Poly (AT) Polymorphism in Intron 11 of the *XPC* DNA Repair Gene Enhances the Risk of Lung Cancer

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Abstract

Reduced DNA repair capacity due to inherited polymorphisms may increase the susceptibility to smoking-related cancers. In this report, we investigate the relationship between xeroderma pigmentosum complementary group C poly (AT) insertion/deletion polymorphism (XPC-PAT) of the *XPC* gene and lung cancer risk in a hospital-based case-control study of 359 newly diagnosed lung cancer patients and 375 control subjects matched on age, sex, and catchment area. The *XPC* genotype was determined by PCR-RFLP, and the results were analyzed using logistic regression, adjusting for relevant covariates. We found that the frequency of the PAT+/+ genotype was higher in the cases (20.6%) than in the controls (14.1%; P = 0.057) and that the PAT+/+ subjects were at significantly increased risk for lung cancer [adjusted odds ratio (OR), 1.60; 95% confidence interval (95% CI), 1.01-2.55]. Stratified analysis revealed that the risk was higher in former smokers (OR, 2.15; 95% CI, 1.07-4.31) and older people (OR, 2.76; 95% CI, 1.02-7.51), although this probably occurs due to 63.4% of cases older than 73 years being ex-smokers. When stratified by histologic type, the variant genotype was associated with statistically significant increased risk for squamous cell carcinoma (OR, 1.93; 95% CI, 1.06-3.51). In conclusion, our findings support the hypothesis that PAT and intron 11 C/A XPC polymorphisms are linked in the Spanish population and may contribute to the risk of developing lung cancer probably due to a higher frequency of deletion of exon 12 and reduced DNA repair capacity of the XPC protein. (Cancer Epidemiol Biomarkers Prev 2004;13(11):1788–93)

Introduction

Lung cancer is the most common malignant neoplasm in the developed world and represents the leading cause of cancer death in men in Europe. The outcome of this tumor is poor, especially for those with advanced disease, and little progress has been made in the last two decades (1). It is generally believed that lung cancer is initiated by tobacco carcinogen–induced DNA damage. However, only a fraction of smokers develop cancer in their lifetime, suggesting that there may be differences in individual susceptibility to cancer and possible gene-environment interactions. DNA repair and maintenance is essential in protecting the genome of the cell from environmental hazards like tobacco smoke. Reduced DNA repair capacity (DRC) can render a higher risk of developing many types of cancer, including lung cancer (2, 3). There is an interindividual variation in DRC among both cancer patients and healthy subjects, and the ability to repair benzo(*a*)pyrene diol epoxide tobacco-induced adducts in peripheral lymphocytes is a significant predictor of risk of lung cancer (4-6). Polymorphisms in DNA repair genes may contribute to variation in DRC in the general population and may lead to genetic instability and carcinogenesis (7, 8). The nucleotide excision repair process includes several steps of damage recognition by a complex of bound proteins including xeroderma pigmentosum complementary group C (XPC), unwinding of the helix, incision and excision of the lesions, gap filling, and ligation, which are done by a group of >35 proteins (7). The *XPC* gene spans 33 kb on chromosome 3 and contains 16 exons and 15 introns (Genbank accession no. AC090645), encoding a 940–amino acid protein uniquely involved in global genome repair (9).

An intronic biallelic poly (AT) insertion/deletion polymorphism (XPC-PAT) of the DNA repair *XPC* gene consisting of an insertion of 83 bases of A and T [poly (AT)] and deletion of 5 bases (GTAAC) at positions 1457 to 1461 in intron 9 has been reported previously (10). PAT+ homozygous subjects exhibited lower DRC than did those with other PAT genotypes; hence, this polymorphism may modulate DRC and could be a useful biomarker for identifying individuals at risk of developing cancer (11, 12). XPC-PAT polymorphism has been reported to be in linkage disequilibrium with a single nucleotide polymorphism in XPC exon 15 that causes an amino acid change (Lys^{939}Gln) that does not

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alter XPC function *in vitro* (10) and with a new common C/A single nucleotide polymorphism located at the −5 position of the XPC intron 11 splice acceptor site (intron 11 C/A polymorphism) that was associated with increased skipping of exon 12. The abnormally spliced XPC mRNA isoform has diminished DNA repair activity and may contribute to cancer susceptibility (9). These three markers are ~9 kb apart in the *XPC* gene and are consistent with a haplotype of PAT−/intron 11 C/exon 15 A in 60% of donors and PAT+/intron 11 A/exon 15 C in 40% of donors (9).

