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Chromosome 15q24-25.1 variants, diet, and lung cancer susceptibility in cigarette smokers

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Abstract

Background—Studying gene-environment interactions may provide insight about mechanisms underpinning the reported association between chromosome 15q24-25.1 variation and lung cancer susceptibility.

Methods—In a nested case-control study comparing 746 lung cancer cases to 1477 controls, all of whom were non-Hispanic white smokers in the β-Carotene and Retinol Efficacy Trial, we examined whether lung cancer risk is associated with single nucleotide polymorphisms (SNPs) tagging the *AGPHD1*, *CHRNA5*, *CHRNA3*, and *CHRNB4* genes and whether such risk is modified by diet and other characteristics. Intake of fruits and vegetables, their botanical groups, and specific nutrients were ascertained generally at baseline by food-frequency questionnaire.

Results—Several sets of SNPs in high linkage disequilibrium were found, one set associated with a 27% to 34% increase and two sets associated with a 13% to 19% decrease in risk per minor allele. Associations were most prominent for the set including the non-synonymous SNP rs16969968. The rs16969968-lung cancer association did not differ by intake level of most dietary factors examined, but was stronger for individuals diagnosed at <70 years of age or having a baseline smoking history of <40 cigarette pack-years.

Conclusions—Our data suggests that diet has little influence on the relation between chromosome 15q24-25.1 variation and lung cancer risk.

Keywords

lung cancer; genetic polymorphism; nicotinic acetylcholine receptors; diet

Genome-wide association (GWA) studies have established chromosome 15q24-25.1 as a susceptibility locus for nicotine dependence and lung cancer (1–5). This region of strong linkage disequilibrium (LD) contains the *CHRNA5*, *CHRNA3*, and *CHRNB4* genes, which encode for the nicotinic acetylcholine receptor (nAChR) subunits α5, α3, and β4, respectively, and three other protein encoding genes *IREB2*, *AGPHD1* (previously known as

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LOC123688), and *PSMA4*. Of these six genes, *CHRNA5, CHRNA3,* and *CHRNB4* have been posited as the most plausible candidates underlying the reported association.

Found in the plasma membrane of all mammalian cells, nAChRs consist of either five identical or non-identical subunits that form ligand-gated ion channels, which regulate neurotransmission and signal transduction (6). High affinity nAChR ligands include nicotine and its carcinogenic derivatives, the nitrosamines N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (7). Long-term exposure to nicotine, NNN, and NNK appears to fuel carcinogenesis by causing an unfavorable imbalance in nAChR regulation of stimulatory over inhibitory neurotransmitters, which induces the synthesis and release of cancer-potentiating factors (6). In lung cancer cells, nAChR expression has been shown to affect regulation of both cell proliferation and apoptosis [reviewed in (6)].

The biological mechanisms by which chromosome 15q24-25.1 variation contribute to lung cancer development are not well established. At least some mechanisms appear to depend on smoking, since associations of 15q24-25.1 variants with lung cancer risk have not been generally detected in never smokers (3,8–11). In addition to smoking, other lifestyle factors, such as diet, may modify the degree to which 15q24-25.1 variants are associated with lung cancer risk. To our knowledge, no studies have addressed this hypothesis. In cohort studies, modest reductions in lung cancer risk have been associated with greater fruit and/or vegetable consumption (12), along with greater variety in fruit and vegetable consumption in current smokers (13). Data suggest that flavonoids found in plant-based foods, such as luteolin and quercetin, can reduce breast cancer cell proliferation by inhibiting nicotine-induced nAChR subunit expression (12). By extrapolation, a diet that is rich in plant-derived compounds possessing anti-carcinogenic properties may also inhibit lung cancer development, similarly through down-regulating nAChR expression. On the other hand, since tobacco-specific nitrosamines bind with strong affinity to nAChRs (7), a diet containing high levels of nitrosamines and possibly other food mutagens may exacerbate smoking-induced damage by up-regulating nAChR expression, further promoting lung cancer development.

In the present investigation, we employed a tag SNP approach to comprehensively examine the extent to which common variation in the *AGPHD1*, *CHRNA5*, *CHRNA3*, and *CHRNB4* genes is associated with lung cancer risk in cigarette smokers and to which such risk is modified by diet and other selected host characteristics. Dietary measures included intake level of fruits and vegetables, their botanical groups, and specific dietary compounds, such as antioxidant micronutrients and nitrosamines. Our study consisted of the same participants in the β-Carotene and Retinol Efficacy Trial (CARET) included in the replication phase of the lung cancer GWA study led by Hung et al. (2).

MATERIALS AND METHODS

Study Population and Setting

The present nested case-control study was comprised of CARET participants who had donated a whole blood specimen between February 1994 and February 1997. Participants diagnosed with primary lung cancer from the date of whole blood collection to September 2005, which marked the end of the follow-up period, were defined as cases. At time of selection, we identified 793 cases, although we have since omitted one who actually had a benign lung tumor. For every case, we selected two controls from those who were free of lung cancer and had completed at least one food-frequency questionnaire (FFQ) by matching on the basis of age (±4 years), sex, race, enrollment year (2-year intervals), baseline smoking status (current or former), history of occupational asbestos exposure, and

length of follow-up. In selecting 20 (of the 1586) controls for 12 cases, matching was retained on sex and race, but relaxed on one or two criteria in the following priority order (counts not mutually exclusive): age (n=10), enrollment year (n=9), smoking status (n=1), history of occupational exposure (n=2), and length of follow-up (n=2).

Details about the design, methods, and primary findings of CARET have been published (14–17). In brief, CARET was a multi-center randomized, double-blinded, placebo-controlled chemoprevention trial assessing the safety and efficacy of daily supplementation with β-carotene (30 mg) plus retinyl palmitate (25,000 IU) for primary prevention of lung cancer in 18,314 high-risk individuals. Eligible trial participants included men and women aged 50–69 years who were former (defined as having quit within six years prior to enrollment) or current smokers with a cigarette smoking history of ≥20 pack-years (n=14,254) and men aged 45–69 years who were former (defined as having quit within fifteen years prior to enrollment) or current smokers with a documented history of occupational asbestos exposure (n=4,060). Participants were enrolled from 1985 to 1994 and followed for lung cancer and other endpoints through regular clinic visits, telephone calls, and mailings. Institutional review boards of the Fred Hutchinson Cancer Research Center and the five other participating institutions approved all study protocols, and all participants provided written informed consent.

