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

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Abstract

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—Stargardt disease or cone-rod dystrophy patients with foveal fixation and with known or suspected disease-causing mutations in the *ABCA4* gene were included. 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, *ABCA4*-RD patients had reduced foveal MPOD and there was 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 patient eyes showed a significant augmentation in MPOD. The retinal responders tended to be female, and have lower serum lutein and zeaxanthin, lower MPOD and greater retinal thickness at baseline. Responding eyes had significantly lower baseline MP concentration compared to non-responding 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 on the natural history of this supplement on macular degenerations require further study.

Keywords

carotenoids; lutein; macular degeneration; Stargardt disease; zeaxanthin

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INTRODUCTION

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 extra-macular retina of patients with known *ABCA4* mutations, we have provided evidence supporting abnormal increase in lipofuscin autofluorescence in the RPE preceding dysfunction and degeneration of the overlying retina[11]. We have also shown that parapapillary retina is relatively spared retinal degeneration[12]. Details of the macular disease sequence remain to be studied.

Macular degenerations, including those caused by *ABCA4* mutations, commonly show a counterintuitive stage when foveal vision and structure are relatively preserved compared to the surrounding parafoveal region[9,10,16–18]. It has been hypothesized that macular pigment (MP), a yellowish carotenoid mainly composed of lutein and zeaxanthin concentrated at the fovea, may contribute 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 recently proposed 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.

METHODS

Subjects

Stargardt disease or cone-rod dystrophy patients (n=17), most of whom had known *ABCA4* gene mutations[10,11,29], were included in this 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 chart. Eyes included in the study had stable foveal fixation, as documented by the correspondence of the center of the anatomical 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 that participated in our previous study[30] were reanalyzed according to the current methods. A subset of the subjects (11 patients and 8 controls) underwent a pilot trial of supplementation with oral lutein for six 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 manuscript. 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, 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 to 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 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' spot. Four different stimuli were used and 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 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 460nm test light needed for a flicker null at this eccentricity was not significantly different in patients compared to normal subjects (171±61 vs. 157±18 counts; P=0.17).

Foveal visual function was measured using a modified[35] automated perimeter (Zeiss Humphrey Instruments, 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 (Zeiss Humphrey Instruments, Dublin, CA). The principles of OCT[36] and our methodology[10,37] have been published. Horizontal scans crossing the anatomical 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, beta-carotene) were measured using high-performance liquid chromatography (Craft Technologies, Inc., Wilson, NC). Dietary information was provided through the Health Habits and History questionnaire (HHHQ) developed by the National Cancer Institute[38]; data were analyzed using the HHHQ Diet System Analysis Software[39].

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. Following two baseline visits (separated by no more than one month; except P4, who had a single baseline visit), subjects supplemented their diet with a commercially-available form of lutein at 20 mg per day (TWIN Laboratories Inc., 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 starting the supplement. 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 values from the two baseline visits were used to describe the study groups and calculate change after lutein supplementation. T-tests were performed to compare means and significance levels for correlation coefficients. Inter-session variability was assessed with signed and absolute differences of measurements between the first and second baseline visit. Means of inter-session differences and person-specific variables were compared with independent t-tests. Proportions were compared using chi-square tests with exact computation of the *P* values.

RESULTS

Patients had a clinical diagnosis of either Stargardt disease (n=14) or cone-rod dystrophy (n=3) and all but three 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 were within normal limits or show abnormalities.

Low macular pigment in *ABCA4*-RD

Patients had significantly lower MPOD compared to normal eyes (Table 2).The distribution of MPOD in patient eyes was shifted toward lower values compared to the 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 patient eyes in which MPOD could be determined 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 were narrower in these 11 patients compared to normals (Table 2). The remaining 6 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[45,50–54] and ocular[55] characteristics in studies of normal populations. Examination of some of these factors (Table 2) showed that patients and normals included in this study were well matched in terms of age, body mass index (BMI), gender, smoking status, race, and color of irides. Mean dietary fat intake was higher in patients compared with our normals but the groups were not significantly different and the patient values were similar to those reported in other studies in normals [45,56,57]. 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 values for 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 to 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, 55].