In the present study, we genotyped PAT polymorphism of the *XPC* gene in a hospital-based case-control study to test the hypothesis that this polymorphism is associated with the risk of developing lung cancer, assessing the main histologic types. We also genotyped C/A intron 11 polymorphism to confirm that these two polymorphisms are in linkage disequilibrium in the Spanish population.

Materials and Methods

Study Subjects. Patients (n = 359) with primary lung cancer diagnosed between October 2000 and January 2004 in three main hospitals of Asturias in northern Spain (Cabueñes Hospital in Gijón, San Agustín Hospital in Avilés, and the Central Hospital in Oviedo) were recruited at the Molecular Epidemiology Unit of the Oncology Institute of the Oviedo University. Eligible participants were ages between 20 and 85 years and had resided in the catchment area of each participating hospital at least 6 months before diagnosis. Patients with primary cancer other than lung cancer in the last 5 years were excluded. All cases were newly diagnosed, previously untreated, and histologically or clinically confirmed (13). Their histologic status was 39.8% squamous cell carcinomas, 29.2% adenocarcinomas, 14.5% small cell carcinomas, 2.8% large cell carcinomas, and 8.9% nondifferentiated carcinomas (Table 1). Their clinical stages were 27% stage I, 4.2% stage II, 27.6% stage III, and 32.9% stage IV (Table 1). Controls were recruited from the same hospitals and matched to cases on gender, age (±5 years), and residential area. We selected one or two controls for each case from the hospital admission database whose pathology matched a list of pathologies including non-cancer-related and non-smoking-related diseases. The main specific pathologies of the 375 controls were 39.2% inguinal and abdominal hernias (*International Classification of Diseases, Ninth Edition* codes 550-553), 30.7% injuries (*International Classification of Diseases, Ninth Edition* codes 800-848, 860-869, and 880-897), 8.0% appendicitis (*International Classification of Diseases, Ninth Edition* code 540), and 10.9% intestinal obstructions (*International Classification of Diseases, Ninth Edition* codes 560, 569, and 574; ref. 13). At recruitment, written informed consent was obtained from each study participant before interview and blood collection for genetic analyses. The Ethical Committee of the Central Hospital of Asturias approved the study procedure.

Data Collection. Epidemiologic data were collected personally through computer-assisted questionnaires by trained interviewers during the first hospital admission. Structured questionnaires collected information on socio-demographic characteristics, recent and prior tobacco use, environmental exposure (air pollution and passive smoking), diet, personal and family history of cancer, and occupational history from each participant. Peripheral blood samples (or mouthwash samples when they refused to donate blood) were collected from all participants.

Table 1. Characteristics of lung cancer cases and control patients in a Spanish population

Variable	Cases (n = 359), n (%)	Controls (n = 375), n (%)	P*
Gender			
Male	314 (87.5)	322 (85.9)	
Female	45 (12.5)	53 (14.1)	0.588
Age (y), mean (SD)	63.91 (11.57)	62.94 (11.81)	0.280
Smoking status			
Never	25 (7.0)	95 (25.3)	
Ever	334 (93.0)	280 (74.7)	<0.001
Former	155 (43.2)	168 (44.8)	
Current	179 (49.9)	112 (29.9)	<0.001
PY,† mean (SD)	64.64 (40.91)	42.97 (43.41)	<0.001
Family history of cancer			
No	188 (54.0)	229 (62.2)	
Lung cancer	40 (11.5)	19 (5.2)	
Other cancers	120 (34.5)	120 (32.6)	0.004
Histologic type			
Squamous cell carcinoma	143 (39.8)		
Adenocarcinoma	105 (29.2)		
Small cell carcinoma	52 (14.5)		
Large cell carcinoma	10 (2.8)		
Nondifferentiated	32 (8.9)		
Others	7 (1.9)		
Clinical diagnosis	6 (1.7)		
Missing	4 (1.1)		
Clinical stage			
Stage I	97 (27.0)		
Stage II	15 (4.2)		
Stage III	99 (27.6)		
Stage IV	118 (32.9)		
Missing	30 (8.4)		

