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Polymorphism rs3819102 in thymidylate synthase and environmental factors: effects on lung cancer in Chinese population

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ABSTRACT

Purpose: Lung cancer is the leading cause of cancer death worldwide, and the predominant risk factor for its development is smoking. Thymidylate synthase (TYMS) is a key enzyme in DNA synthesis that catalyzes the conversion of deoxyuridine monophosphate to dTMP. Rs931794, a single nucleotide polymorphism located in the TYMS gene, was suggested to be associated with cancer risk.

Methods: To analyze the interaction between rs3819102 and environmental factors on the risk of lung cancer in a Chinese population, single nucleotide polymorphismscan was used to genotype this polymorphism in 974 lung cancer cases and 1005 control subjects.

Results: The frequencies of TT, CT, and CC genotypes of TYMS rs3819102 were 61.8%, 32.9%, and 5.3% in controls, and 53.8%, 38.4%, and 7.8% in cases, respectively. Compared with the TT genotype, the CT (odds ratio [OR], 1.380; 95% confidence interval [CI], 1.131-1.683), and CC (OR, 1.786; 95% CI, 1.213-2.644) genotypes were associated with an increased risk of lung cancer after adjustment for age, gender, smoking status, and family history. The C allele of rs3819102 is the risk allele for lung carcinogenesis in a dominant model (OR, 1.435; 95% CI, 1.188-1.735). In a stratified analysis, the risk effects of both the CT and CC genotypes of rs3819102 were more evident in subgroups of smokers and people without a family history of cancer.

Conclusion: The rs3819102 polymorphism in TYMS might increase susceptibility to environmental factors and contribute to the risk of lung cancer. The C allele is a risk allele in lung carcinogenesis.

Introduction

Lung cancer is the most frequent cancer worldwide, with more than 1.8 million new cases (13% of total cancer incidence) and almost 1.6 million deaths (20% of total cancer mortality) estimated in 2012.[1] Similarly in China, lung cancer has the largest number of cancer diagnoses and is the leading cause of cancer death, especially in males.[2]

Nonsmall cell lung cancer (NSCLC), which comprises 85%-95% of all lung cancer cases, is divided into squamous cell carcinoma (SCC) and adenocarcinoma.[3] Smoking remains the predominant risk factor for developing lung cancer, increasing the risk 5- to 10-fold.[4,5] Apart from smoking, there are many internal factors that may be relevant to lung cancer risk, such as gender, age, and family history.[4] However, susceptibility to these risk factors between individuals differs, and is modulated by genetic and epigenetic factors that need further research.[1,6]

Thymidylate synthase (TYMS), encoded by the TYMS gene, catalyzes the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate (dTMP).[7] Abnormal TYMS may induce dysregulation in DNA repair, and the expression of mutated TYMS mRNA might be useful as a prognostic factor for patients with NSCLC.[8-10] TYMS gene polymorphisms have been linked to the risk of colorectal cancer,[11-14] cholangiocarcinoma,[15] and breast cancer.[16,17] Among the single nucleotide polymorphisms (SNPs), rs3819102 has been found to play a role in the development of endometrial cancer, with the increased risk being associated with the internal

milieu.[18] Our study aimed to explore the relationship among SNP rs3819102, the environment, and lung cancer risk.

Patients and methods

Study population

The Ethics Committee for Human Subject Research of Tongji University and Fudan University approved the research protocol. Written informed consent was obtained from all cases and controls before participating in the study. All 974 patients enrolled in the study were Chinese living in Eastern China, and were diagnosed histologically as having lung cancer at the Shanghai Chest Hospital, the Shanghai Tenth People's Hospital, or the Xuhui Central Hospital. The control population consisted of 1005 cancer-free healthy subjects recruited from the Taizhou Longitudinal Study during the same period, with the selection criteria including no individual history of cancer.[19] Each eligible subject was interviewed personally to gather demographic data (such as age, sex, and ethnicity) and environmental exposure history, including radiation exposure, smoking, and alcohol consumption. Control subjects were matched to lung cancer cases by age and gender.

Genotyping

About 3-5 mL of venous blood was collected from each subject. Genomic DNA was extracted from whole blood samples using the Qiagen Blood Kit (Qiagen, Chatsworth, California), according to the manufacturer's instructions. Genotyping quality was determined by a detailed procedure consisting of an over 95% successful call rate, duplicate calling of genotypes, internal positive control samples, and Hardy-Weinberg Equilibrium (HWE) testing. DNA samples (10 ng) were amplified by the polymerase chain reaction according to the standard procedure. SNP genotyping was performed using a custom-by-design 2×48-Plex SNPscan Kit (cat. no. G0104; Genesky Biotechnologies, Shanghai, China). This kit was developed according to patented SNP genotyping technology, which was based on double ligation and the multiplex fluorescence polymerase chain reaction.

