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Genetic susceptibility and dietary patterns in lung cancer

Ya-Yu Tsai^a, Katherine A. McGlynn^b, Ying Hu^a, Anna B. Cassidy^c,
John Arnold^c, Paul F. Engstrom^c, Kenneth H. Buetow^{a,*}

^a *Laboratory of Population Genetics, Center for Cancer Research, National Cancer Institute, NIH, DHHS, Bethesda, MD, USA*

^b *Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, Bethesda, MD, USA*

^c *Division of Population Science, Fox Chase Cancer Center, Philadelphia, PA, USA*

Received 28 November 2002; received in revised form 3 April 2003; accepted 8 April 2003

KEYWORDS

Dietary patterns;
Lung cancer;
Genetic polymorphism;
Detoxification enzymes;
Cluster analysis

Summary Cigarette smoking is the dominant risk factor for lung cancer, but only a minority of smokers ever develops tumors. Though genetic susceptibility is likely to explain some of the variability in risk, results from previous studies of genetic polymorphisms have been inconclusive. As diet may also affect the risk of lung cancer, it is possible that the degree of risk produced by smoking and genetic susceptibility varies, depending on diet. To assess this hypothesis, we conducted a case-control study to examine the effect of cigarette smoking, dietary patterns and variation in genes involved in phase II metabolism. A total of 254 individuals with lung cancer and 184 healthy controls were recruited for the study. To identify persons with similar dietary patterns, cluster analysis was performed using nutrient densities of four major dietary constituents: protein, carbohydrate, animal fat, and dietary fiber. Two groups of individuals were identified with distinct dietary patterns: (1) a group ($n = 2241$) with a high intake of animal fat and protein and a low intake of carbohydrates and dietary fiber (the 'healthy' pattern) (2) a group ($n = 197$) with a high intake of fiber and carbohydrate and a low intake of protein and animal fat (the 'unhealthy' pattern). On stratified analysis, several genotype/dietary pattern combinations were found to affect risk of lung cancer. Smokers who were not homozygous for the most common GSTP1 allele and had a healthy dietary pattern were at significantly lower risk than smokers who were homozygous for the GSTP1 common allele and who had an unhealthy dietary pattern (OR = 0.16, 95%CI: 0.04–0.57). Among smokers who were GSTM1 null, persons with a healthy dietary pattern were at lower risk than persons with an unhealthy dietary pattern (OR: 0.46, 95%CI: 0.21–1.01). Among smokers with an unhealthy dietary patterns, persons with a His/His genotype in the exon 3 polymorphism of EPHX1 were at significantly lower risk than persons who were not homozygous. These data suggest that dietary factors may affect the risk imposed by genetic susceptibility at detoxification loci. Adjustments using dietary pattern may be useful in elucidating the effects of polymorphisms in genes responsible for carcinogen metabolism.

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*Corresponding author. Address: Laboratory of Population Genetics, NCI Center for Bioinformatics, NIH, 6116 Executive Boulevard, Suite 403, Room 4001, Bethesda, MD 20814-9692, USA. Tel.: +1-301-435-1520; fax: +1-301-480-4222.

E-mail address: buetowk@pop.nci.nih.gov (K.H. Buetow).

1. Introduction

In the US, lung cancer is the third most commonly diagnosed cancer and the single greatest contributor to cancer mortality [1]. While cigarette smoking is the dominant risk factor for lung cancer, only 15% of smokers will ever develop a tumor [2]. This lack of uniformity in risk could be related to genetic susceptibility, or the effects of other risk factors, or both. A number of previous studies have examined genetic susceptibility to lung cancer, but the results have been inconsistent [3]. Of particular interest in these studies have been polymorphisms in the phase I (cytochrome p450 enzymes) and phase II (epoxide hydrolases, glutathione *S*-transferases, *N*-acetyltransferases) detoxification loci because of the roles they play in metabolizing the carcinogens in cigarette smoke.

Epidemiologic studies indicate that dietary factors may also play a role in lung cancer etiology [2]. A protective role has been suggested for fruits and vegetables overall [4–7], as well as for micronutrients such as β -carotene, vitamin C, vitamin E and selenium [8,9]. In addition, dietary fat, animal fat, saturated fat and cholesterol have been associated with increased risk of lung cancer in some, but not all studies [2,10]. The discrepant results from diet studies may be due to inter-individual variability in nutrient metabolism. In particular, many dietary constituents can act as inducing agents or substrates for the phase I and II enzymes. For example, brassica vegetables are effective at inducing glutathione *S*-transferase activity of subclasses α and μ [11]. Further, lower consumption of isothiocyanates, non-nutrient compounds in cruciferous vegetables with anti-carcinogenic properties, has been associated with elevated lung cancer risk in current smokers with null genotypes at both *GSTT1* and *GSTM1* [12,13]. In addition, it has been suggested that while male smokers with a *GSTM1* null genotype may have a greater risk of lung cancer than smokers with non-null genotypes, the risk can be attenuated by α -tocopherol supplementation [14]. These studies would suggest that, minimally, it may be important to consider micronutrient intake when examining the relationship between polymorphisms in the phase I and II loci and lung cancer. However, as a number of dietary studies have reported associations between various other dietary constituents and lung cancer risk, it may also be important to include a larger variety of dietary variables. Nutrition studies, though, often have difficulty in discriminating the effects of specific nutrients, since the food sources of the various nutrients are frequently the same. Strong correlations between

