Association of DNA double strand break gene XRCC6 Genotypes and lung cancer in Taiwan

Te-Chun Hsia[1,2,4], Chin-Jung Liu[1,3], Chia-Chen Chu[1,3], Liang-Wen Hang[1,3], Wen-Shin Chang[1,5], Chia-Wen Tsai[1,5], Chao-I Wu[1], Chi-Shun Lien[1], Wen-Ling Liao[1], Chien-Yi Ho[1] and Da-Tian Bau[1,5,6]

[1]Terry Fox Cancer Research Laboratory, and [2]Section of Pulmonary and Critical medicine, Department on Internal Medicine, China Medical University Hospital, Taichung; [3]Department of Respiratory Therapy, [4]Graduate Institute of Integrated Medicine, [5]Graduate Institute of Basic Science, [6]Graduate Institute of Clinical Science, and [7]School of Pharmacy, China Medical University, Taichung, Taiwan

Correspondence to: Da-Tian Bau, and Chien-Yi Ho, Terry Fox Cancer Research Lab, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan.

Tel: +886422053366 Ext 1523, Fax: +886422053366, e-mail: datian@mail.cmuh.org.tw; artbau2@gmail.com.tw

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Abstract. Aim: The DNA repair gene *XRCC6* is thought to play an important role in the repairing of DNA double strand breaks. It is known that defective in double strand break repair capacity can lead to irreversible genomic instability. However, the polymorphic variants of *XRCC6,* has never been reported about their association with lung cancer susceptibility. In this hospital-based case-control study, the association of *XRCC6* promoter T-991C (rs5751129), promoter G-57C (rs2267437), promoter G-31A (rs132770), and intron3 (rs132774) polymorphisms with lung cancer risk in a Taiwanese population was studied. **Materials and Methods:** In total, 358 patients with lung cancer and 716 healthy controls recruited from the China Medical Hospital in Taiwan were genotyped. **Results:** The results showed that there were significant differences between lung cancer and control groups in the distribution of their genotypic (*P*=3.7E-4) and allelic frequency (*P*=2.7E-5) in the *XRCC6* promoter T-991C polymorphism. Individuals who carried at least one C allele (TC or CC) had a 2.03-fold increased odds ratio of developing lung cancer compared to those who carried the TT wild type genotype (95%CI=1.42-2.91, *P*=0.0001). In the other three polymorphisms, there was no difference between the case and control groups in the distribution of either genotypic or allelic frequency. **Conclusion:** In conclusion, the *XRCC6* promoter T-991C, but not the promoter C-57G, promoter G-31A or intron3, is associated with lung cancer susceptibility.

Key Words**:** *XRCC6,* polymorphism, lung cancer, carcinogenesis

Worldwide, lung cancer is the most common cause of cancer-related death in both male and female, and is responsible for more than million deaths annually in recent years [Steward BW. WHO: World Cancer Report 2003. IARC Press, Lyon, 2004; Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin *55*: 74-108, 2005; Jemal A, Siegel R, Ward E, Murray T, Xu J and Thun MJ: Cancer statistics, 2007. CA Cancer J Clin *57*: 43-66, 2007]. In Taiwan, lung cancer is characterized for its high incidence, high mortality, and low 5-year survival rate, especially in female adenocarcinoma cases [Chiu HF, Cheng MH, Tsai SS, Wu TN, Kuo HW and Yang CY: Outdoor air pollution and female lung cancer in Taiwan. Inhal Toxicol *18*: 1025-1031, 2006], and smoking and polluted air are also considered to be the most lung cancer related environmental factors [Chiu HF, Cheng MH, Tsai SS, Wu TN, Kuo HW and Yang CY: Outdoor air pollution and female lung cancer in Taiwan. Inhal Toxicol *18*: 1025-1031, 2006; Yang L, Parkin DM, Ferlay J, Li L and Chen Y: Estimates of cancer incidence in China for 2000 and projections for 2005. Cancer Epidemiol Biomarkers Prev *14*: 243-250, 2005; Zhang H and Cai B: The impact of tobacco on lung health in China. Respirology *8*: 17-21, 2003]. Human genome is insulted by tens to hundreds of thousand times per day, and DNA repair mechanisms protect the genome from these insults both from endogenous and environmental agents. Mutations or defects in the DNA repairing genes and a lower DNA repair capacity are thought to be essential for tumorigenesis [Miller KL, Karagas MR, Kraft

