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Dietary quercetin, quercetin-gene interaction, metabolic gene expression in lung tissue and lung cancer risk

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Epidemiological and mechanistic evidence on the association of quercetin-rich food intake with lung cancer risk and carcinogenesis are inconclusive. We investigated the role of dietary quercetin and the interaction between quercetin and *P450* and glutathione *S*-transferase (*GST*) polymorphisms on lung cancer risk in 1822 incident lung cancer cases and 1991 frequency-matched controls from the Environment And Genetics in Lung cancer Etiology study. In non-tumor lung tissue from 38 adenocarcinoma patients, we assessed the correlation between quercetin intake and messenger RNA expression of the same *P450* and *GST* metabolic genes. Multivariate odds ratios (ORs) and 95% confidence intervals (CIs) for sex-specific quintiles of intake were calculated using unconditional logistic regression adjusting for putative risk factors. Frequent intake of quercetin-rich foods was inversely associated with lung cancer risk (OR = 0.49; 95% CI: 0.37–0.67; *P*-trend < 0.001) and did not differ by *P450* or *GST* genotypes, gender or histological subtypes. The association was stronger in subjects who smoked >20 cigarettes per day (OR = 0.35; 95% CI: 0.19–0.66; *P*-trend = 0.003). Based on a two-sample *t*-test, we compared gene expression and high versus low consumption of quercetin-rich foods and observed an overall upregulation of *GSTM1*, *GSTM2*, *GSTT2*, and *GSTP1* as well as a downregulation of specific *P450* genes (*P*-values < 0.05, adjusted for age and smoking status). In conclusion, we observed an inverse association of quercetin-rich food with lung cancer risk and identified a possible mechanism of quercetin-related changes in the expression of genes involved in the metabolism of tobacco carcinogens in humans. Our findings suggest an interplay between quercetin intake, tobacco smoking, and lung cancer risk. Further research on this relationship is warranted.

Abbreviations: B(*a*)P, benzo(*a*)pyrene; CI, confidence interval; EAGLE, environment and genetics in lung cancer etiology; FFQ, food frequency questionnaire; GST, glutathione *S*-transferase; mRNA, messenger RNA; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; SNP, single-nucleotide polymorphism.

Introduction

The relationship between consumption of fruits and vegetables in relation to lung cancer risk has been investigated and systematically reviewed (1,2). Consumption of fruits and vegetables overall was associated with reduced risk of lung cancer; however, when separated by intake of fruits or vegetables, the evidence was consistent only for fruit intake (2). In recent years, the focus has shifted towards the identification of specific dietary constituents that may be responsible for the observed inverse associations. Emerging evidence has suggested that the anticarcinogenic effects of fruits or vegetables may be partially attributed to polyphenolic and non-nutrient compounds such as crucifer-derived isothiocyanates or flavonoid quercetin (3,4) and that variants of metabolic genes may modulate these associations (4,5). Although the relationship between dietary isothiocyanates/crucifer-derived isothiocyanates and lung cancer risk and their possible interaction with metabolic genes have garnered much attention (6), comparatively fewer epidemiological studies have investigated dietary quercetin.

Quercetin, ubiquitous in certain fruits (apples and grapes) and vegetables (onions and broccoli), is the most abundant naturally occurring flavonoid (7). Its anticarcinogenic and chemopreventive properties may be due to various mechanisms including free radical scavenging, modification of signal transduction pathways, induction of apoptosis, inhibition of Phase I enzymes responsible for activation of carcinogens and induction of Phase II enzymes responsible for the detoxification of carcinogens (8,9). Dietary quercetin inhibits carcinogen-induced tumors in rodents (10,11) and proliferation of human lung carcinoma cells *in vitro* (12). Although quercetin is metabolized mainly in the liver, there is evidence of quercetin's presence in the lungs. In fact, when rats were fed quercetin, the highest tissue concentration of quercetin was found in the lungs (13).

Too few human studies are available to draw conclusions on the relationship between quercetin and lung cancer risk. A major report of the World Cancer Research Fund and the American Institute for Cancer Research concluded that the epidemiological evidence on quercetin-rich food and lung cancer was 'limited' and 'inconclusive' (2). Higher intakes of quercetin have been associated with statistically significantly reduced lung cancer risk in prospective cohort studies (14,15) and case–control studies (16,17), but not all (18,19). The majority of these studies had small sample size and did not examine possible differential associations by histological subtypes or genetic variants of tobacco-related metabolic genes.

Data suggest that individual susceptibility to lung cancer may be modulated by factors that affect the metabolism of tobacco-related carcinogens such as polycyclic aromatic hydrocarbons (PAHs), particularly benzo(*a*)pyrene [B(*a*)P] (20). In humans, B(*a*)P can be activated by a cascading process that is catalyzed by cytochrome P450A1 (CYP1A1) and CYP1B1 into electrophilic metabolites capable of damaging DNA (21). Subsequently, these carcinogenic metabolites can be detoxified or removed by Phase II enzymes, particularly by the superfamily of glutathione *S*-transferases (*GST*s) (22). GSTs catalyze the reduction of electrophilic metabolites with glutathione, which usually results in their elimination and prevention of DNA damage (23). Although association studies on *P450* (24) and *GST* (25) polymorphisms had been conducted, the epidemiological evidence remains unclear on whether variants of these genes do in fact modulate lung cancer risk.

The investigation into the modulating effects of metabolic genes on the relationship between quercetin and lung cancer risk is scarce. Results from a lone case–control study by Le Marchand *et al.* (19) showed that the protective effect of quercetin-rich onion on lung cancer risk was modified by *CYP1A1* genotype among a small Hawaiian population. Although *CYP1A1* is polymorphic in humans,

we know of no study that has extended Le Marchand's investigation to other polymorphisms of *CYP1A1* or other Phase I genes. Additionally, no study has assessed the possible modification by *GST* polymorphisms nor examined the effects of dietary quercetin on gene expression in human lung tissue.

In cell-culture models and experimental studies, quercetin inhibits *CYP1A1*-mediated activities, downregulating gene expression of *CYP1A1* (26) and upregulating the induction of Phase II enzymes (8,9). Moreover, Kang *et al.* (20) also showed reduced B(*a*)P–DNA adducts in B(*a*)P-exposed human HepG2 cells after administration of quercetin. Tang *et al.* (27) observed that PAH–DNA adducts can be used to significantly predict lung cancer risk in a prospective cohort study. Therefore, the inhibition of P450-mediated bioactivation of PAHs and the induction of GST-mediated detoxification by quercetin may be important in the prevention of lung carcinogenesis.

Capitalizing on a large sample size, detailed epidemiological data and clinical information from the Environment And Genetics in Lung cancer Etiology (EAGLE) study (28), we conducted an integrative investigation on the relationship between quercetin-rich foods, the interaction between quercetin and metabolic genes and lung cancer risk. Furthermore, we explored for the first time the effects of quercetin-rich foods on the messenger RNA (mRNA) expression of selected *P450* and *GST* genes in human lung tissues in a small subgroup of subjects with adenocarcinoma.

