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Plasma carotenoid- and retinol-weighted multi-SNP scores and risk of breast cancer in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium

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Abstract

Background—Dietary and circulating carotenoids have been inversely associated with breast cancer risk, but observed associations may be due to confounding. Single nucleotide polymorphisms (SNPs) in β-*carotene 15,15′-monooxygenase 1* (*BCMO1*), a gene encoding the enzyme involved in the first step of synthesizing vitamin A from dietary carotenoids, have been associated with circulating carotenoid concentrations and may serve as unconfounded surrogates for those biomarkers. We determined associations between variants in *BCMO1* and breast cancer risk in a large cohort consortium.

Methods—We used unconditional logistic regression to test four SNPs in *BCMO1* for associations with breast cancer risk in 9,226 cases and 10,420 controls from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3). We also tested weighted multi-SNP scores composed of the two SNPs with strong, confirmed associations with circulating carotenoid concentrations.

Results—Neither the individual SNPs nor the weighted multi-SNP scores were associated with breast cancer risk (odds ratio (95% confidence interval) comparing extreme quintiles of weighted multi-SNP scores =1.04 (0.94–1.16) for β-carotene, 1.08 (0.98–1.20) for α-carotene, 1.04 (0.94–1.16) for β-cryptoxanthin, 0.95 (0.87–1.05) for lutein/zeaxanthin, and 0.92 (0.83–1.02) for retinol). Furthermore, no associations were observed when stratifying by estrogen receptor status, but power was limited.

Conclusions—Our results do not support an association between SNPs associated with circulating carotenoid concentrations and breast cancer risk.

Impact—Future studies will need additional genetic surrogates and/or sample sizes at least three times larger to contribute evidence of a causal link between carotenoids and breast cancer.

Keywords

breast cancer; BCMO1; β-carotene 15,15′-monooxygenase 1; carotenoids; single nucleotide polymorphism

Introduction

Inverse associations with breast cancer for dietary (1) and circulating (2) carotenoids have been observed in two pooled analyses of prospective studies. In particular, higher dietary intakes of α-carotene, β-carotene, and lutein/zeaxanthin have been associated with lower risk of estrogen receptor-negative (ER−) breast cancer, and dietary β-cryptoxanthin has been inversely associated with overall breast cancer risk (1). Several circulating carotenoids have been inversely associated with breast cancer risk with stronger associations observed for α- and β-carotene and ER− breast cancer (2). Together, these analyses suggest carotenoids may reduce breast cancer risk, and α- and β-carotene may be particularly protective against ER− breast cancer.

The major carotenoids in the U.S. are α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin (3,4,5). α-carotene, β-carotene, and β-cryptoxanthin are known as provitamin A carotenoids since they can be converted into retinal, retinol, and other forms of

vitamin A. The first step of this conversion is central cleavage by β-carotene 15,15′-monooxygenase 1 (BCMO1) (6,7,8,9). The resultant retinal, an active form of Vvtamin A, can be reduced to retinol and converted into retinyl esters, such as retinyl palmitate, for storage in the liver. For more detailed description of the carotenoid pathway, the reader is referred to Figure 3 in Ferrucci et al, (10). It is difficult to disentangle potential causal mechanisms behind the observed associations between carotenoids and breast cancer. As the provitamin A carotenoids, including α- and β-carotene, can be converted to vitamin A, protective associations may be due to vitamin A or possibly other metabolic products. Indeed, dietary vitamin A has been weakly inversely associated with breast cancer (11). However, given that more vitamin A activity comes from preformed vitamin A than from provitamin A carotenoids in the western diet, the effect of provitamin A carotenoids on breast cancer risk through their vitamin A activity is probably low.

Because fruits and vegetables are the primary source of carotenoids, both dietary and circulating carotenoids are associated with other phytochemicals and nutrients provided by these same foods. Fruit and vegetable intake is also inversely associated with lifestyle factors such as physical inactivity, smoking, and alcohol consumption (12). These associations thus preclude causal attribution of the reduction in breast cancer risk to carotenoids in the pooled studies of dietary and circulating carotenoids described above. Mendelian Randomization offers one avenue for attempting to circumvent potential confounding (13) and could provide evidence that specific carotenoids are responsible for lower risk of breast cancer. The distribution of genetic polymorphisms is unlikely to be associated with behaviors (13), such as diet and other lifestyle factors that could confound association with dietary and circulating carotenoids. When genetic variants alter the activity of an enzyme involved in nutrient metabolism, those variants can theoretically be used as proxies for different exposure levels of that nutrient (14). Observed associations between the genetic variants and disease risk can thus provide additional evidence of a causal association between a given nutrient and disease risk.

