The Relationship between *BCMO1* Gene Variants and Macular Pigment Optical Density in Persons with and without Age-Related Macular Degeneration

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**Abstract**

**Background:** Recent evidence indicates that gene variants related to carotenoid metabolism play a role in the uptake of macular pigments lutein (L) and zeaxanthin (Z). Moreover, these pigments are proposed to reduce the risk for advanced age-related macular degeneration (AMD). This study provides the initial examination of the relationship between the gene variants related to carotenoid metabolism, macular pigment optical density (MPOD) and their combined expression in healthy humans and patients with AMD.

**Participants and Methods:** Forty-four participants were enrolled from a general population and a private practice including 20 healthy participants and 24 patients with advanced (neovascular) AMD. Participants were genotyped for the three single nucleotide polymorphisms (SNPs) upstream from *BCMO1*, rs11645428, rs6420424 and rs6564851 that have been shown to either up or down regulate beta-carotene conversion efficiency in the plasma. MPOD was determined by heterochromatic flicker photometry.

**Results:** Healthy participants with the rs11645428 GG genotype, rs6420424 AA genotype and rs6564851 GG genotype all had on average significantly lower MPOD compared to those with the other genotypes. When combining *BCMO1* genotypes reported to have “high” (rs11645428 AA/rs6420424 GG/rs6564851 TT) and “low” (rs11645428 GG/rs6420424 AA/rs6564851 GG) beta-carotene conversion efficiency, we demonstrate clear differences in MPOD values in patients with AMD there were no significant differences in MPOD for any of the three *BCMO1* gene variants.

**Conclusion:** In healthy participants MPOD levels can be related to high and low beta-carotene conversion *BCMO1* genotypes. Such relationships were not found in patients with advanced neovascular AMD, indicative of additional processes influencing carotenoid uptake, possibly related to other AMD susceptibility genes. Our findings indicate that specific *BCMO1* SNPs should be determined when assessing the effects of carotenoid supplementation on macular pigment and that their expression may be influenced by retinal disease.

**Introduction**

Macular pigment carotenoids lutein (L), zeaxanthin (Z) and meso-zeaxanthin (MZ) have numerous proposed roles within the eye and brain. These include protection from photochemical damage and oxidative stress [1] and improving visual and cognitive function [2,3,4,5,6,7,8,9]. A standard clinical protocol for measuring macular pigment optical density (MPOD) using heterochromatic flicker photometry (HFP) has been developed [10]. Dietary and supplementary L and Z intake correlates with serum levels [11,12,13] and macular pigment optical density (MPOD) in humans [14,15,16,17,18]. While factors such as gender [19], age [20,21,22], iris colour [23], smoking habits [21] and hereditability [24,25] have been proposed to modulate macular pigment, genetic variants coding for proteins related to carotenoid metabolism have been linked with macular pigment deposition [26,27,28].