Materials and methods

Study population

The EAGLE study has been described previously (28). Briefly, EAGLE is a large population-based case–control study conducted in the Lombardy region of Italy (http://dceg.cancer.gov/eagle). The catchment area covers 216 municipalities, which include five cities (Milan, Monza, Brescia, Pavia and Varese) and surrounding towns and villages. Between April 2002 and June 2005, primary lung cancer cases ($n = 2100$) were enrolled from 13 hospitals that treat ~80% of the incident lung cancer cases in the area. Cases' response rate was 86.6%. The majority of cases (95%) were confirmed pathologically or cytologically and detailed histological classification was recorded. The remaining 5% were confirmed based on clinical history and imaging.

Controls were randomly selected from the Regional Health Service database, which contains demographic information for virtually all Italians from the catchment area, and were frequency matched to cases on gender, age (5 year classes) and residence area (five areas) where cases originated (28). At the study completion, 2120 controls were successfully recruited with a participation rate of 72.4%. The study was approved by the Institutional Review Board of the National Cancer Institute and the local hospitals and universities. Each subject signed an informed consent form prior to study participation.

Exposure assessment

At enrollment, we collected comprehensive information on demographic characteristics and risk factors using both a computer-assisted personal interview and a self-administered questionnaire. Particular attention was given to the collection of data on tobacco exposure including active smoking (number of cigarettes per day averaged over a life time, age at initiation/cessation and pack-years) and passive smoking (during childhood, at work and at home during adulthood).

Dietary information was obtained at baseline from a self-administered 58 item food frequency questionnaire (FFQ) designed to target specific types of foods of interest including meats (and doneness levels), processed meats and fruits and vegetables. The FFQ queried frequency of consumption using 11 possible response categories, from 'never' to '2 or more times a day' in the year prior to the study. A list of relevant food groups queried can be found in supplementary Table 1 (available at *Carcinogenesis* Online). Selection of quercetin-rich food items available in EAGLE's FFQ was based on data published by the United States Department of Agriculture on food-specific quercetin content (29).

Single-nucleotide polymorphism selection and genotyping

Gene selection for the EAGLE study had been described (30). Briefly, at the start of the EAGLE study, single-nucleotide polymorphism (SNP) assays were selected from those available at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics (National Cancer Institute), using our own assessment of linkage disequilibrium between the SNPs from HapMap and previous evidence from the literature. Genotyping was performed on 4050 EAGLE subjects (those with sufficient DNA samples). Duplicate quality control samples (2% of the total) showed 100% agreement. SNP genotyping and quality control were conducted at Core Genotyping Facility using TaqMan® assay as described on the National Cancer Institute SNP500Cancer website (http://snp500cancer.nci.nih.gov). From this original genotype data, a total of 16 SNPs in seven *CYP450* and *GST* genes (supplementary Table 2 is available at *Carcinogenesis* Online) were selected for the present study. Selection was based on genes that have previously been shown or suspected to be associated with quercetin in observational or experimental studies (26) and with a minor allele frequency threshold of 10%.

Lung tissue collection and gene expression analysis

Fresh 'normal' lung tissue (adjacent and distant from the malignant lesion) and tumor samples from a subgroup of adenocarcinoma cases ($n = 49$) were obtained from individuals who underwent surgery and who provided consent (31). After resection, samples were quickly (<20 min) frozen in liquid nitrogen. Selection of tissue samples for this study was based on the amount of tissue, pathologist-defined absence of tumor cells and RNA quality. All cases meeting inclusion criteria with sufficient tissue available were used. Gene expression data were processed and normalized using Bioconductor Affymetrix package, based on the Robust Multichip Average method (32). This report is based on gene expression data from 38 non-tumor tissues of cases with information on quercetin-rich foods intake.

Statistical analysis

Of the 4220 cases and controls, 245 participants (198 cases and 47 controls) did not complete the FFQ and were excluded from this analysis. We further excluded individuals who were identified as outliers for intake of fruits and vegetables ($n = 163$), defined as individuals with combined fruit and vegetable intake exceeding the median intake by more than three times the interquartile range (the difference in values between 25th and 75th percentile) of the controls. These outliers were not associated with lung cancer risk in the study. As a result, the present study consisted of 1822 cases and 1991 controls. Frequency of quercetin intake was divided into sex-specific quintiles using the distribution of the controls separately by sex.

Analyses for intakes of fruits, vegetables and quercetin-rich foods as main effects

Odds ratios (ORs) and 95% confidence interval (CI) were obtained using unconditional logistic regression. All models were adjusted for matching variables (age, sex and area of residence), body mass index, education, dietary consumption of red and processed meats (continuous), cigarette intensity (continuous, 0 for never smokers), smoking duration (continuous, 0 for never smokers) and years since last cigarette smoked (for former smokers only, quartiles based on the controls' distribution). Adjustment for passive smoking and lifetime wine consumption did not substantially alter the results, thus passive smoking and wine consumption were not included in the final models for the results reported here. We chose frequency of intake of quercetin-rich foods as the primary constituent of fruits and vegetables because of laboratory studies suggested that quercetin possesses anticarcinogenic properties. In the analyses of the main effects between intake of quercetin-rich foods and lung cancer risk, total frequency of non-quercetin-rich fruits and vegetables was added to the models to examine the independent effect of quercetin-rich foods on lung cancer risk.

We conducted analyses within subgroups stratified by smoking status (never and ever smokers), smoking intensity (quartiles of cigarettes per day based on controls' distribution), sex, and for various case categories, based on the major histological subtypes (adenocarcinoma, squamous cell carcinoma and small-cell lung cancer) and clinical factors (stage and grade). For histology-, stage-, and grade-specific analyses, ORs and 95% CIs were estimated using unconditional multinomial logistic regression.

Analyses of genes and gene–quercetin interaction

For the analyses of gene and quercetin–gene interaction, we excluded individuals with a genotyping call rate of <90% or without genotype data ($n = 154$). The main effects of the variant genotypes on the risk of lung cancer in three *CYP450* genes (*CYP1A1*, *CYP1A2* and *CYP1B1*) and four *GST* genes (*GSTA1*, *GSTA4*, *GSTM3* and *GSTP1*) were assessed using unconditional logistic regression. The homozygous common allele among controls was used as the referent group.

We evaluated quercetin–gene interaction by examining the risk associated with carrying the variant allele and having consumed the highest quercetin-rich food intake compared with the reference group, individuals who were homozygous for the common allele genotype and had the lowest quercetin intake. Quercetin–gene interactions were assessed with both additive and multiplicative interaction models. On the additive scale, we applied the method described by Rothman (33) and the algorithms by Andersson *et al.* (34). The independent

ORs and 95% CIs for the risk due to the gene alone (OR_{gene}), quercetin-rich diet ($OR_{quercetin}$) and the interaction between gene and quercetin-rich diet ($OR_{gene \times quercetin}$) were first estimated through logistic regression. Biological interaction was then assessed by three measures: (i) the relative excess risk due to interaction (RERI = $OR_{gene \times quercetin} - OR_{gene} - OR_{quercetin} + 1$); (ii) the attributable proportion due to interaction (AP = RERI/$OR_{gene \times quercetin}$) and (iii) the synergy index {$S = (OR_{gene \times quercetin} - 1)/[(OR_{gene} - 1) + (OR_{quercetin} - 1)$}. Lack of interaction was reflected by RERI = AP = 0 and S= 1. Multiplicative interaction was examined using the likelihood ratio test comparing the full model (including the interaction term), the main effect of the genotype and the main effect of intake of quercetin-rich foods versus the reduced model (lacking the interaction term).