Single nucleotide polymorphisms (SNPs) in *BCMO1* have been associated with circulating carotenoid levels and β-carotene conversion efficiency (10,15,16). The rs12934922 *T* allele has been associated with both reduced conversion of β-carotene to retinyl palmitate as well as higher fasting plasma β-carotene (15). The rs6564851 *G* allele was associated with increased circulating levels of α-carotene and β-carotene and decreased levels of lycopene, lutein, and zeaxanthin in a previous genome-wide association study (GWAS) (10). This allele has also been reported to reduce BCMO1 activity (16). In the Nurses' Health Study (NHS), both alleles were significantly associated with higher plasma provitamin A carotenoid concentrations, and the *T* allele for each SNP was associated with higher plasma lutein/zeaxanthin concentrations (17). It is possible that SNPs in *BCMO1* can reduce conversion efficiency to retinol, leading to higher provitamin A carotenoid exposure and theoretically lower retinol exposure. The non-provitamin A carotenoids are not known substrates for BCMO1 (8,9), and Hendrickson and colleagues did not observe associations between *BCMO1* SNPs and plasma lycopene concentrations (17). However, they did observe an association between *BCMO1* SNPs and plasma lutein/zeaxanthin concentrations and hypothesized that the observed association was due to either carotenoid interactions, altered beta, beta-carotete-9',10'-oxygenase (BCDO2) expression or as yet unknown direct activity of BCMO1 on lutein zeaxanthin.

Here, we assessed the association between SNPs in or near *BCMO1* and breast cancer risk in the National Cancer Institute's Breast and Prostate Cancer Cohort Consortium (BPC3). Based on our previous findings that SNPs in or near *BCMO1* predict plasma carotenoid concentrations, we generated weighted multi-SNP scores. Our hypothesis was that the plasma carotenoid-weighted multi-SNP scores, which are positively associated with plasma

carotenoid concentrations, are inversely associated with breast cancer risk. We also tested for possible interactions with menopausal status, smoking status, pack-years of smoking, alcohol intake, and body mass index (BMI).

Materials and Methods

Study Population

Seven prospective cohorts from BPC3, which has been described elsewhere (18), were included in this analysis. The cohorts in this analysis were the Cancer Prevention Study II (CPSII) Nutrition Cohort; European Prospective Investigation into Cancer (EPIC); Multiethnic Cohort (MEC); Nurses' Health Study (NHS); Nurses' Health Study II (NHSII); Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO); and Women's Health Study (WHS). Breast cancer diagnoses were self-reported and confirmed by medical records or tumor registries and/or direct linkage with population-based tumor registries, and controls were selected based on cohort-specific criteria. Informed consent was obtained from all subjects or, in NHS and NHSII, implied by receipt of their blood samples. The project was approved by the Institutional Review Boards for each cohort.

Genotypes for rs6564851, rs12934922, rs7501331, and rs11641417 were determined by Taqman assays with reagents by Applied Bioscience (Foster City, CA). Taqman genotyping failed for rs6564851 in NHS, but data were available for a subset of 2,204 NHS women from Illumina 500K genotyping; in PLCO, rs12925563 was used as a proxy ($r^2 = 0.94$ (19)). Data for rs11641417 was not available for WHS.

In total, 12,642 breast cancer cases and 14,659 controls were included in BPC3. To reduce concerns over population stratification, we excluded 3,539 women of non-European ancestry or who were missing ethnicity. We also excluded 4,116 women missing genotypes, leaving 9,226 cases and 10,420 controls. Genotypes for both rs6564851 and rs12934922 were available for 8,188 cases and 8,660 controls. There was a total of 5,885 ER+; 1,171 ER −; 4,443 PR+; 1,825 PR−; and 931 ER−/PR− breast cancers.

Weighted multi-SNP scores

To test the hypothesis that carotenoids are causally associated with breast cancer risk, we generated multi-SNP scores for carotenoids and retinol based on our previous observations that *BMCO1* SNPs are associated with circulating levels. Based on rs6564851 and rs12934922 genotypes, we created five separate weighted multi-SNP scores that were associated with plasma concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and retinol. We only included rs6564851 and rs12934922 in the multi-SNP score as they have been shown to predict the carotenoids considered here (10,15,16,17). In the weighted multi-SNP scores, the effect allele was defined as the allele associated with higher biomarker concentrations, and the weighted multi-SNP scores were calculated from the number of effect alleles weighted by each SNP's mutually-adjusted association with the relevant plasma biomarker in the NHS. Weights for this analysis were derived among 2,579 NHS participants who were included in a previous analysis of the association between *BCMO1* SNPs and plasma carotenoid and retinol concentrations (17) and for whom rs6564851 and rs12934922 were genotyped. Specifically, we used β-coefficients for each SNP when included simultaneously as additive independent variables (number of effect alleles) in multivariate linear regression models with the natural-log transformed biomarker concentration (μg/L) as the dependent variable. Models for each biomarker included age, case-control status, BMI, cholesterol, menopausal status and postmenopausal hormone use, smoking status, alcohol intake, energy-adjusted fat intake, energy-adjusted intake of the nutrient of interest, and, for the provitamin A carotenoids, energy-adjusted retinol intake