It is believed that macular pigments protect from the development of advanced age-related macular degeneration (AMD) with studies showing that AMD patients have lower MPOD compared to healthy participants [29,30,31,32]. These findings gave rise to numerous studies that aimed to determine the effect of L and Z supplementation on AMD progression with varying results [8,15,33,34,35,36,37]. One of the largest studies was the Age-Related Eye Disease Study (AREDS) [34] that demonstrated no beneficial or harmful effect of L and Z.
supplementation. However, participants in the quintile with the lowest dietary intake of macular carotenoids had less progression to advanced AMD compared to those who had higher dietary intake when supplementing with the AREDS formula (Vitamin A, E, C beta-carotene, copper and zinc) and L and Z. A potential unknown of the study design was identified as being the effect of simultaneous administration of beta-carotene and L and Z, hence potential competitive absorption of carotenoids [38]. Kostic et al. [38] demonstrated that when lutein and beta-carotene are ingested together, serum peak concentration of lutein is lower in the presence of beta-carotene. Borel et al. [27] further proposed that the enzyme beta-carotene monooxygenase (BCMO1) that regulates the conversion from beta-carotene to Vitamin A, may also modulate macular pigment deposition. The conversion activity of BCMO1 is genetically determined and there is evidence that genotype variants upstream from the BCMO1 gene affect conversion efficiency [39]. Lietz et al. [39] highlight the importance of genotyping exonic, intronic and intergenic BCMO1 SNPs to investigate beta-carotene conversion efficiency. In particular, genotypes for three BCMO1 SNPs (rs11645428, rs6420424 and rs6564851) have been identified, with decreased (rs11645428 GG, rs6420424 AA, rs6564851 GG) and increased (rs11645428 AA, rs6420424 GG, rs6564851 TT) beta-carotene conversion efficiency but the relationship with macular pigment levels was not evaluated in that study [39]. A large observational study demonstrated a strong association between MPOD and the BCMO1 SNP (rs11645428) in a healthy cohort of postmenopausal women [26]. Another recent study confirmed these findings but found the strongest association between MPOD and the BCMO1 SNP rs6564851 [28]. However, this study did not show an association between BCMO1 variants and macular pigment levels after L and Z supplementation. Neither study investigated the combined effect of all three BCMO1 SNPs (rs11645428, rs6420424 and rs6564851) that may act synergistically to alter the catalytic activity of BCMO1 and consequently alter pigment deposition in the macula. Moreover, these studies were performed in healthy participants and BCMO1 effects may not only vary in different populations [39] but also with retinal disease.

The aim of this study was therefore to determine the relationship between MPOD and three SNPs (rs11645428 and rs6420424, rs6564851) upstream from the BCMO1 gene. Our hypothesis is that participants with “high” and “low” beta-carotene conversion genotypes will have high and low MPOD levels, respectively. We investigated a group of healthy participants and patients with advanced neovascular macular degeneration. The reason for including the latter group was to determine whether BCMO1 gene variants are also associated with disease progression. Our hypothesis is that given participants have already developed advanced AMD, the BCMO1 SNPs will impact less on MPOD as other environmental and genetic factors may dominate.

**Methods**

**Ethics Statement**

The Queensland University of Technology (QUT) Human Research Ethics Committee approved this study. The study was conducted in accordance with the guidelines of the QUT Human Research Ethics Committee and the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant.

**Participants**

In total, 44 participants were recruited from a general population (healthy participants with no eye disease) and a private practice (patients with age-related macular degeneration) and tested for macular pigment optical density (MPOD). All participants were of European ancestry except for two AMD patients who were of Asian ancestry. Participants were genotyped for the SNPs rs11645428, rs6420424 and rs6564851 upstream of the BCMO1 gene. Of the 44 patients, 20 participants were assigned to the healthy group (mean age 56 ± 5 yrs, 8 female and 12 male). The healthy participants had best corrected visual acuities of greater than 1.0, normal intraocular pressure (<21 mmHg), no signs of anterior segment or retinal or optic nerve disease and normal central retinal thickness within reported normative values as measured with optical coherence tomography (OCT, Stratus, Zeiss, Germany). Twenty-four participants were assigned to the patient group with neovascular AMD (mean age 80 ± 7 yrs, 14 female and 10 male). All AMD patients had steady fixation and.
most of them had a visual acuity of greater than 0.3 (20/50) as suggested by the MPOD manufacturer. Those participants who had a visual acuity less than 0.3 (n = 3) were still able to fixate and perform the MPOD successfully as has been previously demonstrated in patients with reduced visual acuity in AMD [29,40]. All the AMD patients were classified as AREDS level 4b [41] and were undergoing intravitreal injection with anti-vascular endothelial growth factor (anti-VEGF) treatment according to established procedures [42]. None of the participants were taking supplements containing either lutein or zeaxanthin at the time of the study, and none were current smokers.

