

The Contribution of DNA Apurinic/Apyrimidinic Endonuclease Genotype and Smoking Habit to Taiwan Lung Cancer Risk

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Abstract. To evaluate the association and interaction of genotypic polymorphism the gene for DNA-apurinic/apyrimidinic endonuclease (APEX1) with personal smoking habit and lung cancer risk in Taiwan, the polymorphic variants of APEX1, Asp¹⁴⁸Glu (rs1130409), were analyzed in association with lung cancer risk, and their joint effect with personal smoking habits on lung cancer susceptibility was discussed. In this hospital-based case-control study, 358 patients with lung cancer and 716 cancer-free controls, frequency-matched by age and sex, were recruited and genotyped by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). The results showed that the percentages of TT, TG and GG APEX1 Asp¹⁴⁸Glu genotypes were not significantly different at 43.0%, 41.1% and 15.9% in the lung cancer patient group and 39.9%, 46.1% and 14.0% in non-cancer control group, respectively. We further analyzed the genetic-lifestyle effects on lung cancer risk and found the contribution of APEX1 Asp¹⁴⁸Glu genotypes to lung cancer susceptibility was neither enhanced in the cigarette smokers nor in the non-smokers ($p=0.3550$ and 0.8019 , respectively). Our results provide evidence that the non-synonymous polymorphism of APEX1 Asp¹⁴⁸Glu may not be directly associated with lung cancer risk, nor enhance the effects of smoking habit on lung cancer development.

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Lung cancer has kept its throne as the leading cause of cancer death all over the world, and in Taiwan (1). There are many carcinogens contained in cigarette smoke which may produce reactive oxygen species that can induce DNA damage and formation of adducts (2). However, previous studies showed that only 10% to 15% of all smokers actually develop lung cancer during their life, suggesting that individual susceptibility to carcinogens in cigarette smoke varies among the population (3, 4). Individual differences in susceptibility may be inherited in genes encoding DNA repair proteins, which are essential in maintaining genomic integrity of human cells (5).

Among the several DNA repair systems, the base excision repair (BER) system plays a major role in repairing oxidative and small non-helix-distorting base lesions (6). BER plays a central role in removing damaged bases that could otherwise cause DNA mutations via mispairing, or lead to DNA strand breaks during DNA replication. The overall process of BER is initiated by DNA glycosylases, which recognize and remove specific damaged or inappropriate bases, forming apurinic/apyrimidinic (AP) sites. These AP sites are then cleaved by AP endonucleases. The resulting single-strand break can then be processed by either short-patch (where a single nucleotide is replaced), or long-patch BER (where 2-10 new nucleotides are synthesized) (7). The major human AP endonuclease, APEX1 (also known as APE1, HAP1, and REF-1) plays a central role in the BER DNA repair pathway. As a member of AP endonucleases, APEX1 initiates the repair of the AP sites in DNA produced either spontaneously hydrolyzing the 5' phosphodiester bond of the AP site or after enzymatic removal of damaged bases. It can also act as a 3'-phosphodiesterase by initiating the repair of DNA strand breaks with 3'-blocking damage (8). Importantly, APEX1 also functions as a reduction-oxidation activator of some well-known transcription factors closely related to carcinogenesis, such as AP-1 (FOS/JUN), cAMP response element-binding protein (CREB), and p53 (9).

Table I. The sequences of forward and reverse primers, polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) conditions for identifying apurinic/apyrimidic endonuclease DNA-repair gene (*APEX1*) genotypes.

Polymorphism (location)	Primer sequences (5' to 3')	Restriction enzyme	SNP sequence	DNA fragment size (bp)
Asp148Glu (rs1130409)	F: CCAGCTGAACCTCAGGAGCT R: CTCGGCCTGCATTAGGTACA	<i>MnII</i> 37°C for 2 h	T (Asp) G (Glu)	350 252 + 98

*F and R indicate forward and reverse primers, respectively. SNP: single nucleotide polymorphism.

The genetic variations, such as polymorphisms, are most possible explanations for heritable individual susceptibility to various types of cancer. Although the functional influences of single-nucleotide polymorphisms (SNPs) in the *APEX1* gene are not well understood, it is possible that some variants could have regulatory effects on mRNA or protein expression and the subsequent cellular DNA repair capacity, thereby modulating individual susceptibility to lung cancer. In 2012, Lin and colleagues reported that no association with age, smoking status, tumor histology or stage was found for *APEX1* Asp¹⁴⁸Glu (rs1130409) genotype, except that males had a borderline higher frequency than females of the Asp/Asp (TT) genotype (10). Their data also showed that the risk of *p53* mutation was increased in patients with non-small cell lung cancer with the *APEX1* Asp¹⁴⁸Glu Asp/Asp genotype (10). Their investigated population included 217 lung cancer cases and 217 controls. In the present work, we aimed at examining the association of lung cancer susceptibility with the polymorphism *APEX1* Asp¹⁴⁸Glu, in a larger and more representative population (cases/controls=358/716) and to summarize the contribution of the *APEX1* genotype and its joint effect with smoking habit on lung cancer risk in Taiwanese.