(IU/day) as covariates. See reference (17) for further detail. Separate models were run for α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and retinol. We calculated caroteniod-specific weights as follows: α-carotene = (0.06765*rs6564851 *G*) + (0.07195*rs12934922 *T*), β-carotene = (0.1744*rs6564851 *G*) + (0.1167*rs12934922 *T*), β-cryptoxanthin = (0.04268*rs6564851 *G*) + (0.03093*rs12934922 *T*), lutein/zeaxanthin = (0.1294*rs6564851 *T*) + (0.02225*rs12934922 *T*), and retinol = (0.008192*rs6564851 *T*) + (0.01206*rs12934922 *A*). Because neither SNP was significantly associated with plasma lycopene concentrations, a weighted multi-SNP score was not derived for this biomarker. The weighted multi-SNP scores were divided into quintiles based on their distribution among BPC3 controls. Geometric mean plasma biomarker concentrations by weighted multi-SNP score quintiles for NHS subjects are shown in Supplemental Table S1.

Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) were determined by unconditional logistic regression adjusted for study and 5-year age category at baseline or blood draw, depending on cohort. Multi-SNP score quintile indicator variables were included in the models with the lowest quintile as the reference category. Continuous weighted multi-SNP scores were used in tests for trend and interaction. We tested the individual SNPs by including indicator variables for heterozygous and homozygous variant genotypes in the models. For trend and interaction tests, SNPs were additively modeled. We also conducted sub-analyses for ER+, ER−, and ER−/progesterone receptor-negative (PR−) breast cancer risk, data for which were available in 67–76% of the cases. All reported *P*-values are 2-sided and not adjusted for number of tests performed. *P*-values < 0.05 were considered statistically significant. All statistical analyses were performed using SAS version 9 (SAS Institute, Cary, NC).

P-values for interactions were determined by likelihood ratio tests. Interaction cross-product terms were calculated as the weighted multi-SNP score or additive SNP genotype multiplied by indicators for menopausal status (postmenopausal/premenopausal) or smoking status (never/former or current) or by continuous measures of pack-years of smoking, BMI (kg/m^2), or alcohol intake (grams/day). Models stratified by BMI (<25, ≥25–<30, and ≥30 kg/m^2) were adjusted for continuous BMI. Models stratified by alcohol intake (<1, ≥1 drink/day) were adjusted for continuous alcohol intake. Models stratified by pack-years of smoking (0, <14.5 (the median among smokers), and ≥14.5 pack-years) were adjusted for continuous pack-years. Women with implausible BMI (<16 or >55 kg/m^2) (n = 27) or alcohol intake (>100 g/day) (n = 17) were excluded from the respective interaction analyses. Interactions were not tested for ER−/PR− breast cancer due to concerns of limited power.

Expected ORs of breast cancer across extreme α-carotene, β-carotene, β-cryptoxanthin, and lutein/zeaxanthin-weighted multi-SNP score quintiles were determined from the association between each circulating carotenoid and breast cancer risk in the recent pooled analysis (2) and, using methods described in (17), the association between each weighted multi-SNP score and plasma concentrations of the relevant carotenoid in the NHS. Expected ORs for retinol were not determined because the association between circulating retinol and risk of breast cancer was not assessed in the pooled analysis (2). To account for attenuation of the circulating carotenoids and breast cancer OR that may occur from only having one plasma measurement per participant, the intraclass correlation (ICC) for each carotenoid was determined among 839 NHS participants, 804 of whom had the individual plasma carotenoids assayed in two blood samples collected approximately ten years apart. These ICCs were: 0.53 for α-carotene, 0.50 for β-carotene, 0.60 for β-cryptoxanthin, and 0.61 for lutein/zeaxanthin.

Expected ORs comparing extreme weighted multi-SNP score quintiles were determined by exponentiating the following formula: $(\Delta * \hat{\beta} * \hat{\gamma}) / ICC$, where $\hat{\beta}$ = the β-coefficient for each continuous weighted multi-SNP score in relation to the relevant plasma carotenoid in units of natural log-transformed μg/L, $\hat{\gamma}$ = the logistic regression coefficient for each continuous plasma carotenoid as quintile medians among controls in units of natural log-transformed μg/L in relation to breast cancer risk determined from a re-calculation of (2), and Δ = the difference in the median value among BPC3 controls of the relevant weighted multi-SNP score across extreme weighted multi-SNP score quintiles. *P*-values for the test of the hypothesis that the expected and observed ORs are equal were determined from the z-score for the difference between the natural log of the observed OR and the natural log of the expected OR where the standard error of the difference = $\sqrt{\{var[ln(OR_{observed})] + [var(\beta) * \gamma^2] + [var(\gamma) * \beta^2]\}}$. This calculation assumes the estimates of the observed and expected ORs are independent. While MEC, NHS, and WHS contributed to both estimates, several cohorts contributed to only one estimate (2). We thus assumed adequate independence.