Analyses for gene expression

To explore whether a diet rich in quercetin affected metabolic gene expression in the target tissue, we analyzed mRNA expression of 3 *CYP450* and 15 *GST* genes using Affymetrix HG-U133A microarray data from fresh frozen non-tumor lung tissue samples. We compared gene expression between individual consumption above and below the median of quercetin-rich foods as well as in high and low quintiles of quercetin-rich foods (see below). Here, we reported results for the comparison between high versus low consumers as these individuals better represent the two extremes dietary consumption of quercetin. Two sample *t*-tests were used to assess whether the gene expression differed by quercetin consumption status. The same analysis was repeated adjusting for age ($<$ median or $\geq$ median), sex and smoking status (i.e. current, former and never smokers).

For analyses of quercetin–gene interactions and gene expression, we defined high consumers as individuals in the highest fourth or fifth quintile of frequency of quercetin-rich intake and low consumers as individuals in the first quintile of intake. For all other analyses, tests for dose-response trends across different categories of quercetin-rich exposure and variant genotypes were estimated by fitting the ordinal exposure variables as ordered categories. Dose-response tests using the median frequency of intake in each quintile of quercetin intake did not substantially change the results.

All statistical analyses were carried out using STATA version 9.1 (35) with the exception of the gene expression analyses, which were performed using the R-project statistical software version 2.8 (36). A two-tailed P-value of <0.05 was considered to be statistically significant.

Results

Compared with controls, cases were slightly older, consumed lower frequency of fruits and vegetables, higher amount of alcohol and red meat and, among ever smokers, smoked more intensely (Table I). Fruits and vegetables were not correlated with smoking intensity (Spearman correlation: $r = -0.13$) or alcohol consumption ($r = -0.07$). Frequency of quercetin-rich food intake was not highly correlated with frequency of non-quercetin-rich food intake (Spearman correlation: $r = 0.64$).

Fruits, vegetables, quercetin-rich food and lung cancer risk

Individuals in the highest quintile of frequency of intake for total fruits and vegetables had a 30% lower risk of lung cancer compared with the lowest quintile of intake (OR = 0.70; 95% CI: 0.54–0.90; P-trend = 0.007, Table II). When separated by specific fruit or vegetable groups, protective associations, comparing highest versus lowest quintile frequency of intake, were observed for total fruits (OR = 0.79; 95% CI: 0.61–1.0; P-trend = 0.01) and total vegetables (OR = 0.76; 95% CI: 0.59–0.99; P-trend = 0.03). Comparing those who consumed the highest quintile of quercetin-rich food intake with the lowest consumers, a strong statistically significant 53% lower risk of lung cancer (OR = 0.47; 95% CI: 0.35–0.64; P-trend $<$ 0.001) was observed. The inverse associations of quercetin-rich foods were similar in women and men (Table II). Conversely, the beneficial effects from high consumption of fruits and vegetables were strongest in men (Table II). In the analyses stratified by smoking status, ever smokers showed an inverse association for total fruits and vegetables (OR = 0.73; 95% CI: 0.57–0.98; P-trend = 0.03), total fruits (OR = 0.76; 95% CI: 0.58–1.0; P-trend = 0.01) and quercetin-rich foods (OR = 0.46; 95% CI: 0.34–0.64; P-trend $<$ 0.001) (Table III). The association was strongest among the heaviest smokers ($>$1 pack per day) (OR = 0.35; 95% CI: 0.19–0.66; P-trend = 0.003) for quercetin-rich intake compared with similar smokers who eat less quercetin-rich foods. Among never smokers, statistically significant inverse associations were observed for total fruits and vegetables and total vegetables only.

When the analyses were separated by histological subtypes, inverse associations, comparing highest versus lowest quintile of frequency of quercetin-rich foods, were observed for adenocarcinoma (OR = 0.46; 95% CI: 0.34–0.64; P-trend $<$ 0.001), squamous cell carcinoma (OR = 0.43; 95% CI: 0.27–0.70; P-trend = 0.004) and small-cell lung cancer (OR = 0.52; 95% CI: 0.25–1.0; P-trend = 0.13) (Table III). There was no evidence of heterogeneity by histology (P = 0.87, Wald test). The inverse associations conferred by quercetin-rich foods did not differ by stage or grade (data not shown).

With respect to fruit and vegetable intake, highest versus lowest quintile of both combined fruits and vegetables and total vegetables only were associated with a statistically significant lower risk of lung cancer for adenocarcinoma. No statistically significant associations were observed for squamous cell carcinoma and small-cell lung cancer for any of these food groups.

Metabolic gene variants, quercetin–gene interaction and lung cancer risk

None of the examined variants from candidate *P450* and *GST* genes was associated with lung cancer risk in the overall population (supplementary Table 3 is available at *Carcinogenesis* Online). Analyses examining the lung cancer risks associated with consumption of quercetin-rich foods by variants of *P450* and *GST* genes suggested an interaction between high intake of quercetin-rich food and *CYP1B1_18* (rs10175368) on both the additive (RERI = 0.41; CI: 0.17–0.66) and multiplicative scale (P-$value_{lrt}$ = 0.01); however, after accounting for multiple comparisons using Bonferroni correction of P = 0.003 (0.05/16 SNPs), this interaction was no longer statistically significant (Table IV). No further evidence of an effect of genetic variants on quercetin–lung cancer risk association was observed.

Quercetin-rich foods, metabolic gene expression and lung cancer risk

Selected characteristics of the 38 cases with expression data and dietary quercetin are shown in supplementary Table 4 (available at *Carcinogenesis* Online). The comparison between gene expression and high versus low consumption of quercetin-rich foods showed an overall downregulation of *P450* genes and upregulation of *GST* genes for high consumption versus low consumption of quercetin-rich foods (Table V). Differential expression was significant even after age, sex and smoking adjustments for *CYP1A2*, probe 207608_x_at (P-value = 0.025, fold change = 0.856), *CYP1B1*, probe 202434_s_at (P-values = 0.043, fold changes = 0.870), *GSTA3* (P-value = 0.034, fold change = 0.825), *GSTP1* (P-value = 0.028, fold change = 1.206), *GSTM2* (P-value = 0.021, fold change = 1.331), *GSTM1* (P-value = 0.041, fold change = 1.235) and *GSTT2* (P-value = 0.023, fold change = 1.318).