Macular Pigment Optical Density (MPOD) Determination

Macular Pigment Optical Density and ophthalmic routine assessment (visual acuity, indirect ophthalmoscopy, intraocular pressure and OCT) were performed by the same investigators (ophthalmologists BF, AK and optometry staff who were blind to the genotypes of each participant). Macular pigment optical density was determined by heterochromatic flicker photometry (Macular Metrics II, LLC, Providence, USA) with a 0.5° stimulus presented in the fovea and a 2° stimulus presented at 7° in the paracentral area [43]. The stimulus consisted of a flickering light that alternates between a 460 nm light that is maximally absorbed by macular pigment, and a 564 nm light that is not absorbed by macular pigment. All participants performed four measurements at both retinal locations. The flicker frequency was adjusted individually before starting the test. The amount of 460 nm light required to achieve minimum flicker (iso-luminance between the blue and green stimuli) is used to calculate a participant’s MPOD (in density units, D.U.) which is the log ratio of the amount of blue light absorbed centrally to that absorbed peripherally. Patients in the AMD group performed another four measurements at both retinal locations at a second visit (within 3 months of treatment).

Figure 1. MPOD as a function of BCMO1 rs11645428 (error bars indicate+SD). Healthy participants with the GG genotype show on average significantly lower MPOD compared to those with the AA genotype. There were no significant differences between genotypes in the AMD group.

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Figure 2. MPOD as a function of BCMO1 rs6420424 (error bars indicate+SD). Healthy participants with the GG genotype show on average significantly higher MPOD compared to those with the AA genotype. There were no significant differences between genotypes in the AMD group.

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Figure 3. MPOD as a function of BCMO1 rs6564851 (error bars indicate+SD). Healthy participants with the TT genotype show on average significantly higher MPOD compared to those with the GG genotype. There were no significant differences between genotypes in the AMD group.

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Figure 4. MPOD as a function of “high conversion” (high) (rs11645428 AA/rs6420424 GG/rs6564851 TT), “low conversion” (low) (rs11645425 GG/rs6420424 AA/rs6564851 GG) and “medium conversion” (medium) (rs11645425/rs6420424/rs6564851 remaining genotypes) triple genotypes (error bars indicate+SD). Healthy participants with the “high conversion” genotype had significantly higher MPOD compared with the “low conversion” genotype.

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Table 2. Genotype frequencies of BCMO1 triple genotypes (rs11645428, rs6420424 and rs6564851) and mean MPOD.

| Genotype combinations | Frequency (n) | MPOD Mean ± SD |
|-----------------------|--------------|----------------|
| AA/GG/TT              | 24% (n=4)    | 0.72±0.2       |
| GG/AA/GG              | 29% (n=5)    | 0.29±0.2       |
| Remaining genotypes   | 47% (n=8)    | 0.58±0.2       |

Genotyping
The DNA was extracted from saliva samples (Oragene DNA self-collection kit, DNA Genotek Inc, Ottawa, Ontario, Canada) according to established procedures used previously in our laboratories [44,45]. DNA was then genotyped for the SNPs rs11645428, rs6420424 and rs6564581 commercially at the Australian Genome Research Facility (AGRF) using the Sequenom MassARRAY genotyping system.

Statistical Analysis
Data were analysed with a statistical package [IBM SPSS Statistics 21]. Comparisons between MPOD levels and genotypes were made using one-way ANOVA and post-hoc analysis.

Results
Initial data screening indicated the data were normally distributed. Forty-three out of 44 participants were successfully genotyped for rs11645428 and rs6564851 and all 44 participants were genotyped for rs6420424. Genotypes were in Hardy-Weinberg equilibrium (HWE) as computed using Utility Programs for Analysis of Genetic Linkage [46].

Table 1 shows genotype and allele frequencies for the three BCMO1 SNPs and mean MPOD. Alleles were within reported frequencies from other studies [26,39]. For example, this study found a minor allele frequency (MAF) of 35% for rs11645428 and Meyer’s et al. [26] found a MAF of 33%. Lietz et al. [39] summarised allele frequencies of rs6420424 and rs6564581 from different ethnic groups including their own cohort of 28 female volunteers. They found a MAF of 45% for rs6420424 compared to 48% in our study and 48% for rs6564581 compared to 47% in our study.

