Investigation of Genetic Variation in Scavenger Receptor Class B, Member 1 (*SCARB1*) and Association with Serum Carotenoids

Gareth J. McKay, BSc, PhD,[1] Edward Loane, PhD, MRCOphth,[2] John M. Nolan, BSc, PhD,[3] Christopher C. Patterson, BSc, PhD,[1] Kristin J. Meyers, MPH, PhD,[4] Julie A. Mares, MSPH, PhD,[4] Ekaterina Yonova-Doing, BSc,[5] Christopher J. Hammond, MD, FRCOphth,[5] Stephen Beatty, MD, FRCOphth,[3] Giuliana Silvestri, MD, FRCOphth[6]

Objective: To investigate association of scavenger receptor class B, member 1 (*SCARB1*) genetic variants with serum carotenoid levels of lutein (L) and zeaxanthin (Z) and macular pigment optical density (MPOD).

Design: A cross-sectional study of healthy adults aged 20 to 70.

Participants: We recruited 302 participants after local advertisement.

Methods: We measured MPOD by customized heterochromatic flicker photometry. Fasting blood samples were taken for serum L and Z measurement by high-performance liquid chromatography and lipoprotein analysis by spectrophotometric assay. Forty-seven single nucleotide polymorphisms (SNPs) across *SCARB1* were genotyped using Sequenom technology. Association analyses were performed using PLINK to compare allele and haplotype means, with adjustment for potential confounding and correction for multiple comparisons by permutation testing. Replication analysis was performed in the TwinsUK and Carotenoids in Age-Related Eye Disease Study (CAREDS) cohorts.

Main Outcome Measures: Odds ratios for MPOD area, serum L and Z concentrations associated with genetic variations in *SCARB1* and interactions between *SCARB1* and gender.

Results: After multiple regression analysis with adjustment for age, body mass index, gender, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, smoking, and dietary L and Z levels, 5 SNPs were significantly associated with serum L concentration and 1 SNP with MPOD ($P<0.01$). Only the association between rs11057841 and serum L withstood correction for multiple comparisons by permutation testing ($P<0.01$) and replicated in the TwinsUK cohort ($P = 0.014$). Independent replication was also observed in the CAREDS cohort with rs10846744 ($P = 2\times10^{-4}$), an SNP in high linkage disequilibrium with rs11057841 ($r^2 = 0.93$). No interactions by gender were found. Haplotype analysis revealed no stronger association than obtained with single SNP analyses.

Conclusions: Our study has identified association between rs11057841 and serum L concentration (24% increase per T allele) in healthy subjects, independent of potential confounding factors. Our data supports further evaluation of the role for *SCARB1* in the transport of macular pigment and the possible modulation of age-related macular degeneration risk through combating the effects of oxidative stress within the retina.

Financial Disclosure(s): Proprietary or commercial disclosures may be found after the references.
Ophthalmology 2013;xx:xxx *© 2013 by the American Academy of Ophthalmology.*

Age-related macular degeneration (AMD; MIM# 603075) is the most common form of visual impairment among older people of European descent,[1] accounting for more than half of all new cases of registered blindness.[2] The socioeconomic burden associated with AMD continues to challenge our aging society with almost 30% of those aged ≥75 years showing early signs of disease.[3] Although the specifics of the etiology remain largely unresolved, AMD is a common multifactorial disorder of complex etiology with multiple genetic, environmental, and lifestyle risk factors. By definition, AMD specifically affects the macular region of the retina, an area responsible for detailed central vision.

Macular pigment (MP) accumulates in the central retina and is composed of the carotenoids, lutein (L), zeaxanthin (Z) and *meso*-Z, which give the macula its characteristic yellow color. In humans, L and Z are not synthesized de novo and are of dietary origin (mostly fruit and vegetables), whereas *meso*-Z has been reported to be predominantly nondietary and formed after conversion from L in the retina,[4] although the exact process of conversion from L to

© 2013 by the American Academy of Ophthalmology
Published by Elsevier Inc.

ISSN 0161-6420/13/$–see front matter
http://dx.doi.org/10.1016/j.ophtha.2013.01.030

meso-Z has still to be elucidated.[5] Dietary L and Z are absorbed with fats in the gut and are transported to the liver, whereupon they form carotenoid–lipoprotein complexes that facilitate their transport through the vascular system.[6] Macular pigment confers powerful antioxidant protection and also filters actinic short wavelength, blue light, limiting the (photo-)oxidative damage to retinal cells.[7] These properties of MP are believed to limit the development and/or progression of AMD.[8] Although MP is dietary derived, its concentration at the macula is, in part, determined by genetic factors with heritability estimated at 67% to 85% in a cross-sectional study of individual MP levels.[9] More recently, longitudinal response to L and Z supplementation has estimated genetic influence approximating 27%, although this may have been influenced by the composition of the L supplement used.[10]

Genetic variation in scavenger receptor class B, member 1 (*SCARB1*) has previously been reported in association with AMD, implicating a role for cholesterol and MP metabolism in the disease process.[11] The gene, located at 12q24.31, is a region of interest for AMD originating from linkage analysis.[12,13] The *SCARB1* gene encodes a multiligand cell-surface receptor that mediates selective cholesterol uptake and efflux.[14,15] Reverse cholesterol transport is a major process required for the clearance of excess cholesterol from the body and the high-density lipoprotein cholesterol (HDLc) pathway genes, *LIPC* and *CETP*, have been implicated in AMD pathogenesis through large genome-wide association studies.[16,17] Other studies have also reported common variants in *SCARB1* in association with development of coronary heart disease[18] and lipid profiles,[19,20] with several studies providing evidence of a gender-related effect.[21–23] Both coronary heart disease and dyslipidemia have been reported to share common pathogenic pathways with AMD.[24,25] In addition, SRB1, the protein encoded by *SCARB1*, has also been detected in the retinal pigment epithelium[26,27] and in intestine cells where it mediates cholesterol efflux and xanthophyll uptake.[28]

In light of the strong evidence implicating cholesterol metabolism in AMD pathogenesis, coupled with a relative lack of MP in association with a clinically confirmed family history of AMD,[29] we sought to evaluate common genetic polymorphisms within *SCARB1* and how they might contribute to variation of this putatively protective pigment.