Results

Each SNP was in Hardy-Weinberg equilibrium among controls by cohort (all $P > 0.05$). Supplemental Table S2 includes characteristics of the participants by cohort and case-control status.

Individual SNP Results

No SNP was associated with breast cancer overall or with specific subtypes defined by ER status (Supplemental Table S3). When excluding 2,825 cases with *in situ* or unknown stage, rs7501331 was significantly inversely associated with ER− and ER−/PR− breast cancer risk (*P*-trend = 0.02 and 0.04, respectively). We observed a significant interaction between rs7501331 and pack-years of smoking in relation to ER+ breast cancer risk (*P*-interaction = 0.02). There were no significant interactions between individual SNPs and alcohol intake, menopausal status, BMI, or smoking status in relation to total, ER+, or ER− breast cancer risk.

Multi-SNP scores

The multi-SNP scores were associated with plasma concentrations of α- and β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and retinol (Supplemental Table S1). The provitamin A carotenoid-weighted multi-SNP scores were positively correlated with each other and inversely correlated with the lutein/zeaxanthin- and retinol-weighted multi-SNP scores (Supplemental Table S4). The distribution of breast cancer risk factors did not differ by the β-carotene-weighted multi-SNP score (Table 1) or the other carotenoid-weighted multi-SNP scores (data not shown). Median weighted multi-SNP score values by quintile are included in Table 2. No weighted multi-SNP score was associated with risk of overall, ER+, ER−, or ER−/PR− (data not shown) breast cancer. The expected ORs comparing top and bottom quintiles of the weighted multi-SNP scores (data not shown) were not significantly different from the observed ORs presented in Table 2. When excluding 2,825 cases with *in situ* or unknown stage, we observed significant positive and inverse associations comparing extreme quintiles of the α-carotene and retinol-weighted multi-SNP scores, respectively, in relation to ER−/PR− breast cancer, but the trends were non-significant. We conducted a sensitivity analysis by rerunning the Table 2 analyses after removing all NHS women; our results did not change (data not shown). As there was an overlap in NHS subjects used to generate the weights for the multi-SNP score and the breast cancer association analysis, we

reran the association analysis for breast cancer excluding the NHS subjects. The results did not change (data not shown).

There were significant or borderline-significant interactions between alcohol intake and the provitamin A carotenoid- and retinol-weighted multi-SNP scores in relation to ER+ breast cancer risk (Table 3). We observed no significant interactions between alcohol intake and the weighted multi-SNP scores in relation to overall or ER− breast cancer risk (Supplemental Table 5). Significant interactions also were observed between the provitamin A carotenoid- and retinol-weighted multi-SNP scores and pack-years of smoking in relation to overall breast cancer risk (Table 4). Results were similar for ER+ and ER− breast cancer risk, although not all results were significant (Supplemental Table 6).

When we simultaneously included cross-product terms for each provitamin A carotenoid- or retinol-weighted multi-SNP score and both alcohol intake and pack-years of smoking in the models for total breast cancer, only the interaction terms with pack-years of smoking were significant ($P = 0.03$ for all provitamin A carotenoid-weighted multi-SNP scores and 0.04 for the retinol-weighted multi-SNP score). Likelihood ratio tests comparing models with and without the two interaction terms were also significant or borderline-significant ($P = 0.03$ for the provitamin A carotenoid-weighted multi-SNP scores and 0.05 for the retinol-weighted multi-SNP score). When limited to ER+ breast cancer cases, neither interaction term was significant, but the likelihood ratio tests comparing models with and without the two interaction terms were significant ($P = 0.03$ for the α-carotene-weighted multi-SNP score and 0.04 for the β-carotene-, β-cryptoxanthin-, and retinol-weighted multi-SNP scores).

There were no significant interactions between the weighted multi-SNP scores and menopausal status, BMI, or smoking status in relation to total, ER+, or ER− breast cancer risk.

Discussion

In this pooled analysis of 9,226 cases and 10,420 controls using a Mendelian Randomization approach to indirectly assess the hypothesis that higher carotenoid exposure is causally associated with lower risk of breast cancer, we did not observe significant associations between *BCMO1* individual SNPs or weighted multi-SNP scores and breast cancer risk. Because the associations were non-significant, but expected ORs for each weighted multi-SNP score and breast cancer risk were not significantly different from the observed ORs, our null results neither support nor refute a causal association between carotenoids and breast cancer risk.