Low serum lutein in *ABCA4*-RD

Serum levels of lutein and zeaxanthin were significantly lower in patients compared to our group of normals (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[56–59]. When considering the subset of subjects with low serum lutein (<=0.19 μmol/L, lowest quartile of our normal population), patients had significantly lower zeaxanthin levels compared to normals 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 between serum xanthophyll levels of patients based on gender, smoking status and color of irides (data not shown). Examination of concurrent use of dietary supplements revealed that 8/17 patients were using multivitamins containing low doses of carotenoids prior to admission to the study. But there were no differences between those who had used supplements and the rest of the patients in any of the variables measured (data not shown).

Serum lutein was related to MPOD in patients and in normals (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 panels) illustrate the types of abnormalities encountered. Patient 8 shows 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, shows severe central retinal thinning and an adjacent (~1° to 3° eccentric) region with loss of the photoreceptor layer. MPOD in Patient 8 was reduced but measurable at all eccentricities, but in Patient 12 MPOD was only measurable at the foveal locations (Fig 3B, top panels).

Average foveal thickness in patients was reduced to approximately half the normal values, and this was highly significant (Table 2). The relationship between foveal MPOD and retinal thickness was examined in the patients compared with normals (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[30,32,60]. The relationship between retinal thickness and MPOD was much stronger when patients and normal subjects were considered as a single group under the assumption that patient foveas have 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 than 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 patients was not different than normals at 0.2° but it was lower at 0.5° (Table 2). Serum lutein concentration in patients was not significantly correlated to 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 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° was not significantly correlated with visual acuity (r=0.36, P=0.07) and dark-adapted foveal sensitivity (r=0.33, P=0.11).

Effects of Lutein Supplementation

Subset of patients (n=11) and normals (n=8) that took part in the 6-month pilot trial of lutein supplementation (Table 1) were well matched in terms of 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. As a group, the change in serum lutein with supplementation (post-supplementation value minus mean of 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; $P<0.001$). Post-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[53]. However, serum zeaxanthin in patients remained significantly lower than normals post-supplementation (Table 3). There were no changes measured in serum beta-carotene levels in either group (Table 3).

MPOD measurements before and after oral lutein supplementation are illustrated 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) post-supplementation, but showed different retinal responses by MPOD. Patient 8 had increases in MPOD at each eccentricity when compared to the baselines; in contrast, the post-supplementation 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. Inter-visit 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 patients were not significantly different than those in normal subjects (Fig.4B). Previously published inter-visit density differences[21,41,47,61–64] also showed similar ranges suggesting that MPOD could be reproducibly obtained using HFP in maculopathy eyes with foveal fixation.

Sixteen eyes of 10 patients (6 bilateral, 4 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 normals: 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 non-responders in order to seek explanations for their difference. 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 symbols). In the majority, the response occurred at both eccentricities but in some eyes the response was limited to one retinal location. Baseline serum values in responders were on the average lower for lutein and zeaxanthin compared to non-responders, suggesting that changes in the retina were related to initial levels of serum xanthophylls. Accepting the small number of subjects, we asked if there was an association between the MPOD response and some of the general characteristics examined earlier. Interestingly, the 3 female patients (5/5 eyes) who supplemented responded with an increase in MPOD whereas responding in male participants was less frequent (4/7 male patients; 5/11 eyes). Retinal responders and non-responders did not differ significantly in age (36±7 vs. 27±12 yrs), frequency of lighter irides (60% vs. 50%) or smokers (both groups, 20%).

Were there eye-specific variables that could be related to the MPOD response? 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 responders compared to non-responders (Fig.4E) suggesting that MPOD changed in those with the lowest initial MP concentrations. In terms of central visual function, responders and non-responders showed no differences in baseline visual acuity (0.19±0.12 vs. 0.21±0.10 log MAR) 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 6 patients with bilateral measurements. Three patients responded bilaterally whereas one patient did not respond in either eye (Fig. 4D). Two patients showed unilateral responding; in both cases, eyes that responded had thicker retinas compared to the eyes that did not respond.