Materials and Methods

Participants

Three hundred two subjects were recruited for this study, which was carried out in the Macular Pigment Research Group laboratory at Waterford Institute of Technology, Ireland. Subjects were recruited after local advertisement in various media. This study was approved by the Research Ethics Committee of Waterford Institute of Technology, and subjects gave written informed consent before participation. All experimental procedures adhered to the tenets of the Declaration of Helsinki.

Inclusion criteria for participation in this study were the following: age between 20 and 70 years, no clinical evidence of ocular pathology, no dietary supplementation with the MP carotenoids, and visual acuity of ≥20/40. The following information was recorded for each subject: demographic details, family history of AMD (confirmed in writing by the diagnosing ophthalmologist), personal smoking history measured in pack-years, and dietary intake of L and Z, assessed using a validated 170-item food frequency questionnaire (FFQ). Examination included visual acuity (Snellen and logarithm of the minimum angle of resolution), body mass index (BMI [calculated as kg/m^2]), MP optical density (MPOD) measurement by customized heterochromatic flicker photometry (cHFP) using the Macular Densitometer, nonmydriatic fundus photography using a NIDEK AFC-210 nonmydriatic auto fundus camera to screen for ocular pathology, and 12-hour fasting blood samples to quantify serum concentrations of L and Z using high-performance liquid chromatography and for genotyping.

FFQ

Dietary intake of L and Z was assessed by a self-administered, semiquantitative FFQ developed by the Scottish Collaborative Group at the University of Aberdeen, Scotland, UK. This semiquantitative FFQ, including validation, is described in detail in a separate study.[30]

Measurement of MPOD

We measured MPOD psychophysically by cHFP, a technique that has been validated against the absorption spectrum of MP in vitro.[31] The cHFP is based on the fact that MP absorbs short-wavelength blue light, with peak absorption occurring at a wavelength of 458 nm. The subject is required to make isoluminance matches between 2 flickering lights, a green light (not absorbed by MP) and a blue light (maximally absorbed by MP). The log ratio of the amount of blue light absorbed centrally, where MP peaks, to that absorbed at a peripheral retinal locus (the "reference point," where MPOD is assumed to be zero), gives a measure of the subject's MPOD.

In this study, we used the Macular Densitometer (Brown University, Providence, RI),[32] a cHFP instrument that is slightly modified from a device described previously.[32] The subject is required to observe a flickering target, alternating in square-wave counterphase between a green light (with a wavelength of 564 nm) and a blue light (with a wavelength of 460 nm), and to make isoluminance matches between these flickering lights. The luminance of the green and blue lights is varied in a yoked manner, which avoids a change in the overall luminance of the test target. When an isoluminant ("null flicker") match has been made between these flickering lights, flicker is no longer perceived, and this is the desired endpoint of the test. Different sized targets enable measurement of MPOD at 0.25°, 0.5°, 1°, and 1.75° retinal eccentricity, relative to a reference point at 7° retinal eccentricity (where MPOD is assumed to be zero). Targets are presented on a blue background test field (wavelength 468 nm) that saturates the S-cone pathway. A minimum of 3 null-flicker readings, with a coefficient of variance ≤10%, were recorded for each subject at each of the test loci (0.25°, 0.5°, 1°, 1.75°, and 7° retinal eccentricity). Measurement of MPOD at these points of retinal eccentricity enabled us to plot the spatial profile of MP across the macula. We then calculated the area of MPOD under the spatial profile ($MPOD_{Area}$), using the trapezium rule, which approximates the area by a series of trapeziums constructed by assuming linearity in the relationship between MPOD values and retinal eccentricity between adjacent measurement angles:

$$MPOD_{Area} = [0.25 * ((MPOD_{0.25^\circ} + MPOD_{0.5^\circ})/2) + 0.50 * ((MPOD_{0.5^\circ} + MPOD_{1^\circ})/2) + 0.75 * ((MPOD_{1^\circ} + MPOD_{1.75^\circ})/2) + 5.25 * ((MPOD_{1.75^\circ} + MPOD_{7^\circ})/2)].$$

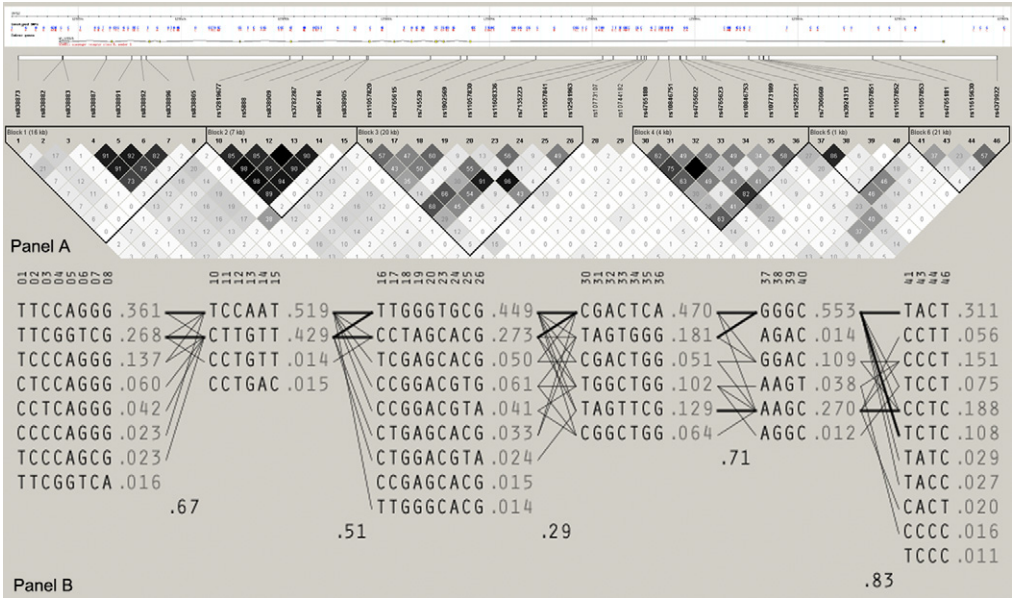

Figure 1. Schematic representation of *SCARB1*, linkage disequilibrium (measured by r^2; **A**) and putative haplotype block structure across the gene (**B**).