Our analysis was limited by the small number of genetic predictors of plasma carotenoid and retinol concentrations. The weighted multi-SNP scores were composed of two SNPs reported to be associated with β-carotene conversion efficiency and circulating β-carotene concentrations (10,15,16). In a previous GWAS in the InCHIANTI study, one of these SNPs was also associated with circulating lutein to a similar magnitude and less strongly associated with circulating α-carotene, lycopene, and zeaxanthin (10). Associations with α-carotene, β-carotene, and lutein/zeaxanthin were confirmed in the NHS (17). However, to be of use, Mendelian Randomization requires a fairly strong association between the genetic variant and the exposure of causal interest (14). The weighted multi-SNP scores explained 5.7% and 6.5% of the variation in plasma β-carotene and lutein/zeaxanthin, respectively, in the NHS (Supplemental Table S1). For α-carotene, β-cryptoxanthin, and retinol, the weighted multi-SNP scores only explained 0.2%–1.4% of variation in the respective plasma biomarker concentrations in the NHS. Given the homeostasis of blood retinol concentrations in the absence of severely low liver stores (20) and the fact that liver vitamin A

concentrations are lower in β-carotene-fed *BCMO1* knockout mice (21,22), associations between SNPs in *BCMO1* and retinol may be stronger in other tissues. Pleiotropy also may have limited the weighted multi-SNP scores as they were correlated with each other. Thus, while the weighted multi-SNP scores likely overcame confounding by non-carotenoid constituents of fruits and vegetables and lifestyle, they may have been confounded by each other. We therefore cannot necessarily attribute observed associations to a specific carotenoid or retinol, and associations may have been nullified if two biomarkers oppositely associated with a given weighted multi-SNP score were similarly associated with breast cancer risk.

Mendelian Randomization relies on three key assumptions. First, the genotype is associated with the intermediate phenotype (here, carotenoid levels). Second, there is no unmeasured common cause of genotype and the outcome (here, breast cancer). Third, every directed pathway from genotype to the outcome passes through the intermediate phenotype (23). The utility of the Mendelian Randomization approach also depends on the strength of the association between the exposure of causal interest and disease risk (14). The strongest association observed in the recent pooled analysis was between circulating β-carotene and risk of ER− breast cancer (OR (95% CI) comparing extreme quintiles = 0.51 (0.35–0.75)) (2). Given this association and the relatively strong association between the β-carotene-weighted multi-SNP score and plasma β-carotene in the NHS, our strongest expected OR was 0.83 for ER− breast cancer comparing extreme β-carotene-weighted multi-SNP score quintiles. Based on calculations provided by Schlesselman (24), 355 ER− cases in the extreme β-carotene-weighted multi-SNP score quintiles, and a 8.6:1 control:case ratio, we had 37% power to detect an OR of 0.83, assuming a causal relation exists between circulating β-carotene and ER− breast cancer risk. To obtain 80% power, we would have needed 1,039 ER− cases and 8,909 controls in the extreme β-carotene-weighted multi-SNP score quintiles. The expected OR for overall breast cancer was 0.94 comparing extreme β-carotene-weighted multi-SNP score quintiles. With 2,811 cases in the extreme β-carotene-weighted multi-SNP score quintiles, and a 1.1:1 control:case ratio, we had 22% power to detect an OR of 0.94, assuming a causal relation exists between circulating β-carotene and breast cancer risk. To obtain 80% power, we would have needed 16,022 cases and 17,350 controls in the extreme β-carotene-weighted multi-SNP score quintiles. Future studies will thus require large sample sizes and/or utilize a genetic surrogate more strongly associated with circulating carotenoids.

We observed significant interactions between specific weighted multi-SNP scores and both alcohol intake and pack-years of smoking, but pleiotropy, common co-occurrence of alcohol intake and smoking, and multiple comparisons render interpretations speculative. Retinoic acid inhibits proliferation in ER+ breast cancer cell lines (25), and chronic alcohol intake decreases hepatic retinoids and increases hepatic expression of *BCMO1* in rats (26). Thus, associations between the α-carotene and retinol-weighted multi-SNP scores (which were inversely correlated with each other) and ER+ breast cancer risk in women consuming ≥ 1 drink/day may be due to higher retinol requirements in these women instead of an adverse effect of α-carotene. We hypothesized carotenoids would protect more strongly against breast cancer risk in smokers due to their increased exposure to oxidative stress. As we conducted many tests, observed interactions may be chance findings and thus require confirmation.

In summary, this study does not provide evidence that genetic variants predicting circulating carotenoids are associated with breast cancer. Because a large-scale randomized trial of β-carotene for reduction of breast cancer risk is unlikely to occur given the outcome of previous β-carotene supplementation trials in relation to lung cancer (27,28), genetic studies may be the best option by which to further study causality in the associations between

carotenoids and breast cancer. Future studies should therefore attempt to identify additional genetic surrogates for carotenoid exposure (29).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of study population at baseline or blood draw by β-carotene-weighted multi-SNP score quintile