Discussion

In a large population-based case–control study from Northern Italy, intakes of combined fruits and vegetables, only fruits and only vegetables were associated with a 30, 21 and 24% lower risk of lung cancer, respectively. A diet rich in quercetin foods was associated with 53% lower risk of lung cancer. The inverse associations for quercetin-rich foods were seen in both women and men, ever smokers, and were strongest in the heaviest smokers. The beneficial effect of a quercetin-rich diet did not differ by histological subtypes. Analyses to examine gene–diet interaction between dietary quercetin and polymorphisms of *P450* and *GST* genes showed no evidence that variants of these metabolic genes modulate the inverse associations between a quercetin-rich diet and lung cancer risk. Notably, in a small subset of cases with dietary information and gene expression data, we observed a downregulation of *P450*s genes and upregulation of *GST* genes in subjects with high frequency of intake of quercetin-rich foods. This finding is consistent with an influence of dietary quercetin on mRNA

Table I. Selected characteristics for cases and controls by quintiles of combined intake of fruits and vegetables[a], EAGLE 2002–2005

	Total		Quintile of total fruit and vegetable intake (frequency per day)									
	Cases, 1822	Controls, 1991	Q1		Q2		Q3		Q4		Q5	
			Cases, 477	Controls, 399	Cases, 407	Controls, 398	Cases, 335	Controls, 399	Cases, 309	Controls, 398	Cases, 294	Controls, 397
Fruits and vegetables, median (frequency per day, IQR)												
Female	3.7 (3.1)	4.2 (3.0)	1.7 (0.88)	1.8 (0.97)	3.2 (0.64)	3.2 (0.69)	4.2 (0.56)	4.2 (0.58)	5.5 (0.85)	5.4 (0.69)	7.0 (1.0)	7.1 (1.2)
Male	3.1 (2.5)	3.5 (2.5)	1.5 (0.82)	1.5 (0.68)	2.5 (0.43)	2.6 (0.42)	3.4 (0.47)	3.5 (0.48)	4.5 (0.51)	4.5 (0.61)	6.2 (1.4)	6.2 (1.4)
	P-value < 0.001											
Quercetin-rich[b] foods, median (frequency per day)												
Female	1.5 (1.3)	1.7 (1.3)	0.62 (0.48)	0.66 (0.44)	1.3 (0.71)	1.2 (0.67)	1.8 (0.66)	1.7 (0.51)	2.1 (0.65)	2.2 (0.68)	2.7 (1.3)	2.9 (0.91)
Male	1.2 (1.1)	1.5 (1.3)	0.59 (0.42)	0.64 (0.43)	1.0 (0.43)	1.0 (0.43)	1.4 (0.56)	1.6 (0.56)	1.9 (0.73)	1.9 (0.68)	2.7 (0.99)	2.7 (1.0)
	P-value < 0.001											
Sex												
Female (%)	365 (20.0)	449 (22.6)	97 (20.3)	90 (22.6)	91 (22.4)	90 (22.6)	55 (16.4)	90 (22.3)	67 (21.7)	90 (22.6)	55 (18.7)	89 (22.4)
Male (%)	1457 (80.0)	1542 (77.5)	380 (79.7)	309 (77.4)	316 (77.6)	308 (77.4)	280 (83.6)	309 (77.4)	242 (78.3)	308 (77.4)	239 (81.3)	308 (77.6)
	P-value $= 0.06$[c]											
Age (years)												
Mean (SD)	66.3 (8.3)	65.4 (8.7)	65.6 (8.7)	65.3 (9.4)	66.7 (8.1)	65.3 (8.7)	66.4 (8.0)	64.8 (8.6)	66.3 (8.3)	66.0 (8.3)	66.7 (8.5)	65.8 (8.2)
	P-value $= 0.003$[d]											
Body mass index (kg/m^2)												
Mean (SD)	25.8 (4.2)	26.0 (4.0)	25.5 (4.4)	25.8 (3.9)	26.2 (4.7)	25.9 (4.6)	25.5 (4.0)	26.1 (3.6)	25.7 (3.6)	26.0 (3.6)	26.1 (4.2)	26.2 (4.0)
	P-value $= 0.10$[d]											
Smoking status (%)												
Never smokers	118 (6.5)	624 (31.4)	22 (4.6)	104 (26.1)	27 (6.7)	114 (28.8)	23 (6.9)	139 (34.9)	29 (9.4)	134 (33.7)	17 (6.1)	129 (33.7)
Former smokers	797 (43.9)	867 (43.6)	177 (37.3)	150 (37.7)	171 (42.2)	164 (41.4)	152 (45.4)	171 (43.0)	140 (45.5)	191 (48.0)	153 (54.5)	184 (48.0)
Current smokers	900 (49.6)	496 (25.0)	275 (58.0)	144 (36.2)	207 (51.1)	118 (29.8)	160 (47.8)	88 (22.1)	139 (45.1)	73 (18.3)	119 (40.6)	73 (18.4)
	P-value ≤ 0.001[c]											
Smoking intensity (py per day) in ever smokers												
Median (IQR)	1.0 (0.62)	0.75 (0.56)	1.0 (0.75)	0.75 (0.50)	1.0 (0.50)	0.75 (0.50)	1.0 (0.58)	0.75 (0.50)	1.0 (0.51)	0.75 (0.70)	1.0 (0.75)	0.75 (0.60)
	P-value ≤ 0.001[e]											
Smoking duration (years) in ever smokers												
Median (IQR)	44.0 (15.0)	33.0 (23.0)	45.0 (14.0)	38.0 (23.0)	45.0 (14.0)	33.8 (21.0)	44.0 (16.0)	32.0 (22.0)	42.0 (16.0)	30.5 (22.5)	43.0 (18.0)	30.0 (22.0)
	P-value ≤ 0.001[e]											
Lifetime alcohol consumption (g/day)												
Median (IQR)	22.4 (32.4)	17.2 (31.9)	25.3 (33.1)	16.7 (34.4)	22.8 (32.5)	15.9 (31.4)	20.3 (30.3)	18.4 (31.8)	21.9 (31.4)	17.8 (30.3)	19.9 (32.9)	17.2 (30.6)
	P-value ≤ 0.001[e]											
Total red meat consumption[f] (frequency per day)												
Median (IQR)	1.0 (0.91)	0.85 (0.76)	0.69 (0.77)	0.63 (0.74)	0.95 (0.76)	0.79 (0.75)	1.1 (0.95)	0.88 (0.75)	1.2 (1.0)	0.91 (0.72)	1.4 (1.1)	1.0 (0.79)
	P-value ≤ 0.001[e]											

IQR, interquartile range; py, pack-years; SD, Standard Deviation.

[a]Total fruits and vegetables: summary measure of apples, pears, bananas, kiwis, oranges/grapefruits, mandarins/clementines, grapes, peaches/clingstones, apricots, plums, strawberries, melons, fruit cocktails, tomatoes, peppers, carrots, salad, peas, beans/chick peas, mushrooms, broccoli, turnips, savoy, black cabbage, onions, cooked spinach/Swiss chard/beets/rabes, cooked eggplants/zucchini/string beans, artichokes/fennel and beets.

[b]Quercetin-rich foods: Summary measure of apples, grapes, onions, artichoke/fennel/celery, beans, apricots, plums, turnips, peppers, strawberries, tomatoes and broccoli.

[c]Chi-square test.

[d]t-test.

[e]Non-parametric Wilcoxon's test for two independent samples.

[f]Total red meat: summary measure of beef steak, hamburger, pork chops, veal chop/cutlet, cooked ham (prosciutto cotto), smoked ham (prosciutto crudo), cured ham (speck), salami, baloney (mortadella), wurstel, salted sliced beef, coppa, pancetta and other types of processed meats.

