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MTHFR Polymorphisms, Folate Intake, and Carcinogen DNA Adducts in the Lung

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Abstract

The methylenetetrahydrofolate reductase (*MTHFR*) genes and folate in one-carbon metabolism are essential for DNA methylation and synthesis. However, their role in carcinogen DNA damage in target lung tissue, a dosimeter for cancer risk, is not known. Our study aimed to investigate the association between genetic and nutritional one-carbon metabolism factors and DNA adducts in target lung. Data on 135 lung cancer cases from the Massachusetts General Hospital were studied. Genotyping was completed for *MTHFR* C677T (rs1801133) and A1298C (rs1801131). Information on dietary intake for one-carbon related micronutrients, folate and other B vitamin, was derived from a validated food frequency questionnaire. DNA adducts in lung were measured by ^{32}P-postlabeling. After adjusting for potential confounders, DNA adduct levels in lung significantly increased by 69.2% [95% confidence interval (CI), 5.5% to 171.5%] for the *MTHFR* 1298AC+CC genotype. The high risk group, combining the A1298C (AC+CC) plus C677T (CT +TT) genotypes, had significantly enhanced levels of lung adducts by 210.7% (95% CI, 21.4% to 695.2%) in contrast to the A1298C (AA) plus C677T (CC) genotypes. Elevation of DNA adduct was pronounced - 111.3% (95% CI, −3.0 to 360.5%) among 1298AC+CC patients who consumed the lowest level of folate intake as compared with 1298AA individuals with highest tertile of intake. These results indicate that DNA adducts levels are influenced by *MTHFR* polymorphisms and low folate consumption, suggesting an important role of genetic and nutritional factors in protecting DNA damage from lung carcinogen in at-risk populations.

Keywords

MTHFR; folate; genetic polymorphisms; DNA adducts; one carbon metabolism

INTRODUCTION

DNA adducts serve as a reliable marker of tobacco-associated exposure and cancer [1]. DNA adducts caused by tobacco smoking carcinogen such as polycyclic aromatic hydrocarbons (PAHs), listed as group 1 (carcinogenic to human) by International Agency for Research on Cancer (IARC), are formed by covalently binding to DNA. If the DNA adducts are left unrepaired, the resultant DNA damage can result in mutation that may ultimately lead to

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development of lung cancer. The methylenetetrahydrofolate reductase (*MTHFR*)genes and folate in one-carbon metabolism are essential for DNA synthesis, repair, and methylation. A few human studies have examined their role in DNA damage in non-target tissue, peripheral blood [23], but none have explored in carcinogen DNA damage in the target tissue, lung, as a molecular dosimeter for lung cancer.

Folate, a water soluble vitamin B_9, has been considered as an 'essential vitamin' because it modulates potential DNA damage and the risk of developing cancer, although not consistently [4]. *In vivo* and *in vitro* evidences suggest that folate deficiency results in DNA damage and instability, and altered DNA methylation, and eventually result in cell death via apoptosis, all of which may promote tumor initiation [5–9]. The MTHFR protein, a central enzyme in folate metabolism, has been implicated with lung cancer risk [10–13], because it is involved in methyl group synthesis process by catalyzing the irreversible conversion of 5, 10-methylenetetrahydrofolate (THF) to 5-methyl THF, which serves as methyl donor for the remethylation of homocystein to methionine and the precursor of *S*-adenosylmethionine (SAM). The polymorphisms in the *MTHFR* gene: C677T and A1298C are known to have functional relevance and thus variation of *MTHFR* in the folate metabolic pathway may be associated with variable folate levels [14] and is suspected of influencing the risk of lung cancer.

In this study, we aimed to investigate independent and combined effects of two polymorphisms of a key one-carbon pathway gene, *MTHFR* C677T and A1298C, and as well as dietary folate intake on DNA damage in target lung tissue. As DNA adducts in target lung tissue are mainly formed by cigarette smoking, the main source of exposure that contribute to the increased risk of lung cancer in smokers [15], DNA adducts were analyzed in uninvolved lung tissues in lung cancer patients with exposed to cigarette smoking.