*Two-sided χ^2 test and Mann-Whitney where appropriate.
† PY for ever smokers.

Tobacco Exposure Information. Participants were defined as never smokers if they had not smoked >100 cigarettes in their lifetime and had not smoked cigars or pipes once per month for >6 months. A former smoker was one who had stopped smoking at least 1 year before the interview. Smoking intensity [pack-years (PY)] was defined as the number of packs of cigarettes smoked per day (assuming the equivalency of a cigar to four cigarettes and a pipe to two cigarettes) multiplied by the number of years smoking. The cut point for PY to classify as light and heavy smokers was based on the median of the distribution among all of the control participants who smoked.

XPC Genotyping. Laboratory personnel were blinded to case and control status. Genomic DNA was extracted from peripheral blood samples (96.2% of total) or exfoliated buccal cells (3.8% of total) using a previously reported procedure (14). For quality control, a random

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5% of the samples were repeated, and two of the authors independently reviewed all results. A quality control of 50 blood and mouthwash samples from the same participants ensured the reliability of genotyping results of mouthwash samples.

To determine the XPC-PAT polymorphism (Genbank accession no. AF156539), intron 9 of the *XPC* gene was amplified by PCR using the primers and protocol described previously (10). The PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide. After electrophoresis, homozygous PAT−/− genotypes were represented by a 266-bp DNA band, whereas homozygous PAT+/+ genotypes were represented by a 344-bp fragment. Heterozygous displayed a combination of both alleles (266 and 344 bp; Fig. 1A).

The determination of XPC intron 11 C/A polymorphism (Genbank accession no. AF261900) was done by RFLP. The PCR primers used for amplifying this polymorphism were forward 5′-GCCAAATGCT-GACTTGCTCACCgG-3′ and reverse 5′-GCCACG CGGTGTAGATTGGG-3′. The forward primer was specially designed to introduce a recognition site of the restriction enzyme *Hae*III by replacing a C with a G (small letter). The wild-type allele has this recognition site (GGCC), whereas the variant allele destroys the recognition site by inserting an adenine. PCR was done in a 25-μL mixture containing 20 ng genomic DNA, 1.6 mmol/L $MgCl_2$, 0.25 mmol/L of each deoxynucleotide triphosphate, 0.5 unit Taq polymerase, and 2.5 pmol of each primer in 1× PCR buffer. The PCR running conditions were 5 minutes at 94°C followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 65°C, and 30 seconds at 72°C with a final step at 72°C for 7 minutes. The PCR products were digested overnight with *Hae*III at 37°C, converting the 128-bp fragment into two fragments of 24 and 104 bp in the wild-type allele. DNA fragments were resolved on 3% agarose gels and stained with ethidium bromide (Fig. 1B).

Statistical Analysis. Tests for Hardy-Weinberg equilibrium among controls were conducted using observed genotype frequencies and a χ^2 test with 1 *df*. Univariate analysis was first done to compare the distribution of age and sex and the frequencies of alleles and genotypes. The differences in the distribution between cases and controls were tested using the χ^2, Fisher exact, and Mann-Whitney *U* tests, where appropriate. The crude odds ratios (OR) were calculated by Woolf's method (15). Multivariate logistic regression analysis with adjustment for age, sex, family history of cancer, and smoking status was done to calculate adjusted ORs and 95% confidence intervals (CI), which were estimated using the SPSS program (version 11).