Table II. ORs[a] and 95% CIs for lung cancer by quintiles[b] of dietary intake, EAGLE

Quintile	Case/ control	Q1	Case/ control	Q2	Case/ control	Q3	Case/ control	Q4	Case/ control	Q5	*P*-trend
Food group											
Total fruit and vegetable[c]											
All	477/399	1.0 (ref)	407/398	0.93 (0.73–1.2)	335/399	0.86 (0.68–1.1)	309/398	0.85 (0.66–1.1)	294/397	0.70 (0.54–0.90)	0.007
Female	97/90	1.0 (ref)	91/90	1.1 (0.66–1.8)	55/90	0.74 (0.43–1.3)	67/90	0.89 (0.52–1.4)	55/89	0.85 (0.49–1.5)	0.38
Male	380/309	1.0 (ref)	316/308	0.88 (0.67–1.1)	280/309	0.89 (0.68–1.2)	242/308	0.84 (0.63–1.1)	239/308	0.67 (0.50–0.90)	0.01
Total fruits[d]											
All	459/399	1.0 (ref)	407/398	1.1 (0.87–1.4)	385/399	1.1 (0.86–1.4)	286/398	0.82 (0.63–1.0)	281/397	0.79 (0.61–1.0)	0.01
Female	98/90	1.0 (ref)	82/90	1.1 (0.66–1.8)	77/90	1.1 (0.66–1.9)	54/90	0.84 (0.49–1.4)	51/89	0.86 (0.50–1.5)	0.38
Male	361/309	1.0 (ref)	325/308	1.1 (0.84–1.4)	308/309	1.1 (0.83–1.4)	232/308	0.82 (0.62–1.1)	230/308	0.76 (0.57–1.0)	0.02
Total vegetables[e]											
All	435/399	1.0 (ref)	398/398	1.0 (0.82–1.3)	347/399	0.87 (0.68–1.1)	341/398	0.93 (0.73–1.2)	301/397	0.76 (0.59–0.99)	0.03
Female	95/90	1.0 (ref)	78/90	0.89 (0.52–1.4)	62/90	0.76 (0.45–1.3)	65/90	0.74 (0.43–1.3)	65/89	0.75 (0.44–1.3)	0.24
Male	340/309	1.0 (ref)	320/308	1.1 (0.84–1.4)	285/309	0.92 (0.70–1.2)	276/308	1.0 (0.78–1.4)	236/38	0.78 (0.58–1.0)	0.10
Quercetin-rich foods[f,g]											
All	496/399	1.0 (ref)	408/398	0.89 (0.70–1.1)	336/398	0.72 (0.56–0.93)	297/398	0.68 (0.52–0.90)	263/397	0.47 (0.35–0.64)	<0.001
Female	102/89	1.0 (ref)	76/90	0.80 (0.47–1.4)	64/90	0.81 (0.46–1.4)	78/90	1.1 (0.60–2.0)	42/89	0.53 (0.27–1.0)	0.24
Male	394/309	1.0 (ref)	332/308	0.91 (0.69–1.2)	272/308	0.70 (0.52–0.94)	219/308	0.59 (0.43–0.82)	221/308	0.45 (0.32–0.63)	<0.001

[a]All models adjusted for age, gender, area of residence, education, body mass index, alcohol consumption, total red meat intake (continuous), cigarette intensity in packs per day (continuous, 0 for non-smokers), duration of cigarettes smoking (continuous, 0 for non-smokers) and years since last cigarette smoked (former smokers only).
[b]Quintiles of intake: sex specific, based on the distribution of controls.
[c]Total fruits and vegetables: total vegetables + total fruits.
[d]Total fruits: summary measure of apples, pears, bananas, kiwis, oranges/grapefruits, mandarins/clementines, grapes, peaches/clingstones, apricots, plums, strawberries, melons and fruit cocktails.
[e]Total vegetables: summary measure of tomatoes, peppers, carrots, salad, peas, beans/chick peas, mushrooms, broccoli, turnips, savoy, black cabbage, onions, cooked spinach/Swiss chard/beets/rabes, cooked eggplants/zucchini/string beans, artichokes/fennel and beets.
[f]Quercetin-rich foods: summary measure of apples, grapes, onions, artichoke/fennel/celery, beans, apricots, plums, turnips, peppers, strawberries, tomatoes and broccoli.
[g]OR[a] additionally adjusted for non-quercetin-rich foods.

expression of key metabolic genes in human lung tissues and suggests a possible mechanism for the protective effect of quercetin-rich food consumption against lung cancer risk. Importantly, the metabolic genes affected by quercetin intake are key regulators of the metabolism of tobacco carcinogens, suggesting an interplay between quercetin intake, tobacco smoking and risk of lung cancer.

The vast epidemiological evidence showing that fruit intake lower the risk of lung cancer is convincing, whereas the evidence is not consistent for vegetables (2). Recently, the National Institutes of Health–American Association of Retired Persons prospective cohort study in the USA reported no relationship between combined fruits and vegetables or either fruits or vegetables alone and lung cancer risk (37). However, high intake of foods belonging to the Rosacea botanical family, which included some quercetin-rich foods, but not all, reduced the risk of lung cancer (37).

The current literature on the relationship between quercetin-rich foods and lung cancer risk is limited and equivocal. Our data corroborate the results from prospective cohort studies (14,15) and some case–control studies (16,17), but not others (18,19). The two prospective cohort studies were conducted in Finland. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (14), including only smokers, and the Finnish Mobile Clinic Health Examination (15) showed statistically significant 44 and 58% lower risks of lung cancer comparing highest versus lowest quartile of intake, respectively. Our finding of an inverse association among ever smokers corroborates recent results from a population-based case–control study in the USA that found a 37% lower risk of lung cancer among tobacco smokers but no relationship among never smokers (17). The two studies showing discrepant results were either very small (103 cases/206 controls) (18) or relied on proxy interview in 30% of the cases (19). The latter study, however, although did not find an association for total quercetin intake, did observe a protective association with quercetin-rich onions.

Quercetin has been observed to inhibit carcinogen-induced tumors in rats (11) and mice (10) and cell proliferation in human lung cancer cells (38). The mechanisms by which quercetin exerts anticarcinogenic properties are multi-fold (8) and have been shown in both animal and experimental studies. Of interest, quercetin may prevent carcinogenesis by inhibiting expression of P450 enzymes (26) and has been shown to inhibit hepatic *CYP1A1* in rats (39). Furthermore, experimental studies showed that quercetin also inhibited B(*a*)P-induced DNA damage in human Hep G2 cells by altering *CYP1A1* gene expression (20). There are some data showing that quercetin may influence gene expression of GST enzymes, although it is unclear whether quercetin induces (26) or inhibits *GSTP1* expression (40).

The precise mechanism by which quercetin influences metabolic gene expression is speculative. It has been suggested that quercetin competes with PAHs-like B(*a*)P for binding to the aryl hydrocarbon receptor, a transcription factor that regulates expression of the *CYP1* family, including *CYP1A1*, *CYP1A2* and *CYP1B1* (26). These genes are involved in activating tobacco-related procarcinogens into carcinogenic metabolites (41). For Phase II genes, quercetin may interact with the antioxidant-responsive element, a promoter factor, and mediate the induction of Phase II genes, like *GST*s (9,42). In a previous study by our group, we observed an upregulation of gene expression with polymorphisms of *CYP1A1* and *CYP1B1* in current smokers (30). In the present study, we found that frequent dietary intake of quercetin resulted in an upregulation of several *GST* genes, including *GSTM1*, *GSTM2*, *GSTM3* and *GSTP1* as well as a downregulation of several *P450* genes in human non-tumor lung tissues. If confirmed, this finding may illustrate a mechanism of quercetin-related protection against tobacco-induced lung carcinogenesis.