In the healthy group, there was a significant difference between rs11645428 genotypes (F2,19 = 7.1, p<0.01) with the mean MPOD values being on average lower for those with the GG genotype (0.38±0.2 D.U.) compared to those with the GA (0.51±0.1 D.U.) and the AA genotypes (0.71±0.2 D.U.) (Figure 1). Post-hoc analysis demonstrated that the rs11645428 GG genotype was significantly lower compared to the AA genotype (p=0.002) and that the GA genotype was significantly lower than the AA genotype (p=0.05). There was also a significant difference between the rs6420424 genotypes (F2,19 = 7.8, p<0.01) where those with the GG genotype had higher MPOD levels compared to those with the AA genotype. The average MPOD for the GG, GA and AA genotypes were 0.67±0.2 D.U., 0.48±0.1 D.U. and 0.33±0.2 D.U., respectively. Post-hoc analysis demonstrated that participants with the GG genotype had significantly higher MPOD levels compared to GA (p=0.03) and AA (p<0.01) (Figure 2). Analysis of rs6564851 in healthy participants demonstrated significant differences in mean MPOD (F2,19 = 15.5, p<0.001), with the TT genotype showing significantly higher MPOD (0.72±0.2 D.U.) compared to the GT (0.48±0.1 D.U.) and GG (0.29±0.02 D.U.) genotypes. Post-hoc analysis revealed that the TT genotype was significantly higher than the GT and GG genotype (p<0.01) (Figure 3).

In the patients with age-related macular degeneration there was on average no significant difference in MPOD between the two visits within the three months of anti-VEGF treatment (visit one: mean MPOD = 0.47±0.1 D.U. and visit 2: mean MPOD = 0.44±0.2 D.U., p = 0.4). The average MPOD of these two time points was taken for further analysis. There was no significant difference in MPOD between the rs11645428 genotypes (F2,22 = 0.4, p = 0.7), the rs6420424 genotypes (F2,22 = 1.6, p = 0.2) or the rs6564581 genotypes (F2,22 = 1.0, p = 0.3) in the AMD patients. The average MPOD in the rs11645428 GG, rs6420424 GG, GA and AA genotypes were 0.47±0.2 D.U., 0.45±0.1 D.U., 0.39±0.1 D.U. and 0.4±0.1 D.U., 0.46±0.1 D.U., 0.52±0.2 D.U., respectively. The average MPOD for the rs6564581 TT, GT and GG genotypes was 0.38±0.1 D.U., 0.46±0.1 D.U. and 0.48±0.2 D.U., respectively (Figure 1-3) (Table 1).

We determined rs11645428, rs6420424 and rs6564581 “triple genotypes” based on the previously reported BCMO1 beta-carotene conversion efficiency, that is, those who should have high- or low-conversion efficiency based on their BCMO1 genotypes [39]. Interestingly, the healthy participants MPOD values reflected plasma conversion efficiency, with the GG/AA/GG (rs11645428/rs6420424/rs6564581) low conversion triple genotypes having low MPOD values, those with the AA/GG/TT high conversion triple genotype had significantly higher MPOD values while the remaining genotypes had intermediate MPOD values (Figure 4). There was a significant difference between the three high, low and intermediate MPOD groups (F2,16 = 6.8, p = 0.01). Data show that healthy persons with the AA/GG/TT genotypes have on average significantly higher MPOD levels (0.72±0.2 D.U.) compared to those with the GG/AA/GG genotypes (MPOD = 0.29±0.2 D.U.) (p<0.01). Those healthy participants with the remaining genotypes showed significantly higher MPOD (0.58±0.2 D.U.) compared to the GG/AA/GG genotype with the lowest MPOD (p = 0.02) (Figure 4) (Table 2).

Discussion
The data demonstrate that BCMO1 SNPs and their predicted effects on beta-carotene conversion efficiency in the plasma [39] can be related to MPOD. We show that healthy participants fall into high, low and intermediate MPOD values based on combined BCMO1 rs11645428/rs6420424/rs6564581 genotypes. We demonstrate that healthy participants with rs11645428 GG, rs6420424 AA and rs6564581 GG “low conversion” genotypes had also low MPOD levels and those with rs11645428 AA, rs6420424 GG and rs6564581 TT “high conversion” genotypes had high MPOD levels. All the remaining homozygous and heterozygous genotypes had MPOD levels that were between the “high” and “low” conversion genotype groups. Hence the findings support our hypothesis that the increased BCMO1 β-carotene conversion efficiency in the plasma is reflected in increased MPOD and that decreased conversion results in decreased MPOD.