Assessment of Diet and Smoking History

As described previously (18), a self-administered FFQ was used to assess dietary intake over the past year at baseline and every two years thereafter. Specifically designed to ascertain intake of fruits, vegetables, and their nutrients, the FFQ included queries about (a) food and preparation techniques; (b) the frequency of consumption (using predefined categories from “never or less than once per month” to “2+ per day” for solid foods and from “never or less than once per month” to “6+ per day” for beverages) and corresponding portion size (small, medium, or large) of 110 food items, including mixed dishes; and (c) usual consumption of fruits and vegetables. Nutrient values were determined using the University of Minnesota Nutrition Coordinating Center (NCC) database (19), which included the 1999 USDA-NCC carotenoid database for U.S. foods and its more recent updates (20). Botanical groups were defined by grouping the FFQ line item responses for fruits, vegetables, and mixed foods containing vegetables as follows: rosaceae (apples, peaches, pears, apricots, strawberries); rutaceae (oranges, grapefruit, orange juice, grapefruit juice); cruciferae (broccoli, cauliflower, Brussels sprouts, cole slaw, cabbage, sauerkraut, mustard greens, turnip greens, collards); apiaceae (carrots, carrot juice, mixed foods with carrots); curcurbitaceae (squash, pumpkin, watermelon, cantaloupe); leguminosae (peas, green beans, other beans, tofu, peanuts, mixed foods with beans); chenopidiaceae (spinach); and solonaceae (potatoes, tomatoes, tomato juice, mixed foods and condiments with tomatoes).

For this study, these dietary measures were obtained from FFQ data collected at baseline or, if not available, at the next earliest time point. Data from any FFQ that was incomplete or that reflected implausible values for total energy intake (<800 or >5000 kcal/day for men; <600 and >4000 kcal/day for women) were judged as invalid and excluded from analysis. Data from a single rather than multiple FFQs for each participant were used to ensure dietary exposures were estimated with similar precision for cases and controls. Averaging measures across multiple FFQs would have provided relatively less precise estimates for the earlier diagnosed cases, since they completed fewer FFQs than the later diagnosed cases. Only FFQ data obtained at least one year prior to lung cancer diagnosis were used for cases. With these imposed restrictions, dietary data were available for 771 cases and 1,586 controls. Data were collected at baseline from 81% of the cases and 79% of the controls and, by 2.5 years post-baseline, from 90% of the cases and 89% of the controls. Data were collected >3 years before lung cancer diagnosis from 97% of the cases.

Information about smoking history, including age at initiation, total number of years smoked, average number of cigarettes per day, and current smoking status, was also collected at baseline by questionnaire. Total pack-years of smoking were calculated as the product of the average number of cigarette packs per day and the total number of years smoked at baseline.

SNP Selection and Genotyping

With the Genome Variation Server (http://gvs.gs.washington.edu/GVS), the ldSelect algorithm (21) was employed using HapMap CEU I and II population data (dbSNP build 129) to identify tag SNPs for the region spanning ±2,500 base pairs of each candidate gene. One SNP was chosen from each tag bin for a gene region in the following order of priority: 1) non-synonymous SNP; 2) SNP in the 5' promoter region; 3) SNP in the 3' untranslated region; 4) synonymous SNP; 5) intronic SNP in a splice site; 6) intronic enhancer SNP; and 7) intronic SNP with no known function. The tag SNPs chosen captured >99% of markers with minor allele frequencies of >5% at $r^2 \geq 0.8$ in each gene region. Additional SNPs that were identified either as putatively functional (rs8192475), as a reported marker of lung cancer risk (rs1051730), or as a tag SNP for the *AGPHD1-CHRNA5-CHRNA3-CHRNB4* gene region (rs569207) were also selected. Online bioinformatic resources, specifically SIFT (sift.jcvi.org/), PolyPhen (genetics.bwh.harvard.edu/pph/), PolyDoms (polydoms.cchms.org/polydoms), and FastSNP (fastsnp.ibms.sinica.edu/tw), were used to annotate the function of SNP candidates.

Genomic DNA samples for 792 cases, 1568 controls, and 83 blind duplicates were typed for twenty SNPs (design scores: 0.6–1.1) using a custom 384-plex Illumina GoldenGate assay and for four SNPs (rs6495307, rs3885951, rs569207, rs12441088) using individual Applied Biosystem TaqMan assays. Samples for 18 originally selected controls were not typed due to limited quantity of available DNA. The Illumina platform failed to assay seven samples (two cases, four controls, and one blind duplicate) and identified three gender-mismatched samples (one case, two controls). Data collected on individuals who provided these ten samples were not included in our final analyses. Genotyping for rs8034191 and rs16969968 was previously conducted by Hung et al. (2) in an effort to validate lung cancer GWA study results, but these data were not analyzed as part of the present study.

Observed genotype frequencies in study controls were consistent with those expected under Hardy-Weinberg equilibrium. Genotype call success for each SNP ranged from 98.8% to 100%. Among the blind duplicates, genotype concordance for all 24 SNPs was 100%.

Statistical Analysis

To minimize population stratification bias, analyses were restricted to non-Hispanic whites (746 cases, 1477 controls) who comprised about 94% of study participants. Unless otherwise specified, analyses were conducted using Stata® 10 (StataCorp, College Station, TX).

Genotype Analysis—Using logistic regression, odds ratios (OR) and 95% confidence intervals (95% CI), adjusted for the case-control matching variables (age, sex, enrollment year, baseline smoking status, and occupational asbestos exposure), were calculated to estimate the relative risk of lung cancer associated with SNP genotype. The most common homozygous genotype served as the reference group. SNP genotype was also coded according to the number of minor alleles carried (0, 1, or 2), allowing estimation of the per-allele risk of lung cancer.

Subgroup analyses were performed to examine whether lung cancer risk associated with SNP genotype varied by age (dichotomized using the median value: <70, ≥70 years), sex (male, female), baseline smoking status (former, current), the number of pack-years smoked (thirds of the distribution among controls: <40, 40–53, ≥54), occupational asbestos exposure (yes, no), trial arm assignment (intervention, placebo), and tumor histology (non-small cell lung cancer, small cell lung cancer). For those SNPs associated with overall lung cancer risk, subgroup analyses were additionally performed to assess whether such associations differed according to dietary intake level of vitamin C, vitamin E, folate, nitrosamine-containing foods, total carotenoids, total polyunsaturated fatty acids (PFAs), total fruits, total vegetables, and each of the botanical groups studied. Cutpoints for each dietary variable were defined as thirds of the intake distribution among controls. To assess departure from a multiplicative relation, p-values for the Wald test of the cross product term for SNP genotype and level of a given exposure were calculated.