Foveal absolute sensitivity as a measure of central visual function was little changed after supplementation from the mean baseline value of 26.2±6.3 dB to 26.0±6.7 dB. Pre- and post-supplementation results were highly correlated (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 (black symbols, Fig. 4F) was not different that the mean change in non-responding 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 than the mean change in non-responding 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[65]. MPs originate from dietary consumption of lutein, and lutein-free diets in non-human primates can result in abnormalities of foveal photoreceptors and RPE[66,67,68]. Epidemiological studies have shown an association between lower dietary and serum levels of lutein and higher risk of age-related maculopathy[69,70], but questions about causality remain[65] 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]. In order 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 to surrounding parafoveal retina. Pathogenesis of human *ABCA4* disease involves a dramatic increase in A2-PE and A2E[3,13,14]. Consistent with earlier reports[71,72], 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 a role of MP 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 [73]. 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 to 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[74] delineation of Henle fiber layer thickness and MP imaging[42] may allow a better estimate of the maximal 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[75]; alternatives include retinal reflectance[71,76], lipofuscin fluorescence[77], Raman spectroscopy[78] and other psychophysical methods[79,80]. All psychophysical methods, including HFP, require stable foveal fixation, and patient eyes were selected accordingly. HFP-based MPOD values in our patients were highly repeatable with an inter-session variability that was comparable to normal subjects from this study and from other published work[21,41,47,61–64]. Repeatability does not necessarily imply validity and assumptions implicit in the use of 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[22,81]. 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 population of *ABCA4*-lipofuscinopathy patients 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[81–85] 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[56–59]. 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/uptake not revealed by the dietary questionnaire. Analysis of patients with and without 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[86]. 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 such as hypothesized in age-related macular degeneration[69]. 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. Means of systemic signaling from the retina has been previously proposed to explain reduced blood levels of docosahexaenoic acid observed in many hereditary retinal degenerations[87].

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[88,89]. We supplemented our *ABCA4* patients with lutein for six months to evaluate short-term effects as a prelude to longer term studies. Serum lutein increased significantly in supplemented patients consistent with previous observations[30,32,64,90,91]. Retinal MPOD and MP concentration also increased significantly in more than half of the “responding” eyes of patients, consistent with previous studies[30,32,64,90]. 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 MPOD increase upon supplementation. Whether other

techniques of measuring MPOD would detect higher percentages of responders to supplementation or capable of detecting accumulation at cellular compartments such as the RPE[92] 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. The present study builds upon available knowledge and uses clinically-feasible techniques to evaluate molecularly-identified patients at a specific disease stage. In this disease subset, we tried to understand whether or not 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 theoretically decrease the 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.