The BCMO1 gene encodes an enzyme that cleaves β-carotenoids to produce vitamin A. It has been hypothesised that its reduced activity due to variants in the BCMO1 gene has an effect on retinal levels of L and Z and that BCMO1 may modulate concentration of lutein in the macula [27]. The mechanism by which a change in enzymatic activity affects carotenoid uptake however, is not clear. During et al. [47] suggested an antagonising effect by showing differential absorption patterns of carotenoids in
a cellular in vitro model. Our findings suggest that decreased conversion of β-carotene and hence increased β-carotene availability may cause competition with lutein uptake, thereby decreasing MPOD. Conversely, increased β-carotene conversion and reduced availability may improve uptake of macular pigments in healthy humans.

In the cohort with age-related macular degeneration, MPOD did not differ between genotypes or triple genotypes. This is consistent with our hypothesis and suggests that other factors may be involved. In particular, in advanced AMD where there is a change in retinal anatomy, retinal carotenoid binding proteins [48,49,50] may be altered, hence affecting MPOD. Additional environment and genetic factors are influential in the evolution of AMD and while macular pigments may protect from environmental risk, this function may be “overridden” by a strong genetic predisposition for AMD. All our patients had advanced neovascular AMD and macular pigment was not a protective factor in this cohort. Complement factor H (CFH) genotypes have been identified as major contributors to the risk of developing AMD [51,52] and we found that the AMD patients with the homozygous CFH high risk genotype had on average lower MPOD (0.43±0.1 D.U.) compared to the low risk genotypes (0.47±0.1 D.U.) (data not shown, p = 0.2). This is in support of another study that demonstrated reduced macular pigment in a small cohort of healthy participants who were homozygous for CFH risk genotypes [53]. Our findings are further supported by a recent large study in women that investigated the relationship of BCMO1 genotypes with macular pigment optical density in a sample of patients who predominantly had early AMD [54]. While Meyers et al. [54] did not investigate combined BCMO1 genotypes as done in this study, they demonstrated a relationship between MPOD and one of the BCMO1 genotypes we investigated. The strongest relationship however, was found between MPOD and other genes related to carotenoid status (ABCA1, ALDH3A2 and BCO2) [54]. Meyers et al. [54] acknowledge that carotenoid pathway genes need to be evaluated in relation to early and advanced AMD risk in prospective studies. Our study therefore provides evidence of a differing impact of BCMO1 genotypes on MPOD based on AMD stage.

Another finding was that repeated anti-VEGF treatment did not affect MPOD levels over a short period of three months. Anti-VEGF therapy can cause toxic photoreceptor damage [55], and macular pigment can be found in outer photoreceptor segments. The effect of anti-VEGF on MPOD however, is unknown and studies have only considered the effects of laser treatment on MPOD [29]. Our findings may have importance for recovery of vision in patients undergoing anti-VEGF treatment given the role of macular pigment in visual performance with studies demonstrating that macular pigments enhance contrast sensitivity, chromatic discrimination and mesopic visual function [2,5,6,56]. However, it remains to be shown whether supplementation of macular pigments is useful in advanced AMD for improvement of visual function rather than protection, in particular when considering that the retinal uptake of pigments may be affected.

Our study reflects emerging evidence of the significant effect of gene variants modifying MPOD levels and that this effect may be different in patients with retinal disease. Our study confirms large association studies of BCMO1 gene variants with MPOD in healthy participants [26] [28]. It is known that these SNPs are associated with high or low serum beta-carotene levels [39] but none of these studies investigated the combined effects of these SNPs on MPOD. Also, these effects were not evaluated in different cohorts with and without AMD. Additionally, our study suggests that competitive uptake of carotenoids requires further assessment in different population cohorts with and without retinal disease.

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Author Contributions

Conceived and designed the experiments: BF AZ. Performed the experiments: BF. Analyzed the data: BF CPM JV AZ. Contributed reagents/materials/analysis tools: BF CPM JV AK AZ. Wrote the paper: BF CPM JV AZ.

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