Haplotype Analysis—Pairwise LD patterns between *AGPHD1*, *CHRNA5*, *CHRNA3*, and *CHRNB4* SNPs were visualized using Haploview, version 4.1 (22). Haplotype imputation from genotype data was conducted using PHASE, version 2.1 (23).

Since strong correlations between multiple SNPs were observed, we inferred common haplotypes for the entire *AGPHD1-CHRNA5-CHRNA3-CHRNB4* gene region instead of each candidate gene separately. Haplotypes were constructed with the following eleven tag SNPs that were identified by applying the ldSelect algorithm to HapMap I and II CEU data for the gene region (dbSNP build 129; position 76586961–76720642) with parameters of $r^2 \geq 0.8$ and a minor allele frequency ≥5%: rs3885951, rs588765, rs569207, rs16969968, rs578776, rs1948, rs7178270, rs950776, rs12440014, rs12441088, and rs1316971. The algorithm considered a total of 106 SNPs, which were allocated into 15 bins [Supplementary Table 1]. Thirteen of the 24 SNPs genotyped were identified as tag SNPs for 11 of the 15 bins. The SNP rs16969968 was selected to represent the one bin for which several SNPs were genotyped (i.e., rs8034191, rs16969968, rs1051730, rs1317286, rs17487223). Tag SNPs for the remaining four bins were not genotyped, due to high coverage by the 11 tag SNPs (95.3%, 101 of the 106 SNPs captured). We also inferred haplotypes limited to the three tag SNPs rs588765 (bin 1), rs569207 (bin 2), and rs16969968 (bin 3); each SNP represented one of three distinct sets of highly correlated SNPs that together tagged about 83% of the common SNPs in this region.

ORs and 95% CIs, adjusted for the matching variables, were calculated under an additive model to estimate lung cancer risk associated with imputed haplotypes of the *AGPHD1-CHRNA5-CHRNA3-CHRNB4* gene region. The most common haplotype, serving as the reference group, was compared to each haplotype with a frequency of >1%. Similar subgroup analyses were conducted for haplotypes as described above for genotypes.

RESULTS

Selected baseline characteristics of the non-Hispanic white cases and controls are presented in Table 1. Participants were predominantly male (67%) and current smokers (72%). Relative to controls, cases were slightly older and reported more pack-years of cigarette smoking. Cases smoked an average of 26.7 cigarettes per day and 54.9 cigarette pack-years, and controls smoked an average of 25.1 cigarettes per day and 49.2 cigarette pack-years.

The majority of SNPs genotyped in the *AGPHD1-CHRNA5-CHRNA3-CHRNB4* gene region were modestly associated with lung cancer risk [Table 2], and there were three distinct sets of SNPs in strong LD [Supplementary Figure 1]. The SNPs in two of these sets (bins 1 and 2, see Table 2 footnotes) were associated with a 13% to 19% decrease in risk per minor

allele. For SNPs in bin 2, however, inverse associations were most evident for comparisons of the heterozygous, and not the minor homozygous, genotype to the major homozygous genotype. The SNPs in the remaining set (bin 3) were associated with a 27% to 34% increase in risk per minor allele. These results did not differ materially after further adjustment for the number of pack-years smoked at baseline (data not shown). Haplotype analyses indicated that, of the three sets of highly correlated SNPs, the latter set (represented by rs16969968) was the most strongly related to lung cancer susceptibility [Table 3]. Irrespective of the number of SNP markers considered in haplotype construction – either all eleven tag SNPs or only those representing the top three bins – the risk estimates were consistently highest for those haplotypes that included the minor allele for rs16969968 in reference to the most common haplotype (that included the major allele for rs16969968).

In view of these findings, the results of the subgroup analyses have been presented for rs16969968 only. Differences in lung cancer risk associated with this SNP were found by age at diagnosis and baseline smoking history [Table 4]. The per-allele OR was higher for individuals diagnosed with lung cancer at ages <70 (1.5; 95% CI: 1.2–1.8) than ages ≥70 (1.2; 95% CI: 0.96–1.4). With respect to smoking history, the association was more pronounced among those who had reported smoking <40 pack-years (per-allele OR, 1.9; 95% CI: 1.5–2.4) than those who had reported smoking 40–53 (per-allele OR, 1.2; 95% CI: 0.93–1.5) or ≥54 pack-years (per-allele OR, 1.1; 95% CI: 0.84–1.3). The increase in lung cancer risk per minor allele of rs16969968 was marginally greater for former than current smokers and for those occupationally exposed than unexposed to asbestos, yet was equivalent across sex, tumor histology, and trial arm assignment subgroups.

There was little difference in the association between rs16969968 genotype and lung cancer risk by level of most dietary factors examined, including intake of total fruits, total vegetables, and nitrosamine-containing foods [Table 4]. The few suggestive trends observed, specifically across strata of total PFAs, cruciferae, apiaceae, and curcurbitaceae, showed a stronger association among persons with higher intake of PFAs and lower intake of each botanical group. Since previously published data showed a lower risk of lung cancer associated with greater fruit and vegetable intake in CARET participants assigned to the placebo arm only (18), analyses were stratified further by trial arm assignment. The described risk patterns for PFAs, apiaceae, and curcurbitaceae were more apparent in the placebo arm, while that for cruciferae was more apparent in the intervention arm (data not shown). Additional analyses limited to persons with valid FFQ data collected at baseline only were conducted to evaluate whether these patterns were influenced by the timing of dietary assessment. The same suggestive trends remained for PFAs, cruciferae, and apiaceae, but not for curcurbitaceae (data not shown).

Since certain combinations of dietary nutrients may modify the extent to which lung cancer risk is associated with rs16969968 genotype, given our observations, we examined whether this risk varied by intake of total PFAs, cruciferae, apiaceae, and curcurbitaceae combined. Subgroups were formed using a dietary score (range: 4–12) calculated for each participant by summing points assigned to the intake levels of these four dietary factors, with 1, 2, and 3 points allocated respectively to the highest, middle, and lowest third of total PFAs and to the lowest, middle, and highest third of each botanical group. In this post-hoc analysis, the rs16969968-lung cancer association was strongest among those with the lowest dietary scores, who consumed the highest levels of PFAs and the lowest levels of cruciferae, apiaceae, and curcurbitaceae foods [Table 4].