Statistical analysis

Differences in demographic variables, smoking status, family history of cancer, and grouped allele and genotype frequencies between cases and controls were compared by the χ^2 test. The HWE was determined using the standard χ^2 test to compare the observed with the expected frequency in controls. To estimate the association between the gene polymorphism and the risk of lung cancer, the odds ratio (OR) and 95% confidence interval (CI) were calculated using unconditional logistic regression with adjustments for age, gender, smoking status, and family history. Furthermore, we performed stratified analyses for the gene polymorphism by age, gender, smoking status, and family history. SPSS 17.0 (IBM, Chicago, Illinois) was used for all statistical analyses. Data are expressed as means $\pm$ standard deviation. All P values presented are two-sided, and a level of $P < 0.01$ was considered statistically significant.

Results

Characteristics of the study population

There were 974 lung cancer cases and 1005 control subjects, the average patient ages were 62.2 ± 10.8 in cases and 62.2 ± 10.7 in controls. There were 710 males and 264 females in cases and 698 males and 307 females in controls. Differences in the distributions of gender and age

Table 1
Frequency distribution of selected characteristics of study subjects by the case–control status.

Variable	Cases (974)	Controls (1005)	*P* value
Age (mean ± SD, y)	62.2 ± 10.8	62.2 ± 10.7	0.944†
Age (y)			0.772*
≤60	411(42.2%)	432(43.0%)	
>60	562(57.8%)	573(57.0%)	
Gender			0.101*
Male	710(72.9%)	698(69.5%)	
Female	264(27.1%)	307(30.5%)	
Smoking status			<0.001*
Nonsmoker	294(30.2%)	503(50.0%)	
Eversmoker	680(69.8%)	502(50.0%)	
Family history of cancer			<0.001*
Have	337(34.6%)	147(14.6%)	
Have not	637(65.4%)	858(85.4%)	

* *P* value for pearson χ^2 test.
† *P* value for independent-samples *t* Test.

Table 2
Analysis of association between rs3819102 and risk of lung cancer.

Rs3819102	Controls no. (%)	All lung cancer			
		No. (%)	*P*	OR (95 % CI)*	*P**
Alleles					
T	1573(78.3%)	1401(73.0%)		1.000 (reference)	
C	437(21.7%)	519(27.0%)	<0.001	1.368 (1.174-1.596)	<0.001
Genotypes					
Additive model					
TT	621(61.8%)	516(53.8%)		1.000 (reference)	
CT	331(32.9%)	369(38.4%)	0.002	1.380 (1.131-1.683)	0.002
CC	53(5.3%)	75(7.8%)	0.005	1.786 (1.213-2.644)	0.003
Dominant model					
TT	621(61.8%)	516(53.8%)		1.000 (reference)	
CT + CC	384(38.2%)	444(46.3%)	<0.001	1.435 (1.188-1.735)	<0.001
Recessive model					
TT + CT	952(94.7%)	885(92.2%)		1.000 (reference)	
CC	53(5.3%)	75(7.8%)	0.023	1.577 (1.080-2.317)	0.019

* Adjusted for age, gender, smoking status, and family history by using unconditional logistic regression analysis.

between cases and controls were not statistically significant ($P = 0.101$ and 0.772, respectively). In cases, 680 individuals were smokers and 294 were nonsmokers. In controls, 502 individuals were smokers and 503 were nonsmokers. The groups who had never smoked represented 30.2% of cases and 50.0% of controls. The cases were more likely to report a family history of cancer than controls (34.6 vs 14.6%, $P < 0.01$). For the 974 lung cancer cases, there were 427 adenocarcinomas, 319 SCCs, 83 small cell lung carcinomas, and 113 other pathologic types. Details of selected characteristics of the 974 lung cancer cases and 1005 control subjects are shown in Table 1.