Characteristic[a,b]	**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**
N	3218	3626	4152	3215	2637
Age, years, mean (SD)	55.9 (8.8)	56.1 (8.8)	56.2 (8.7)	56.3 (8.6)	56.4 (8.7)
BMI, kg/m^2, mean (SD)	25.8 (4.8)	25.7 (4.9)	25.7 (4.8)	25.5 (4.6)	25.7 (4.8)
Alcohol ≥ 1 drink/day, %	16.6	17.0	16.3	16.3	17.8
Ever smoker, %	47.6	45.0	45.4	46.8	46.4
Pack-years in ever smokers, mean (SD)	18.9 (17.3)	18.2 (16.8)	18.4 (17.2)	19.1 (16.9)	18.6 (18.2)
Family history of breast cancer, %	16.2	14.3	15.8	17.0	15.5
Age at menarche ≤ 11 years, %	20.1	17.3	17.4	18.3	17.2
Nulliparous, %	12.7	11.8	12.9	13.2	11.8
Postmenopausal, %	76.2	76.7	77.5	78.8	79.2
Ever hormone therapy in postmenopausal, %	57.7	57.2	59.4	58.6	63.0
Age at menopause ≥ 50 years, %	52.8	52.5	50.0	51.7	51.9

[a] Mean (SD) or percent of non-missing data

[b] Percent missing = 1.2% BMI, 3.0% alcohol, 1.0% ever smoker, 4.6% pack-years in ever smokers, 28.1% family history, 2.2% age at menarche, 2.5% parity, 10.2% menopausal status (missing or perimenopausal), 1.8% ever HT in postmenopausal, 8.8% age at menopause

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2014 May 01.

Table 2

Odds ratios of breast cancer and 95% confidence intervals by weighted multi-SNP score and estrogen receptor status

Weighted multi-SNP score[a]	Quintile[b]	All cases		ER+ cases		ER− cases	
		Cases/ controls	OR (95% CI)[c]	Cases[d]	OR (95% CI)	Cases	OR (95% CI)
β-carotene	1 (0.117)	1530/1688	1.00	972	1.00	203	1.00
	2 (0.174)	1739/1887	1.02 (0.92,1.12)	1116	1.02 (0.92,1.14)	217	0.97 (0.79,1.18)
	3 (0.291)	2037/2115	1.06 (0.97,1.17)	1333	1.08 (0.97,1.20)	259	1.03 (0.84,1.25)
	4 (0.408)	1601/1614	1.10 (1.00,1.21)	1038	1.11 (0.99,1.24)	204	1.06 (0.86,1.31)
	5 (0.466)	1281/1356	1.04 (0.94,1.16)	824	1.04 (0.93,1.17)	152	0.95 (0.76,1.19)
	P-trend[e]		0.12		0.12		0.85
α-carotene	1 (0.068)	1698/1864	1.00	1075	1.00	221	1.00
	2 (0.072)	1787/1879	1.05 (0.95,1.15)	1141	1.05 (0.95,1.17)	226	1.00 (0.82,1.22)
	3 (0.140)	2037/2115	1.06 (0.96,1.16)	1333	1.09 (0.98,1.20)	259	1.02 (0.84,1.24)
	4 (0.207)	1491/1606	1.01 (0.92,1.12)	967	1.04 (0.93,1.16)	171	0.89 (0.72,1.10)
	5 (0.212)	1175/1196	1.08 (0.98,1.20)	767	1.12 (0.99,1.26)	158	1.11 (0.90,1.39)
	P-trend		0.14		0.10		0.80
β-cryptoxanthin	1 (0.031)	1530/1688	1.00	972	1.00	203	1.00
	2 (0.043)	1739/1887	1.02 (0.92,1.12)	1116	1.02 (0.92,1.14)	217	0.97 (0.79,1.18)
	3 (0.074)	2037/2115	1.06 (0.97,1.17)	1333	1.08 (0.97,1.20)	259	1.03 (0.84,1.25)
	4 (0.105)	1601/1614	1.10 (1.00,1.21)	1038	1.11 (0.99,1.24)	204	1.06 (0.86,1.31)
	5 (0.116)	1281/1356	1.04 (0.94,1.16)	824	1.04 (0.93,1.17)	152	0.95 (0.76,1.19)
	P-trend		0.12		0.11		0.84
Lutein/zeaxanthin	1 (0.022)	1707/1774	1.00	1095	1.00	198	1.00
	2 (0.129)	1529/1637	0.98 (0.89,1.08)	973	0.98 (0.87,1.09)	198	1.09 (0.89,1.35)
	3 (0.152)	2037/2115	1.00 (0.91,1.10)	1333	1.03 (0.93,1.14)	259	1.09 (0.90,1.33)
	4 (0.174)	1344/1423	0.98 (0.89,1.09)	869	1.01 (0.90,1.13)	181	1.14 (0.92,1.41)
	5 (0.281)	1571/1711	0.95 (0.87,1.05)	1013	0.98 (0.87,1.09)	199	1.02 (0.83,1.26)
	P-trend		0.21		0.48		0.97
Retinol	1 (0.008)	1175/1196	1.00	767	1.00	158	1.00
	2 (0.012)	1491/1606	0.94 (0.84,1.04)	967	0.93 (0.82,1.05)	171	0.80 (0.63,1.00)
	3 (0.020)	2037/2115	0.97 (0.88,1.08)	1333	0.97 (0.87,1.09)	259	0.92 (0.74,1.14)