nutrients (colinearity) result in unstable relationships, and may cause attenuation of diet-disease associations if these elements are simultaneously entered into the same model. Using cluster analysis to define dietary patterns, however, may turn this colinearity into an analytical advantage. Cluster analysis has previously been used to identify groups of individuals with similar patterns of food energy consumption and, more recently, to classify genes with related function or expression in microarray assays [15–19]. Cluster analysis creates non-overlapping easily interpretable categories and potentially allows identification of subgroups with specific dietary behaviors. Examining dietary patterns rather than specific nutrients may have an advantage in that many epidemiological studies have provided evidence that dietary or lifestyle patterns, rather than individual constituents, play a role in cancer prevention [20]. In addition, the approach is well-suited to studies that lack the statistical power to examine a large number of dietary factors separately. Consequently, using dietary patterns identified by cluster analysis presents a viable approach to examining the effects of polymorphisms on cancer risk, while adjusting for diet. We utilized this approach in a case-control study which jointly examine the effects of 15 polymorphisms in 14 genes and dietary pattern on the risk of lung cancer.

2. Materials and methods

2.1. Study population

A total of 254 individuals with lung cancer (cases) and 184 healthy individuals (controls) were recruited for the study. The cases were recruited from among all newly diagnosed lung cancer patients seen at Fox Chase Cancer Center and at community hospitals of the Fox Chase Cancer Center Network (Community Regional Cancer Center, Toms River, NJ; Virtua Memorial Hospital, Mt. Holly, NJ; Hunterdon Regional Cancer Center, Flemington, NJ; St. Francis Medical Center, Trenton, NJ) over a twelve month period (April 1995–March 1996). In order to participate, cases were asked to sign an informed consent document, donate a 20-ml venous blood sample and complete validated questionnaires concerning cigarette smoking, dietary intake and family history of cancer and/or lung diseases. The blood sample was collected prior to the initiation of therapy whenever possible. Controls were recruited from among individuals attending cancer screening clinics or wellness programs at the same hospitals.

Individuals with a prior diagnosis of lung cancer, mesothelioma or chronic obstructive pulmonary disease were ineligible to participate as controls. No exclusions were made based on sex or ethnicity. In order to participate, controls were asked to sign an informed consent document and donate a 20-ml venous blood sample. In addition, control participants completed validated study questionnaires concerning cigarette smoking habits, dietary intake and family history of cancer and/or lung diseases. The 61-item semi-quantitative food frequency questionnaire has been previously described in detail and its validity and reproducibility have been reported [21,22].

Pack-years of tobacco use were calculated as the product of the average number of packs per day (i.e. number of cigarettes per day divided by 20) and the total number of years smoked. Individuals were categorized into non-smokers, moderate smokers (lower two quartiles), and heavy smokers (upper two quartiles), according to the quartiles specified by reported pack-years of smoking. Individuals who reported extreme calorie intakes (< 750 or > 3500 kcal) were excluded from the analysis.

Height and weight of all participants was obtained by self-report. Cases were asked to report their usual height and weight 1-year prior to their diagnosis. Controls were asked to report their usual height and weight 1 year prior to agreeing to participate in the study. Body mass index (BMI) was calculated as the ratio of weight in kilograms to height in meters squared.

The 20 ml whole blood specimens were separated into components and divided into multiple aliquots. Nucleic acid preparation was performed utilizing a salt extraction protocol. This research was approved by the Institutional Review Boards of all participating hospitals.

2.2. Lung cancer susceptibility genes

A total of 15 variants in 14 genes involved in phase II metabolism were evaluated in this study (Appendix A). These genes were members of four families: (1) Epoxide hydrolases (EPHX): epoxide hydrolase 1, exon 3 (EPHX1-3E); epoxide hydrolase 1, exon 4 (EPHX1-4E); and epoxide hydrolase 2 (EPHX2); (2) Glutathione *S*-transferases (GST): glutathione *S*-transferase pi 1 (GSTP1); glutathione *S*-transferase theta 1 (GSTT1); glutathione *S*-transferase theta 2 (GSTT2); microsomal glutathione *S*-transferase 1 (MGST1); glutathione *S*-transferase alpha 1 (GSTA1); glutathione *S*-transferase alpha 4 (GSTA4); glutathione *S*-transferase mu 1 (GSTM1); glutathione *S*-transferase mu 2 (GSTM2); and glu-

tathione *S*-transferase mu 3 (GSTM3); (3) *N*-acetyltransferases (NAT): *N*-acetyltransferase 1 (NAT1); and *N*-acetyltransferase 2 (NAT2); (4) NAD(P)H: quinone oxidoreductase, exon 6 (NQO1Exon6). Variants used were either previously described in the literature, or obtained through data-mining publicly available sequence data utilizing the SNPpipeline of the NCI's CGAP Genetic Annotation Initiative [23].