There is evidence that variants of metabolic genes may modulate the association between specific dietary constituents, particularly crucifer-derived isothiocyanates, and lung cancer risk (43). With respect to quercetin, Le Marchand *et al.* previously reported on the modifying effect of *CYP1A1 MspI* (*CYP1A1*2A*) polymorphism on the association between onions and lung cancer in 72 cases and 453 controls (19). We extended the quercetin-gene interaction investigation beyond the

Table III. ORs[a] and 95% CIs for lung cancer risk by smoking status and histological subtypes, EAGLE

	Quintile[b] of dietary intake					
	Q1	Q2	Q3	Q4	Q5	*P*-trend
Smoking status						
Ever smokers (n = 3061)						
Total fruit and vegetable[c]	1.0 (ref)	0.92 (0.72–1.2)	0.87 (0.68–1.1)	0.84 (0.64–1.1)	0.75 (0.57–0.98)	0.03
Total fruits[d]	1.0 (ref)	1.1 (0.82–1.4)	1.0 (0.81–1.3)	0.80 (0.61–1.0)	0.76 (0.58–1.0)	0.01
Total vegetables[e]	1.0 (ref)	1.1 (0.83–1.4)	0.93 (0.71–1.2)	0.95 (0.73–1.2)	0.86 (0.65–1.1)	0.19
Quercetin-rich foods[f]	1.0 (ref)	0.93 (0.72–1.2)	0.69 (0.53–0.91)	0.67 (0.49–0.90)	0.46 (0.34–0.64)	<0.001
Never smokers (n = 742)						
Total fruit and vegetable[c]	1.0 (ref)	0.82 (0.41–1.6)	0.63 (0.31–1.3)	0.81 (0.41–1.6)	0.39 (0.18–0.84)	0.04
Total fruits[d]	1.0 (ref)	1.3 (0.62–2.7)	1.3 (0.61–2.6)	0.84 (0.39–1.8)	0.91 (0.43–1.9)	0.38
Total vegetables[e]	1.0 (ref)	0.75 (0.38–1.5)	0.50 (0.25–1.0)	0.77 (0.40–1.5)	0.31 (0.14–0.66)	0.008
Quercetin-rich foods[f]	1.0 (ref)	0.61 (0.30–1.2)	0.91 (0.45–1.9)	0.70 (0.32–1.5)	0.46 (0.19–1.2)	0.23
Histology						
Adenocarcinoma (n = 740)						
Total fruit and vegetable[c]	1.0 (ref)	1.0 (0.78–1.4)	0.96 (0.71–1.3)	0.78 (0.57–1.1)	0.75 (0.55–1.0)	0.02
Total fruits[d]	1.0 (ref)	1.3 (0.96–1.7)	1.2 (0.87–1.6)	0.91 (0.67–1.2)	0.82 (0.59–1.1)	0.06
Total vegetables[e]	1.0 (ref)	1.1 (0.86–1.5)	0.91 (0.67–1.2)	1.1 (0.82–1.5)	0.68 (0.49–0.95)	0.06
Quercetin-rich foods[f]	1.0 (ref)	0.92 (0.69–1.2)	0.74 (0.54–1.0)	0.60 (0.42–0.85)	0.46 (0.32–0.67)	<0.001
Squamous cell carcinoma (n = 470)						
Total fruit and vegetable[c]	1.0 (ref)	1.1 (0.73–1.5)	0.91 (0.62–1.4)	1.0 (0.69–1.6)	0.99 (0.67–1.5)	0.93
Total fruits[d]	1.0 (ref)	1.2 (0.82–1.7)	1.2 (0.79–1.7)	0.84 (0.56–1.3)	0.84 (0.56–1.3)	0.18
Total vegetables[e]	1.0 (ref)	1.2 (0.81–1.7)	1.1 (0.72–1.6)	1.1 (0.76–1.7)	1.2 (0.77–1.7)	0.57
Quercetin-rich foods[f]	1.0 (ref)	0.86 (0.58–1.3)	0.67 (0.44–1.0)	0.88 (0.57–1.4)	0.43 (0.27–0.70)	0.004
Small cell carcinoma (n = 189)						
Total fruit and vegetable[c]	1.0 (ref)	0.76 (0.46–1.3)	0.87 (0.51–1.5)	0.94 (0.54–1.6)	0.59 (0.32–1.1)	0.24
Total fruits[d]	1.0 (ref)	0.77 (0.46–1.3)	0.98 (0.59–1.6)	0.62 (0.34–1.1)	0.77 (0.44–1.3)	0.24
Total vegetables[e]	1.0 (ref)	0.86 (0.51–1.5)	1.2 (0.74–2.0)	0.72 (0.40–1.3)	0.92 (0.53–1.6)	0.64
Quercetin-rich foods[f]	1.0 (ref)	0.90 (0.53–1.5)	0.95 (0.54–1.7)	0.91 (0.48–1.7)	0.52 (0.26–1.0)	0.13

[a]All models adjusted for age, gender, area of residence, education, body mass index, alcohol consumption (continuous), total red meat intake (continuous), cigarette intensity in packs per day (continuous, 0 for non-smokers), duration of cigarettes smoking (continuous, 0 for non-smokers), years since last cigarette smoked (continuous, 0 for non-smokers and current smokers) and passive smoking (work and home); For quercetin-rich foods: additionally adjusted for total intake of non-quercetin-rich foods (continuous).
[b]Quintile of intake: sex specific, based on distribution of the controls for each gender.
[c]Total fruits and vegetables: total vegetables + total fruits.
[d]Total fruits: summary measure of apples, pears, bananas, kiwis, oranges/grapefruits, mandarins/clementines, grapes, peaches/clingstones, apricots, plums, strawberries, melons and fruit cocktails.
[e]Total vegetables: summary measure of tomatoes, peppers, carrots, salad, peas, beans/chick peas, mushrooms, broccoli, turnips, savoy, black cabbage, onions, cooked spinach/Swiss chard/beets/rabes, cooked eggplants/zucchini/string beans, artichokes/fennel and beets.
[f]Quercetin-rich foods: summary measure of apples, grapes, onions, artichoke/fennel/celery, beans, apricots, plums, turnips, peppers, strawberries, tomatoes and broccoli.

single polymorphism of *CYP1A1* to include additional variants of *CYP450* and *GST* genes in a much larger population. In our study, gene variants were not associated with lung cancer risk after accounting for multiple comparisons using Bonferroni correction. We note that this correction is conservative and may lead to false negative results (44). Without this adjustment, we observed a suggestive gene–quercetin interaction for *CYP1B1_18* (rs10175368; $P\text{-value}_{lrt}$ = 0.01). The gene–diet analyses as well as the smoking- and histology-stratified results did not replicate Le Marchand's results. The effect of high dietary intake of quercetin-rich foods on P450s and GSTs activities, lowering their ability to biotransform procarcinogens to carcinogenic electrophiles and increasing xenobiotic elimination, respectively, may overcome the effect of individual variants of these metabolic genes.