The $MPOD_{Area}$, a weighted average of the MPOD value at the various points of retinal eccentricity, should offer improved accuracy of the quantity of MP across the macula compared with a single eccentricity measurement alone. We measured MPOD under conditions of dimmed light (ambient illuminance: 4 lux, as measured with an Iso-Tech ILM 350 Lux Meter; RS Components, Corby, UK) at a viewing distance of 18.5 inches (47 cm).

The major advantage of cHFP over standard HFP instruments is that the flicker frequency of each test target is customized for each individual subject, minimizing variance between consecutive measurements and thus increasing the accuracy and ease of use of the test. Further information on the technique and advantages of cHFP has been published previously.[33]

Blood Sample Collection

A 12-hour fasting blood sample was collected from each subject in a 4-ml Z Serum Sep Clot Activator Vacuette tube (Greiner Bio-One GmbH, Kremsmünster, Austria) at the beginning of the study visit. This whole blood sample was immediately refrigerated at 2°C to 8°C, before centrifugation at approximately 1800*g* for 10 minutes. Centrifugation was performed within 4 hours of phlebotomy. After centrifugation, the supernatant serum sample was aliquoted into 1.5-ml amber (light-sensitive) microcentrifuge tubes (Brand GmbH, Wertheim, Germany) and stored at minus 70°C before analysis.

Serum L and Z Analysis

Serum L and Z were quantified using reverse phase high-performance liquid chromatography. We used an Agilent 1200 series LC system (Agilent Technologies Ireland Ltd, Dublin, Ireland), with photodiode array detection at 295 nm (detection of the internal standard, alpha tocopherol acetate) and 450 nm (detection of L and Z). A 5-μm analytical/preparative 4.6×250 mm 201TP specialty reverse phase column (Vydac, Hesperia, CA) was used with an in-line guard column. The mobile phase consisted of 97% methanol and 3% tetrahydrofuran, and was degassed using an in-line degasser. The flow rate was 1 ml/min, and the total run time was 15 minutes. All carotenoid peaks were integrated and quantified using Agilent Chem Station software. Further detail on the methodology used for this analysis is provided in a separate publication.[30]

Serum Lipoprotein Analysis

Lipoprotein analysis (HDLc, LDLc, triglycerides [TGs]) was performed using the ACE Clinical Chemistry System (Alfa Wassermann, Woerden, The Netherlands) with reagents and consumables supplied by Randox Laboratories Ltd (Antrim, UK). Analyses were performed on 200-μL serum samples by spectrophotometric analysis at 37°C. The analysis module uses a holographic diffraction grating spectrophotometer to measure absorbance at 16 different wavelengths. Measurements for each assay were recorded at wavelengths and times preprogrammed for each test with results calculated as specified by the test parameters. The protocol used included daily, 2-level quality control assessments and duplicate analyses on 50 (16.6%) samples, to ensure the precision of the results.

SNP Selection and Genotyping

Common variants were selected from Phase III, release 2 HapMap (available at http://www.hapmap.org; accessed October 30, 2009) CEPH data (Utah residents with ancestry in northern and western Europe; CEU) using Haploview (available at http://www.broadinstitute.org/haploview; accessed October 30, 2009) to visualize linkage disequilibrium (Fig 1A). Tag SNPs were selected using multimarker tagging where $r^2>0.8$ (log of odds threshold 3.0) for all downloaded SNPs with a minor allele frequency ≥5%, genotype call rate ≥95%, and no significant deviation from Hardy–Weinberg equilibrium.

Genotyping was performed by MassARRAY iPLEX (Sequenom, San Diego, CA) assays according to the manufacturer's instructions. Quality filters for exclusion of SNPs included call rates below 95% and deviation from Hardy–Weinberg equilibrium ($P<0.001$). DNA samples were excluded if missing genotypes exceeded 10%. Other quality control measures included duplicates