Forward and reverse PCR primers flanking the candidate susceptibility polymorphisms were designed using the PRIMER program (obtained from the Whitehead Genome Center). Restriction fragment length polymorphism (RFLP) assays were designed for each polymorphism and evaluated. The results of the RFLP assays were validated by direct sequencing of DNA obtained from 10 parents of 5 Centre d'Etude du Polymorphisme Humain (CEPH) families (1331, 1332, 1347, 1362, 1413) representing 20 independent alleles.

PCR reactions were performed in a 5 µl final volume containing 20 ng DNA, 5 µM forward and reverse primers, 0.1 mM dNTPs, and 0.005 U AmpliTaq Gold (Perkin-Elmer, Boston, MA) in 10 × reaction (Perkin-Elmer) buffer. Following denaturation for 10 min at 95 °C, the reactions were cycled in a Peltier Thermal Cycler (PTC-225, MJ Research, Waltham, MA) 35 times at 94 °C for 30 s (denaturation), 64 °C for 30 s (annealing temperatures listed in Table 1), and 72 °C for 30 s (extension). This was followed by final extension for 10 min at 72 °C. Reactions were held at 15 °C. Restriction digests were performed using 5 µl of the PCR products, added to 3.5 µl H₂O, 1.0 µl 10 × buffer, and 0.5 µl restriction enzyme, and incubated 2 h at 37 °C. For most reactions, 2 µl of the total digest was added to 3 µl loading buffer and run in 2% NuSieve agarose (FMC, Philadelphia, PA) in 1 × TAE buffer for about 30 min., at 125 V. Gel bands were visualized by GelStar (FMC) staining and ultraviolet transillumination. Images were captured with a Kodak DC120 Zoom Digital Camera and the Electrophoresis Documentation and Analysis System 120 (Kodak Digital Science, Rochester, NY).

2.3. Statistical methods

To identify groups of individuals with similar dietary patterns, cluster analyses were performed using the nutrient densities of four nutrient groups: protein, carbohydrate, animal fat and dietary fiber. The nutrient density of each nutrient was calculated by dividing the intake of the nutrient (in grams) by total energy intake (in 1000 kcal). Ward's minimum-variance method was used, where the

Table 1 Demographic distributions of variables of interest and genotypes in cases and controls

Variables	Category	Case (%)	Control (%)	P-value
Sex	Male	127(50.00)	42(22.83)	< 0.0001
	Female	127(50.00)	142(77.17)	
Race	Caucasian	246(96.85)	180(97.83)	0.5370
	Others	8(3.15)	4(2.17)	
Smoking	Never	19(7.48)	90(48.91)	< 0.0001
	Light	55(21.65)	57(30.98)	
	Heavy	180(70.87)	37(20.11)	
Pack-years	54.74 ± 32.09	30.37 ± 25.54		< 0.0001
Age	63.13 ± 9.26	50.87 ± 14.79		< 0.0001
BMI	25.85 ± 4.98	26.49 ± 5.88		0.2273

distance between two clusters is the ANOVA sum of squares between the two clusters added up over all the variables [24]. At each generation, the within-cluster sum of squares is minimized over all partitions obtainable by merging two clusters from the previous generation. A pseudo *F*-statistic is used as the method of judging the best number of clusters. Relatively large values indicate a stopping point.

To verify that the groups identified by cluster analysis represented distinct dietary patterns, discriminant analysis was performed. Generalized squared distance was used to determine proximity, and the classification criteria were based on the individual within-group covariance matrices. The total population was divided into two groups for discriminant analyses: one third was randomly selected as the training sample, and the remainder was used as the test sample. The error-count estimate was calculated by applying the classification criterion derived from the training sample to the test sample, and then counting the number of misclassified observations. The relationship between actual and predicted group allocation was inspected using error-count estimates, and the percentage of subjects correctly classified by the clustering procedure was determined. Differences between clusters in total calorie consumption, intake of carbohydrate, protein, animal fat, dietary fibers, other dietary constituents, and BMI were examined using *t*-tests.

χ^2 -tests were used to examine the differences in distributions of genotypes between cases and controls. Associations between genotypes and lung cancer risk were examined using logistic regression models to calculate the odds ratios and 95% confidence intervals (CIs), adjusted for gender and age. Effect modifications of the genotypes

with dietary patterns were estimated by stratified analyses using the high-risk or the common genotype and the unhealthy dietary pattern as a common reference group. All analyses were performed using SAS statistical software, version 8.0 (SAS Institute, Cary, NC).

3. Results

There were 254 lung cancer cases and 184 controls in this study. Ninety-seven percent of the study population identified themselves as being of European ancestry. There were significantly more heavy smokers (70.9%) among the cases than the controls (20.1%, $P < 0.0001$). In addition, mean pack-years of smoking among cases were significantly greater than that among the controls (54.7 vs. 30.4, $P < 0.0001$). There was no significant difference in mean BMI between the cases and the controls (25.9 vs. 26.5; $P = 0.23$) (Table 1).

