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Candidate gene study of macular response to supplemental lutein and zeaxanthin

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ABSTRACT

Supplementation with carotenoids is proposed to protect against age-related macular degeneration. There is, however, considerable variability in retinal macular pigment response, which may be due to underlying genetic variation. The purpose of this study was to determine whether genetic factors, which have been previously associated with cross-sectional macular pigment levels in the retina or serum lutein, also influence response to supplementation.

To this end we conducted an association study in 310 subjects from the TwinsUK cohort between variants in 8 candidate genes and serum lutein and retinal macular pigment optical density (MPOD) levels before and after supplementation. Four variants were associated with MPOD response to supplementation ($p < 0.05$): rs11057841 (*SCARB1*), rs4926339 (*RPE65*), rs1929841 (*ABCA1*) and rs174534 (*FADS1*). We also confirmed previous associations between rs6564851 near *BMCO1* ($p < 0.001$) and rs11057841 within *SCARB1* ($p = 0.01$) and baseline measures of serum lutein; while the latter was also associated with MPOD response, none of the *BMCO1* variants were. Finally, there was evidence for association between variants near *RPE65* and *ELOVL2* and changes in lutein concentration after supplementation.

This study is the first to show association between genetic variants and response to carotenoids supplementation. Our findings suggest an important link between MP response and the biological processes of carotenoids transport and fatty acid metabolism.

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries. Age is the most important determinant, and common genetic polymorphisms are important in its etiology (Evans, 2001; Fritsche et al., 2013; McCarty et al., 2001). Oxidative stress has been implicated in AMD pathogenesis, together with other risk factors such as smoking, obesity and hypertension (Beatty et al., 2000; Evans, 2001; Hyman et al., 2000; Johnson, 2005; Smith et al., 2001). Macular pigment (MP) may play an important role in protecting the eye from accumulation of oxidative species by absorbing blue light and thus reducing light-induced oxidative stress, as well as having a direct antioxidant effect (Beatty et al., 2000; Margrain et al., 2004). MP represents the accumulation of the carotenoids lutein (L) and zeaxanthin (Z), as well as meso-zeaxanthin, a derivative of lutein. Lutein and zeaxanthin (LZ) are xanthophyll carotenoid pigments which cannot be synthesized *de novo* in mammals and are predominantly derived from fruits and vegetables. Therefore, carotenoids-rich diets and LZ dietary supplements have been proposed to protect against AMD (Delcourt et al., 2006), and supplements have been tested in the

Abbreviations: CAREDS, Carotenoids in Age Related Eye Disease Study; MP, macular pigment; L, lutein; MPOD, macular pigment optical density; LZ, lutein and zeaxanthin; Z, zeaxanthin.

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Age-Related Eye Disease Study 2 (AREDS2) (AREDS2, 2013). Although additional LZ supplementation to the AREDS1 formulation did not significantly reduce progression of AMD in the AREDS2 primary analysis, secondary analysis suggested that LZ supplementation was protective against AMD progression in individuals with low LZ dietary intake. Moreover, when LZ was given without β-carotene there was a statistically significant reduction in AMD progression. This, and the fact that addition of β-carotene increases the risk of lung cancer in former smokers, led the authors of the AREDS2 study to conclude that LZ could be an appropriate carotenoid substitute in the AREDS formulation (AREDS2, 2013).

The accumulation of LZ in the macula is dependent on a variety of factors – absorption, digestion, transport, retinal uptake, macular storage, degradation and secretion. However, little is known about the extent to which genetic variation influences these mechanisms. To our knowledge there are no studies on heritability of serum xanthophyll levels, but there is data on heritability of serum LZ concentration (between 67% and 85%) and on heritability of macular pigment optical density (MPOD) response to LZ supplementation (27%) (Hammond et al., 2012; Liew et al., 2005). Genetic studies, both genome-wide association studies (GWAS) and studies at a candidate gene level, have explored genetic factors that underlie variation in serum LZ and MPOD measures, with some success. There have been GWAS on plasma levels of carotenoids alone (Ferrucci et al., 2009) and on AMD and circulating carotenoid levels (Cipriani et al., 2012; Kopplin et al., 2010; Yu et al., 2011). Ferrucci et al. (2009), in a GWAS of 1190 people, found significant associations between plasma levels of the carotenoids β-carotene and lutein and SNPs mapping to *BCMO1*; the SNP rs6564851 explained 1.9% of variance. In a candidate gene study examining plasma LZ levels in 302 healthy adult subjects, McKay et al. (2013) found 5 of 47 variants in *SCARB1* associated with serum L; only one survived correction for multiple testing by permutation.

