc Am A*. 1997;14:1187–1196.
42. Delori FC, Goger DG, Keilhauer C, Salvetti P, Staurenghi G. Bimodal spatial distribution of macular pigment: evidence of a gender relationship. *J Opt Soc Am A*. 2006;23:521–538.
43. Schalch W, Dayhaw-Barker P, Barker FM II. The carotenoids of the human retina. In: Taylor A, ed. *Nutritional and Environmental Influences on the Eye.* Boca Raton: CRC Press;1999:215–250.
44. Johnson EJ, Hammond BR, Yeum K-J, et al. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am J Clin Nutr*. 2000;71:1555–1562.
45. Nolan J, O'donovan O, Kavanagh H, Stack J, Harrison M, Muldoon A, Mellerio J, Beatty S. Macular pigment and percentage of body fat. *Invest Ophthalmol Vis Sci*. 2004;45:3940–3950.
46. Hammond BR Jr, Curran-Celentano J, Judd S, et al. Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. *Vision Res*. 1996;36:2001–2012.
47. Snodderly DM, Mares JA, Wooten BR, Oxton L, Gruber M, Ficek T; CAREDS Macular Pigment Study Group. Macular pigment measurement by heterochromatic flicker photometry in older subjects: the carotenoids and age-related eye disease study. *Invest Ophthalmol Vis Sci.* 2004;45:531–538.
48. Ciulla TA, Hammond BR Jr. Macular pigment density and aging, assessed in the normal elderly and those with cataracts and age-related macular degeneration. *Am J Ophthalmol*. 2004;138:582–587.
49. Hammond BR Jr, Wooten BR, Snodderly DM. Cigarette smoking and retinal carotenoids: implications for age-related macular degeneration. *Vision Res*. 1996;36:3003–3009.
50. Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM. Nutritional manipulation of primate retinas, III: effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. *Invest Ophthalmol Vis Sci*. 2005;46:692–702.
51. Broekmans WMR, Berendschot TTJM, Klöpping-Ketelaars IAA, et al. Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. *Am J Clin Nutr*. 2002;76:595–603.
52. Bone RA, Landrum JT, Guerra LH, Ruiz CA. Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans. *J Nutr*. 2003;133:992–998.
53. Beatty S, Nolan J, Kavanagh H, O'Donovan O. Macular pigment optical density and its relationship with serum and dietary levels of lutein and zeaxanthin. *Arch Biochem Biophys*. 2004;430:70–76.
54. Hammond BR Jr, Fuld K, Snodderly DM. Iris color and macular pigment optical density. *Exp Eye Res*. 1996;62:293–297.
55. Ford ES. Variations in serum carotenoid concentrations among United States adults by ethnicity and sex. *Ethn Dis*. 2000;10:208–217.
56. Curran-Celentano J, Hammond BR Jr, Ciulla TA, Cooper DA, Pratt LM, Danis RB. Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population. *Am J Clin Nutr*. 2001;74:796–802.
57. Mares-Perlman JA, Brady WE, Klein R, et al. Serum antioxidants and age-related macular degeneration in a population-based case-control study. *Arch Ophthalmol*. 1995;113:1518–1523.
58. Gruber M, Chappell R, Millen A, et al. Correlates of serum lutein + zeaxanthin: findings from the third national health and nutrition examination survey. *J Nutr*. 2004;134:2387–2394.
59. Liew SH, Gilbert CE, Spector TD, et al. Central retinal thickness is positively correlated with macular pigment optical density. *Exp Eye Res*. 2006;82:915–920.
60. Hammond BR Jr, Fuld K. Interocular differences in macular pigment density. *Invest Ophthalmol Vis Sci*. 1992;33:350–355.
61. Koh HH, Murray IJ, Nolan D, Carden D, Feather J, Beatty S. Plasma and macular responses to lutein supplement in subjects with and without age-related maculopathy: a pilot study. *Exp Eye Res*. 2004;79:21–27.
62. Nolan JM, Stack J, Mellerio J, et al. Monthly consistency of macular pigment optical density and serum concentrations of lutein and zeaxanthin. *Curr Eye Res*. 2006;31:199–213.
63. Hammond BR Jr, Johnson EJ, Russell RM, et al. Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci*. 1997;38:1795–1801.
64. Davies NP, Morland AB. Macular pigments: their characteristics and putative role. *Prog Retin Eye Res*. 2004;23:533–559.
65. Malinow MR, Feeney-Burns L, Peterson LH, Klein ML, Neuringer M. Diet-related macular anomalies in monkeys. *Invest Ophthalmol Vis Sci*. 1980;19:857–863.
66. Leung IY, Sandstrom MM, Zucker CL, Neuringer M, Snodderly DM. Nutritional manipulation of primate retinas, II: effects of age, n-3 fatty acids, lutein, and zeaxanthin on retinal pigment epithelium. *Invest Ophthalmol Vis Sci*. 2004;45:3244–3256.