The genotypic distributions of the 15 polymorphisms in 14 loci were examined in the controls to assess Hardy–Weinberg equilibrium. All polymorphisms were in equilibrium. The polymorphism distributions were then contrasted in the cases and controls using χ^2 -tests. No significant differences between the cases and the controls were apparent (data not shown).

Cluster analysis identified two categorized non-overlapping dietary groups based on similarities in the consumption of the four macronutrients of interest: protein, carbohydrate, animal fat, and dietary fiber (data not shown). Discriminant analysis verified that the population was well discriminated by the clusters. The agreement between actual and predicted cluster allocation ranged from 91.8 to 95.5%. Due to the large value of the pseudo

F-statistic in cluster analysis and the low error rate generated by discriminant analysis, we classified the 438 individuals into two distinct dietary patterns using the four main dietary components. One group consisted of 241 individuals (151 cases and 90 controls) who consumed a relatively greater amount of animal fat and protein, and a smaller amount of carbohydrates and dietary fiber (referred to as the 'unhealthy' diet pattern). In contrast, 197 individuals (103 cases and 94 controls) consumed a 'healthy' diet pattern that was distinguished by a relatively greater intake of carbohydrates and dietary fiber and a lower intake of animal fat and protein. There was no significant difference between the two groups in intake of total kilocalories ($P = 0.73$). A borderline significant difference in BMI was observed ($P = 0.09$), with the individuals in the unhealthy diet group having a slightly higher BMI than those in the healthy diet group (26.6 vs. 25.6, respectively). Individuals in the unhealthy diet group were significantly more likely to smoke (82.6 vs. 66.0%, $P < 0.0001$), while individuals in the healthy diet group were more likely to be female (68.5 vs. 55.6%; $P = 0.006$) (Table 2).

To further characterize the two dietary patterns, other dietary constituents collected from the food frequency questionnaire were evaluated between the clusters. The unhealthy diet group had significantly lower levels of folate, carotene, vitamin A, calcium, magnesium, potassium and copper intake, and significantly higher levels of alcohol, animal protein, saturated fat and cholesterol intake than did the healthy diet group (data not shown).

Logistic regression modeling showed that a healthy dietary pattern had a protective effect against lung cancer (OR: 0.65, 95%CI: 0.45–0.96). The effect, however, was no longer statistically

significant after smoking was added to the model (OR: 0.93, 95%CI: 0.59–1.44).

In order to examine the effects of genotype and diet, independent of smoking, logistic regression analysis was performed using only the data from the smokers ($N = 329$). A comparable analysis among the non-smokers could not be performed due to the small number of non-smokers ($N = 109$). Individuals with the putative high-risk or most common genotype and an unhealthy diet were defined as the referent group for each genotype/diet comparison. All analyses were adjusted for sex and age. Odds ratios and 95%CIs were calculated for each combination of genotype and dietary pattern.

Among the 329 smokers, a healthy diet reduced the risk of lung cancer, but the odds ratio was not statistically significant (OR = 0.74, 95%CI: 0.46–1.20). As was seen in the whole population, none of the genotypes were significantly associated with risk among smokers, although the odds ratios were generally in the direction predicted by prior studies (Table 3). When diet and genotypes were examined together, however, several associations became apparent (Table 4).

In contrast to persons who were homozygous for the most common allele of GSTP1 and who had an unhealthy dietary pattern, persons with other GSTP1 genotypes who had a healthy diet pattern were at significantly decreased risk (OR: 0.16, 95%CI: 0.05–0.58). A combined effect of GSTM1 genotype and diet was also suggested. In comparison with persons who were GSTM1 null and had an unhealthy diet, persons who were GSTM1 null and had a healthy diet were at a non-significantly reduced risk (OR = 0.46, 95%CI: 0.21–1.01). Other relationships among the glutathione-S-transferases, dietary pattern and risk were not apparent.

Table 2 Descriptive analyses of dietary constituents between the two dietary clusters

Variable	Unhealthy high-fat low-fiber	Healthy high-fiber low-fat	Test statistic	<i>P</i> -value
Cases/controls	151/90	103/94	4.786	0.0287
Male/female	107/134	62/135	7.645	0.0057
Smoking (yes vs. no)	199/42	130/67	15.946	< 0.0001
BMI	26.50 ± 5.66	25.65 ± 4.99	1.67	0.0940
Dietary components				
Total calorie (kcal)	1441.800 ± 465.980	1426.400 ± 464.850	0.35	0.7299
Carbohydrate (g)	157.030 ± 58.499	212.330 ± 75.594	−8.41	< 0.0001
Animal fat (g)	40.635 ± 16.126	25.776 ± 11.507	11.23	< 0.0001
Protein (g)	68.838 ± 24.439	58.773 ± 22.924	4.44	< 0.0001
Dietary fiber (g)	12.111 ± 5.598	16.981 ± 7.793	−7.36	< 0.0001