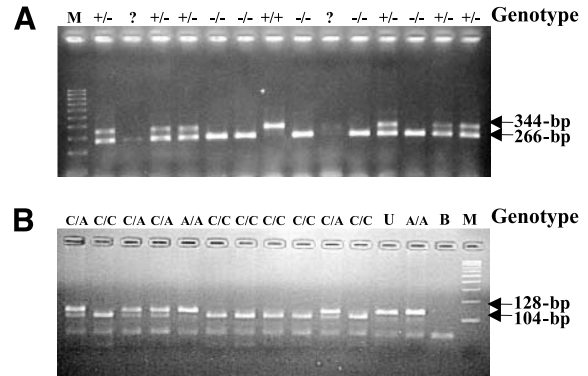

Figure 1. A. PCR products of XPC-PAT alleles. PCR generated two fragments: a 344-bp XPC-PAT+ fragment and a 266-bp XPC-PAT− fragment, permitting detection of PAT+/+ (+/+), PAT+/− (+/−), and PAT−/− (−/−) genotypes. **B.** RFLP assay for detection of the C/A intron 11 polymorphism. *Hae*III cuts the 128-bp sequence generated by PCR into fragments of 24 and 104 bp if the C allele is present in the sequence, permitting detection of C/C, C/A, and A/A genotypes. *U,* undigested PCR amplification; *M,* 100-bp ladder (10 bands: 100-1,000 bp in exact 100-bp increments); *B,* no genomic DNA in the PCR amplification.

Results

The analysis included 359 lung cancer cases and 375 controls from the Caucasian population of Asturias, northern Spain. The participation rates for cases and controls were 88.5% and 90%, respectively. Population characteristics of the participant subjects such as age, sex, smoking status and intensity, first-degree family history of cancer, and histologic type and clinical stage data for the cases are summarized in Table 1. There were no statistically significant differences among cases and controls in terms of mean age and sex distributions, suggesting that the frequency matching was adequate. However, there were more current smokers and more heavy smokers (number of PY) between cases than among controls ($P < 0.001$). Although the mean PY of smoking in the control population (42.97 PY) is high compared with the control population in other studies, there is no difference with the Asturian normal population of that gender and age (16). Case patients were significantly more likely than control subjects to report a family history of any cancer ($P = 0.004$), especially lung cancer, in their first-degree relatives.

The XPC-PAT genotype distribution for cases and controls is shown in Table 2. The distribution of the genotypes among the control population was consistent with Hardy-Weinberg equilibrium ($\chi^2 = 1.3678$; $P = 0.242$). The cases exhibited a borderline statistically significant higher frequency of the PAT+/+ genotype (20.6%) than the controls (14.1%; $P = 0.057$). The XPC PAT+ allele was very common, with a frequency of 45.0% in cases and 39.5% in controls, the difference being statistically significant ($P = 0.032$). Table 2 also shows the association between XPC genotypes and lung cancer risk. Those individuals with the PAT+/+ genotype presented a significantly higher lung cancer risk (OR, 1.68; 95% CI, 1.09-2.59). After adjustment by age, gender, smoking status, and family history of cancer, the OR (95% CI) was 1.66 (1.05-2.63) and 1.60 (1.01-2.55) for individuals with the polymorphic genotype. The distribution of XPC-PAT polymorphism was compared with XPC intron 11 C/A polymorphism results in 150 cases and 150 controls. These markers are linked in our Spanish population and are consistent with a haplotype of PAT−/intron 11 C and PAT+/intron 11 A.

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Table 2. Analysis of XPC-PAT polymorphism and lung cancer risk estimates

XPC-PAT genotype	Cases (n = 359), n (%)	Controls (n = 375), n (%)	P*	Crude OR (95% CI)	Adjusted† OR (95% CI)	Adjusted‡ OR (95% CI)
−/−	110 (30.6)	132 (35.2)		Reference	Reference	Reference
+/−	175 (48.7)	190 (50.7)		1.11 (0.80-1.53)	1.08 (0.76-1.53)	1.05 (0.74-1.50)
+/+	74 (20.6)	53 (14.1)	0.057	1.68 (1.09-2.59)	1.66 (1.05-2.63)	1.60 (1.01-2.55)
PAT+ allele frequency (%)	45.0	39.5	0.032			

*Two-sided χ^2 test.
†Adjusted by age, gender, smoking status (never and ever) and family history of cancer.
‡Adjusted by age, gender, smoking status (never, former, and current) and family history of cancer.