MATERIALS AND METHODS

Study population

The study population consisted of 142 lung cancer patients at Massachusetts General Hospital(MGH, Boston, MA), as described previously [16, 17]. Among the participants, nonsmokers (n = 7) were excluded. Surgically resected non-involved lung tissue was sampled from the same lobe, distal to tumor in patients. Lung tissue specimens were frozen immediately on dry ice and stored deep-frozen at −80 until DNA adduct analysis. Blood samples for genotyping, socio-demographic information(including age, gender, and smoking status), and dietary intake were collected at the time of recruitment by trained personnel. Informed consent was obtained from all study participants. This study was approved by the Committees on the Use of human Subjects in Research at the MGH and the Harvard School of Public Health.

DNA adduct analysis

We used previously reported data on DNA adducts in lung samples determined by the ^{32}P-postlabeling assay [18, 19]. These DNA adducts are considered primarily to represent tobacco-derived aromatic hydrophobic adducts, mainly polycyclic aromatic hydrocarbon(PAH)-DNA adducts [18, 19]. The half-life of DNA adduct in the lung tissue of lung cancer patients has been reported to be approximately 1.7 years, indicating that DNA adduct persist longer in lung tissues than other tissues [20]. Total relative DNA adducts were measured in the diagonal reactive zone plus discrete adducts as in prior studies [18, 19]. Each sample was repeated at least twice as a validation analysis and average adducts levels were obtained from the combination of all experiments of the relative adduct levels. The coefficient of variation for the repeated measurements was 14% for the positive control sample [19].

Genotyping

DNA was extracted from peripheral blood samples using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). Of the 135 patients, the *MTHFR* polymorphisms, C677T (rs1801133) and A1298C (rs1801131), were genotyped in 96 and 102 patients using the TaqMan method with an ABI7900HT sequence detection system (Applied Biosystems, Foster City, CA) and a random 5% of the samples were repeated for the validation of genotyping procedures. Genotypes were verified by two independent readers.

Assessment of Dietary Intake

Dietary folate intake was assessed from the Harvard-Willet validated food frequency questionnaire (FFQ) of each patient at the time of recruitment [21, 22], asking how often on average the subject have used the amount specified during the past one year. The FFQ, designed to estimate an individual's habitual intake over a defined period of time (e.g., a year), has been used extensively as the standard tool for dietary assessment in epidemiologic studies. The 126-food item FFQ, developed by the Nutrition Department at the Harvard School of Public Health, has been validated in a group of female Caucasian nurses [22] and male health professionals [21] living in Boston. In the FFQ, a commonly used unit or portion size was specified for each food item or supplement, and subjects were asked about their average consumption over the past year before enrollment. Estimated average intakes for each specific food were obtained and nutrient intake was computed using the Harvard database, which is a modification of the U.S. Department of Agriculture Nutrition Composition Laboratory's food composition database.

Statistical analysis

The dependent variable, DNA lung adduct per 10^{10} nucleotides, was transformed using natural logarithm to improve normality and to stabilize the variance. Genotypes were coded as wild type (major-allele homozygote) and variant genotype (minor-allele homozygote + heterozygote). Dietary folate intake values were fist adjusted for total energy intake using multivariate nutrient-density model based on an established method [23]. Because nutrient intake highly depends on total energy intake, adjustment for total energy intake is necessary. Energy-adjusted folate categorized into tertile with the highest tertile as the reference group. Potential confounders including age at diagnosis(continuous), gender(male and female), smoking status (ex-smoker and current smoker), and pack-years of smoking (continuous)were adjusted in the multivariate analysis. We estimated the percent change in DNA lung adduct levels for the risk genotype compared with the common allele as $[e^{\beta} - 1] \times 100\%$, with 95% CI $[e^{(\beta \pm 1.96 \times SE)} - 1] \times 100\%$, where β and SE are the estimated regression coefficient and its standard error from multiple regression analysis. To examine the combined effects of C677T and A1298C, we constructed a model that included all possible combinations between C677T and A1298C polymorphisms, with the low-risk combination of the homozygous wild-type genotype, 677CC plus 1298AA, as a reference category. To analyze whether DNA adduct level changed with the tertile of folate intake, trend tests were conducted by treating each category as a continuous variable in a regression model. We also assessed the nonlinear relationship by fitting the energy-adjusted folate intake using a natural cubic spline with 2 degrees of freedom. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Carry, NC, USA) and R (version 2.11.0; The R Foundation for Statistical Computing 2010).