Weighted multi-SNP score[a]	Quintile[b]	All cases		ER+ cases		ER− cases	
		Cases/ controls	OR (95% CI)[c]	Cases[d]	OR (95% CI)	Cases	OR (95% CI)
	4 (0.028)	1787/1879	0.96 (0.87,1.07)	1141	0.94 (0.84,1.06)	226	0.90 (0.72,1.12)
	5 (0.032)	1698/1864	0.92 (0.83,1.02)	1075	0.89 (0.79,1.01)	221	0.90 (0.72,1.12)
	P-trend		0.18		0.10		0.78

[a] α-carotene = (0.06765*rs6564851 *G*)+(0.07195*rs12934922 *T*), β-carotene = (0.1744*rs6564851 *G*)+(0.1167*rs12934922 *T*), β-cryptoxanthin = (0.04268*rs6564851 *G*)+(0.03093*rs12934922 *T*), lutein/zeaxanthin = (0.1294*rs6564851 *T*)+(0.02225*rs12934922 *T*), and retinol = (0.008192*rs6564851 *T*)+(0.01206*rs12934922 *A*)

[b] Values are median weighted multi-SNP scores by quintile

[c] Adjusted for 5-year age category and study

[d] Control numbers are equal across case subtypes

[e] Trend for continuous weighted multi-SNP score adjusted for 5-year age category and study

Table 3

Odds ratios[a] of ER+ breast cancer and 95% confidence intervals by weighted multi-SNP score quintile and alcohol intake

Weighted multi-SNP score[b]	Cases/ controls	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P*-trend[c]	*P*-int.[d]
β-carotene[e]		0.117	0.174	0.291	0.408	0.466		
< 1 drink/day	4106/7146	1.00	1.02 (0.91,1.16)	1.08 (0.96,1.22)	1.08 (0.95,1.22)	1.01 (0.88,1.15)	0.52	0.05
≥ 1 drink/day	1018/1248	1.00	1.16 (0.89,1.51)	1.11 (0.86,1.44)	1.28 (0.97,1.68)	1.18 (0.89,1.57)	0.08	
α-carotene		0.068	0.072	0.140	0.207	0.212		
< 1 drink/day	4106/7146	1.00	1.04 (0.92,1.17)	1.07 (0.95,1.20)	0.99 (0.87,1.12)	1.05 (0.92,1.20)	0.58	0.03
≥ 1 drink/day	1018/1248	1.00	1.08 (0.83,1.40)	1.10 (0.86,1.41)	1.20 (0.92,1.56)	1.37 (1.03,1.83)	0.04	
β-cryptoxanthin		0.031	0.043	0.074	0.105	0.116		
< 1 drink/day	4106/7146	1.00	1.02 (0.91,1.16)	1.08 (0.96,1.22)	1.08 (0.95,1.22)	1.01 (0.88,1.15)	0.53	0.05
≥ 1 drink/day	1018/1248	1.00	1.16 (0.89,1.51)	1.11 (0.86,1.44)	1.28 (0.97,1.68)	1.18 (0.89,1.57)	0.07	
Lutein/zeaxanthin		0.022	0.129	0.152	0.174	0.281		
< 1 drink/day	4106/7146	1.00	0.99 (0.88,1.13)	1.04 (0.93,1.17)	0.99 (0.87,1.13)	0.98 (0.86,1.10)	0.54	0.45
≥ 1 drink/day	1018/1248	1.00	0.98 (0.75,1.29)	0.98 (0.76,1.26)	1.04 (0.79,1.37)	1.02 (0.79,1.33)	0.86	
Retinol		0.008	0.012	0.020	0.028	0.032		
< 1 drink/day	4106/7146	1.00	0.94 (0.82,1.08)	1.02 (0.90,1.16)	0.99 (0.87,1.13)	0.96 (0.84,1.09)	0.63	0.03
≥ 1 drink/day	1018/1248	1.00	0.87 (0.65,1.17)	0.80 (0.61,1.06)	0.78 (0.59,1.05)	0.73 (0.55,0.97)	0.03	

[a] Adjusted for 5-year age category, study, and continuous alcohol intake

[b] α-carotene = (0.06765*rs6564851 *G*)+(0.07195*rs12934922 *T*), β-carotene = (0.1744*rs6564851 *G*)+(0.1167*rs12934922 *T*), β-cryptoxanthin = (0.04268*rs6564851 *G*)+(0.03093*rs12934922 *T*), lutein/zeaxanthin = (0.1294*rs6564851 *T*)+(0.02225*rs12934922 *T*), and retinol = (0.008192*rs6564851 *T*)+(0.01206*rs12934922 *A*)

[c] Trend for continuous weighted multi-SNP score adjusted for 5-year age category, study, and continuous alcohol intake

[d] *P*-value for interaction from likelihood ratio tests comparing models with and without the continuous weighted multi-SNP score by continuous alcohol intake (g/day) cross-product term adjusted for age category, study, and continuous alcohol intake

[e] Values are median weighted multi-SNP scores by quintile

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2014 May 01.