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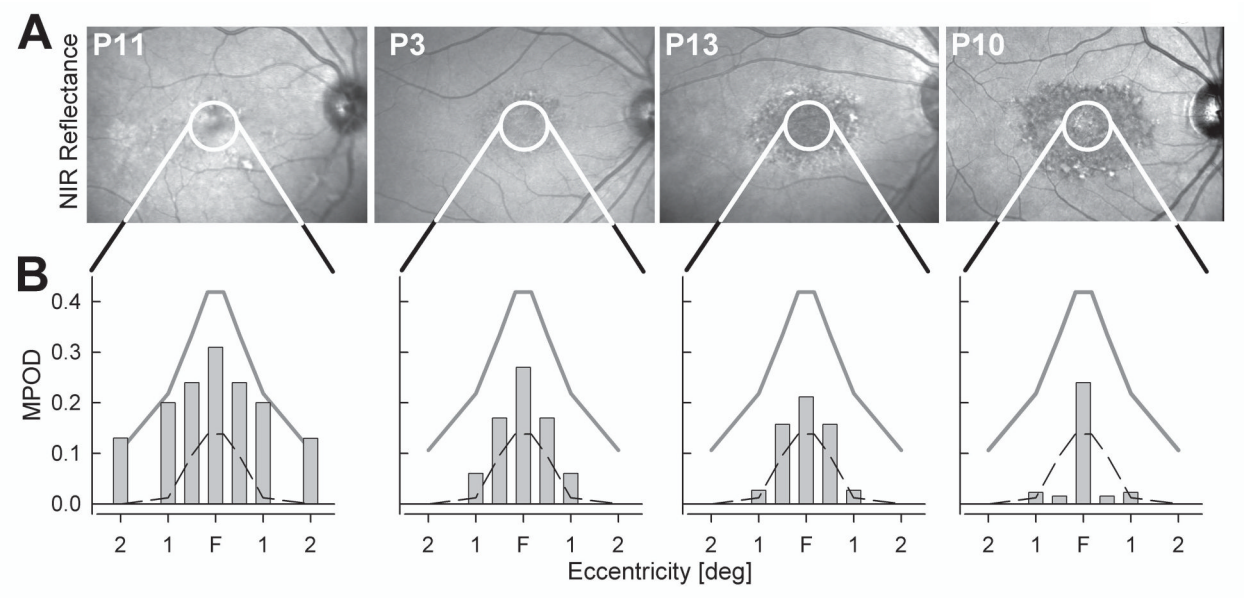

Figure 1. Patients with *ABCA4-RD* representing the spectrum of fundus appearance and MPOD

(A) Near-infrared (NIR) reflectance images of the central retina in four patients. Overlaid white circles delimit the central 5° diameter region within which MPOD measurements were performed.**(B)** MPOD in patients shown in Panel A. Foveal center (F) corresponds to MPOD determined with a 0.2° radius stimulus; MPOD determined with circular stimuli of 0.5°, 1°, 2° radius are plotted with symmetric duplicatation from F. Gray lines represent mean normal MPOD and dashed lines represent lower limit (mean-2SD) of normal.

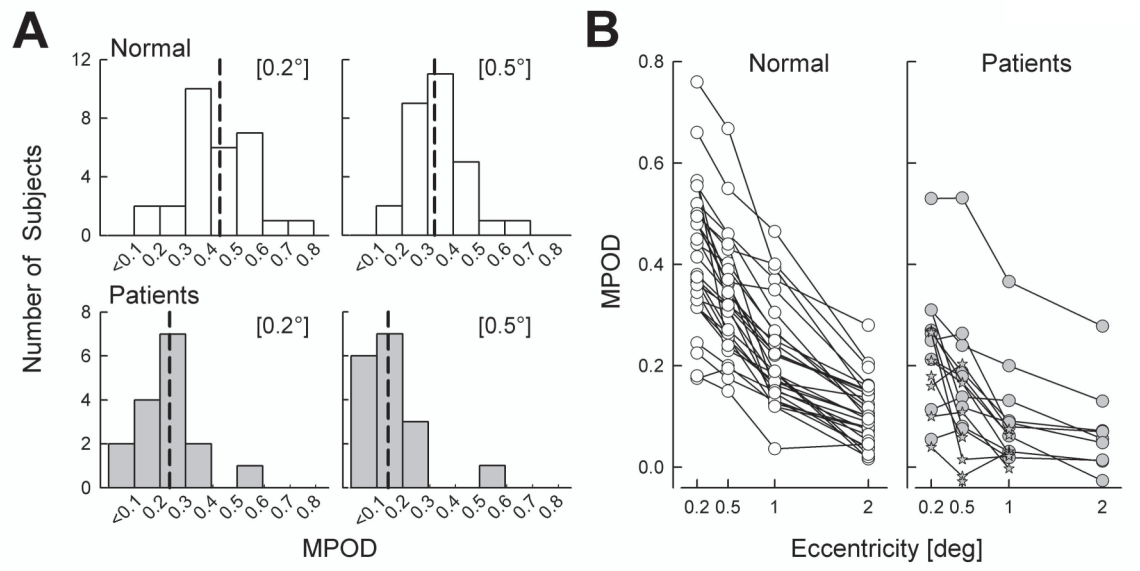

Figure 2. MPOD in patients compared to normal subjects

(A) Frequency distribution histograms of MPOD measured at foveal locations (0.2° and 0.5°). Vertical dashed lines indicate median value of each distribution. **(B)** All individual MPOD values measured in patients and normals. Full profiles are shown with circles. Stars represent patient eyes in which MPOD could only be determined in a subset of the four eccentricities.