Table 3 Distributions of genotypes in cases and controls among 329 smokers

Gene	Genotype	Case (%)	Control (%)	OR ^a (95%CI)
EPHX1-3E	His/His	65(27.78)	35(37.23)	0.65
	Others	169(72.22)	59(62.77)	(0.39–1.08)
EPHX1-4E	Arg/Arg	70(29.79)	26(27.66)	1.11
	Others	165(70.21)	68(72.34)	(0.65–1.89)
EPHX2	1/1	135(57.45)	54(57.45)	1.00
	Others	100(42.55)	40(42.55)	(0.62–1.62)
GSTP1	252/252	54(23.18)	20(21.28)	1.12
	Others	179(76.82)	74(78.72)	(0.62–1.99)
GSTT1	Null	36(15.52)	19(20.43)	0.72
	Others	196(84.48)	74(79.57)	(0.39–1.33)
MGST1	2/2	205(87.23)	82(87.23)	1.00
	Others	30(12.77)	12(12.77)	(0.49–2.05)
GSTA1	1/2	116(49.36)	54(57.45)	0.72
	Others	119(50.64)	40(42.55)	(0.45–1.17)
GSTA4	1/2	126(53.62)	43(45.74)	1.37
	Others	109(46.38)	51(54.26)	(0.85–2.22)
GSTM1	Null	137(58.30)	48(51.06)	1.34
	Others	98(41.70)	46(48.94)	(0.83–2.17)
GSTM2	2/2	208(88.51)	79(84.04)	1.46
	Others	27(11.49)	15(15.96)	(0.74–2.89)
GSTM3	1/1	167(71.06)	70(74.47)	0.84
	Others	68(28.94)	24(25.53)	(0.49–1.45)
GSTT2	1/2	103(44.40)	43(46.74)	0.91
	Others	129(55.60)	49(53.26)	(0.56–1.48)
NAT1	1/1	122(53.98)	52(56.52)	0.90
	Others	104(46.02)	40(43.48)	(0.55–1.47)
NAT2	1/1	91(38.89)	46(48.94)	0.66
	Others	143(61.11)	48(51.06)	(0.41–1.08)
NQO1EXON6	1/1	154(65.53)	56(59.57)	1.29
	Others	81(34.47)	38(40.43)	(0.79–2.11)

^a All odds ratios are age and sex adjusted.

Individuals with an unhealthy dietary pattern who were homozygous for the Tyr113His polymorphism in exon 3 of EPHX1 were at significantly reduced risk when compared to individuals with an unhealthy diet who were not homozygous for the Tyr/113His polymorphism (OR: 0.44, 95%CI: 0.20–0.96). In contrast, there was no combined diet-genotype effect on risk with the EPHX1 exon 4 polymorphism (His139Arg) or with the EPHX2 polymorphism.

NAT1, NAT2 and the NQO1 exon 6 polymorphisms were not significantly related to lung cancer risk when stratified by diet.

4. Discussion

A sizable number of studies have examined associations between genetic polymorphisms in the detoxification loci and the risk of lung cancer, often reporting contradictory results [3,25,26]. A

lesser number of studies have examined associations among genetic polymorphisms, specific dietary components and lung cancer risk [12,13,27]. Choosing which dietary components to include in such studies, though, is always challenging as the dietary risk factors are themselves not certain and many dietary factors are highly correlated. Therefore, in the research reported in this paper, we have examined associations among polymorphisms, dietary patterns and the risk of lung cancer. The findings suggest that there may be combined effects on risk of diet and polymorphisms in two loci; GSTP1 and EPHX1. Diet and GSTM1 genotype may also have a combined effect on risk.

GSTP1 is the most abundantly expressed GST in human lung tissue [28]. Previous studies have examined two single nucleotide polymorphisms in GSTP1 in association with lung cancer; one in codon 104 (A to G; Ile to Val) and one in codon 113 (C to T; Ala to Val) [29]. Both polymorphisms affect enzymatic catalytic activity and specificity