Table 4

Odds ratios[a] of overall breast cancer and 95% confidence intervals by weighted multi-SNP score quintile and pack-years of smoking

Weighted multi-SNP score[b]	Cases/ controls	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P*-trend[c]	*P*-int.[d]
β-carotene[e]		0.117	0.174	0.291	0.408	0.466		
Never smoker	4201/4775	1.00	1.11 (0.97,1.26)	1.18 (1.04,1.34)	1.16 (1.01,1.33)	1.02 (0.88,1.18)	0.56	
Smoker, <14.5 pack-years	1737/1790	1.00	0.97 (0.79,1.20)	0.98 (0.80,1.20)	0.99 (0.80,1.23)	0.97 (0.78,1.22)	0.93	0.02
Smoker, ≥14.5 pack-years	1775/1626	1.00	0.91 (0.73,1.13)	0.95 (0.77,1.17)	1.14 (0.92,1.42)	1.14 (0.91,1.44)	0.04	
α-carotene		0.068	0.072	0.140	0.207	0.212		
Never smoker	4201/4775	1.00	0.96 (0.85,1.09)	1.07 (0.95,1.21)	0.93 (0.82,1.07)	1.02 (0.88,1.18)	0.70	
Smoker, <14.5 pack-years	1737/1790	1.00	1.07 (0.88,1.32)	1.03 (0.84,1.25)	1.04 (0.85,1.28)	1.00 (0.80,1.27)	0.95	0.01
Smoker, ≥14.5 pack-years	1775/1626	1.00	1.21 (0.98,1.49)	1.04 (0.85,1.27)	1.10 (0.88,1.37)	1.27 (1.00,1.61)	0.06	
β-cryptoxanthin		0.031	0.043	0.074	0.105	0.116		
Never smoker	4201/4775	1.00	1.11 (0.97,1.26)	1.18 (1.04,1.34)	1.16 (1.01,1.33)	1.02 (0.88,1.18)	0.58	
Smoker, <14.5 pack-years	1737/1790	1.00	0.97 (0.79,1.20)	0.98 (0.80,1.20)	0.99 (0.80,1.23)	0.97 (0.78,1.22)	0.93	0.02
Smoker, ≥14.5 pack-years	1775/1626	1.00	0.91 (0.73,1.13)	0.95 (0.77,1.17)	1.14 (0.92,1.42)	1.14 (0.91,1.44)	0.05	
Lutein/zeaxanthin		0.022	0.129	0.152	0.174	0.281		
Never smoker	4201/4775	1.00	1.03 (0.91,1.18)	1.10 (0.97,1.24)	1.03 (0.89,1.18)	0.94 (0.82,1.08)	0.36	
Smoker, <14.5 pack-years	1737/1790	1.00	0.95 (0.77,1.18)	0.98 (0.81,1.19)	0.96 (0.77,1.19)	1.02 (0.83,1.26)	0.89	0.22
Smoker, ≥14.5 pack-years	1775/1626	1.00	0.88 (0.71,1.09)	0.84 (0.69,1.03)	0.90 (0.72,1.13)	0.87 (0.71,1.08)	0.09	
Retinol		0.008	0.012	0.020	0.028	0.032		
Never smoker	4201/4775	1.00	0.92 (0.79,1.06)	1.05 (0.92,1.21)	0.94 (0.82,1.09)	0.98 (0.85,1.13)	0.81	
Smoker, <14.5 pack-years	1737/1790	1.00	1.04 (0.82,1.32)	1.03 (0.82,1.29)	1.07 (0.85,1.35)	1.00 (0.79,1.26)	0.97	0.02
Smoker, ≥14.5 pack-years	1775/1626	1.00	0.87 (0.68,1.11)	0.82 (0.65,1.03)	0.96 (0.76,1.20)	0.79 (0.62,1.00)	0.09	

[a] Adjusted for 5-year age category, study, and continuous smoking pack-years

[b] α-carotene = (0.06765*rs6564851 *G*)+(0.07195*rs12934922 *T*), β-carotene = (0.1744*rs6564851 *G*)+(0.1167*rs12934922 *T*), β-cryptoxanthin = (0.04268*rs6564851 *G*)+(0.03093*rs12934922 *T*), lutein/zeaxanthin = (0.1294*rs6564851 *T*)+(0.02225*rs12934922 *T*), and retinol = (0.008192*rs6564851 *T*)+(0.01206*rs12934922 *A*)

[c] Trend for continuous weighted multi-SNP score adjusted for 5-year age category, study, and continuous smoking pack-years

[d] *P*-value for interaction from likelihood ratio tests comparing models with and without the continuous weighted multi-SNP score by continuous smoking pack-years cross-product term adjusted for age category, study, and continuous smoking pack-years

[e] Values are median weighted multi-SNP scores by quintile