Materials and Methods

Study population and sample collection. Three hundred and fifty-eight patients diagnosed with lung cancer were recruited at the Outpatient Clinics of General Surgery between 2005-2008 at the China Medical University Hospital, Taichung, Taiwan. The clinical characteristics of patients, including histological details, were all graded and defined by expert surgeons. All participants voluntarily completed a self-administered questionnaire and provided their peripheral blood samples. Twice as many non-lung cancer healthy volunteers as controls were selected by matching for age, gender and personal habits after initial random sampling from the Health Examination Cohort of our hospital. The exclusion criteria of the controls included previous malignancy, metastasized cancer from other or unknown origin, and any genetic or familial diseases. Our study was approved by the Institutional Review Board of the China Medical University Hospital (DMR100-IRB-284) and written-informed consent was obtained from all participants.

Genotyping assays. Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous studies (11-14). The polymerase chain reaction (PCR) cycling conditions were as

followed: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The resultant 350 bp PCR product was mixed with 2 U *MnII*. The G form PCR products could be further digested while the T form could not. Two fragments of 252 and 98 bp were present if the product was the digestible C form. The reaction mixture was incubated for 2 h at 37°C. Subsequently, 10 µl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The pairs of PCR primer sequences and specific restriction enzyme for identifying the DNA products are summarized in Table I.

Statistical analyses. All of the 716 of the controls and 358 cases with genotypic and clinical data were analyzed. To ensure that the selected controls represented the general population of Taiwan and to exclude the possibility of genotyping error, the deviation of the *APEX1* Asp¹⁴⁸Glu genotypic frequencies in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test was used to compare the distribution of the genotypes between cases and controls. Data were recognized as significant when the statistical *p*-value was less than 0.05. The lung cancer risk associated with the genotypes was estimated as an odds ratio (ORs) and 95% confidence intervals (CIs) by unconditional logistic regression with adjustment for the effect of possible confounders such as age, gender, and pack-years of smoking.

Results

The investigated population of the current study included 358 patients with lung cancer and 716 non-cancer controls, and the frequency distributions of selected characteristics of patients and controls are shown in Table II. Since we used frequency matching to select the non-cancer controls, none of the differences between the groups were statistically significant (*p*>0.05) (Table II).

The distributions of genotypic and allelic frequencies of the *APEX1/Ref-1* Asp¹⁴⁸Glu polymorphism in lung cancer cases and controls are presented in Table III. The ORs after adjusting for the confounding factors (age, gender, smoking and alcohol drinking status) for those carrying TG and GG genotypes were 0.82 (95% CI=0.58-1.17) and 1.08 (95% CI=0.65-1.73), respectively, compared to those carrying the TT wild-type genotype. The *p*-value for trend was not significant (*p*=0.2819). In the dominant (TG plus GG *versus* TT) and recessive (GG *versus* TT plus TG) models, the association between *APEX1* Asp¹⁴⁸Glu polymorphism with the risk for lung cancer was not statistically significant either (*p*=0.3570 and 0.4101,

Table II. Characteristics of investigated patients with lung cancer and controls.

Characteristic	Controls (n=716)			Patients (n=358)			p-Value ^a
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			64.8 (6.8)			64.0 (6.9)	0.58
Gender							0.36
Male	488	68.1%		254	70.9%		
Female	228	31.9%		104	29.1%		
Cigarette habit							
Smokers	563	78.6%		293	81.8%		0.23
Non-smokers	153	21.4%		65	18.2%		

^aBased on Chi-square test.

respectively) (Table III). To sum up, these data indicated that individuals carrying variant G allele at *APEX1* Asp¹⁴⁸Glu do not appear to have a higher or lower risk of lung cancer than those carrying the T allele.

The interaction of genotype of *APEX1* Asp¹⁴⁸Glu and the smoking habits of participants was of great interest since lung cancer is known to be a smoking-related disease. The genotypic distribution of the different genetic polymorphisms of *APEX1* Asp¹⁴⁸Glu was not significantly different between lung cancer and control groups who have a smoking habit ($p=0.3550$), nor was that for non-smokers ($p=0.8011$) (Table IV). Overall, there was no differential distribution of genotypic frequency of *APEX1* Asp¹⁴⁸Glu among smokers in patient and control groups. Nor was there any differential genotypic distribution of *APEX1* Asp¹⁴⁸Glu in the non-smoking lung cancer and control groups.