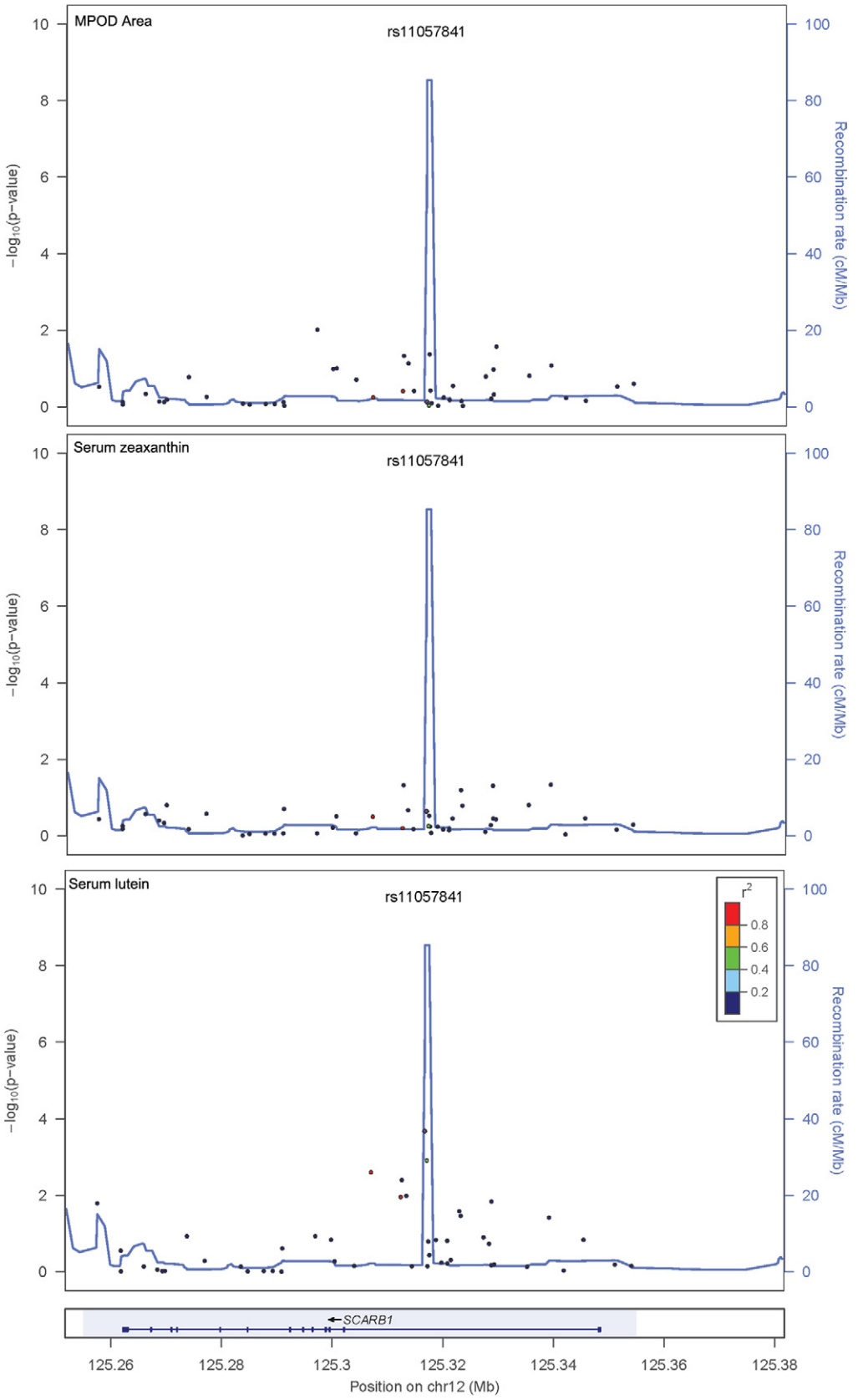
MPOD Area
rs11057841
Serum zeaxanthin
rs11057841
Serum lutein
rs11057841
r²
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
SCARB1
125.26
125.28
125.3
125.32
125.34
125.36
125.38
Position on chr12 (Mb)

Table 2. Anthropometric and Lifestyle Data for All Genotyped Subjects in the Discovery Sample for rs11057841

Characteristic	All Samples (n = 298)	CC (n = 228)	CT (n = 64)	TT (n = 6)	P
Age (yrs)	48.0 (11.1)	47.8 (11.4)	48.4 (10.2)	51.2 (10.4)	0.50
Male gender, n (%)	91 (31%)	66 (29%)	22 (34%)	3 (50%)	0.21
BMI (kg/m^2)	26.8 (4.6)	26.6 (4.5)	27.3 (5.0)	24.7 (2.4)	0.75
MPOD area	0.718 (0.431)	0.713 (0.436)	0.734 (0.411)	0.735 (0.507)	0.73
MPOD 0.25°	0.482 (0.207)	0.477 (0.208)	0.494 (0.201)	0.500 (0.231)	0.54
MPOD 0.5°	0.379 (0.175)	0.376 (0.171)	0.392 (0.185)	0.348 (0.255)	0.76
MPOD 1°	0.239 (0.128)	0.239 (0.128)	0.240 (0.129)	0.203 (0.132)	0.76
MPOD 1.75°	0.126 (0.097)	0.126 (0.097)	0.125 (0.095)	0.138 (0.106)	0.93
Pack-years smoked	5.7 (11.3)	6.0 (11.9)	4.8 (9.2)	5.7 (9.1)	0.53
Serum lutein (μg/ml)	0.079 [0.060–0.104]	0.075 [0.057–0.102]	0.093 [0.066–0.127]	0.111 [0.077–0.160]	2×10^{-4}
Serum zeaxanthin (μg/ml)	0.013 [0.009–0.020]	0.013 [0.009–0.020]	0.014 [0.009–0.020]	0.016 [0.010–0.027]	0.14
Dietary lutein (mg)	1.063 [0.698–1.725]	1.077 [0.683–1.806]	1.061 [0.738–1.630]	0.679 [0.403–0.922]	0.34
Dietary zeaxanthin (mg)	0.157 [0.109–0.231]	0.158 [0.109–0.238]	0.158 [0.111–0.215]	0.127 [0.087–0.198]	0.66
LDLc (mmol/L)	3.22 (0.92)	3.21 (0.91)	3.27 (0.97)	2.78 (1.02)	0.83
HDLc (mmol/L)	1.50 (0.38)	1.50 (0.38)	1.51 (0.37)	1.72 (0.51)	0.31
Triglycerides (mmol/L)	1.19 [0.88–1.51]	1.17 [0.87–1.49]	1.28 [0.88–1.73]	0.94 [0.63–1.24]	0.57
Total cholesterol (mmol/L)	5.41 (1.10)	5.38 (1.09)	5.52 (1.15)	5.55 (0.93)	0.36
Positive family history of AMD, n (%)	118 (40%)	83 (36%)	31 (48%)	4 (67%)	0.03

AMD = age-related macular degeneration; BMI = body mass index; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; MPOD = macular pigment optical density.
Data presented as mean (standard deviation), geometric mean [interquartile range] or n (%).

on plates, random sample allocation to plates, independent scoring of problematic genotypes by 2 individuals, and resequencing of selected DNAs to validate genotypes.