We next did stratified analysis by selected variables (Table 3). Age was categorized by the first and third quartile values of controls. In older subjects (≥73 years), the adjusted OR (95% CI) for the homozygous variant was 2.76 (1.02-7.51). However, no significant risk was found in younger subjects (≤54 years; OR, 2.07; 95% CI, 0.84-5.16) or for the group ages between 55 and 72 years (OR, 1.10; 95% CI, 0.56-2.16). The interaction between XPC genotypes and cigarette smoking in lung cancer risk was examined separately by stratifying according to smoking status (never, ever, former, and current; Table 3). Among the nonsmokers and current smokers, the variant PAT+/+ genotype was not associated with an elevated risk of lung cancer (adjusted OR, 1.59; 95% CI, 0.37-6.81 and OR, 1.26; 95% CI, 0.62-2.54, respectively). However, former smokers carrying two PAT+ alleles had twice more risk of lung cancer than those with two PAT− alleles (adjusted OR, 2.15; 95% CI, 1.07-4.31).

We also examined according to family history of cancer (Table 3). The variant genotype was associated with increased risk for lung cancer in patients with a first-degree relative with another type of cancer (OR, 2.40; 95% CI, 1.08-5.31). The number of controls with first-degree relatives with lung cancer was too small to calculate the risk associated with this variable.

When stratified by histologic type, the variant PAT+/+ genotype was associated with significantly increased risk for squamous cell carcinoma (OR, 1.93; 95% CI, 1.06-3.51) but not for adenocarcinoma and small cell carcinoma (Table 3).

Discussion

In this report, we have examined PAT polymorphism in the *XPC* gene and its association with the risk of lung cancer in a Caucasian population from Asturias, northern Spain. We found that the subjects with the XPC-PAT+/+ genotype were at an elevated risk for lung cancer, suggesting that this polymorphism may contribute to

Table 3. Analysis of XPC-PAT genotype frequencies, OR, and 95% CI for lung cancer stratified by selected variables

XPC-PAT genotype	Cases, n (%)			Controls, n (%)			Adjusted OR (95% CI)*			P for trend†
	−/−	+/−	+/+	−/−	+/−	+/+	−/−	+/−	+/+	
Sex										
Male	91 (29.0)	156 (49.7)	67 (21.3)	108 (33.5)	166 (51.6)	48 (14.9)	1.00	1.05 (0.72-1.54)	1.55 (0.94-2.55)	0.185
Female	19 (42.2)	19 (42.2)	7 (15.6)	24 (45.3)	24 (45.3)	5 (9.4)	1.00	1.01 (0.38-2.66)	2.26 (0.57-9.05)	0.484
Age (y)										
≤54	22 (25.6)	45 (52.3)	19 (22.1)	40 (38.5)	46 (44.2)	18 (17.3)	1.00	1.98 (0.96-4.07)	2.07 (0.84-5.16)	0.137
55-72	56 (32.6)	83 (48.3)	33 (19.2)	57 (32.9)	89 (51.4)	27 (15.6)	1.00	0.85 (0.51-1.42)	1.10 (0.56-2.16)	0.676
≥73	32 (31.7)	47 (46.5)	22 (21.8)	35 (35.7)	55 (56.1)	8 (8.2)	1.00	0.97 (0.49-1.94)	2.76 (1.02-7.51)	0.080
Smoking status										
Never	11 (44.0)	10 (40.0)	4 (16.0)	36 (37.9)	47 (49.5)	12 (12.6)	1.00	0.81 (0.28-2.31)	1.59 (0.37-6.81)	0.658
Ever	99 (29.6)	165 (49.4)	70 (21.0)	96 (34.3)	143 (51.1)	41 (14.6)	1.00	1.12 (0.77-1.62)	1.69 (1.04-2.75)	0.098
Former	50 (32.3)	69 (44.5)	36 (23.2)	59 (35.1)	90 (53.6)	19 (11.3)	1.00	0.85 (0.51-1.42)	2.15 (1.07-4.31)	0.022
Current	49 (27.4)	96 (53.6)	34 (19.0)	37 (33.0)	53 (47.3)	22 (19.6)	1.00	1.40 (0.80-2.46)	1.26 (0.62-2.54)	0.497
Family history of cancer										
No	60 (31.9)	90 (47.9)	38 (20.2)	80 (34.9)	116 (50.7)	33 (14.4)	1.00	0.96 (0.61-1.51)	1.44 (0.79-2.63)	0.362
Lung cancer‡	16 (40.0)	19 (47.5)	5 (12.5)	3 (15.8)	14 (73.7)	2 (10.5)				
Other cancers	30 (25.0)	61 (50.8)	29 (24.2)	46 (38.3)	56 (46.7)	18 (15.0)	1.00	1.72 (0.90-3.29)	2.40 (1.08-5.31)	0.080
Histologic type										
Squamous cell carcinoma	44 (30.8)	63 (44.1)	36 (25.2)				1.00	0.90 (0.56-1.47)	1.93 (1.06-3.51)	0.026
Adenocarcinoma	28 (26.7)	58 (55.2)	19 (18.1)	132 (35.2)	190 (50.7)	53 (14.1)	1.00	1.51 (0.88-2.58)	1.81 (0.90-3.64)	0.192
Small cell carcinoma	18 (34.6)	23 (44.2)	11 (21.2)				1.00	0.88 (0.44-1.75)	1.31 (0.55-3.11)	0.634