P, Hunter DJ, Catalano PJ, Byler SH, et al. XPA, haplotypes, and risk of basal and squamous cell carcinoma. Carcinogenesis. 2006;27:1670-1675; Vogelstein B, Alberts B, Shine K. Genetics. Please don't call it cloning! Science. 2002;295:1237]. Therefore, it is logical to suspect that genetic variants of DNA repair genes, such as polymorphisms, might contribute to lung cancer susceptibility.

DNA double strand breaks (DSBs) are repaired by the two important DNA DSB repair subpathways, homologous recombination (HR) and nonhomologous end-joining (NHEJ) [Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet. 2001;27:247-254]. In humans, NHEJ is the predominant repair system in all the cell cycle phases. In recent years, several proteins involved in the NHEJ pathway have been identified, including ligase IV, XRCC4, XRCC6 (Ku70), XRCC5 (Ku80), DNA-PKcs, Artemis and XLF [Jackson SP. Sensing and repairing DNA double-strand breaks. Carcinogenesis. 2002;23:687-696; Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem. 2011;79:181-211]. Genetic variation in DNA repair genes has been postulated as an important contributor to the aetiology of cancer [Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev. 2002;11:1513-1530]. Also, Inappropriate NHEJ can lead to translocations and telomere fusion, hallmarks of tumor cells [Espejel S, Franco S,

Rodriguez-Perales S, Bouffler SD, Cigudosa JC, Blasco MA. Mammalian Ku86 mediates chromosomal fusions and apoptosis caused by critically short telomeres. EMBO J. 2002 May 1;21(9):2207-19]. However, there is seldom information regarding lung cancer and NHEJ gene polymorphisms. As for NHEJ, some genetic polymorphisms were reported to influence DNA repair capacity and confer predisposition to several types of cancers, including skin [Han J, Colditz GA, Samson LD, Hunter DJ. Polymorphisms in DNA double-strand break repair genes and skin cancer risk. Cancer Res. 2004;64:3009-3013], breast [Bau DT, Fu YP, Chen ST, Cheng TC, Yu JC, Wu PE, et al. Breast cancer risk and the DNA double-strand break end-joining capacity of nonhomologous end-joining genes are affected by BRCA1. Cancer Res. 2004;64:5013-5019; Bau DT, Mau YC, Ding SL, Wu PE, Shen CY. DNA double-strand break repair capacity and risk of breast cancer. Carcinogenesis. 2007;28:1726-1730; Chiu CF, Wang HC, Wang CH, Wang CL, Lin CC, Shen CY, et al. A new single nucleotide polymorphism in XRCC4 gene is associated with breast cancer susceptibility in Taiwanese patients. Anticancer Res. 2008;28:267-270], bladder [Chang CH, Chang CL, Tsai CW, Wu HC, Chiu CF, Wang RF, et al. Significant association of an XRCC4 single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res. 2009;29:1777-1782; Chang CH, Wang RF, Tsai RY, Wu HC, Wang CH, Tsai CW, et al. Significant association of XPD codon 312 single nucleotide polymorphism with bladder cancer susceptibility in

Taiwan. Anticancer Res. 2009;29:3903-3907], and oral cancers [Bau DT, Tseng HC, Wang CH, Chiu CF, Hua CH, Wu CN, et al. Oral cancer and genetic polymorphism of DNA double strand break gene Ku70 in Taiwan. Oral Oncol. 2008;44:1047-1051; Chiu CF, Tsai MH, Tseng HC, Wang CL, Wang CH, Wu CN, et al. A novel single nucleotide polymorphism in XRCC4 gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol. 2008;44:898-902].