Table 4 Stratified analyses by genotype and dietary pattern among smokers

Gene	Genotype	Dietary pattern	Cases	Controls	OR (95%CI) ^a
EPHX1-3E	Others	Unhealthy	113	31	1.00
		Healthy	56	28	0.65 (0.27–1.53)
	His/His	Unhealthy	34	21	0.44 (0.20–0.96)
		Healthy	31	14	0.51 (0.25–1.04)
EPHX1-4E	Arg/Arg	Unhealthy	35	14	1.00
		Healthy	35	12	1.00 (0.36–2.80)
	Others	Unhealthy	112	38	1.19 (0.51–2.76)
		Healthy	53	30	0.74 (0.30–1.84)
EPHX2	1/1	Unhealthy	89	30	1.00
		Healthy	46	24	0.53 (0.24–1.20)
	Others	Unhealthy	58	22	0.72 (0.34–1.53)
		Healthy	42	18	0.74 (0.35–1.56)
GSTP1	252/252	Unhealthy	40	10	1.00
		Healthy	14	10	0.47 (0.19–1.21)
	Others	Unhealthy	105	42	0.42 (0.17–1.04)
		Healthy	74	32	0.16 (0.04–0.57)
GSTT1	Null	Unhealthy	21	12	1.00
		Healthy	15	7	1.15 (0.45–2.96)
	Others	Unhealthy	124	40	1.72 (0.69–4.29)
		Healthy	72	34	1.24 (0.34–4.51)
MGST1	2/2	Unhealthy	126	44	1.00
		Healthy	79	38	0.61 (0.16–2.30)
	Others	Unhealthy	21	8	0.87 (0.32–2.44)
		Healthy	9	4	0.73 (0.39–1.34)
GSTA1	1/2	Unhealthy	70	31	1.00
		Healthy	46	23	1.16 (0.52–2.60)
	Others	Unhealthy	77	21	1.77 (0.84–3.73)
		Healthy	42	19	0.79 (0.36–1.70)
GSTA4	1/2	Unhealthy	70	24	1.00
		Healthy	56	19	0.50 (0.22–1.17)
	Others	Unhealthy	77	28	0.87 (0.42–1.81)
		Healthy	32	23	0.85 (0.38–1.89)
GSTM1	Null	Unhealthy	87	29	1.00
		Healthy	50	19	0.46 (0.21–1.01)
	Others	Unhealthy	60	23	1.18 (0.56–2.50)
		Healthy	38	23	1.22 (0.56–2.66)
GSTM2	2/2	Unhealthy	130	42	1.00
		Healthy	78	37	0.55 (0.14–2.19)
	Others	Unhealthy	17	10	0.47 (0.17–1.29)
		Healthy	10	5	0.66 (0.35–1.21)
GSTM3	1/1	Unhealthy	104	39	1.00
		Healthy	63	31	0.79 (0.31–2.03)
	Others	Unhealthy	43	13	1.41 (0.62–3.22)
		Healthy	25	11	0.81 (0.41–1.57)
GSTT2	1/2	Unhealthy	68	22	1.00
		Healthy	35	21	0.88 (0.38–2.02)
	Others	Unhealthy	76	30	0.74 (0.35–1.56)
		Healthy	53	19	0.47 (0.20–1.11)
NAT1	1/1	Unhealthy	83	29	1.00
		Healthy	39	23	0.71 (0.31–1.60)
	Others	Unhealthy	58	23	0.65 (0.31–1.37)

Table 4 (Continued)

Gene	Genotype	Dietary pattern	Cases	Controls	OR (95%CI) ^a
NAT2	1/1	Healthy	46	17	0.59 (0.27–1.29)
		Unhealthy	64	25	1.00
	Others	Healthy	27	21	1.11 (0.51–2.42)
		Unhealthy	83	27	1.34 (0.64–2.80)
NQO1EXON6	1/1	Healthy	60	21	0.55 (0.22–1.33)
		Unhealthy	100	31	1.00
	Others	Healthy	54	25	0.63 (0.27–1.46)
		Unhealthy	47	21	0.77 (0.36–1.64)
		Healthy	34	17	0.68 (0.33–1.40)

^a All odds ratios are sex and age adjusted.

[30,31]. While these polymorphisms have been associated with lung cancer risk in some studies, they have not been associated with risk in others [32–35]. Similarly, a relationship between the polymorphisms and lung DNA adducts levels has been observed only inconsistently [36–38]. In regard to diet, GSTP1 protein expression has been associated with the intake of specific dietary components in both animal and human studies. An animal study found a strong induction of lung GSTP1 expression levels in rats receiving organo-sulfur compounds, which are abundant in garlic and onions [39]. A human study reported increased expression of GSTP1 protein after 2 weeks of vegetable consumption in 26% of participants, although protein induction was not correlated with genotype [40].

Unlike previous studies, we examined the relationship between lung cancer and a simple tandem repeat polymorphism of GSTP1. This polymorphism was evaluated by examining the number of pentanucleotide repeats (ATAAA) in the promoter region [41]. We designated homozygosity for the 20-repeat allele of the pentanucleotide as the referent group. We observed that individuals who were not homozygous for the 20-repeat allele were at significantly decreased risk only when they consumed a healthy diet. The importance of this association is not clear as the functional significance of the pentanucleotide polymorphism has yet to be examined. Studies of other GSTP1 polymorphisms, however, indicate that some variants are more active than others in conjugation reactions towards the carcinogenic diol epoxides of polycyclic aromatic hydrocarbons [42]. If components of a healthy dietary pattern are able to induce GSTP1 expression, it is conceivable that the

20-repeat allele codes for a less-inducible form of the enzyme.

In addition to a diet/GSTP1 effect, we also found a borderline diet/GSTM1 effect. In our study, individuals who were GSTM1 null were at decreased risk of lung cancer when they had a healthy dietary pattern, rather than an unhealthy one. Though the effect did not attain statistical significance, it was in line with previous findings concerning GSTM1 and lung cancer [24,43,44]. Possible effects of α -tocopherol and isothiocyanates among GSTM1 null individuals have both been reported. The α -tocopherol, β -carotene trial found that α -tocopherol may attenuate the risk of lung cancer associated with cumulative tobacco exposure among individuals who are GSTM1 null [14]. Similarly, data from Chinese and American populations suggest that isothiocyanates, a major component of cruciferous vegetables, make reduce lung cancer risk among persons who are GSTM1 null [12,13,27]. A likely explanation for these latter observations is that glutathione S-transferase mu 1 conjugates isothiocyanates to excrete them, thereby resulting in lower isothiocyanate levels among individuals who are not GSTM1 null and higher levels among individuals who are GSTM1 null. Thus, although GSTM1 null individuals may be more susceptible to the damaging effects of tobacco, they may also be able to compensate by not inactivating protective isothiocyanates in a healthy diet. Individuals who are null for both GSTM1 and GSTT1 may have an even greater reduction in risk when consuming isothiocyanates [12,13], however small numbers limited our power to examine this hypothesis.