DISCUSSION

In our population comprised largely of heavy, long-term smokers, we detected the strongest associations, with increases of 27% to 34% in lung cancer risk per minor allele, for a set of highly correlated SNPs: *AGPHD1* rs8034191, *CHRNA5* rs16969968, *CHRNA3* rs1051730, *CHRNA3* rs1317286, and *CHRNB4* rs17487223. Corresponding estimates in prior studies have likewise ranged from increases of 17% to 33% (2–5,8,9,11,24–26). Using rs16969968 as a representative marker, we found that the strength of this association varied by age at diagnosis and smoking quantity, but not by sex, tumor histology, or intake level of fruits, vegetables, and other dietary factors thought to influence lung cancer risk. Although two other sets of SNPs, one tagged by *CHRNA5* rs588765 and the other by *CHRNA5* rs569207, were individually associated with modest reductions in lung cancer risk per minor allele, neither of these tag SNPs appeared to contribute to risk beyond that of rs16969968 when examined jointly.

Due to aspects of the study design, including the examination of ever smokers only, insight into whether the mechanisms by which variants at 15q24-25.1 might induce lung carcinogenesis are direct or indirect through smoking was limited. In accord with some studies (2,3,5,8,9,11), individual SNP associations persisted after adjusting for smoking quantity. Our adjustment for the number of cigarette pack-years smoked at baseline, however, may not have sufficiently captured the true magnitude of association between smoking behavior and lung cancer, since the average duration from baseline to lung cancer diagnosis was 9.1 years. The evidence thus far has been mostly suggestive of a stronger association for carriage of the minor allele of rs1051730, rs16969968, or rs8034191 in earlier diagnosed cases [defined as <50 years in refs. (9,11,25) and <61 years in ref. (8)] and lighter smokers [defined as <20 cigarettes per day in ref. (8) and ≤20 cigarettes per day or ≤35 pack-years in ref. (3)]. Nonetheless, in a recent pooled analysis of 21 case-control studies (11), the association between rs16969968 genotype and lung cancer risk did not vary by the number of pack-years smoked, and it appeared stronger in current than former smokers. Because of the CARET eligibility criteria for age and smoking history, the cutpoint values of the lowest categories of age at diagnosis and total pack-years smoked were higher in our analysis.

For reasons that are not clear, we were unable to reproduce the findings of Wang et al. (27) and Liu et al. (28) suggesting that a second variant (represented by *CHRNA5* rs588765 or rs481134) is independently associated with lung cancer risk. In our haplotype analysis of the SNPs rs16969968, rs588765, and rs569207, the haplotype containing the rs16969968 minor allele was associated with increased risk, but that containing the rs569207 minor allele was only nominally associated with decreased risk, when compared to the most common haplotype containing the rs588765 minor allele. Interestingly, even though Wang et al. (27) found positive and negative associations for haplotypes equivalent to our first two, they did not detect associations for each individual SNP alone. Their haplotype analysis included rs16969968, rs6495306 (HapMap CEU: r^2=1 with rs588765), and rs3743078 (HapMap CEU: r^2=0.9 with rs569207). The LD patterns that we observed were generally similar to those described by Wang et al. (27) and others (29,30) in prior studies of nicotinic receptor gene variants and susceptibility to heavy smoking, nicotine dependence, and lung cancer. Also, our haplotype analyses considering all eleven tag SNPs, in contrast to those of the three top bins only, did not identify any additional risk alleles, despite improved coverage of common HapMap CEU variants in the *AGPHD1-CHRNA5-CHRNA3-CHRNB4* region compared to the GWA analysis by Hung et al. (2). The genotyping of four tag SNPs (for bins 4, 11, 14, and 15; Supplementary Table 1), which were not included in the GWA analysis, increased coverage from 89% to 95%.

Current evidence supports the premise that susceptibility to lung cancer is determined by multiple *CHRNA5* variants that act through different mechanisms, specifically altering α5 subunit function and regulating *CHRNA5* expression. The amino acid substitution of aspartic acid to asparagine at position 398 (D398N) in the α5 subunit encoded by rs16969968 has been shown to diminish the response of α4β2α5-containing nAChRs to a nicotine agonist in vitro (29). In the aforementioned study by Wang et al. (27), the major alleles of *CHRNA5* rs588765 and *CHRNA3* rs3743078 were correlated with low mRNA expression of *CHRNA5* (not *CHRNA3* or *CHRNB4*) in human brain tissue. Furthermore, the two haplotypes of rs16969968, rs6495306 (or rs588765), and rs3743078 that were associated with lung cancer risk, each containing the major allele of rs6495306, were found to correlate with low *CHRNA5* mRNA expression. Four polymorphisms in the *CHRNA5* promoter region, which together form three haplotypes, have also been shown to influence CHRNA5 transcript levels in normal lung tissue (31).

Due to the extent of LD at the 15q24-25.1 locus, it should be noted that the reported associations could be explained by variants in the neighboring genes as well. Recent in vitro studies examining the functional role of the six candidate genes at 15q24-25.1 in human lung tissue have pointed to the involvement of *CHRNA3* and *PSMA4*, in addition to *CHRNA5*, in lung carcinogenesis (32,33). Only variation in the *IREB2* gene was not captured by the SNPs that we genotyped.

Nesting our study in the CARET cohort was advantageous to investigate whether specific dietary factors modulate lung cancer risk associated with variants at 15q24-25.1. For most participants, detailed dietary data were ascertained using a standardized FFQ within 2.5 years post-baseline. In a prior analysis evaluating the reliability of FFQ data collected at baseline and two years later, intra-individual variation estimates of the dietary measures examined were reasonably low (18). Also, for the majority of cases, FFQ administration occurred more than three years prior to lung cancer diagnosis, signifying that usual diet was likely assessed before indications of disease onset.