Polymorphism rs3819102 and the risk of lung cancer

The call rate of genotyping was 99.3%. Allele and genotype frequencies, and associated ORs (95% CI) for cases and controls, are presented in Table 2. The observed frequency of the C allele of rs3819102 in controls was 21.7%, which was similar to that reported in HapMap for Han Chinese in Beijing, China (22.2%). Genotype frequencies of rs3819102 among controls ($P = 0.16$) and cases ($P = 0.16$) did not differ significantly from those expected under HWE. The C allele

Table 3
Stratified analysis of associations between rs3819102 alleles and risk of lung cancers by age, gender, smoking status, family history of cancer, and histological types.

Stratification	Cases/controls		OR (95%CI)*	P*
	T(ref)	C		
Age (y)				
≤60	595/678	213/186	1.351 (1.071-1.707)	0.011
>60	804/895	306/251	1.365 (1.112-1.677)	0.003
Gender				
Male	1030/1105	370/291	1.367 (1.134-1.649)	0.001
Female	371/468	149/146	1.382 (1.050-1.820)	0.021
Smoking status				
Nonsmoker	414/778	166/228	1.352 (1.057-1.728)	0.016
Eversmoker	987/795	353/209	1.368 (1.122-1.671)	0.002
Family history of cancer				
Have	491/227	165/67	1.172 (0.843-1.643)	0.35
Have not	910/1346	354/370	1.431 (1.204-1.701)	<0.001
Histology types				
Adenocarcinoma	626/1573	228/437	1.300 (1.072-1.575)	0.007
Squamous cell cancer	453/1573	185/437	1.503 (1.205-1.872)	<0.001
Small cell lung cancer	122/1573	44/437	1.349 (0.925-1.937)	0.112

* Adjusted for age, gender, smoking status, and family history by using unconditional logistic regression analysis.

frequencies were significantly higher in the cases (27.0%) than in the controls (21.7%, $P < 0.001$). Frequencies of the TT, CT, and CC genotypes of rs3819102 were 61.8, 32.9, and 5.3% in controls, and 53.8, 38.4, and 7.8% in cases, respectively. Compared with the TT genotype, logistic regression analysis showed that the CT (OR, 1.380; 95% CI, 1.131-1.683) and CC (OR, 1.786; 95% CI, 1.213-2.644) genotypes were associated with an increased risk of lung cancer in the overall population after adjustment for age, gender, smoking status, and family history. The C allele of rs3819102 is the risk allele for lung carcinogenesis in the dominant model (OR, 1.435; 95% CI, 1.188-1.735, Table 2).

Stratified analyses of associations between genotypes and the risk of lung cancer

A stratified analysis for rs3819102 by epidemiological characteristics was performed. As shown in Table 3, individuals with the C allele had an increased risk of lung cancer (adjusted OR, 1.368; 95% CI, 1.174-1.596), especially males (adjusted OR, 1.367; 95% CI, 1.134-1.649), elderly patients (adjusted OR, 1.365; 95% CI, 1.112-1.677), ever smokers (adjusted OR, 1.368; 95% CI, 1.122-1.671), and people without a family history of cancer (adjusted OR, 1.431; 95% CI, 1.204-1.701). Individuals with the C allele were more likely to develop adenocarcinoma (adjusted OR, 1.300; 95% CI, 1.072-1.575) or SCC (adjusted OR, 1.503; 95% CI, 1.205-1.872) than small cell lung cancer. As shown in Table 4, compared with the TT genotype, the risk of lung cancer in the CT (adjusted OR, 1.453; 95% CI, 1.161-1.821) and CC (adjusted OR, 1.918; 95% CI, 1.246-2.966) genotype carriers was significantly increased for those without a family history of cancer. Individuals with the CT genotype (adjusted OR, 1.414; 95% CI, 1.103-1.812) had an increased risk of suffering from adenocarcinoma, while people with the CC genotype (adjusted OR, 2.455; 95% CI, 1.453-4.125) had an increased risk of suffering from SCC, compared with the TT genotype.

In the dominant model, the C allele of rs3819102 was the risk allele for lung carcinogenesis, especially for males (adjusted OR, 1.419; 95% CI, 1.131-1.783), age over 60 years (adjusted OR, 1.436; 95% CI, 1.115-1.852), those who had been smokers (adjusted OR, 1.407; 95% CI, 1.105-1.793), and people without a family history of cancer (adjusted OR, 1.518; 95% CI, 1.225-1.881) (Tables 5 and 6). After stratifying by histological subtypes, no significant increased risk for individual pathological types of lung cancer was found.

Table 4
Stratified analysis of associations between rs3819102 genotypes and risk of lung cancers by age, gender, smoking status, family history of cancer, and histological types.