RESULTS

Details of demographic and clinical characteristic of the study population are presented in Table 1. The geometric mean (GM) of DNA adduct levels was 86.2 adducts per 10^{10} nucleotides in lung tissue(mean, 172 adducts per 10^{10} nucleotides). The mean daily intake

was 487.5μg for folate and 2,207 kcal for total energy, respectively. To check for possible selection bias due to lack of *MTHFR* genotyping data on 25% of the population, we analyzed whether differences exist between the groups, classified by cases with MTHFR genotypes (n = 94) and cases without MTHFR genotype (n = 41). We found no significant differences in variables including age, gender, smoking status, histology, pack-years, energy-adjusted folate, and lung DNA adduct levels.

Genotype distribution of the *MTHFR* polymorphisms and their association with DNA lung adduct levels are given in Table 2. The DNA adduct levels in lung tissue significantly increased by 69.2% (95% CI, 5.5% to 171.5%) for A1298C AC+CC genotypes, but no association was founded with C667T CT+TT genotypes. In addition to the independent effect of each *MTHFR* genes, we also examined combined effect of A1298C and C677T genotype, in which the low-risk combination of the homozygous wild-type genotype, 677CC plus 1298AA, as the reference group in comparison. The high risk group, 677CT+TT plus 1298AC+CC, was highly significantly associated with an enhanced DNA adduct level in the lung of 210.7% (95% CI, 21.4 % to 695.2%, $P = 0.02$) compared with the low-risk reference group. There was a trend towards increased level of DNA lung adducts for increased risk combination of *MTHFR* genotypes (P for trend = 0.012).

Table 3 reports joint associations of *MTHFR* genotypes and dietary folate intake with DNA adduct levels. The DNA adducts levels in the lung significantly decreased by −92.6% (95% CI, −99.2% to −30.3%) with increased level of dietary folate intake (μg/kcal) among patients with 1298AC+CC genotypes, but no associations were found with C677T genotypes. The DNA adduct levels were elevated by 111.3% (95% CI, −3.0 to 360.5%) among 1298AC+CC patients who consumed the lowest tertile of folate intake compared with 1298AA individuals with highest tertile of intake (P for trend = 0.067), whereas no associations were found with C677T polymorphism. The interaction terms between folate intake and *MTHFR* polymorphisms in relation to DNA adduct in the lung were not statistically significant. When stratifying by smoking status, similar and consistent decreases in DNA adduct levels in the lung were observed among former (−90.2%, 95% CI −99.8 to 423.2), and current smokers (−91.5%, 95% CI −99.7 to 122.8) with increased level of folate among cases with 1298AC+CC genotypes, but our analysis with small sample size suffers from a lack of statistical power to find associations. We further analyzed the effects of vitamin B_6 and B_{12} on DNA adduct levels in the lung. No associations were found in overall subjects. A weak association was found with vitamin B_6 among patients with 1298 A+CC genotypes(−74.9%, 95% CI, −92.4 to −17.4), but no significant trend was observed (P for trend = 0.68).

We examined the assumption of the nonlinear association between folate intake and DNA adduct in the lung, as shown in Figure 1. The DNA adducts levels in lung decreased with increasing dietary folate consumption through 0.4 μg/kcal, but it plateaued with wide 95% CIs after 0.4 μg/kcal.

DISCUSSION

In our study, increased levels of lung DNA adducts were associated with the *MTHFR* risk genotypes, A1298C variantor in combination with C677T variant. This suggests that a clustering of *MTHFR* risk genotypes increases DNA adduct levels in lung. In addition, an inverse association between dietary folate intake and lung adduct levels was observed among individuals with the A1298C variant genotype, implying that the combination of low folate intake and impaired folate metabolic polymorphisms may be implicated in DNA damage in target lung tissue.