In the CARET cohort, lung cancer risk was inversely associated with consumption of total fruit, rosaceae fruit, cruciferous vegetables, and vitamin C in persons assigned to the placebo arm (18). We thus hypothesized that these dietary factors, along with nitrosamine-containing foods, would be most likely to modify any of the genetic associations observed. The fact that only weak monotonic trends in lung cancer risk associated with the rs16969968 genotype were found across intake levels of cruciferae, apiaceae, curcurbitaceae, and PFAs suggest that none of the individual dietary factors examined greatly modify the rs16969968-lung cancer association. Nevertheless, we noted a stronger, albeit not statistically significant, monotonic trend in risk across subgroups based on the collective intake of cruciferae, apiaceae, curcurbitaceae, and PFAs, indicating that a specific combination of nutrients found in vegetables may offset the risk of lung cancer linked to carriage of the rs16969968 minor allele in smokers. In contrast to diet composition, diet diversity of fruit and vegetable consumption has no clear impact on the relation between rs16969968 genotype and lung cancer. In our study, we were unable to confirm evidence of an inverse relation between variety of fruit and vegetable consumption and lung cancer risk (13). However, most participants had relatively high diet diversity scores [calculated as the total number of botanical groups represented in one's diet], which may have precluded detection of this possible association (data not shown).

For several reasons, the hypothesis of interplay between 15q24-25.1 variants, diet, and lung cancer susceptibility in smokers should not be dismissed on the basis of our data alone. First, the suggestive risk patterns were in the expected direction and more evident in participants assigned to a specific trial arm. Therefore, although such findings may have arisen by

chance, a lack of power may have hampered the detection of differential effects for specific dietary factors, particularly if modest and/or present only in certain subgroups. Second, diet may not have been assessed at the most etiologically relevant time point, given that most participants started smoking much earlier in life. Lastly, since the CARET FFQ was primarily designed to estimate usual consumption of fruits and vegetables and their nutrients, and not of nitrosamines and other food mutagens, a lack of sensitivity in estimating dietary nitrosamine intake may have prohibited the detection of its modifying effect as posited. Our data do suggest, however, that greater intake of nitrosamine-containing foods is associated with increased lung cancer risk (>3.6 versus ≤1.2 servings per week: OR=1.3, 95% CI: 0.99–1.6), a finding consistent with the only known study to have examined the link between dietary nitrosamines and lung cancer (34).

In conclusion, the results of this study substantiate chromosome 15q24-25.1 as a susceptibility locus for lung cancer in long-term smokers. Additional research characterizing the functional effects of identified risk variants, along with those in strong LD, should help to resolve whether lung cancer susceptibility is determined by multiple markers in this genomic region, and if so, which ones. Further evaluation of whether diet has any influence on lung cancer risk associated with variants at 15q24-25.1 should be conducted in large studies with detailed, high-quality data on diet and other lifestyle factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

AGPHD1	aminoglycoside phosphotransferase domain containing 1
CARET	β-Carotene and Retinol Efficacy Trial
CHRNA5	cholinergic receptor, nicotinic, alpha 5
CHRNA3	cholinergic receptor, nicotinic, alpha 3
CHRNB4	cholinergic receptor, nicotinic, beta 4
CI	confidence interval
FFQ	food-frequency questionnaire
IREB2	iron-responsive element binding protein 2
LD	linkage disequilibrium
nAChR	nicotinic acetylcholine receptor
PFA	polyunsaturated fatty acid
PSMA4	proteasome subunit, alpha type 4
OR	odds ratio
SNP	single nucleotide polymorphism

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Table 1

Selected baseline characteristics of non-Hispanic white cases and controls

	Cases (n=746)		Controls (n=1477)	
Characteristic	**N**	**%**	**n**	**%**
Age, years*				
<50	11	1.5	24	1.6
50 – 54	133	17.8	364	24.6
55 – 59	201	26.9	376	25.5
60 – 64	237	31.8	445	30.1
≥65	164	22.0	268	18.1
Sex*				
Male	502	67.3	984	66.6
Female	244	32.7	493	33.4
Smoking status*				
Former smoker	206	27.6	407	27.6
Current smoker	540	72.4	1070	72.4
No of pack-years smoked				
<40	180	24.1	511	34.6
40 – 53	250	33.5	493	33.4
≥54	316	42.4	473	32.0
Occupational asbestos exposure*				
Yes	125	16.8	247	16.7
No	621	83.2	1230	83.3
Trial arm assignment				
Intervention	403	54.0	775	52.5
Placebo	343	46.0	702	47.5

*Case-control matching variable

Table 2

AGPHD1-CHRNA5-CHRNA3-CHRNB4 SNPs and lung cancer risk among non-Hispanic white study participants