Stratification	Cases and/or controls			CT VS TT		CC VS TT	
	TT (ref)	CT	CC	OR (95%CI)*	P*	OR (95%CI)*	P*
Age (y)							
≤60	219/266	157/146	28/20	1.370 (1.017-1.849)	0.038	1.776 (0.961-3.338)	0.069
>60	296/355	212/185	47/33	1.384 (1.059-1.810)	0.017	1.734 (1.050-2.892)	0.033
Gender							
Male	382/441	266/223	52/34	1.358 (1.070-1.726)	0.012	1.832 (1.135-2.996)	0.014
Female	134/180	103/108	23/19	1.418 (0.984-2.046)	0.061	1.782 (0.915-3.504)	0.090
Smoking status							
Nonsmoker	148/304	118/170	24/29	1.417 (1.025-1.958)	0.035	1.662 (0.898-3.053)	0.102
Eversmoker	368/317	251/161	51/24	1.336 (1.037-1.724)	0.025	1.887 (1.135-3.215)	0.016
Family history of cancer							
Have	185/88	121/51	22/8	1.178 (0.772-1.810)	0.451	1.351 (0.586-3.422)	0.499
Have not	331/533	248/280	53/45	1.453 (1.161-1.821)	0.001	1.918 (1.246-2.966)	0.003
Histology types							
Adenocarcinoma	226/621	174/331	27/53	1.414 (1.103-1.812)	0.006	1.422 (0.848-2.341)	0.172
Squamous cell cancer	167/621	119/331	33/53	1.354 (1.009-1.814)	0.042	2.455 (1.453-4.125)	<0.001
Small cell lung cancer	44/621	34/331	5/53	1.500 (0.926-2.413)	0.096	1.449 (0.478-3.583)	0.462

* Adjusted for age, gender, smoking status, and family history by using unconditional logistic regression analysis

Table 5
Stratified analysis of associations between dominant model of rs3819102 and risk of lung cancers by age, gender, smoking status, family history of cancer, and histological types.

stratification	Cases and/or controls		OR (95%CI)*	P*
	TT (ref)	CT + CC		
Age (y)				
≤60	219/266	185/166	1.420 (1.067-1.891)	0.016
>60	296/355	259/218	1.436 (1.115-1.852)	0.005
Gender				
Male	382/441	318/257	1.419 (1.131-1.783)	0.003
Female	134/180	126/127	1.473 (1.042-2.087)	0.029
Smoking status				
Nonsmoker	148/304	142/199	1.452 (1.067-1.978)	0.012
Eversmoker	368/317	302/185	1.407 (1.105-1.793)	0.006
Family history of cancer				
Have	185/88	143/59	1.201 (0.801-1.810)	0.377
Have not	331/533	301/325	1.518 (1.225-1.881)	<0.001
Cancer subtype				
Adenocarcinoma	226/621	201/384	1.415 (1.116-1.796)	0.004
Squamous cell cancer	167/621	152/384	1.501 (1.139-1.978)	0.004
Small cell lung cancer	44/621	39/384	1.493 (0.940-2.363)	0.087

* Adjusted for age, gender, smoking status, and family history by using unconditional logistic regression analysis.

Discussion

We collected a total of 1979 blood samples and found that C carriers in the TYMS gene rs3819102 were associated with an increased risk of lung cancer. Associations were also significant in the cases of smokers, males, the elderly (over 60 years of age), and those without a family history of cancer. Similar results were found in various histological types; for example, both adenocarcinoma and SCC.

SNP rs3819102 is located in the TYMS 3′ flanking region and an intron of the ENOSF1 gene. The ENOSF1 gene codes for two proteins (rTSα and rTSβ), and the 3′-untranslated region of rTSα RNA is a naturally occurring antisense to TYMS mRNA.[20] The ribonuclease protection assay revealed that the antisense region of rTSα RNA is necessary and sufficient for

Table 6
Stratified analysis of associations between recessive model of rs3819102 and risk of lung cancers by age, gender, smoking status, family history of cancer, and histological types.