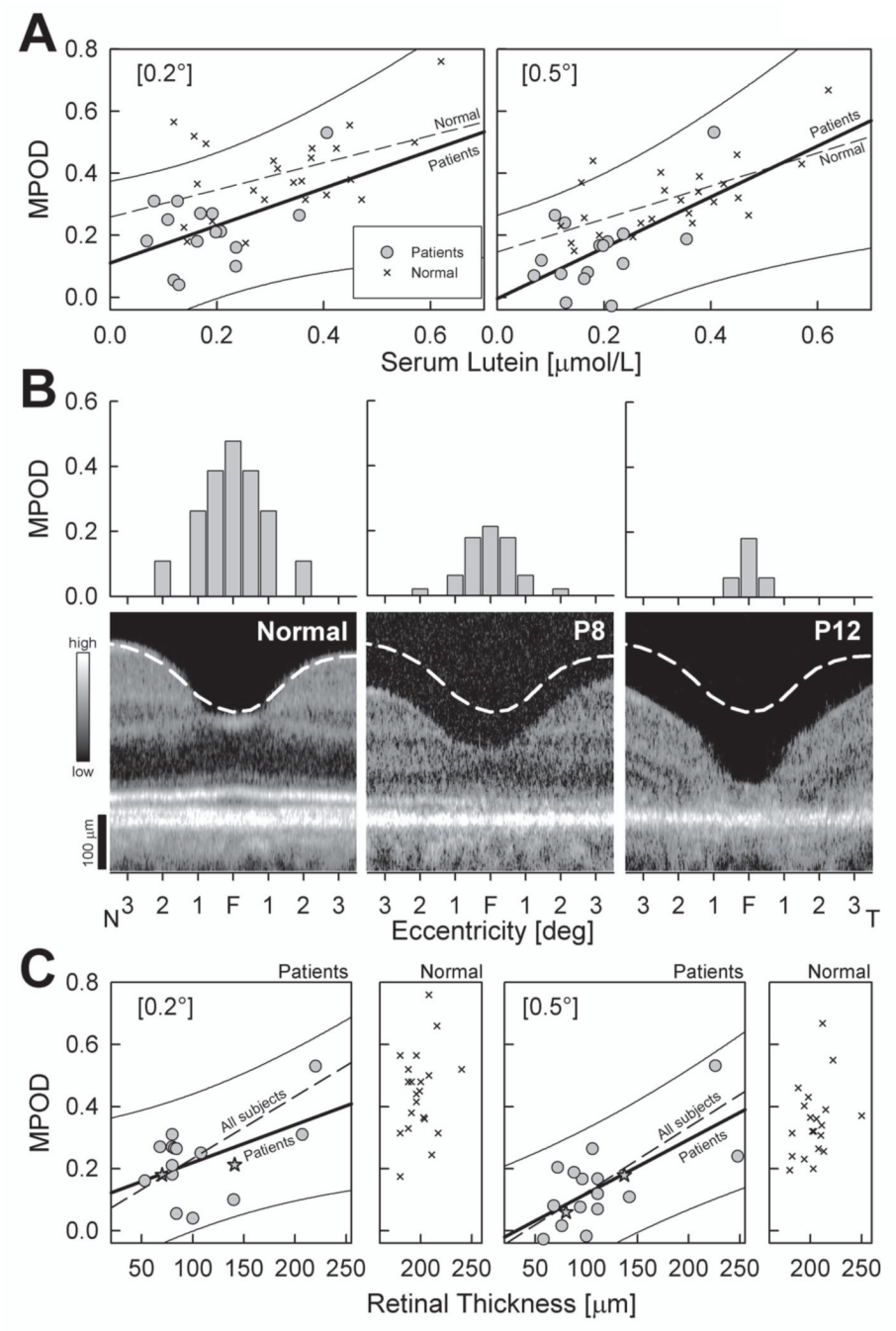

Figure 3. Systemic and ocular factors in relationship to MPOD

(A) Foveal MPOD (0.2° and 0.5°) as a function of serum lutein concentrations in patients (gray circles) compared to normal subjects (crosses). Linear regressions for patients (thick line) and normals (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 panels; plotted as in Fig. 1B) and corresponding foveal microstructure by OCT (bottom panels) in two patients (P8 and P12) compared to a normal subject. OCT scans are crossing the anatomic foveal center (F) horizontally, from 3.5° temporal (T) to 3.5° nasal (N). Images are displayed with logarithm of reflectivity mapped to a gray scale (left). Dashed white lines on OCTs represent mean normal location of the vitreo-retinal boundary. **(C)** Foveal MPOD (0.2° and 0.5°) as a function of

retinal thickness in patients (gray symbols) compared to normal subjects (crosses; smaller panels). 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 specify the patients P8 and P12 shown in Panel B.