SNP	Gene	Major Allele (A1)	Minor Allele (A2)	MAF [a] (%)	No of cases (n=746)			No of controls (n=1477)			$OR_{A1/A2}$ (95% CI)[b,c]	$OR_{A2/A2}$ (95% CI)[b,c]	$OR_{per\ A2}$ (95% CI)[b,c]
					A1/A1	A1/A2	A2/A2	A1/A1	A1/A2	A2/A2			
rs7164594[e]	AGPHD1	G	A	21.8	486	229	30	890	524	60	0.79 (0.65–0.96)	0.91 (0.58–1.44)	0.85 (0.73–1.00)
rs8034191[f]	AGPHD1	A	G	34.2	258	369	119	625	691	159	1.31 (1.08–1.59)	1.82 (1.38–2.41)	1.34 (1.18–1.53)
rs952216[d]	AGPHD1	G	A	43.0	269	368	108	472	738	265	0.87 (0.72–1.06)	0.72 (0.55–0.94)	0.85 (0.75–0.97)
rs3885951	AGPHD1	A	G	9.3	596	144	6	1207	263	6	1.10 (0.87–1.38)	1.94 (0.62–6.09)	1.13 (0.91–1.40)
rs588765[d]	CHRNA5	G	A	43.1	264	368	113	468	744	263	0.87 (0.72–1.07)	0.76 (0.58–1.00)	0.87 (0.77–0.99)
rs569207[e]	CHRNA5	C	T	22.3	486	229	31	884	527	66	0.78 (0.64–0.94)	0.85 (0.55–1.33)	0.83 (0.71–0.98)
rs637137[e]	CHRNA5	A	T	22.2	486	228	30	884	526	65	0.78 (0.64–0.94)	0.83 (0.53–1.31)	0.83 (0.71–0.97)
rs16969968[f]	CHRNA5	G	A	34.4	258	370	115	624	689	163	1.32 (1.08–1.60)	1.72 (1.30–2.28)	1.31 (1.15–1.50)
rs615470[d]	CHRNA3	G	A	38.3	319	344	83	550	719	205	0.83 (0.68–1.00)	0.70 (0.52–0.94)	0.83 (0.73–0.95)
rs578776	CHRNA5	G	A	27.2	422	278	44	777	591	105	0.86 (0.71–1.03)	0.76 (0.52–1.10)	0.86 (0.75–1.00)
rs6495307[d]	CHRNA3	C	T	42.8	268	366	112	476	739	262	0.88 (0.72–1.07)	0.76 (0.58–0.99)	0.87 (0.77–0.99)
rs1051730[f]	CHRNA3	G	A	34.2	255	373	117	625	690	160	1.35 (1.11–1.63)	1.80 (1.36–2.39)	1.34 (1.18–1.53)
rs1317286[f]	CHRNA3	A	G	34.3	252	372	118	621	686	161	1.35 (1.11–1.65)	1.82 (1.37–2.41)	1.35 (1.18–1.54)
rs938682[e]	CHRNA3	A	G	22.6	484	225	30	870	531	67	0.75 (0.62–0.91)	0.80 (0.51–1.25)	0.80 (0.69–0.94)
rs8192475	CHRNA3	G	A	4.4	670	73	3	1341	126	2	1.15 (0.84–1.56)	2.91 (0.48–17.6)	1.19 (0.89–1.59)
rs6495309[e]	CHRNB4	G	A	20.9	510	208	28	921	496	60	0.75 (0.62–0.91)	0.83 (0.52–1.33)	0.81 (0.69–0.95)
rs1948	CHRNB4	G	A	34.3	339	342	64	633	673	169	0.95 (0.79–1.14)	0.71 (0.51–0.97)	0.88 (0.77–1.01)
rs7178270	CHRNB4	G	C	40.1	275	372	99	517	733	226	0.95 (0.78–1.15)	0.82 (0.62–1.08)	0.92 (0.80–1.04)
rs17487223[f]	CHRNB4	G	A	36.9	242	376	124	565	707	183	1.26 (1.04–1.54)	1.61 (1.22–2.12)	1.27 (1.11–1.45)
rs950776	CHRNB4	A	G	34.6	332	342	71	627	679	171	0.95 (0.79–1.14)	0.79 (0.58–1.07)	0.91 (0.79–1.04)
rs12440014	CHRNB4	C	G	23.7	477	234	34	859	530	84	0.79 (0.65–0.95)	0.72 (0.48–1.09)	0.81 (0.70–0.95)
rs12441088	CHRNB4	T	G	23.8	456	254	36	861	529	87	0.91 (0.75–1.10)	0.77 (0.52–1.16)	0.90 (0.77–1.04)
rs1316971	CHRNB4	G	A	19.2	508	215	20	968	447	59	0.91 (0.75–1.11)	0.65 (0.38–1.09)	0.87 (0.74–1.03)
rs11633223	CHRNB4	A	G	39.6	275	370	93	531	712	226	0.99 (0.82–1.20)	0.79 (0.60–1.05)	0.91 (0.80–1.04)

[a] Minor allele frequency among controls

[b] Adjusted for matching factors (age, sex, smoking status, occupational asbestos exposure, and enrollment year)

[c] Compared to the reference category, A1/A1

[d] Highly correlated variants in bin 1: rs952216, rs588765, rs615470, rs6495307

[e] Highly correlated variants in bin 2: rs7164594, rs569207, rs637137, rs938682, rs6495309

[f] Highly correlated variants in bin 3: rs8034191, rs16969968, rs1051730, rs1317286, rs17487223

Table 3

Chromosome 15q24-25.1 region haplotypes and lung cancer risk among non-Hispanic whites

Haplotypes[a]													
rs3885951	**rs588765**	**rs569207**	**rs16969968**	**rs578776**	**rs1948**	**rs7178270**	**rs950776**	**rs12440014**	**rs12441088**	**rs1316971**	**Cases (%)**	**Controls (%)**	**OR (95% CI)[b]**
1	2	1	1	1	2	2	2	1	1	1	24.6	27.1	1.00 (reference)
1	1	1	2	1	1	1	1	1	1	1	28.6	24.2	1.32 (1.12–1.57)
1	1	2	1	2	1	1	1	2	2	2	13.3	15.1	0.97 (0.78–1.19)
2	1	1	2	1	1	1	1	1	1	1	9.9	8.8	1.23 (0.97–1.57)
1	1	2	1	2	1	1	1	2	1	1	4.0	4.6	0.95 (0.68–1.32)
1	2	1	1	2	1	2	1	1	1	1	4.3	3.9	1.19 (0.85–1.67)
1	2	1	1	1	2	2	2	1	2	1	3.7	3.6	1.13 (0.80–1.61)
1	2	1	1	1	1	1	1	1	1	1	1.2	2.0	0.68 (0.40–1.17)
1	1	2	1	2	1	1	1	1	1	1	1.4	1.4	1.14 (0.67–1.93)
1	2	1	1	1	1	2	1	2	2	2	1.1	1.4	0.87 (0.50–1.52)
All other haplotypes											7.8	7.9	1.08 (0.84–1.38)
	2	1	1								39.8	43.0	1.00 (reference)
	1	1	2								40.5	34.3	1.30 (1.12–1.50)
	1	2	1								19.5	22.2	0.95 (0.80–1.13)
	All other haplotypes[c]										0.2	0.5	0.44 (0.13–1.50)

[a] 1=major allele; 2=minor allele

[b] Adjusted for matching factors (age, sex, smoking status, occupational asbestos exposure, and enrollment year)

[c] Haplotypes 212, 111, 122

Table 4

Stratum-specific odds ratios (OR) and 95% confidence intervals (95% CI) for lung cancer risk associated with *CHRNA5* SNP rs16969968