stratification	Cases and/or controls		OR (95%CI)*	*P**
	TT + CT (ref)	CC		
Age (y)				
≤60	376/412	28/20	1.571 (0.860-2.919)	0.145
>60	508/540	47/33	1.531 (0.937-2.526)	0.091
Gender				
Male	648/664	52/34	1.634 (1.021-2.651)	0.043
Female	237/288	23/19	1.544 (0.807-2.981)	0.190
Smoking status				
Nonsmoker	266/474	24/29	1.441 (0.790-2.607)	0.228
Eversmoker	619/478	51/24	1.695 (1.029-2.866)	0.042
Family history of cancer				
Have	306/139	22/8	1.268 (0.559-3.170)	0.587
Have not	579/813	53/45	1.658 (1.087-2.540)	0.019
Cancer subtype				
Adenocarcinoma	400/952	27/53	1.240 (0.746-2.019)	0.395
Squamous cell cancer	286/952	33/53	2.189 (1.312-3.630)	0.002
Small cell lung cancer	78/952	5/53	1.237 (0.415-2.979)	0.665

* Adjusted for age, gender, smoking status, and family history by using unconditional logistic regression analysis.

down-regulating TYMS mRNA.[21] Furthermore, rTSβ is associated with decreased TYMS protein expression through a methionine-based signaling pathway.[22]

Some studies indicated that the risk of lung cancer may be associated with the expression of TYMS.[23] TYMS mRNA levels were many times higher in tumor samples of NSCLC patents than in paired normal lung tissues.[24,25] NSCLC tissues with higher TYMS staining intensities had significantly higher percentages of proliferative cell nuclear antigen (mean 48.2 vs 34.4, $P = 0.020$). In contrast, the percentages were low in tissues with low staining for TYMS.[26,27] A clinical study of 193 NSCLC patients found that TYMS-positive tumor tissues were more frequent in patients who were smokers, male, and elderly.[28] The expression level of TYMS mRNA ($P < 0.0001$) and protein ($P = 0.0269$) was significantly higher in SCC compared with adenocarcinoma in formalin-fixed and paraffin-embedded specimens, and a strong association was observed between mRNA and protein expression ($P = 0.00017$).[25]

There are more than 20 compounds in smoke classified as lung carcinogens that result in the formation of DNA adducts.[29] DNA adducts can cause miscoding during DNA replication as bypass polymerases catalyze insertion of the wrong base opposite the adduct, resulting in a permanent mutation. DNA adducts have also been shown to inhibit DNA repair processes, again resulting in permanent mutations.[29,30] TYMS is the enzyme that catalyzes the methylation of deoxyuridine monophosphate to dTMP. This enzymatic reaction provides the sole de novo intracellular source of dTMP, an essential nucleotide precursor for DNA replication and repair. Our study found that smokers with TYMS gene rs3819102 had a higher risk of lung cancer than nonsmokers. It is possible that the accumulation of DNA damage may be the cause of this increased risk.

In our study, elderly individuals exhibited a high risk of lung cancer. This might result from the time-dependent accumulation of DNA damage that occasionally provides aberrant advantages to certain cells and eventually produces cancer.[31] The accumulation of DNA damage throughout life is a common denominator of aging, and a decrease in total DNA repair capacity coincides well with that damage.[32] Consistent with the accumulation of DNA damage, studies have shown that deficiencies in DNA repair mechanisms can cause accelerated aging in mice and underlie several human progeroid syndromes.[31] TYMS is pivotal in DNA repair, and rs3819102 may influence the expression of this enzyme, weakening the ability of cells to repair DNA damage.

Since the first report by Tokuhata and Lilienfeldon noting the familial aggregation of lung cancer,[33] many studies have focused on the risk for people with a family history of this

disease. A case-control study in Eastern and Central Europe, and meta-analyses, demonstrated an increased risk (OR, 1.72; 95% CI, 1.56-1.88) for people whose family members were lung cancer patients.[34] The consistent finding of familial lung cancer could result from shared environmental as well as genetic factors. In our study, individuals without a family history of cancer also had an increased risk of lung cancer, suggesting there may be environmental factors that contribute to carcinogenesis (eg, smoking), apart from genetic factors.

Several limitations exist in the present study. First, although our study involved a relatively large number of patients, the small sample size in the different histological subtypes might have limited the statistical power to detect significant associations for each of the pathological types. Second, because patients were recruited in hospitals, there might be inherent biases resulting in the selection of a non-representative population. Third, the functional change of SNP rs3819102 on TYMS expression was based on a bioinformatics presumption, and no biochemical data were used to test the hypothesis. Thus, further prospective population-based studies and biochemical experiments are needed to verify the conclusion.

In summary, our findings suggest that polymorphism rs3819102 in the TYMS gene might contribute to the risk of lung cancer in Chinese populations, and the risk is more remarkable in smokers. However, establishing the underlying biological relevance among rs3819102, environmental factors, and lung carcinogenesis requires further study.

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