Although this present study hypothesizes on one possible mechanism by which quercetin may exert its anticarcinogenic properties against lung cancer risk by influencing expression of *P450* and *GST* genes, additional mechanisms have been proposed to account for the putative anticarcinogenic effect of quercetin, including scavenging free radicals (45,46), inhibiting proliferation by via cell cycle arrest (47) and apoptosis (38,48).

The findings of beneficial effects with a high quercetin-rich diet could also be attributed to other dietary components found in fruits and vegetables, such as isothiocyanates (found in cruciferous vegetables). In a recent meta-analysis, consumption of cruciferous vegetables was associated with lower risk of lung cancer (6). In our study, intake of cruciferous vegetables was not statistically associated with lung cancer risk (supplementary Table 5 is available at *Carcinogenesis* Online) possibly because there was a limited consumption of these dietary components in this population. Several factors suggest that quercetin may be an independent protective factor in lung cancer etiology. In the present study, the analyses for quercetin-rich foods were adjusted for other fruits and vegetables; moreover, the effective size observed for quercetin-rich foods compared with the findings for combined fruits and vegetables, as well as fruits and vegetables separately, was stronger and persisted for both men and women as well as across histological subtypes.

Study limitations include the possibility of recall bias due to the case–control study design, although the rapid recruitment protocol that allowed study enrollment and interview at the time of the diagnosis and not when the patients were in terminal conditions was designed to minimize such issues. Dietary data derived from FFQs are subject to measurement errors that may be random or systematic (49). Moreover, because the FFQ in the EAGLE study was targeted to obtain information on specific foods, categories of interest were limited in scope and did not include portion size. The lack of information on portion size limited our assessment of quercetin intake to frequency of quercetin-rich foods consumption and not quercetin intake. Additionally, we were unable to calculate and adjust for total energy intake. Energy adjustment, although not perfect for addressing measurement error in FFQs, has been shown to be a reasonable method by some (50), whereas others have proposed adjustment for body weight and physical activity

Table IV. Lung cancer risk associated with highest versus lowest intake of quercetin[a]-rich foods, by P450 and GST genotypes in EAGLE

Gene (rs #)	Quercetin[a]-rich foods OR[b], (95% CI)				Additive interaction			Multiplicative interaction
	Case/control	Lowest[c]	Case/control	Highest[d]	RERI[e] (LL, UL)[f]	AP[g] (LL, UL)[f]	*S*[h] (LL, UL)[f]	*P*-value$_{lrt}$[i]
CYP450s								
CYP1A1_14 (rs2606345)								
GG	205/154	1.0 (ref)	228/311	0.50 (0.34–0.72)	0.19	0.40	0.74	
GT + TT	268/227	0.79 (0.57–1.1)	207/447	0.47 (0.33–0.68)	(−0.10, 0.48)	(−0.28, 1.1)	(0.50, 1.1)	0.41
CYP1A1_78 (rs2198843)								
GG	330/259	1.0 (ref)	363/526	0.50 (0.37–0.69)	0.27	0.58	0.66	
CG+CC	142/122	0.69 (0.49–0.98)	172/234	0.47 (0.32–0.67)	(−0.01, 0.55)	(−0.06, 1.2)	(0.47, 0.95)	0.20
CYP1A1_81 (rs2472299)								
CC	215/192	1.0 (ref)	257/340	0.67 (0.47–0.96)	−0.34	−0.57	5.8	
CT+TT	257/191	1.23 (0.92–1.7)	277/421	0.59 (0.42–0.83)	(−0.78, 0.11)	(−1.3, 0.14)	(0.0, 8293)	0.11
CYP1A1_114 (rs2470893)								
GG	294/233	1.0 (ref)	328/480	0.54 (0.39–0.75)	0.02	0.03	0.96	
GT+TT	179/149	1.0 (0.74–1.4)	205/277	0.59 (0.41–0.84)	(−0.35, 0.39)	(−0.59, 0.65)	(0.41, 2.2)	0.76
CYP1A2_79 (rs11072508)								
AA	170/152	1.0 (ref)	205/272	0.64 (0.43–0.94)	−0.20	−0.35	2.0	
AB+BB	302/230	1.2 (0.84–1.6)	327/486	0.59 (0.41–0.85)	(−0.63, 0.22)	(−1.0, 0.32)	(0.20, 20.5)	0.39
CYP1B1_07 (rs1800440)								
AA	288/247	1.0 (ref)	344/463	0.63 (0.46–0.88)	−0.33	−0.60	3.5	
AG+GG	185/135	1.2 (0.89–1.7)	188/296	0.54 (0.38–0.77)	(−0.78, 0.12)	(−1.4, 0.21)	(0.08, 147.9)	0.12
CYP1B1_18 (rs10175368)								
GG	252/183	1.0 (ref)	264/411	0.42 (0.30–0.60)	0.41	0.80	0.55	
GA+AA	220/199	0.68 (0.49–0.93)	271/347	0.51 (0.36–0.72)	(0.17, 0.66)	(0.23, 1.4)	(0.40, 0.74)	0.01
CYP1B1_27 (rs162556)								
CC	129/128	1.0 (ref)	170/210	0.75 (0.49–1.2)	−0.41	−0.65	−7.5	
CT+TT	341/253	1.3 (0.92–1.8)	361/546	0.64 (0.43–0.94)	(−0.94, 0.11)	(−1.4, 0.07)	—	0.07
CYP1B1_31 (rs162562)								
CC	336/284	1.0 (ref)	376/539	0.55 (0.40–0.76)	−0.03	−0.05	1.1	
CT+TT	136/98	1.1 (0.75–1.5)	158/222	0.59 (0.41–0.84)	(−0.39, 0.33)	(−0.65, 0.55)	(0.41, 2.8)	0.96
CYP1B1_42 rs162557)								
AA	333/273	1.0 (ref)	358/517	0.54 (0.40–0.75)	0.04	0.07	0.91	
AC+CC	141/110	0.99 (0.70–1.4)	176/243	0.57 (0.40–0.82)	(−0.34, 0.42)	(−0.58, 0.73)	(0.41, 2.0)	0.81
GSTs								
GSTA1_01 (rs3957357)								
TT	156/118	1.0 (ref)	161/251	0.41 (0.27–0.63)	0.30	0.65	0.46	
TC + CC	317/260	0.75 (0.53–1.1)	371/506	0.46 (0.31–0.68)	(0.53,1.1)	(−0.70, 2.0)	(0.32, 1.3)	0.08
GSTA4_05 (rs1051535)								
CC	148/123	1.0 (ref)	157/232	0.57 (0.37–0.85)	−0.01	−0.02	1.0	
CA+AA	326/259	0.96 (0.68–1.36)	376/528	0.52 (0.36–0.77)	(−0.4, 0.37)	(−0.74, 0.70)	(0.45, 2.3)	0.80
GSTM3_01 (rs7483)								
GG	258/192	1.0 (ref)	262/384	0.52 (0.37–0.73)	0.14	0.27	0.78	
GA+AA	205/187	0.86 (0.62–1.2)	251/362	0.51 (0.36–0.73)	(−0.17, 0.45)	(−0.36, 0.90)	(0.48, 1.3)	0.37
GSTM3_02 (rs1799735)								
+/+	312/265	1.0 (ref)	381/496	0.60 (0.44–0.82)	−0.15	−0.31	1.4	
+/− and −/−	162/117	1.0 (0.74–1.5)	154/263	0.49 (0.34–0.70)	(−0.54, 0.25)	(−1.1, 0.49)	(0.46, 4.3)	0.28
GSTM3_06 (rs1537234)								
TT	188/143	1.0 (ref)	183/305	0.50 (0.34–0.73)	0.08	0.50	0.84	
GT + GG	284/234	1.0 (0.70–1.4)	347/451	0.57 (0.40–0.82)	(−0.75, 0.91)	(−1.3, 1.6)	(0.17, 4.3)	0.48
GSTP1_01 (rs1695)								
AA	236/172	1.0 (ref)	258/386	0.45 (0.32–0.65)	0.26	0.47	0.63	
AG+GG	238/210	0.84 (0.61–1.2)	274/372	0.56 (0.39–0.79)	(−0.02, 0.55)	(−0.10, 1.0)	(0.42, 0.96)	0.08