To date, there is no clinical and epidemiological evidence regarding the effects of *MTHFR* polymorphisms and/or dietary folate intake on DNA adducts in lung. A few human studies have evaluated their influence on DNA adducts in peripheral leukocyte [2, 3] used as a surrogate for the target lung tissue [17]. In the European Prospective Investigation into Cancer and Nutrition (EPIC)-Italy cross-sectional study, reduced DNA adduct levels in peripheral white blood cells were associated with the frequent consumption of fresh fruit and vegetables, which are the major sources of one-carbon nutrients including folate and the intake of antioxidants. No associations were seen with folic acid [2] and *MTHFR* C677T polymorphisms [3]. Several studies have shown the association of an increased DNA adducts levels and the risk of lung cancer [24–28], and *MTHFR* genetic polymorphisms and dietary folate in one-carbon metabolism factors may modulate their link. *MTHFR*, a fundamental enzyme in one-carbon (methyl group) metabolism, balances the folate pool involved in DNA synthesis and methylation. The *MTHFR* A1298C polymorphism is associated with an increased risk of lung cancer in women, but not in men [11], and with diminished DNA methylation [29]. No association was also observed between the *MTHFR* C677T and A1298C polymorphisms and risk of lung cancer [13]. Our study found the association between DNA adducts in lung tissue with A1298C polymorphisms, but not with C677T polymorphisms, which is consistent with the previous study [3]. It is still unclear why and how *MTHFR* C677T and A1298C genes play a role in DNA damage in the lung. This may probably be due to complex *MTHFR* polymorphisms that many other factors such as environmental exposure to chemicals and diet, and other genes in the same pathway, may contribute to these inconsistent results in smaller data sets [11]. More extensive studies with large sample size are needed to confirm and interpret of these findings.

Folate deficiency can lead to DNA damage by causing chromosomal breaks, oxidative lesions, or both, which could contribute to the increased risk of cancer [30]. Experimental studies have shown that dietary folate deficiency increases DNA damage and initiates tumor development in mice, and that *MTHFR* mutations play a role in this phenomenon [5]. An *in vitro* study showed that folate depletion induces DNA instability [8] and increases chromosomal breakage (measured by micronuclei frequency) and abnormalities in human lymphocytes [31]. Recent epidemiologic evidence showed that increasing serum folate levels were inversely associated with the risk of lung cancer among former and current smokers, but not among nonsmokers [32]. Although low dietary folate intake was weakly correlated with lung DNA adducts levels ($P = 0.096$ in Table 3)among overall subjects, enhanced DNA adducts was observed with increased levels of dietary folate among individuals with *MTHFR* A1298C variant genotypes ($P = 0.029$). Marginally significant trend of increased levels of lung DNA adducts with decreased dietary folate was also observed (P for trend = 0.067).

Although the biological mechanism remains unclear for the observed associations, possible mechanisms may include the alteration of DNA methylation in one-carbon (methyl group) metabolism pathways. The disruption of homeostasis in one-carbon metabolism affects the risk of cancer [6]. PAHs such as benzo[a]pyrene are metabolized *in vivo* to form highly genotoxic and tumorigenic diol epoxides that bind to DNA at the guanine residues forming adducts. These PAH-DNA adducts have been served as markers of biologically effective doses from exposure to tobacco smoke. The state of methylation enhances carcinogen DNA adduct formation for a particular mutation site in the *p53* gene in human lung cancer [33, 34]. In coke oven workers, DNA methylation was associated not only with PAHs exposure, assessed by urinary 1-hydroxypyrene, but also with anti-benzo[a]pyrene diolepoxide (BPDE)-DNA adduct levels in peripheral blood leukocytes, suggesting that the epigenetic effect of PAHs exposure involve changes in DNA methylation status [35]. Folate as a methyl donor can lead to elevated DNA damage and altered DNA methylation. *MTHFR* is a critical gene in folate-mediated one-carbon metabolism that is responsible for DNA remethylation

and DNA synthesis pathways. DNA methylation might be impaired in individuals with carrying the variant *MTHFR* genotypes, particularly with low folate status [6, 36], providing biological plausible link between low-folate diet and *MTHFR* genotypes involved in DNA methylation [37], which may modulate adduct formation [35].

A significant gene-gene and gene-nutrient interactions between *MTHFR* A1298C and C677T and between A1298C polymorphism and folate intake were observed in the Poison regression model (*P* for interaction < 0.0001) [18]. The Recommended Dietary Allowance (RDA) of folate for US adults aged ≥ 19 years is 400 μg dietary folate equivalent per day [38]. In this study, 48% of the subjects had folate below RDA. As a sensitivity analysis, when we re-analyzed the data using this criterion, the subjects with folate levels below RDA had significantly increased levels lung adducts by 88% (95% CI, 3.3% to 243%) as compared with individuals with folate above RDA, taking *MTHFR* polymorphisms into account as well as other covariates.