	No of cases			No of controls						
Stratum	**GG**	**GA**	**AA**	**GG**	**GA**	**AA**	**OR_{GA} (95% CI)[a]**	**OR_{AA} (95% CI)[a]**	**$OR_{per\ A\ allele}$ (95% CI)[a]**	***p-value for interaction***
Age										*0.05*
<70 years	117	209	66	316	379	82	1.54 (1.17–2.03)	2.18 (1.48–3.23)	1.49 (1.24–1.80)	
≥70 years	141	161	49	308	310	81	1.12 (0.85–1.48)	1.38 (0.91–2.08)	1.15 (0.96–1.40)	
Sex										*0.90*
Male	173	247	79	406	471	107	1.25 (0.99–1.59)	1.77 (1.25–2.49)	1.31 (1.11–1.54)	
Female	85	123	36	218	218	56	1.47 (1.05–2.07)	1.66 (1.02–2.71)	1.33 (1.06–1.67)	
Smoking status										*0.18*
Former smokers	68	103	34	185	180	42	1.56 (1.08–2.26)	2.21 (1.29–3.76)	1.50 (1.17–1.93)	
Current smokers	190	267	81	439	509	121	1.23 (0.98–1.54)	1.56 (1.12–2.17)	1.24 (1.06–1.45)	
No. of pack-years smoked										*0.001*
<40	55	91	33	252	215	44	2.02 (1.37–2.98)	3.37 (1.96–5.80)	1.88 (1.45–2.43)	
40–53	91	124	34	204	233	55	1.18 (0.84–1.64)	1.37 (0.83–2.26)	1.17 (0.93–1.48)	
≥54	112	155	48	168	241	64	0.97 (0.70–1.33)	1.15 (0.73–1.80)	1.05 (0.84–1.30)	
Occupational asbestos exposure										*0.27*
Yes	40	63	22	109	114	24	1.47 (0.91–2.38)	2.59 (1.29–5.19)	1.57 (1.13–2.18)	
No	218	307	93	515	575	139	1.28 (1.04–1.59)	1.60 (1.18–2.18)	1.27 (1.10–1.47)	
Histologic subtype										
Non-small cell lung cancer	159	217	72	624	689	163	1.26 (0.99–1.59)	1.75 (1.26–2.43)	1.31 (1.12–1.53)	
Small cell lung cancer	42	54	21	624	689	163	1.15 (0.76–1.76)	1.98 (1.13–3.45)	1.35 (1.02–1.79)	
Trial arm assignment										*0.82*
Intervention	142	195	63	339	343	93	1.36 (1.04–1.77)	1.61 (1.10–2.35)	1.29 (1.08–1.54)	
Placebo	116	175	52	285	346	70	1.26 (0.95–1.68)	1.83 (1.20–2.79)	1.33 (1.09–1.62)	
Vitamin C										*0.64*
≤46.1 mg/d	79	125	38	187	245	59	1.22 (0.86–1.72)	1.51 (0.93–2.47)	1.23 (0.97–1.55)	
46.2–85.2 mg/d	83	129	33	211	229	53	1.45 (1.04–2.04)	1.62 (0.98–2.70)	1.32 (1.05–1.67)	
>85.2 mg/d	90	111	38	226	215	51	1.30 (0.93–1.83)	1.80 (1.10–2.94)	1.33 (1.06–1.68)	
Vitamin E										*0.57*
≤6.5 mg/d	72	104	38	192	238	61	1.18 (0.82–1.69)	1.65 (1.01–2.70)	1.26 (1.00–1.60)	

Stratum	No of cases			No of controls			OR_{GA} (95% CI)[a]	OR_{AA} (95% CI)[a]	$OR_{per\ A\ allele}$ (95% CI)[a]	*p-value for interaction*
	GG	GA	AA	GG	GA	AA				
6.6–9.4 mg/d	86	133	30	212	226	55	1.49 (1.06–2.09)	1.33 (0.79–2.23)	1.24 (0.98–1.57)	
>9.4 mg/d	94	128	41	220	225	47	1.33 (0.96–1.85)	2.04 (1.25–3.33)	1.40 (1.11–1.76)	
Folate										*0.99*
≤175.4 mcg/d	65	115	32	203	230	58	1.62 (1.12–2.33)	1.79 (1.06–3.01)	1.40 (1.09–1.78)	
175.5–256.0 mcg/d	95	122	40	200	234	59	1.10 (0.79–1.54)	1.45 (0.90–2.34)	1.18 (0.94–1.48)	
>256.0 mcg/d	92	128	37	221	225	46	1.42 (1.02–1.97)	1.91 (1.15–3.16)	1.39 (1.10–1.76)	
Nitrosamine-containing foods										*0.82*
≤1.2 servings/wk	77	103	27	224	222	46	1.34 (0.94–1.90)	1.73 (1.00–3.00)	1.32 (1.03–1.70)	
1.3–3.6 servings/wk	90	140	39	205	234	58	1.38 (0.99–1.92)	1.54 (0.95–2.49)	1.27 (1.02–1.59)	
>3.6 servings/wk	85	122	43	195	223	59	1.25 (0.89–1.76)	1.74 (1.08–2.80)	1.30 (1.04–1.64)	
Total polyunsaturated fatty acids										*0.17*
≤11.5 g/d	90	113	33	205	225	61	1.14 (0.81–1.59)	1.24 (0.76–2.04)	1.12 (0.89–1.41)	
11.5–16.7 g/d	71	122	37	196	245	52	1.42 (0.99–2.02)	1.96 (1.18–3.25)	1.40 (1.10–1.79)	
>16.7 g/d	91	130	39	223	219	50	1.46 (1.05–2.04)	1.91 (1.17–3.13)	1.40 (1.12–1.76)	
Total carotenoids[b]										*0.76*
≤6890.4 mcg/d	76	119	39	194	246	62	1.22 (0.86–1.73)	1.56 (0.96–2.54)	1.24 (0.98–1.57)	
6890.5–10802.1 mcg/d	74	121	31	217	217	48	1.74 (1.22–2.47)	2.01 (1.18–3.42)	1.50 (1.17–1.91)	
>10802.1 mcg/d	102	125	39	213	226	53	1.16 (0.84–1.61)	1.53 (0.94–2.47)	1.22 (0.97–1.52)	
Total fruits										*0.91*
≤3.5 servings/wk	94	149	47	191	272	56	1.14 (0.83–1.58)	1.76 (1.10–2.79)	1.28 (1.02–1.60)	
3.6–8.4 servings/wk	76	112	31	216	193	56	1.72 (1.20–2.46)	1.63 (0.97–2.74)	1.38 (1.08–1.75)	
>8.4 servings/wk	83	104	31	217	224	51	1.22 (0.86–1.73)	1.64 (0.97–2.76)	1.26 (0.99–1.61)	
Rosaceae[c]										*0.67*
≤2.1 servings/wk	97	129	46	188	249	51	1.03 (0.74–1.44)	1.81 (1.12–2.92)	1.26 (1.00–1.58)	
2.2–5.9 servings/wk	64	109	28	217	213	65	1.87 (1.29–2.72)	1.41 (0.82–2.42)	1.32 (1.03–1.69)	
>5.9 servings/wk	91	127	35	219	227	47	1.35 (0.97–1.88)	1.83 (1.10–3.04)	1.35 (1.07–1.71)	
Rutaceae[d]										*0.35*
≤0.8 servings/wk	81	116	32	168	221	56	1.12 (0.79–1.60)	1.20 (0.72–2.02)	1.10 (0.87–1.41)	
0.9–4.0 servings/wk	79	137	41	217	246	50	1.54 (1.10–2.16)	2.26 (1.38–3.68)	1.51 (1.20–1.90)	
>4.0 servings/wk	91	112	36	239	222	57	1.39 (0.99–1.94)	1.75 (1.07–2.86)	1.34 (1.06–1.68)	