*Adjusted for the other covariates presented in this table in a logistic regression model for each stratum.
†Adjusted for the other covariates presented in this table in a logistic regression model for each stratum.
‡Insufficient number of controls to calculate the ORs.

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the etiology of lung cancer. We also found that PAT and intron 11 C/A polymorphisms are fully associated due to linkage disequilibrium. Therefore, considering that the PCR-based intron 9 PAT assay is more rapid and economical than the intron 11 C/A RFLP assay, as it does not involve the additional step of restriction enzyme digestion, it may be preferred for use as a risk marker in population studies. We chose these two polymorphisms as candidates because the other described polymorphism in the exon 15 of the *XPC* gene, which causes an amino acid change, does not alter the function of the *XPC* gene in an *in vitro* assay (10). The results reported above are biologically plausible, as being homozygous for a variant PAT+ allele in the *XPC* gene is associated with a reduced capacity to repair UV-induced damage as assayed by the host reactivation assay in a cohort of healthy subjects (11, 12). In addition, the PAT+ allele is in linkage disequilibrium with the A allele of intron 11 splice acceptor polymorphism, which is associated with a higher frequency of deletion of exon 12. This exon 12 deleted XPC mRNA isoform has reduced DNA repair activity, presenting a dominant negative effect (9). Skipping of exon 12 creates a premature stop codon, generating a truncated XPC protein lacking the carboxyl-terminal 125 amino acids, which are essential for the recruitment of transcription factor IIH to the lesion following initial damage recognition (17). Transcription factor IIH is essential for the nucleotide excision repair reaction, so it is conceivable that loss of interaction between XPC and transcription factor IIH results in complete abrogation of the repair process (17). Furthermore, in two newly diagnosed Turkish families with the autosomal recessive disorder xeroderma pigmentosum, mutations of the XPC intron 3 resulting in alternative splicing that impaired DNA repair function have also been identified (18).