Our study limitations include its relatively small sample size and the inclusion of Caucasian population which limited generalizability across other populations, not unlike many other molecular epidemiologic studies. In addition, we did not see associations with other B vitamins, such as vitamin B_6 and B_{12} involved in one-carbon metabolism in overall subjects, but only a weak association with vitamin B_6 among patients with 1298 A+CC genotypes. We speculate that this is probably due to 90% and 76% of the total subjects who met current recommendations intake for B_{12} (2.4 μg/d)and B_6 [1.3 mg/d for ages 19 to 50, 1.7 mg/d (men) and 1.3 mg/d (women) for age ≥ 51), respectively. DNA adducts may lead to lesions that are expressed as micronuclei (MN), which reflects chromosome breakage and abnormal chromosome segregation [39]. Although we found increased DNA adduct levels in target lung with decreased dietary folate in small dataset, further studies using prospective designs with measures of DNA adducts, as a marker of biological effective dose, and MN frequency, as an indicator of early biological effect, are needed to confirm our findings. Further studies with large sample size are needed to determine other micronutrients that can affect the one-carbon metabolic pathways underlying the role of gene and nutrient and their interactions on DNA damage.

In summary, our results provide evidence that DNA adduct levels in target lung tissue are influenced by *MTHFR* polymorphisms and low folate intake, implying a significant role of genetic and nutritional factors in prevention of DNA damage from lung carcinogen in at-risk populations.

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Abbreviations

FFQ	food frequency questionnaire
GM	geometric mean
MTHFR	methylenetetrahydrofolate reductase
PAHs	polycyclic aromatic hydrocarbons
RDA	recommended dietary allowance

SAM	*S*-adenosylmethionine
THF	5, 10-methylenetetrahydrofolate

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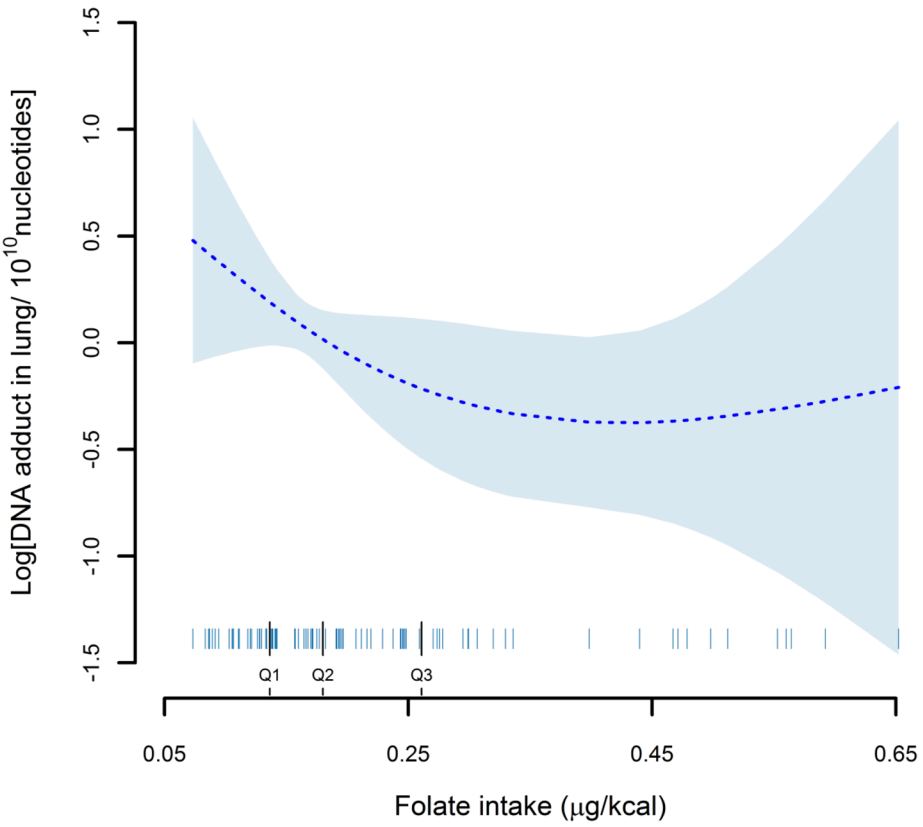

Figure 1.
Hypothesized nonlinear association between folate intake and the changes in DNA lung adduct level adjusting for age, gender, smoking status, pack-years, total energy and *MTHFR* A1298C polymorphism. The predicted values are indicated by the dashed line and their 95% confidence intervals by the blue shades. Folate intakes of all individual subjects are indicated by short vertical lines on the abscissa.