67. Leung IY, Sandstrom MM, Zucker CL, Neuringer M, Max Snodderly D. Nutritional manipulation of primate retinas. IV. Effects of n-3 fatty acids, lutein, and zeaxanthin on S-cones and rods in the foveal region. *Exp Eye Res*. 2005;81:513–529.
68. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA*. 1994;272:1413–1420.
69. Beatty S, Boulton M, Henson D, Koh H-H, Murray IJ. Macular pigment and age related macular degeneration. *Br J Ophthalmol*. 1999;83:867–877.
70. Zhang X, Hargitai J, Tammur J, et al. Macular pigment and visual acuity in Stargardt macular dystrophy. *Graefes Arch Clin Exp Ophthalmol*. 2002;240:802–809.
71. Zhao D-Y, Wintch SW, Ermakov IV, Gellermann W, Bernstein PS. Resonance Raman measurement of macular carotenoids in retinal, choroidal, and macular dystrophies. *Arch Ophthalmol*. 2003;121:967–972.
72. Snodderly DM, Auran JD, Delori FC. The macular pigment. II: spatial distribution in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:674–685.
73. Cense B, Chen TC, Park BH, Pierce MC, de Boer JF. Thickness and birefringence of healthy retinal nerve fiber layer tissue measured with polarization-sensitive optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2004;45:2606–2612.
74. Hammond BR Jr, Wooten BR, Smollon B. Assessment of the validity of in vivo methods of measuring human macular pigment optical density. *Optom Vis Sci*. 2005;82:387–404.
75. Kilbride PE, Alexander KR, Fishman M, Fishman GA. Human macular pigment assessed by imaging fundus reflectometry. *Vision Res*. 1989;29:663–674.
76. Delori FC, Goger DG, Hammond BR, Snodderly DM, Burns SA. Macular pigment density measured by autofluorescence spectrometry: comparison with reflectometry and heterochromatic flicker photometry. *J Opt Soc Am A*. 2001;18:1212–1230.
77. Bernstein PS, Yoshida MD, Katz NB, McClane RW, Gellermann W. Raman detection of macular carotenoid pigments in intact human retina. *Invest Ophthalmol Vis Sci*. 1998;39:2003–2011.
78. Moreland JD, Robson AG, Soto-Leon N, Kulikowski JJ. Macular pigment and the colour-specificity of visual evoked potentials. *Vision Res*. 1998;38:3241–3245.
79. Davies NP, Morland AB. Color matching in diabetes: optical density of the crystalline lens and macular pigments. *Invest Ophthalmol Vis Sci*. 2002;43:281–289.
80. Werner JS, Bieber ML, Schefrin BE. Senescence of foveal and parafoveal cone sensitivities and their relations to macular pigment density. *J Opt Soc Am A*. 2000;17:1918–1932.

81. Morse PH, Smith VC, Pokorny JM, Burch JV. Fundus flavimaculatus with cystoid macular changes and abnormal Stiles-Crawford effect. *Am J Ophthalmol*. 1981;91:190–196.
82. van Meel GJ, van Norren D. Foveal densitometry as a diagnostic technique in Stargardt's disease. *Am J Ophthalmol*. 1986;102:353–362.
83. Kilbride PE, Fishman M, Fishman GA, Hutman LP. Foveal cone pigment density difference and reflectance in retinitis pigmentosa. *Arch Ophthalmol*. 1986;104:220–224.
84. Elsner AE, Burns SA, Weiter JJ. Cone photopigment in older subjects: decreased optical density in early age-related macular degeneration. *J Opt Soc Am A*. 2002;19:215–222.
85. Kostic D, White WS, Olson JA. Intestinal absorption, serum clearance, and interactions between lutein and β-carotene when administered to human adults in separate or combined oral doses. *Am J Clin Nutr*. 1995;62:604–610.
86. Anderson RE, Maude MB, Alvarez RA, Acland G, Aguirre GD. A hypothesis to explain the reduced blood levels of docosahexaenoic acid in inherited retinal degenerations caused by mutations in genes encoding retina-specific proteins. *Lipids*. 1999;34(suppl)S235–S237.
87. Dagnelie G, Zorge IS, McDonald TM. Lutein improves visual function in some patients with retinal degeneration: a pilot study via the Internet. *Optometry*. 2000;71:147–164.
88. Bahrami H, Melia M, Dagnelie G. Lutein supplementation in retinitis pigmentosa: PC-based vision assessment in a randomized double-masked placebo-controlled clinical trial. *BMC Ophthalmol*. 2006;6:23.
89. Hininger IA, Meyer-Wenger A, Moser U, et al. No significant effects of lutein, lycopene or beta-carotene supplementation on biological markers of oxidative stress and LDL oxidizability in healthy adult subjects. *J Am Coll Nutr*. 2001;20:232–238.
90. Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL, Sprague KE. A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Exp Eye Res*. 1997;65:57–62.
91. Rapp LM, Maple SS, Choi JH. Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. *Invest Ophthalmol Vis Sci*. 2000;41:1200–1209.