Microsomal epoxide hydrolase, encoded by EPHX1, is highly expressed in several human tissues including the lung [45]. EPHX1 is an essential Phase

II detoxification enzyme which catalyzes the hydrolysis of reactive arene, alkene and aliphatic epoxides to more water soluble dihydrodiols [46]. This hydrolysis generally results in less reactive species, though this is not always the case as EPHX1 can metabolize tobacco-associated polycyclic aromatic hydrocarbons into carcinogenic diol epoxides [47,48]. The contrasting roles played by EPHX1 make an effect on lung cancer risk difficult to predict and perhaps dependent on the presence of other factors in the at-risk smoker. Two polymorphisms in EPHX1 have been widely examined and both have been demonstrated to affect enzyme activity. The Tyr113His substitution in exon 3 results in 40% lower enzymatic activity while the His139Arg substitution in exon 4 results in 25% increased activity [49]. A recent meta-analysis of both polymorphisms and lung cancer reported an odds ratio of 0.98 (95%CI: 0.72–1.35) for the exon 3 polymorphism and an odds ratio of 1.00 (95%CI: 0.71–1.41) for the exon 4 polymorphism [50]. In contrast, the same report also presented the results of pooled analysis of the polymorphisms and found that the exon 3 polymorphism significantly decreased the risk of lung cancer (OR = 0.70, 95%CI: 0.51–0.96). Our data suggest that these conflicting findings may be related to the effect of diet. In our study, we found that among smokers who had an unhealthy diet pattern, individuals who were homozygous for the low activity allele (His/His) were at significantly decreased risk in comparison to individuals of other genotypes. These results may suggest that an unhealthy diet is further contributing components, like PAH or even PAH itself, that undergo phase I metabolism by EPHX1. A possible candidate component may be cooked meat as several studies of the EPHX1 exon 3 polymorphism and colorectal polyps have reported an increased risk among persons with high activity genotypes who consume greater amounts of cooked meat [51,52]. Further study of meat consumption, the EPHX1 polymorphism and lung cancer is warranted.

This case-control study has several limitations. The control population was not necessarily repre-

sentative of the general population in that they were individuals who were attending cancer screening or wellness clinics. An advantage of enrolling this group was that they were drawn from the same general population as the cases and tended to be in the same age range as the patients. A disadvantage, however, was that they were likely to be more health conscious than the average person, a fact that may be reflected in their BMI being equivalent to that of the case group. The caloric intake of the case and control groups did not differ, but persons attending screening clinics may have healthier diets than the average person. In addition, it is possible that persons are motivated to attend cancer screening clinics because they are concerned that they might already have cancer or are more likely to develop cancer due to past exposures or family history. It is uncertain whether these motivations were those of our control group, though it is certain that none of our controls had cancer detected at the screening visit. An additional limitation is that our study, like many others, lacked sufficient power to examine the effects of combined genotypes. These concerns suggest that it would be prudent to replicate our results in a population utilizing an alternative choice of controls and a very large sample size.

In summary, our results strengthened the previous findings that genetic susceptibility to lung cancer may depend on polymorphisms in some phase II detoxification loci. They are also consistent with the general dietary-lung cancer finds of increased risk associated with total fat, saturated fat, animal fat, cholesterol and alcohol and decreased risk associated with fruits, vegetables, carotenoids, vitamin C, vitamin E and selenium [2]. More importantly, our results indicate that diet may affect the relationship of GSTP1, EPHX1 and GSTM1 polymorphisms to lung cancer risk. Future epidemiologic studies of lung cancer genetic susceptibility may benefit by consideration of and control for the influence of diet.

Appendix A: Detailed information on the 15 polymorphisms in 14 detoxification loci