[a]Total quercetin-rich foods: summary measure of apples, grapes, onions, artichoke/fennel/celery, beans, apricots, plums, turnips, peppers, strawberries, tomatoes and broccoli.
[b]All models adjusted for age, gender, area of residence, education, body mass index, alcohol consumption, total red meat intake (continuous), total intake of non-quercetin-rich fruits and vegetables (continuous), cigarette intensity in packs per day (continuous, 0 for non-smokers), duration of cigarettes smoking (continuous, 0 for non-smokers) and years since last cigarette smoked.
[c]Lowest—first quintile.
[d]Highest—fourth and fifth quintile.
[e]RERI, relative excess risk due to interaction.
[f]Lower limit (LL) and upper limit (UL) using the delta method.
[g]AP, attributable proportion due to interaction.
[h]S, synergy index.
[i]*P*-value$_{lrt}$, *P*-value likelihood ratio test.

as more appropriate methods (51). Although we adjusted for body mass index, used sex-specific quintile of quercetin-rich food intake and conducted analyses stratified by sex, we cannot totally exclude residual measurement error, as in all dietary studies.

Cigarette smoking has been correlated with a less healthy lifestyle, including higher alcohol consumption, poor diet and lower socioeconomic status (52). The extensive data available in our study enabled rigorous control for cigarette smoking, alcohol and other factors in the

Table V. Association between consumption of quercetin-rich foods and gene expression in lung tissues, EAGLE

Gene Symbol	Probe	Unadjusted			Adjusted[a]		
		Coefficient	Fold change	*P*-value	Coefficient	Fold change	*P*-value
CYP1A1	205749_at	−0.42	0.84	**0.04**	−0.40	0.85	0.21
CYP1A2	207608_x_at	−0.20	0.92	0.13	−0.38	0.86	**0.03**
CYP1A2	207609_s_at	−0.07	0.97	0.33	−0.13	0.95	0.25
CYP1B1	202437_s_at	−0.84	0.71	0.06	−0.31	0.88	0.52
CYP1B1	202436_s_at	−0.85	0.71	**0.02**	−0.39	0.85	0.26
CYP1B1	202435_s_at	−0.76	0.74	**0.01**	−0.46	0.83	0.21
CYP1B1	202434_s_at	−0.24	0.91	**0.02**	−0.34	0.87	**0.04**
GSTA1	215766_at	−0.12	0.95	0.20	−0.18	0.93	0.20
GSTA2	203924_at	0.19	1.08	0.52	0.19	1.08	0.70
GSTA3	222102_at	−0.24	0.91	0.08	−0.47	0.83	**0.03**
GSTA4	202967_at	0.09	1.04	0.67	0.38	1.17	0.24
GSTK1	217751_at	0.26	1.11	0.08	0.38	1.17	0.12
GSTM1	204550_x_at	0.41	1.18	**0.01**	0.52	1.23	**0.04**
GSTM1	215333_x_at	0.26	1.11	0.08	0.31	1.13	0.21
GSTM2	204418_x_at	0.54	1.24	**0.00**	0.71	1.33	**0.02**
GSTM3	202554_s_at	0.42	1.19	**0.03**	0.46	1.20	0.15
GSTM4	210912_x_at	0.00	1.00	0.99	−0.12	0.95	0.21
GSTM4	204149_s_at	0.04	1.02	0.56	0.09	1.04	0.47
GSTM5	205752_s_at	0.18	1.07	0.10	−0.12	0.95	0.45
GSTO1	201470_at	0.16	1.07	0.33	0.18	1.07	0.52
GSTP1	200824_at	0.43	1.19	**0.00**	0.46	1.21	**0.03**
GSTT1	203815_at	−0.28	0.89	0.53	−1.01	0.66	0.17
GSTT2	205439_at	0.41	1.18	**0.02**	0.68	1.32	**0.02**
GSTZ1	209531_at	0.12	1.05	0.35	0.23	1.10	0.25

[a]Adjusted for age (<median, ≥median), sex and smoking status (current, former and never smokers). Bolded *P*-values: statistically significant.

analyses, although residual confounding can never be completely ruled out. Finally, red wine is a rich source for quercetin, whereas white wine is not (29). The EAGLE's FFQ did not collect information on red and white wine consumption separately; thus, we could not include red wine consumption as part of the summary measure for quercetin-rich foods. However, we verified that consumption of wine overall did not modify the association between quercetin-rich food and lung cancer risk by adjusting for total wine consumption. This suggested that, if any, the effect of quercetin contained in wine was modest.

To our knowledge, our study examined the largest combination of SNPs in *P450* genes and the first to examine the role of *GST* polymorphisms in the relationship between dietary quercetin and lung cancer risk. However, there are other plausible candidate genes that could be explored, e.g. genes involved in glucuronidation and sulfation, which may lead to novel findings in future studies. Lastly, the microarray expression results were based on a small sample of adenocarcinoma cases only. Moreover, mRNA expression may not predict protein expression levels due to posttranscriptional and posttranslational modifications as well as other factors. Therefore, this finding requires confirmation in a larger population with protein expression data.

Our study has several strengths. It is a large population-based case–control study with high participation rates and detailed information on smoking history as well as many other risk factors. The large sample size permitted investigation by histological subtypes and smoking status with adequate power. The comprehensive genotype data on metabolic genes permitted selection of specific candidate genes for an investigation of gene–diet interaction that extends beyond previous studies on one or a couple of genes. Additional data on mRNA expression from human lung tissues enabled an investigation into the influence of dietary quercetin on expression of metabolic genes. And lastly, cases were rapidly ascertained and surrogate participants were not needed.

In conclusion, higher frequencies of intake of quercetin-rich foods were associated with lower risk of lung cancer in this Italian population. The inverse association did not differ by histological subtypes, were stronger among heavy smokers, and was not affected by variants of *P450* and *GST* genes. Downregulation of several *P450* genes and upregulation of *GST* genes involved in the metabolism of tobacco carcinogens were observed in human lung tissues of subjects consuming high quercetin-rich foods. This finding provides potential provocative mechanistic insights into the role of dietary quercetin in tobacco-induced lung carcinogenesis. Further studies exploring this relationship are warranted.

Supplementary material

Supplementary Tables 1–5 can be found at http://carcin.oxfordjournals.org/

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