A recent study, in 1585 female participants of the Carotenoids Age Related Eye Disease Study (CAREDS), investigated associations between 440 SNPs in 26 candidate genes and MPOD (Meyers et al., 2013). Variants in 11 genes were associated with MPOD and collectively explained 5.1% of variance, though only rs11645428 in *BCMO1* survived Bonferroni correction for multiple testing.

Given that there is considerable variability in MPOD response in the (generally small) supplementation studies (Connolly et al., 2011) and that AREDS2 showed LZ supplementation to be effective in absence of β-carotene (AREDS2, 2013), understanding the pharmacogenetics of supplementation response is important to determine which groups of people might benefit most from taking supplements. Therefore, the aims of the current study were to determine whether any of 12 variants in 8 genes previously associated with MPOD or serum LZ influence MPOD and/or serum response to LZ supplementation, and to replicate these associations.

2. Methods

2.1. Subjects

We studied 310 Caucasian female twins (79 monozygotic (MZ) and 76 dizygotic (DZ) pairs), aged 20–50 years (mean: 39.72, SD: 8.40), free of ocular pathology, who were previously enrolled in a prospective, non-randomized supplement study that aimed to estimate the heritability of macular response to LZ supplementation (Hammond et al., 2012). Briefly, participants were asked to take LZ supplements with food (3 tablets per day of "Macuvite" (Springfield®, Oud-Beijerland, The Netherlands)) for a period of 6 months. Each tablet contained 18 mg lutein and 2.4 mg zeaxanthin. The compliance was 90% at 3 months and 82% at six months (Hammond et al., 2012). All individuals were unaware of eye research interests at the time of enrollment and provided written informed consent. The research was approved by the local ethics committee and conducted in accordance with the tenets of the Declaration of Helsinki.

2.2. Response measurements

Serum lutein and zeaxanthin levels were measured and analyzed separately using reverse phase high performance liquid chromatography while MPOD was measured by autofluorescence (AF). We assessed response to supplementation as change of serum L and Z levels after 3 months of supplement intake and as change in MPOD after 6 months of supplementation. These changes were expressed as a rate of change as defined by the ratio of the difference between the second and baseline measurements, divided by the baseline measurement. MPOD at baseline and MPOD response were approximately normally distributed and were not transformed, but serum L and Z and the change in their levels underwent logarithmic transformation. As L and Z were correlated (correlation coefficient of 0.61), we chose to analyze L only.

2.3. Genotyping data

The TwinsUK cohort, a subset of which was used in this study, was previously genotyped using Illumina 610K/Illumina 317K chips and later imputed against the CEU HapMap2 panel using IMPUTE 2 (Howie et al., 2009). All SNPs passed strict quality control (QC). Briefly, SNPs with minor allele frequency less than 5% and SNPs showing deviation from Hardy–Weinberg equilibrium ($p < 10^{-4}$) or high missingness were excluded. Individuals with non-Caucasian ancestry, as detected by principal components analysis, were excluded. After imputation, poorly imputed SNPs were also excluded. The final number of SNPs after the quality control step was 2,287,998.

2.4. Statistical analysis

Ten of the 13 CAREDS MPOD-associated SNPs from their final multivariate model were tested (there were no data on rs6078 within *LIPC* and rs675679 within *GSTP1*; rs10744182 was excluded because of low imputation quality) (Meyers et al., 2013). SNPs in *BCMO1* overlapped with SNPs in the GWAS of serum L (Ferrucci et al., 2009), only rs6564851 was added to the analysis. Finally, the SNP most strongly associated with serum LZ in the *SCARB1* candidate gene study, rs11057841, was included (McKay et al., 2013). This resulted in 12 SNPs in 8 genes being tested for association for both MPOD and serum L response to supplementation (Table 1). Where more than one SNP was tested per gene, those SNPs were independent of one another ($R^2 < 0.80$). Eight of the analyzed SNPs were genotyped while rs11057841, rs838879, rs10179921 and rs174534 were imputed. Age-adjusted association analyses taking zygosity into account were performed using the likelihood ratio test implemented in the Merlin software package. SNPs with $p < 0.05$ were regarded as nominally significant and SNPs with $p < 0.0019$ as significant after Bonferroni correction for multiple testing (0.05/number of SNPs × 2 where 2 is the number of independent traits tested i.e. L and MPOD).

3. Results

At baseline, the mean MPOD was 0.41 (SD = 0.15) density units and the mean lutein concentration in the blood was 0.121 μg/ml (SD = 0.05). The mean increase in MPOD after 6 months of supplementation was 3.7% (0.015 density units (SD = 0.06)). The lutein concentration at 3 months after supplementation had increased by