Discussion

In order to reveal the role of the *APEX1* genotype in lung cancer, we examined the non-synonymous SNP of the *APEX1* gene, Asp¹⁴⁸Glu (Table I), and investigated its association with the risk for lung cancer in a 358/716 case/control study in central Taiwan (Table II). We found that the distributions of TT, TG and GG genotypes of *APEX1* Asp¹⁴⁸Glu were 43.0, 41.1 and 15.9% among patients with lung cancer, which were not associated with lung cancer risk in Taiwan (Table III). This is consistent with a previous study conducted among patients with lung cancer in Taiwan with a smaller population (10). As for the investigation of joint effects of genetic (the genotype of *APEX1* Asp¹⁴⁸Glu) and lifestyle (personal smoking status) factors on lung cancer risk, there was no statistically positive association observed in this study. There are still many further studies needed to reveal any contribution of *APEX1* to lung carcinogenesis. One is to investigate the expression alterations at mRNA and protein levels for *APEX1* in tumor sites from the patients with lung cancer and another is to investigate the contribution of other SNPs of *APEX1* to lung carcinogenesis. For instance, Lo and colleagues provided evidence to suggest that the GG and GT genotypes of

Table III. Distributions of apurinic/apyrimidic endonuclease DNA-repair gene (*APEX1*) Asp¹⁴⁸Glu genotypic and allelic frequencies and their association with risk of lung cancer.

	Lung cancer cases (%)	Controls (%)	Adjusted OR ^a (95% CI)	p-Value
<i>APEX1</i> Asp ¹⁴⁸ Glu				
TT	154 (43.0)	286 (39.9)	1.00 (ref)	
TG	147 (41.1)	330 (46.1)	0.82 (0.58-1.17)	0.1819
GG	57 (15.9)	100 (14.0)	1.08 (0.65-1.73)	0.7713
p-Value for trend				0.2819
(TG+GG) vs. TT			0.86 (0.67-1.24)	0.3570
GG vs. (TT+TG)			1.14 (0.80-1.89)	0.4101

^aAdjusted by age, gender, smoking and alcohol drinking status.Table IV. Distribution of apurinic/apyrimidic endonuclease DNA-repair gene (*APEX1*) Asp¹⁴⁸Glu genotypes among patients with lung cancer after stratification by personal smoking habits.

Variable	<i>APEX1</i> Asp ¹⁴⁸ Glu genotype			p-Value ^a
	TT (%)	TG (%)	GG (%)	
Smokers				0.3550
Controls	225 (40.0%)	259 (46.0%)	79 (14.0%)	
Patients	126 (43.0%)	120 (41.0%)	47 (16.0%)	
Non-smokers				0.8011
Controls	61 (39.9%)	71 (46.4%)	21 (13.7%)	
Patients	28 (43.1%)	27 (41.5%)	10 (15.4%)	

^aBased on Chi-square test.

APEX1 T-656G (rs1760944) are protective against lung cancer risk in Taiwan (15). They also showed that GG and GT variant promoter had higher transcriptional activity than that with the TT genotype in lung cancer cells.

Literature investigating the association between *APEX1* Asp¹⁴⁸Glu and lung cancer is inconsistent among different populations. For example, it was found that lung cancer risk

was increased among those carrying the Glu variant of *APEX1* compared to those with Asp/Asp genotype in a Belgian population (16). However, those carrying the Glu variant of *APEX1* were reported to have reduced risk of lung cancer in a southeast China population (17). In addition, the studies have found no association between *APEX1* Asp¹⁴⁸Glu genotype and lung cancer risk in Japanese (18) and Germans (19). Clinically, patients with advanced non-small cell lung cancer carrying *APEX1*-Glu variants are reported to have poorer responses to chemotherapy and radiotherapy than those with the Asp/Asp genotype. (20). Functionally, the *in vitro* endonuclease activity and DNA binding activity were not significantly different among *APEX1* Asp¹⁴⁸Glu polymorphic variants (21). We also found that the mRNA and protein levels for *APEX1* Asp¹⁴⁸Glu polymorphic variants were similar among patients with renal cell carcinoma in Taiwan (11).

To sum up, we have reviewed on the contribution of *APEX1* genotype to lung cancer, and discussed the joint effects of *APEX1* Asp¹⁴⁸Glu with personal smoking habit on lung cancer risk in Taiwan. The current and previous findings together may indicate that while *APEX1* T-656G genotype may play a major role, in tumorigenesis of lung cancer in Taiwan, the non-synonymous Asp¹⁴⁸Glu genotype appears to have no role.

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