In the stratified analysis, the elevated risk of lung cancer associated with PAT polymorphism was observed in older subjects (≥73 years), ex-smokers, patients with a family history of cancer, and patients with squamous cell carcinoma. Although our findings about subgroups are preliminary due to the relatively small number of subjects in each stratum and possibly as a result of multiple tests, it is surprising that the association was observed in former smokers only, not for never smokers and current smokers. A possible explanation may be that the risk is higher in individuals who had less exposure to tobacco smoke; the mean number of years smoking is 39.8 and 43.7 years in former smoker cases and current smoker cases, respectively ($P < 0.001$). Another possible explanation might be that smoking also alters the level by triggering and up-regulating DNA repair enzymes, flattening or even reversing the difference among the XPC-PAT genotypes with possibly different repair efficiency (19). Indeed, Shen et al. (20) showed that current smokers exhibited the highest DRC as compared with former and nonsmokers both among patients and control subjects. On the other hand, in the group of never smokers, we only have four cases with the PAT+/+ genotype, so the results in this group are not reliable. Moreover, the effect that we observed in the group of older people is possibly due to the fact that 63.4% of people older than 73 years are former smokers. We also observed that the risk is higher in squamous lung carcinoma probably because this histologic type is the one most related with tobacco smoking.

Recently, one epidemiologic study investigated the association between PAT genetic polymorphism in the *XPC* gene and susceptibility to lung cancer in Chinese population, but they do not find any association (21). In contrast, the PAT+/+ genotype was associated with a 1.85-fold increase in squamous cell carcinoma of the head and neck (22). Moreover, and in line with our results, the subgroup of individuals who were at the highest risk associated with the PAT+ allele were older subjects, male subjects, and those who had only slight exposure to smoking and alcohol. Furthermore, another study found that the frequency of the variant allele for A/C polymorphism in exon 15 of the *XPC* gene, in linkage disequilibrium with the PAT polymorphism, was significantly higher in bladder cancer cases than in controls (OR, 1.49; 95% CI, 1.16-1.92; ref. 23).

The main limitation of the present study is the small sample size, thus lacking sufficient power to examine the effect of stratification. It would thus be prudent to replicate our results with a very large sample size. Due to case-control design, we are not able to exclude the possibility that DNA repair is also associated with some of the diseases selected for controls. By matching on age, sex, and residence, potentially confounding factors may be minimized. Furthermore, the additional adjustment of the data analysis would help to eliminate any inadequacy in matching.

In conclusion, we found for the first time in Caucasian population that PAT of the DNA repair *XPC* gene predisposes to lung cancer. Increased susceptibility to lung cancer might be related to a reduced level of repair of tobacco-damaged DNA in PAT+/+ patients who would have some truncated nonfunctional XPC protein.

In complex diseases like lung cancer, the presence or absence of disease is attributable to environmental exposure, polymorphisms of multiple interacting genes, and gene-environment interactions. Therefore, successful investigation requires the evaluation of specific functional genetic variants (single nucleotide polymorphisms) as cancer susceptibility markers in well-designed epidemiologic studies. With the rapid improvement in high-throughput genotyping methods (24), we are in an excellent position to translate genetic susceptibility information into health behavior promotion, as genetically susceptible subpopulations may be more motivated to seek screening and intervention. It is also hoped that association data will help to elucidate presently unidentified biological pathways related to lung cancer, ultimately yielding practical applications in drug discovery and early diagnosis. Lung cancer is the paradigm of a disease attributable to the interaction between genes and environmental exposure. Although cigarette smoking is the major risk factor for lung cancers, individual susceptibility to tobacco carcinogenesis plays a role in the etiology of lung cancer. Molecular epidemiologic approaches to identify individuals at high risk, especially those who have difficulty in quitting smoking, are an important step forward in lung cancer prevention. The type of descriptive work presented here will be critical in deciding which genetic polymorphisms to include in future risk assessment studies and statistical modeling. Additional studies of this polymorphism in other populations will be necessary to confirm these

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results, and larger sample sizes will be needed to examine potentially relevant gene-gene and gene-environment interactions. Furthermore, looking at the single nucleotide polymorphisms of other nucleotide excision repair genes would help to more fully understand the role of DNA repair and lung cancer.