Stratum	No of cases			No of controls			OR_{GA} (95% CI)[a]	OR_{AA} (95% CI)[a]	$OR_{per\ A\ allele}$ (95% CI)[a]	*p-value for interaction*
	GG	GA	AA	GG	GA	AA				
Total vegetables										*0.98*
≤8.4 servings/wk	70	122	44	186	239	62	1.32 (0.93–1.89)	1.83 (1.13–2.97)	1.35 (1.07–1.70)	
8.5–13.3 servings/wk	84	122	30	205	238	57	1.29 (0.92–1.81)	1.32 (0.79–2.21)	1.19 (0.94–1.50)	
>13.3 servings/wk	98	121	35	233	212	44	1.37 (0.98–1.90)	1.84 (1.11–3.06)	1.36 (1.08–1.71)	
Cruciferae[e]										*0.26*
≤1.0 servings/wk	90	131	45	213	224	54	1.43 (1.02–1.99)	1.97 (1.23–3.16)	1.41 (1.13–1.76)	
1.1–2.3 servings/wk	70	120	28	192	241	45	1.39 (0.97–1.98)	1.73 (1.00–3.01)	1.34 (1.04–1.72)	
>2.3 servings/wk	92	113	36	219	224	64	1.23 (0.88–1.72)	1.36 (0.84–2.20)	1.18 (0.94–1.48)	
Apiaceous[f]										*0.26*
≤0.5 servings/wk	69	130	38	189	242	51	1.50 (1.05–2.13)	2.03 (1.22–3.40)	1.44 (1.13–1.84)	
0.6–1.0 servings/wk	93	117	41	216	225	58	1.18 (0.84–1.66)	1.68 (1.04–2.71)	1.27 (1.01–1.59)	
>1.0 servings/wk	90	118	30	219	222	54	1.32 (0.94–1.85)	1.32 (0.79–2.20)	1.20 (0.95–1.51)	
Curcurbitaceae[g]										*0.37*
≤0.8 servings/wk	79	105	44	187	207	53	1.22 (0.85–1.76)	2.03 (1.25–3.28)	1.38 (1.09–1.75)	
0.9–2.3 servings/wk	75	132	36	217	263	60	1.48 (1.05–2.08)	1.69 (1.03–2.78)	1.34 (1.06–1.69)	
>2.3 servings/wk	98	128	29	220	219	50	1.32 (0.95–1.84)	1.27 (0.75–2.14)	1.19 (0.94–1.50)	
Legumes[h]										*0.97*
≤2.3 servings/wk	81	114	46	196	229	60	1.27 (0.89–1.81)	2.00 (1.25–3.20)	1.39 (1.10–1.74)	
2.4–4.6 servings/wk	85	127	30	211	231	56	1.36 (0.97–1.91)	1.33 (0.79–2.23)	1.21 (0.96–1.53)	
>4.6 servings/wk	86	124	33	217	229	47	1.39 (0.99–1.95)	1.78 (1.06–2.99)	1.35 (1.07–1.71)	
Chenopodiaceae[i]										*0.91*
0 servings/wk	94	144	43	262	277	67	1.49 (1.08–2.04)	1.79 (1.13–2.82)	1.37 (1.11–1.70)	
0.1–0.5 servings/wk	74	91	26	140	185	40	0.92 (0.63–1.35)	1.21 (0.68–2.15)	1.04 (0.80–1.37)	
>0.5 servings/wk	84	129	40	220	225	56	1.56 (1.11–2.18)	1.81 (1.12–2.94)	1.40 (1.11–1.75)	
Solonaceae[j]										*0.30*
≤4.8 servings/wk	81	109	39	187	250	55	1.01 (0.71–1.43)	1.68 (1.03–2.75)	1.22 (0.96–1.55)	
4.9–8.5 servings/wk	90	142	35	209	223	60	1.50 (1.08–2.09)	1.33 (0.81–2.19)	1.24 (0.99–1.55)	
>8.5 servings/wk	81	114	35	228	216	48	1.55 (1.10–2.19)	2.06 (1.24–3.44)	1.47 (1.16–1.86)	
Dietary score[k]										

Stratum	No of cases			No of controls			OR_{GA} (95% CI)[a]	OR_{AA} (95% CI)[a]	$OR_{per\ A\ allele}$ (95% CI)[a]	*p-value for interaction*
	GG	GA	AA	GG	GA	AA				
Score 4–6	59	85	32	138	160	33	1.28 (0.85–1.93)	2.33 (1.30–4.19)	1.47 (1.11–1.94)	*0.07*
Score 7–9	115	199	57	334	375	91	1.55 (1.18–2.04)	1.85 (1.24–2.75)	1.40 (1.16–1.69)	
Score 10–12	78	80	20	152	154	39	1.01 (0.68–1.49)	0.99 (0.54–1.82)	1.00 (0.76–1.32)	

[a] Adjusted for age, sex, smoking status, occupational asbestos exposure, and enrollment year, as applicable

[b] Alpha-carotene, beta-carotene, beta-crytoxanthin, lutein, zeaxanthin, and lycopene

[c] Apples, peaches, pears, apricots, and strawberries

[d] Oranges, grapefruit, orange juice, and grapefruit juice

[e] Broccoli, cauliflower, brussel sprouts, cole slaw, cabbage, sauerkraut, mustard greens, turnip greens, and collards

[f] Carrots, carrot juice, and mixed foods with carrots

[g] Squash, pumpkin, watermelon, and cantaloupe

[h] Peas, green beans, other beans, tofu, peanuts, and mixed foods with beans

[I] Spinach

[j] Potatoes (including french fries; boiled, mashed, and baked potatoes), tomatoes, tomato juice, and mixed foods and condiments with tomatoes

[k] Derived from levels of cruciferae, apiaceae, curcurbitaceae, and total PFA intake combined