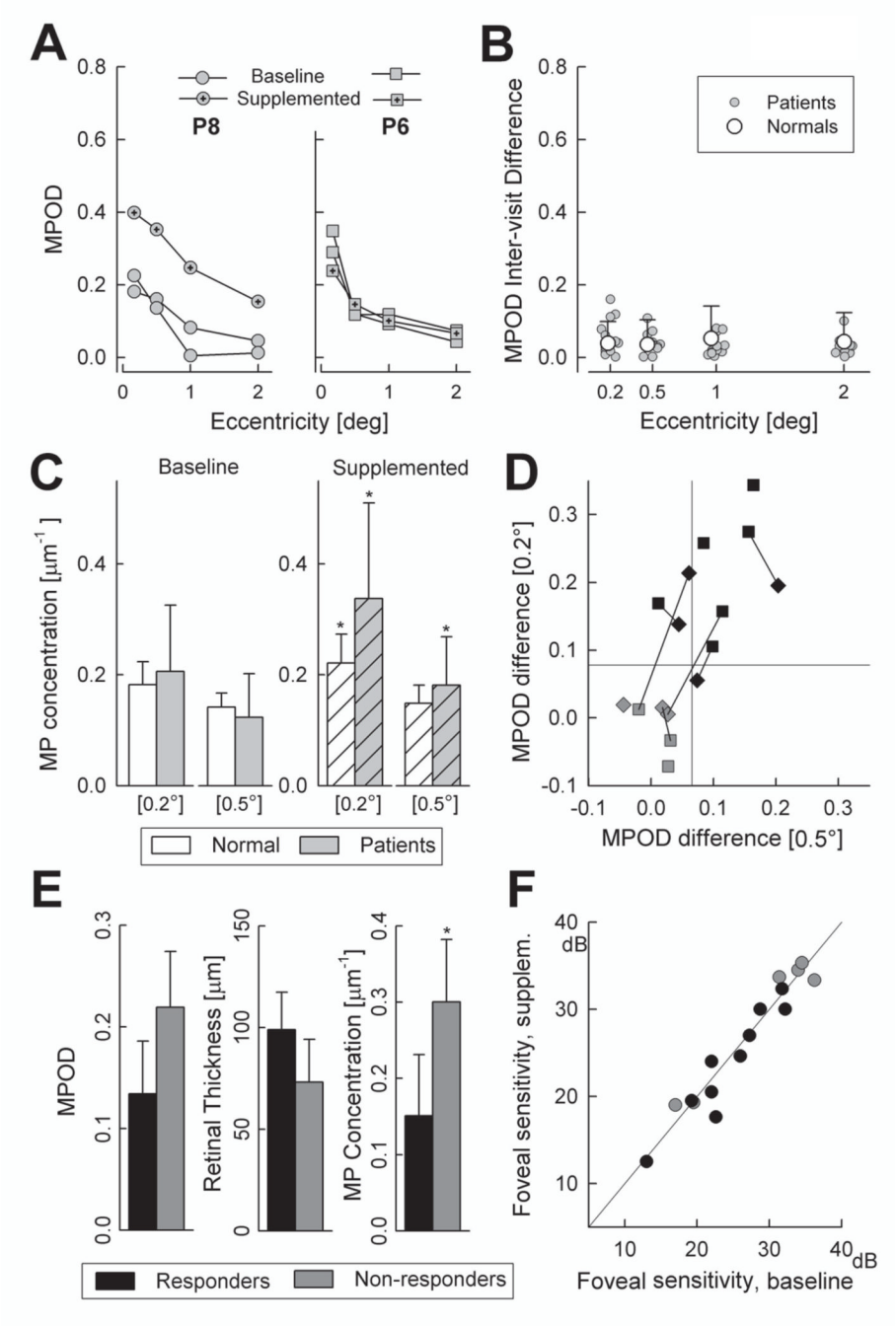

Figure 4. Effects of lutein supplementation

(A) MPOD profiles at two baseline visits and following supplementation in two patients. **(B)** Inter-visit absolute MPOD differences for each eccentricity in individual patients (gray circles) compared to normal mean+2SD (white circles and bar). Some of the patient 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 to normal subjects (white bars) at baseline (uniform filled) and 6-month post-supplementation (hatched). Error bars define 1SD from mean; asterisk denote significant (P<0.05) change compared to 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 “non-responders”; 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 fill) and “non-responders” (gray fill); asterisk denotes statistically significant difference. **(F)** Foveal sensitivity (650nm, 1.7° diameter, dark-adapted) in patients at baseline and after 6 months of lutein supplementation. Diagonal line represents no change.

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	14/M	G863A/R943Q	20/32	20/32	−0.50	−0.50	109	105	Y
2	17/F	E1087K/G1961E	20/25	20/25	−1.00	−1.25	103	104	N
3	18/M	IVS48+21C>T	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 equals 90%.

§Clinical diagnosis of cone-rod dystrophy; remaining patients had a clinical diagnosis of Stargardt disease.

‖Mutation unknown.

#Patients are siblings.