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References

1. Tyczynski JE, Bray F, Parkin DM. Lung cancer in Europe in 2000: epidemiology, prevention, and early detection. Lancet Oncol 2003;4:45–55.
2. Benhamou S, Sarasin A. Variability in nucleotide excision repair and cancer risk: a review. Mutat Res 2000;462:149–58.
3. Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. J Natl Cancer Inst 2000;92:874–97.
4. Wei Q, Cheng L, Hong WK, Spitz MR. Reduced DNA repair capacity in lung cancer patients. Cancer Res 1996;56:4103–7.
5. Wei Q, Cheng C, Amos CI, et al. Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiology study. J Natl Cancer Inst 2000;92:1764–72.
6. Li D, Firozi PF, Wang L, et al. Sensitivity to DNA damage induced by benzo(*a*)pyrene diol epoxide and risk of lung cancer: a case-control analysis. Cancer Res 2001;61:1445–50.
7. Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:1513–30.
8. Hu JJ, Mohrenweiser HW, Bell DA, Leadon SA, Miller MS. Symposium overview: genetic polymorphisms in DNA repair and cancer risk. Toxicol Appl Pharmacol 2002;185:64–73.
9. Khan SG, Muñiz-Medina V, Shahlavi T, et al. The human *XPC* DNA repair gene: arrangement, splice site information content and influence of a single nucleotide polymorphism in a splice acceptor site on alternative splicing and function. Nucleic Acids Res 2002;30:3624–31.
10. Khan SG, Metter EJ, Tarone RE, et al. A new xeroderma pigmentosum group C poly (AT) insertion/deletion polymorphism. Carcinogenesis 2000;21:1821–5.
11. Qiao Y, Spitz MR, Shen H, et al. Modulation of repair of ultraviolet damage in the host-cell reactivation assay by polymorphic XPC and XPD/ERCC2 genotypes. Carcinogenesis 2002;23:295–9.
12. Qiao Y, Spitz MR, Guo Z, et al. Rapid assessment of repair of ultraviolet DNA damage with a modified host-cell reactivation assay using a luciferase reporter gene and correlation with polymorphisms of DNA repair genes in normal human lymphocytes. Mutat Res 2002;509:165–74.
13. OPS-OMS. International Classification of Diseases, Ninth Revision. Scientific Publication No. 353. Washington (DC): OMS; 1978.
14. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
15. Wolf FM. Meta-analysis: quantitative methods for research synthesis. Newbury Park (CA): Sage Publications; 1986.
16. Encuesta Nacional de Salud; 2001. Available from: http://www.msc.es/Diseno/sns/sns_sistemas_informacion.htm.
17. Uchida A, Sugasawa K, Masutani C, et al. The carboxy-terminal domain of the XPC protein plays a crucial role in nucleotide excision repair through interactions with transcription factor IIH. DNA Repair 2002;1:449–61.
18. Khan SG, Metin A, Gozukara E, et al. Two essential splice lariat branchpoint sequences in one intron in a xeroderma pigmentosum DNA repair gene: mutations result in reduced XPC mRNA levels that correlate with cancer risk. Hum Mol Genet 2004;13:343–52.
19. Matullo G, Palli D, Peluso M, et al. XRCC1, XRCC3, XPD gene polymorphisms, smoking and ^{32}P-DNA adducts in a sample of healthy subjects. Carcinogenesis 2001;22:1437–45.
20. Shen H, Spitz MR, Qiao Y, et al. Smoking, DNA repair capacity and risk of nonsmall cell lung cancer. Int J Cancer 2003;107:84–8.
21. Wang YG, Xing DY, Tan W, Wang LJ, Tang PZ, Lin DX. Poly (AT) polymorphism in DNA repair gene XPC and lung cancer risk. Zhonghua Zhong Liu Za Zhi 2003;25:555–7.
22. Shen H, Sturgis EM, Khan SG, et al. An intronic poly (AT) polymorphism of the DNA repair gene XPC and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Res 2001;61:3321–5.
23. Sanyal S, Festa F, Sakano S, et al. Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 2004;25:729–34.
24. Kirk BW, Feinsod M, Favis R, Kliman RM, Barany F. Single nucleotide polymorphism seeking long term association with complex disease. Nucleic Acids Res 2002;30:3295–311.

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