Replication Cohorts

Replication of the most significant SNP was undertaken in the TwinsUK adult twin cohort (n = 199) and in the Carotenoids in Age-Related Eye Disease Study (CAREDS; n = 1643), an ancillary study of the Women's Health Initiative Observational Study.[34] TwinsUK genotyping had been undertaken previously using an Illumina HumanHap 610 Quad array[35] and the CAREDS cohort using a customized Illumina array. In CAREDS, MPOD was estimated using cHFP,[36] using protocols similar to those implemented in the discovery cohort described, and serum samples were analyzed for concentrations of *trans*-L and -Z at Tufts University by a reverse phase high-performance liquid chromatography analysis.[37] The TwinsUK study used HFP (Maculometer) and an imaging method (2-wavelength fundus autofluorescence) to estimate MPOD with serum analysis in the same laboratory as that implemented in the discovery cohort.[10] All procedures conformed to the Declaration of Helsinki and were approved by the institutional review board at each university.

Statistical Analysis

Chi-square tests and 1-way analysis of variance tests for trend were used to investigate differences in qualitative and quantitative traits, respectively, between genotype subgroups. Analyses were performed using an additive genotypic model (SPSS, version 18, SPSS, Inc, Chicago, IL). Multiple regression analysis was used to adjust for potential confounders (age, gender, smoking status in pack years, BMI, dietary L and Z estimates, LDLc, HDLc, and TGs). Serum and dietary L and Z concentrations and TGs were log base 10 transformed in light of heavy positive skew in their distributions and were summarized using geometric means and interquartile ranges. The genotype regression coefficients (and their confidence limits) for these transformed quantitative traits were anti-logged and interpreted as proportionate changes in concentration per allele. Given previous evidence suggesting a gender-related effect at *SCARB1*,[21–23] a term for genotype by gender interaction was also included within the regression model. Correction of the quantitative trait comparisons for multiple SNPs was performed in PLINK (version 1.07)[38] by permutation testing (n = 100 000). The level of statistical significance used to assess the permutation test *P* values was 5/3 = 1.67% to allow for the analysis of the 3 quantitative traits (serum L, serum Z, and $MPOD_{Area}$).

Results

Genotype data were available for 47 SNPs from 301 of the 302 (99.7%) subjects included in the study (Table 1; available at http://aaojournal.org). A total of 7 SNPs were excluded for failing to meet the quality filters of call rates below 95% or deviation from Hardy–Weinberg equilibrium ($P<0.001$). The average call rate for the remaining SNPs was 98.8%. No duplicate inconsistencies were observed.

←

Figure 2. Regional association plots are presented for single nucleotide polymorphisms (SNPs) investigated for the outcome measures (1) macular pigment optical density (MPOD area), (2) serum zeaxanthin, and (3) serum lutein. The color of the SNP symbol indicates the linkage disequiliblium (r^2) with the index SNP, rs11057841, which is colored purple (http://csg.sph.umich.edu/locuszoom/; accessed July 30, 2012). Points on the plot relate to the *P* values (indicated on the left side scale) and the lines relate to the recombination rates (right side scale).

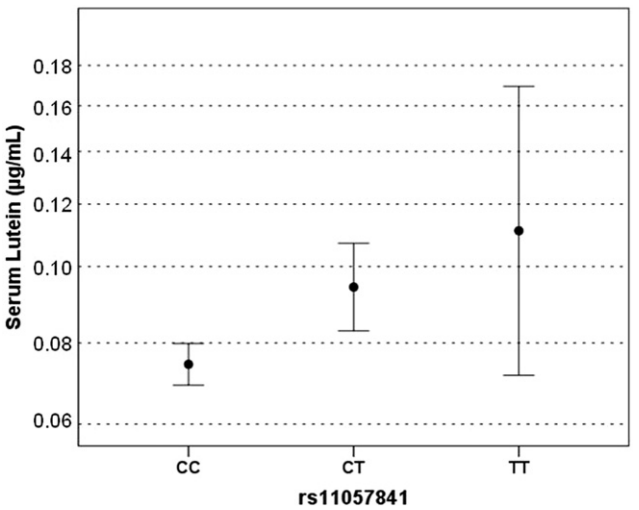

Figure 3. Plots of serum lutein showing geometric means and 95% confidence intervals against rs11057841 genotype.

In a univariate analysis, 5 SNPs were significantly associated with serum L (rs11057841, $P = 2\times10^{-4}$; rs10773109, $P = 0.001$; rs11057830, $P = 0.002$; rs11608336, $P = 0.001$; rs12581963, $P = 0.003$), although only rs11057841 was significant ($P<0.0167$) after correction for multiple testing by permutation. Multiple regression analysis was performed on MPOD area, serum L and Z concentrations both before and after adjustment for age, BMI, gender, HDLc, LDLc, TGs, smoking status, and dietary L and Z estimates (Table 1; Fig 2; MPOD area, serum Z and serum L). One SNP (rs11057820, $P = 0.008$) was associated with MPOD, although this did not remain significant after correction for multiple testing. No significant interactions by gender were found. Haplotype analysis revealed no stronger associations than were obtained with the single SNP analysis. No associations were identified for serum Z.

Anthropometric and lifestyle data are presented in relation to rs11057841 genotype for all 298 successfully genotyped subjects (Table 2). The mean values ± standard deviation (range) for age was 48.0±11.1 years (range, 21–66) and 69% were female. There was no significant difference between rs11057841 genotypes for any of the following variables: age, BMI, cigarette smoking, dietary L intake, dietary Z intake, serum Z concentration, HDLc, LDLc, and TGs (1-way analysis of variance $P>0.05$). Neither was there any significant difference in the male:female ratio between the genotype groups. Significant differences in serum L concentration (increase in geometric mean per T allele by a factor of 1.24 [95% confidence interval, 1.11–1.39]; $P = 2\times10^{-4}$) and in the ratio of positive:negative family history of AMD ($P = 0.03$) were detected between the 3 rs11057841 genotypes (Table 2; Fig 3).