Table 1

General and clinical characteristics of patients of lung cancer (N=135)

	N (%) or mean ±SD
Age at diagnosis, y	66.3 ± 10.6
Gender (male)	78 (57.8)
Smoking	
Current	56 (41.5)
Former	79 (58.5)
Histology	
Adenocarcinoma	65 (48.1)
Squamous	44 (32.6)
Others	26 (19.3)
Pack-years, y	63.5 ± 40.6
Folate, μg/d	487.5 ± 259.5
Total energy intake, kcal/d	2,207.7 ± 738.5
Lung DNA adduct levels, adducts per 10^{10} nucleotides†	86.2 ± 4.7†
MTHFR C677T (rs1801133)‡	
CT+TT	47 (46.1)
CC	55 (53.9)
MTHFR A1298C(rs1801131)‡	
AC+CC	58 (60.4)
AA	38 (39.6)

†Geometric mean ± geometric SD

‡The reference SNP identification numbers (ref SNP ID) are from the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP).

Table 2

Adjusted geometric mean (GM)[†] and estimated percent changes (95% CIs)[†] in DNA adducts in lung by MTHFR polymorphisms

	N (%)	Adjusted GM	% Change (95% CI)[†]
MTHFR A1298C			
AC+CC	47 (46.1)	336	69.2 (5.5 to 171.5)[*]
AA	55 (53.9)	199	Reference
MTHFR C677T			
CT+TT	58 (60.4)	240	1.2 (−41.5 to 75.2)
CC	38 (39.6)	237	Reference
Combined *MTHFR*			
A1298C (AC+CC) and C677T (CT+TT)	18 (19.2)	376	210.7 (21.4 to 695.2)[*]
A1298C (AC+CC) and C677T (CC)	27 (28.7)	313	159.0 (6.4 to 530.7)[*]
A1298C (AA) and C677T (CT+TT)	38 (40.4)	216	78.5 (−23.8 to 318.0)
A1298C (AA) and C677T (CC)	11 (11.7)	121	Reference
P for trend			0.012

[†]Adjusted for age, gender, smoking status, pack-years, and total energy adjusted-folate intake.

[*]$P < 0.05$

Table 3

Estimated percent changes (95% CIs)[†] in DNA lung adducts associated with folate intake by *MTHFR* polymorphisms

			***MTHFR* A1298C**				***MTHFR* C677T**			
	Overall[‡]		**AC+CC**		**AA**		**CT+TT**		**CC**	
	Adjusted GM	**% Change (95% CI)**	**Adjusted GM**	**% Change (95% CI)**	**Adjusted GM**	**% Change (95% CI)**	**Adjusted GM(95% CI)**	**% Change (95% CI)**	**Adjusted GM**	**% Change (95% CI)**
Folate, μg/kcal		−77.0 (−97.0 to 75.1)		−92.6 (−99.2 to −30.3) *		−65.9 (−98.6 to 726.7)		−77.8 (−98.7 to 287.2)		−91.8 (−99.8 to 176.8)
Folate, μg/kcal, tertile										
Low	356	76.7 (−8.9 to 242.7)	495	111.3 (−3.0 to 360.5)	180	81.9 (−29.5 to 369.4)	327	72.3 (−32.5 to 340.0)	448	167.9 (−19.2 to 788.4)
Medium	212	8.3 (−42.9 to 105.3)	290	24.5 (−43.2 to 173.1)	170	13.7 (−55.1 to 187.7)	232	31.3 (−50.4 to 247.7)	177	5.9 (−61.4 to 190.6)
High	199	Reference	235	Reference	153	Reference	182	Reference	170	Reference
P for trend		0.094		0.067		0.214		0.188		0.103

Note: Percentchange represents per unit change in DNA adduct levels (DNA adduct in lung/10^{10} nucleotides) with per unit increase in folate level (μg/kcal)or decreasing tertile of folate level (μg/kcal).

[†]Adjusted for age, gender, smoking status, and pack-years.

[‡]Adjusted for age, gender, smoking status, pack-years, and *MTHFR* A1298C and C677T.

*$P < 0.05$

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