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TABLE 2

Baseline Group Summary Statistics*

	Patients [n=17]	Normal [n=29]	$P^{\dagger}$
General			
Age [years]	32±12	30±12	N.S.
BMI [$kg.m^{-2}$]	24±5	23±3	N.S.
Female Gender [%]	47	55	N.S.
Smoker [%]	18	14	N.S.
Caucasian Race [%]	82	90	N.S.
Light Irides [%]	44	48	N.S.
Diet			
Lutein [mg/day]	2.6±1.7	2.8±2.1	N.S.
Fat [g/day]	89±51	66±41	N.S.
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	N.S.
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	N.S.
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	N.S.
Half-width at half-peak [deg]	0.98±0.63‡	1.28±0.33	0.045

* Non-categorical variables are specified as mean ± s.d.

† Not significant (N.S.) values correspond to $P>0.05$.

‡ Determined in subset of 9 patients with full spatial profiles.

TABLE 3

Supplemented Group Summary Statistics*

	Patients [n=11]	Normal [n=8]	p†
General			
Age [years]	30±11	27±8	N.S.
BMI [kg.m^{-2}]	23±4	24±4	N.S.
Female Gender [%]	36	50	N.S.
Smoker [%]	27	13	0.042
Caucasian Race [%]	82	75	N.S.
Light Irides [%]	55	38	N.S.
Serum (pre-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	N.S.
Serum (post-supplementation)			
Lutein [μmol/L]	0.84±0.50	1.06±0.41	N.S.
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	N.S.

* Non-categorical variables are specified as mean ± s.d.

† Not significant (N.S.) values correspond to $P>0.05$.