Genes	Accession number	SNP: position	Size	Primer 1	Primer 2	Annealing temperature	Scoring
EPHX1-3E	L29766	Exon 3, codon 113	147	GCTGCTTCCAC- TATGGCTTC	GGCGTTTTGCAAACA- TACCT	66 °C	11 (CC)
	AF253417	T to C (Tyr to His) T/C: 17673					12 (CT) 22 (TT)
EPHX1-4E	L29766	Exon 4, odon 139	381	CAGAGCCTGACCGTG- CAG	GGTCACCCCGCCG- GAAGG	67 °C	11 (AA)
	AF253417	A to G (His to Arg) A/G: 24448					22 (GG) 12 (GA)
EPHX2	L05779	A/G: 1742	291	CGGTGGTCTCAAAGATG- TAGA	TGTCCTTACAGGA- CACTA	60 °C	11 (AA)
							22 (GG) 12 (AG)
GSTP1	M37065	STRs (AAAAT): 1856–1942	230–295	CACGCACCTATAATTC- CACC	GCTTAGAGGAAAG- GAAATTGC	54 °C	NA
GSTT1	Z67376	STRs (CA repeats): 110–155	268	CAACTTCATCCACGTT- CACC	GAAGAGCCAAGGA- CAGTTAC	54 °C	NA
GSTT2	Z84718	G/T: 66094	527	TAAACACTGATGA- CATTTGCC	AGGTGACACTGGCT- GATCTC	56 °C	11 (TT)
							22 (GG) 12 (TG)
MGST1	J03746	G/A: 560	325	TTCCATGGCTTA- CAGGTTG	AGTGAGGTGTTGTGT- GAATGTT	66 °C	11 (AA)
							22 (GG) 12 (AG)
GSTA1	L13269	C/T: 3962	287	CCAACCTTGAAAAGGAA- CAC	CTAGACAGGAGGGTG- TAAGGC	66 °C	11 (CC)
							22 (TT) 12 (CT)
GSTA4	AL121969	T/G: 97161	397	TGTAACGACGGC- CAGTGCCATAAAACAA- CACATCC	CAGGAAACAGCTAT- GACCGAGAGCAGAAA- GACGCTCAG	62 °C	11 (TT)
							22 (GG) 12 (TG)

Appendix A (Continued)

Genes	Accession number	SNP: position	Size	Primer 1	Primer 2	Annealing temperature	Scoring
GSTM1	X68676	C/G: 534	132	GCTTCACGTGTTAT- GAAGGTTTC	TTGGGAAGGCGTC- CAAGCGC/ TTGGGAAGGCGTC- CAAGCAG	56 °C	11 (CC) GSTM1B
							22 (GG) GSTM1A 12 (GC) GSTM1AB
GSTM2	M63509	G/A: 905	284	GCCCTTTAAAGCAGACA- CAA	GAGTGAGGAGCCCA- TACTCA	56 °C	11 (AA)
							22 (GG) 12 (GA)
GSTM3	AF043105	AGG/-: 4596-4598	273	CCTCAGTACTTGGAA- GAGCT	CACATGAAAGCCTT- CAGGTT	58 °C	11 GSTM3*A
							22 GSTM3*B 12 GSTM3*AB
NAT1	D90041	T/A: 1088	224	TTTGTCCATCCAGCTCAC- CAG	TTCCAAGATAACCA- CAGGCC	66 °C	11 (TT:AA)
		C/A: 1095					12 (TA:CA) 13 (TA+STRs) 22 (AA:CC) 23 (AC+STRs) 33 (STRs)
NAT2	D10870	G/A: 590	735	CTGGGTCTGGAAGCTCC- TC	AGGCCATCCT- TAAAATGCCT	66 °C	11 (GG:GG)
		G/A: 857					12 (GG:GA) 13 (GA:GG) 22 (GG:AA) 23 (GA:AG) 33 (AA:GG)
NQO1EXON6	M81600	Exon 6	156	TTRACTGAGAAGCCCA- GACCA	TGTGCCCAATGCTA- TATGTCA	56 °C	11 (CC)
	HUMNQO105	C/T: 609					22 (TT) 12 (CT)

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CORRIGENDUM

Corrigendum to ‘Genetic susceptibility and dietary patterns in lung cancer’ [Lung Cancer 41 (2003) 269–281][☆]

Ya-Yu Tsai^a, Katherine A. McGlynn^b, Ying Hu^a, Anna B. Cassidy^c,
John Arnold^c, Paul F. Engstrom^c, Kenneth H. Buetow^{a,*}

^a *Laboratory of Population Genetics, Center for Cancer Research, National Cancer Institute, NIH, DHHS, Bethesda, MD 20814-9692, USA*

^b *Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, Bethesda, MD, USA*

^c *Division of Population Science, Fox Chase Cancer Center, Philadelphia, PA, USA*

The authors regret that a sentence in the abstract of their article is in error. Please note that the sentence should read as follows:

‘Two groups of individuals were identified with distinct dietary patterns: (1) a group ($n = 241$) with a high intake of animal fat and protein and a low intake of carbohydrates and dietary fiber (the ‘unhealthy’ pattern) and (2) a group ($n = 197$) with a high intake of fiber and carbohydrate and a low intake of protein and animal fat (the ‘healthy’ pattern).’ The dietary groups and numbers of individuals are correctly noted in the text of the article and the tables.

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[☆] doi of original article 10.1016/S0169-5002(03)00238-1.

* Corresponding author. Address: Laboratory of Population Genetics, NCI Center for Bioinformatics, NIH, 6116 Executive Boulevard, Suite 403, Room 4001, Bethesda, MD 20814-9692, USA. Tel.: +1-301-435-1520; fax: +1-301-480-4222.

E-mail address: buetowk@pop.nci.nih.gov (K.H. Buetow).