Evaluation of rs11057841 in the Independent Replication Cohorts

Replication of association for rs11057841 with serum L concentration was undertaken in the TwinsUK adult cohort (n = 199) using a likelihood ratio chi-square test accounting for familial relatedness with support for the association detected in the discovery cohort (Table 3; beta coefficient = 0.252, $P = 0.014$). Although CAREDS did not genotype rs11057841 directly, there was data available for rs10846744, a SNP in high LD with rs11057841 ($r^2 = 0.93$; available at http://www.1000genomes.org/; accessed July 30, 2012), providing further support to the size and direction of effect observed at this locus (Table 3; beta coefficient = 0.0395, $P = 2\times10^{-4}$).

Discussion

Age-related macular degeneration is a common, complex disease; 1 strategy proposed for a reduction in associated risk includes modification of nutrient intake. Several studies have shown that an increased intake of the macular carotenoids L and Z through foods rich in these nutrients (e.g., spinach and egg yolk) tends to reduce the risk of the development and/or progression of late AMD.[39,40] This study has investigated the relationship between common variants in *SCARB1*, MPOD, and serum concentrations of L and Z in 302 healthy subjects aged between 21 and 66 years. The mean MPOD of all genotyped subjects recorded at 0.5° retinal eccentricity was 0.38±0.17 optical density units, a value comparable with previous studies that used cHFP to measure MPOD at this eccentricity.[8,29] Genotype data were available on 99.7% of our sample and association of rs11057841 with serum L concentration survived correction by permutation for multiple testing, showing a 24% increase per T allele, further supported after replication in the independent TwinsUK and CAREDS studies. Whereas Wang et al[41] reported transportation of serum L preferentially on HDLc, we found association of rs11057841 and serum L independent of HDLc and other factors such as BMI, smoking, gender, LDLc, and TGs.

Table 3. Genotype Effect Size for rs11057841 on Serum Lutein Concentration

Cohort	Allele	MAF	n	Coefficient	SE	Change Per Allele (CI)*	P
Waterford (Discovery)	T	0.13	298	0.094	0.025	1.24 (1.11–1.39)	2×10^{-4}
TwinsUK (Replication)	T	0.09	199	0.252	0.102	1.79 (1.13–2.83)	0.014
CAREDS (Replication)†	C	0.15	1640	0.040	0.011	1.10 (1.04–1.15)	2×10^{-4}

CI = 95% confidence intervals; MAF = minor allele frequency; SE = standard error.
The effect on serum lutein concentration (log base 10 scale) was estimated from an additive genetic model without adjustment for covariates in discovery and replication cohorts.
*Genotype regression coefficients (and their confidence limits) were anti-logged and interpreted as proportionate serum lutein changes in concentration per allele.
†Genotype data were not available for rs11057841 for the Carotenoids in Age-Related Eye Disease Study (CAREDS) dataset. Effect size and direction was estimated using rs10846744, reported to be in high linkage disequilibrium ($r^2 = 0.93$) with rs11057841 according to the 1000 Genomes dataset (http://www.1000genomes.org/; accessed July 30, 2012). Note the T allele at rs11057841 is in linkage disequilibrium with the C allele at rs10846744.

Early stage AMD is characterized by hallmark lesions of cholesterol and lipid-rich drusen and basal linear deposits, which accumulate with age between the retinal pigment epithelium and the choroid.[42,43] Bruch's membrane forms the inner margin of the choroid, effectively acting as a vessel wall.[43] During the atherosclerotic process, lipoproteins traverse the vascular endothelium and accumulate in the arterial wall on binding to proteoglycans, culminating in deleterious processes, which include inflammation and neovascularization.[44] In many respects, these atherosclerotic plaques mimic the accumulation of drusen and basal laminar deposits in a manner similar to that observed in early AMD. Most studies of early AMD are often predominated by younger adults and have reported either a protective association or no association with elevated HDLc levels, in contrast with studies of late-stage AMD, characterized by elderly participants, which have tended to report the opposite.[45] As such, although HDLc has been widely studied and implicated in AMD disease etiology, the mechanisms and timing involved with respect to participant age and disease status remain unresolved.

Previously reported associations of *SCARB1* SNP rs5888 with AMD implicated a role for cholesterol and antioxidant metabolism, identifying L in particular, in AMD disease etiology.[11] Recent identification of the hepatic lipase (*LIPC*) and cholesterylester transfer protein (*CETP*) genes[16,17] provide further evidence in support of cholesterol metabolism and AMD pathogenesis, particularly given previous association of these genes with HDLc levels in blood.[45–47] Although multiple common alleles near *CETP* and *LIPC* have been independently associated with HDLc levels,[45] they have also shown modest association in a smaller AMD cohort with less power.[16]

Alternatively, the associations with *SCARB1* variants in this and other studies might reflect variation in carotenoid uptake into the body and eye. There is evidence that transport of carotenoids in the retina[27] and intestine[28] is a facilitated process mediated by the scavenger receptor class B type I. The direct association of rs11057841 genotypes with increasing T alleles and level of L and Z in the serum are consistent with this possibility.

Several studies have provided evidence of a gender-related effect at this locus.[21–23] In a community-based cohort of postmenopausal women, *SCARB1* polymorphisms were associated with decreased HDLc and elevated TG levels in an estrogen-dependent manner.[21,22] Although the majority of female participants in our study were likely to be premenopausal, we found no evidence to suggest a gender-specific interaction between serum L concentration or MPOD and *SCARB1* SNPs.

Interestingly, analyses originating from the Multi-Ethnic Study of Atherosclerosis has shown association between rs10846744 and common carotid intimal-medial artery thickness, a surrogate marker for subclinical atherosclerosis, and increased risk of cardiovascular disease.[23] The association observed in our study between rs11957841 and increased serum L concentration is a variant known to share high linkage disequilibrium with rs10846744, a proxy SNP tested in the CAREDS replication study ($r^2 = 0.93$). Manichaikul et al[23] suggest that genetic variants within *SCARB1* may exert *cis* or *trans* regulatory effects, possibly influencing endothelial function or inflammatory pathways. In view of the common pathways shared between AMD and cardiovascular disease,[24,25] these data implicate *SCARB1* in both disease processes.

Although our study was limited in that participants were too young to characterize symptoms associated with AMD, identification of a positive family history of AMD showed a positive correlation with serum L concentration and rs11957841. Although our study has identified common variants in *SCARB1* associated with serum L concentration, we were unable to assess potential effects exerted by rare or low frequency variants. Despite copy number variation reported at *SCARB1* (Database of Genomic Variants, available at http://projects.tcag.ca/variation/; accessed November 22, 2012), its rare observation is unlikely to have a significant impact at a population level.

Given that genetic variation attenuates cholesterol levels, which may influence drusen formation and modulate AMD risk, and given the poor correlation observed between serum L and Z concentration and MPOD in this study and elsewhere,[10] further studies on the influence of genetic variants in *SCARB1* on cholesterol transport and AMD are warranted. Accumulation of MP in the central retina is reliant on a complex process originating from ingested foodstuff, digestion, absorption, and transport in the serum, and ultimately capture by and stabilization in the retina.[10] Although our study examines a key gene implicated in this process, assessment of additional genetic components of this pathway should be undertaken and their potential influence evaluated. Our data indicates an important role for *SCARB1* in serum L concentration, but this seems to be a poor surrogate for MPOD. It would be particularly interesting to examine the genetic influence of *SCARB1* polymorphisms in an elderly population and their respective influences on serum L concentration and associated AMD risk.

Acknowledgments. The authors thank Dr. S.H. Melissa Liew, who performed the TwinsUK phenotyping.

References

1. U.S. Centers for Disease Control and Prevention (CDC). Prevalence of visual impairment and selected eye diseases among persons aged ≥ 50 years with and without diabetes-United States, 2002. MMWR Morb Mortal Wkly Rep 2004; 53:1069–71.
2. Bunce C, Xing W, Wormald R. Causes of blind and partial sight certifications in England and Wales: April 2007-March 2008. Eye (Lond) 2010;24:1692–9.
3. Klein R, Klein B, Linton K. Prevalence of age-related maculopathy: the Beaver Dam Eye study. Ophthalmology 1992;99:933–43.
4. Bone RA, Landrum JT, Hime GW, et al. Stereochemistry of the human macular carotenoids. Invest Ophthalmol Vis Sci 1993;34:2033–40.
5. Thurnham DI, Trémel A, Howard AN. A supplementation study in human subjects with a combination of mesozeaxanthin, (3R,3′R)-zeaxanthin and (3R,3′R,6′R)-lutein. Br J Nutr 2008;100:1307–14.
6. Parker RS. Absorption, metabolism, and transport of carotenoids. FASEB J 1996;10:542–51.

7. Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am J Clin Nutr 1995;62(suppl):1448S–61S.
8. Beatty S, Murray IJ, Henson DB, et al. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. Invest Ophthalmol Vis Sci 2001;42:439–46.
9. Liew SH, Gilbert CE, Spector TD, et al. Heritability of macular pigment: a twin study. Invest Ophthalmol Vis Sci 2005; 46:4430–6.
10. Hammond CJ, Liew SM, Van Kuijk FJ, et al. The heritability of macular response to supplemental lutein and zeaxanthin: a classical twin study. Invest Ophthalmol Vis Sci 2012;53: 4963–8.
11. Zerbib J, Seddon JM, Richard F, et al. rs5888 variant of SCARB1 gene is a possible susceptibility factor for age-related macular degeneration. PLoS One [serial online] 2009; 4:e7341. Available at: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0007341. Accessed December 12, 2012.
12. Weeks DE, Conley YP, Mah TS, et al. A full genome scan for age-related maculopathy. Hum Mol Genet 2000;22:1329–49.
13. Schick JH, Iyengar SK, Klein BE, et al. A whole-genome screen of a quantitative trait of age-related maculopathy in sibships from the Beaver Dam Eye Study. Am J Hum Genet 2003;72:1412–24.
14. Ji Y, Jian B, Wang N, et al. Scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux. J Biol Chem 1997;272:20982–5.
15. Acton S, Rigotti A, Landschulz KT, et al. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science 1996;271:518–20.
16. Neale BM, Fagerness J, Reynolds R, et al. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc Natl Acad Sci U S A 2010;107:7395–400.
17. Chen W, Stambolian D, Edwards AO, et al, Complications of Age-Related Macular Degeneration Prevention Trial (CAPT) Research Group. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. Proc Natl Acad Sci U S A 2010;107:7401–6.
18. Rodríguez-Esparragón F, Rodríguez-Pérez JC, Hernández-Trujillo Y, et al. Allelic variants of the human scavenger receptor class B type 1 and paraoxonase 1 on coronary heart disease: genotype-phenotype correlations. Arterioscler Thromb Vasc Biol 2005;25:854–60.
19. Acton S, Osgood D, Donoghue M, et al. Association of polymorphisms at the SR-BI gene locus with plasma lipid levels and body mass index in a white population. Arterioscler Thromb Vasc Biol 1999;19:1734–43.
20. Morabia A, Ross BM, Costanza MC, et al. Population-based study of SR-BI genetic variation and lipid profile. Atherosclerosis 2004;175:159–68.
21. McCarthy JJ, Somji A, Weiss LA, et al. Polymorphisms of the scavenger receptor class B member 1 are associated with insulin resistance with evidence of gene by sex interaction. J Clin Endocrinol Metab 2009;94:1789–96.
22. Chiba-Falek O, Nichols M, Suchindran S, et al. Impact of gene variants on sex-specific regulation of human Scavenger receptor class B type 1 (SR-BI) expression in liver and association with lipid levels in a population-based study. BMC Med Genet [serial online] 2010;11:9. Available at: http://www.biomedcentral.com/1471–2350/11/9. Accessed December 12, 2012.
23. Manichaikul A, Naj AC, Herrington D, et al. Association of SCARB1 variants with subclinical atherosclerosis and incident cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. Arterioscler Thromb Vasc Biol 2012;32: 1991–9.
24. Snow KK, Seddon JM. Do age-related macular degeneration and cardiovascular disease share common antecedents? Ophthalmic Epidemiol 1999;6:125–43.
25. Klein R, Deng Y, Klein BE, et al. Cardiovascular disease, its risk factors and treatment, and age-related macular degeneration: Women's Health Initiative Sight Exam ancillary study. Am J Ophthalmol 2007;143:473–83.
26. Duncan KG, Bailey KR, Kane JP, Schwartz DM. Human retinal pigment epithelial cells express scavenger receptors BI and BII. Biochem Biophys Res Commun 2002;292:1017–22.
27. During A, Doraiswamy S, Harrison EH. Xanthophylls are preferentially taken up compared with beta-carotene by retinal cells via a SRBI-dependent mechanism. J Lipid Res 2008;49:1715–24.
28. Reboul E, Abou L, Mikail C, et al. Lutein transport by Caco-2 TC-7 cells occurs partly by a facilitated process involving the scavenger receptor class B type I (SR-BI). Biochem J 2005; 387:455–61.
29. Nolan JM, Stack J, O'Donovan O, et al. Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. Exp Eye Res 2007;84:61–74.
30. Loane E, McKay GJ, Nolan JM, Beatty S. Apolipoprotein E genotype is associated with macular pigment optical density. Invest Ophthalmol Vis Sci 2010;51:2636–43.
31. Bone RA, Landrum JT, Cains A. Optical density spectra of the macular pigment in vivo and in vitro. Vision Res 1992;32:105–10.
32. Wooten BR, Hammond BR Jr, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. Invest Ophthalmol Vis Sci 1999;40:2481–9.
33. Nolan JM, Stringham JM, Beatty S, Snodderly DM. Spatial profile of macular pigment and its relationship to foveal architecture. Invest Ophthalmol Vis Sci 2008;49:2134–42.
34. Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. Control Clin Trials 1998;19:61–109.
35. Hysi PG, Young TL, Mackey DA, et al. A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25. Nat Genet 2010;42:902–5.
36. Mares JA, LaRowe TL, Snodderly DM, et al, CAREDS Macular Pigment Study Group and Investigators. Predictors of optical density of lutein and zeaxanthin in retinas of older women in the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. Am J Clin Nutr 2006;84:1107–22.
37. Yeum KJ, Booth SL, Sadowski JA, et al. Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. Am J Clin Nutr 1996;64:594–602.
38. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
39. Seddon JM, Ajani UA, Sperduto RD, et al, Eye Disease Case-Control Study Group. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. JAMA 1994;272:1413–20.
40. Loane E, Kelliher C, Beatty S, Nolan JM. The rationale and evidence base for a protective role of macular pigment in age-related maculopathy. Br J Ophthalmol 2008;92:1163–8.
41. Wang W, Connor SL, Johnson EJ, et al. Effect of dietary lutein and zeaxanthin on plasma carotenoids and their transport in lipoproteins in age-related macular degeneration. Am J Clin Nutr 2007;85:762–9.
42. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated

with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J 2000;14:835–46.
43. Curcio CA, Johnson M, Rudolf M, Huang JD. The oil spill in ageing Bruch membrane. Br J Ophthalmol 2011;95: 1638–45.
44. Tabas I, Williams KJ, Borén J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation 2007;116: 1832–44.
45. Klein R, Cruickshanks KJ, Nash SD, et al. The prevalence of age-related macular degeneration and associated risk factors. Arch Ophthalmol 2010;128:750–8.
46. Willer CJ, Sanna S, Jackson AU, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 2008;40:161–9.
47. Kathiresan S, Willer CJ, Peloso GM, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 2009;41:56–65.

Footnotes and Financial Disclosures

Originally received: October 8, 2012.
Final revision: January 7, 2013.
Accepted: January 7, 2013.
Available online: ●●●. Manuscript no. 2012-1525.

[1] Centre for Public Health, Royal Victoria Hospital, Queen's University Belfast, Belfast, Northern Ireland.

[2] Ophthalmology Department, Royal Victoria Eye and Ear Hospital, Dublin, Ireland.

[3] Macular Pigment Research Group, Department of Chemical and Life Sciences, Waterford Institute of Technology, Waterford, Ireland.

[4] Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, Wisconsin.

[5] Department of Twin Research and Genetic Epidemiology, King's College London, London, UK.

[6] Centre for Vision and Vascular Science, Royal Victoria Hospital, Queen's University Belfast, Belfast, Northern Ireland.

Financial Disclosure(s):
The author(s) have made the following disclosure(s): John M. Nolan and Stephen Beatty do consultancy work for nutraceutical companies, in a personal capacity, and as directors of Nutrasight Consultancy Limited.

Supported in part by Bausch and Lomb, Ireland, and EU Strand 1 research funding. The TwinsUK authors received funding from the Wellcome Trust with genotyping supported in part by US National Institutes of Health (NIH)/National Eye Institute (NEI) grant 1RO1EY018246 and performed by the NIH Center for Inherited Disease Research. The TwinsUK study also received support from the National Institute for Health Research comprehensive Biomedical Research Centre award to Guy's and St. Thomas' National Health Service Foundation Trust partnering with King's College London. The CAREDS authors received funding from the NIH, NEI [grants EY013018, EY016886], Research to Prevent Blindness, and the Women's Health Initiative (WHI), of which CAREDS is ancillary to, and is supported by contracts from the National Heart, Lung and Blood Institute, NIH.

Correspondence:
Gareth McKay, Centre for Public Health, The Queen's University of Belfast, Institute of Clinical Science, Royal Victoria Hospital, Grosvenor Road, Belfast BT12 6BJ, Northern Ireland. E-mail: g.j.mckay@qub.ac.uk.