In our previous study, we have found that one polymorphism of *XRCC4* NHEJ repair gene is associated with lung cancer susceptibility in Taiwan [Hsu NY, Wang HC, Wang CH, Chang CL, Chiu CF, Lee HZ, et al. Lung cancer susceptibility and genetic polymorphism of DNA repair gene XRCC4 in Taiwan. Cancer Biomark. 2009;5:159-165]. In this study, we assumed that the upstream gene *XRCC6* in NHEJ, like *XRCC4*, its polymorphisms may also contribute to lung cancer susceptibility. To test this hypothesis, we determined the genotypic frequency of four polymorphisms of the *XRCC6* gene at promoter T-991C (rs5751129), promoter G-57C (rs2267437), promoter G-31A (rs132770), and intron3 (rs132774), using a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method. To the best of our knowledge, this is the first study carried out to evaluate the contribution of *XRCC6* genotypes in lung cancer risk.

Materials and Methods

Study population and sample collection. Three hundred and fifty-eight cancer 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 patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. Twice as many non-lung cancer healthy volunteers as controls were selected by matching for age, gender and smoking habits after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups completed a short questionnaire which included questions related to smoking habits. The study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Genotyping conditions. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and stored as previously published [Chang CH, Chiu CF, Wang HC, Wu HC, Tsai RY, Tsai CW, Wang RF, Wang CH, Tsou YA and Bau DT: Significant association of *ERCC6* single nucleotide polymorphisms with

bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 5121-5124, 2009; Chang CH, Wang RF, Tsai RY, Wu HC, Wang CH, Tsai CW, Chang CL, Tsou YA, Liu CS and Bau DT: Significant association of *XPD* codon 312 single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 3903-3907, 2009; Liu CJ, Hsia TC, Wang RF, Tsai CW, Chu CC, Hang LW, Wang CH, Lee HZ, Tsai RY and Bau DT: Interaction of *cyclooxygenase 2* genotype and smoking habit in Taiwanese lung cancer patients. Anticancer Res *30*: 1195-1199, 2010; Liu CS, Tsai CW, Hsia TC, Wang RF, Liu CJ, Hang LW, Chiang SY, Wang CH, Tsai RY, Lin CC and Bau DT: Interaction of *methylenetetrahydrofolate reductase* genotype and smoking habit in Taiwanese lung cancer patients. Cancer Genomics Proteomics *6*: 325-329, 2009; Wang HC, Chiu CF, Tsai RY, Kuo YS, Chen HS, Wang RF, Tsai CW, Chang CH, Lin CC and Bau DT: Association of genetic polymorphisms of *EXO1* gene with risk of breast cancer in Taiwan. Anticancer Res *29*: 3897-3901, 2009; Wang HC, Liu CS, Chiu CF, Chiang SY, Wang CH, Wang RF, Lin CC, Tsai RY and Bau DT: Significant association of DNA repair gene *Ku80* genotypes with breast cancer susceptibility in Taiwan. Anticancer Res *29*: 5251-5254, 2009]. The primers used for *XRCC6* promoter C-991T were: forward 5’-AACTCATGGACCCACGGTTGTGA-3’, and reverse 5’-CAACTTAAATACAGGAATGTCTTG-3’; for promoter G-57C were: forward 5’-AACTCATGGACCCACGGTTGTGA-3’, and reverse 5’-CAACTTAAATACAGGAATGTCTTG-3’; for promoter G-31A were: forward

5'-TACAGTCCTGACGTAGAAG-3', and reverse 5'-AAGCGACCAACTTGGACAGA-3'; for intron3 were forward 5'-GTATACTTACTGCATTCTGG-3', and reverse 5'-CATAAGTGCTCAGTACCTAT-3'. The following cycling conditions were performed: 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.

RFLP conditions. As for the *XRCC6* promoter C-991T, the resultant 301 bp PCR product was mixed with 2 U *Dpn II*. The restriction site was located at -991 with a C/T polymorphism, and the C form PCR products could be further digested while the T form could not. Two fragments 101 bp and 200 bp were present if the product was digestible C form. The reaction was incubated for 2 h at 37°C. Then, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was categorized as either (a) C/C homozygote (digested), (b) T/T homozygote (undigested), or (c) C/T heterozygote. As for the *XRCC6* promoter G-57C, the resultant 298 bp PCR products were mixed with 2 U *Hae II*. The restriction site was located at -57 with a C/G polymorphism, and the G form PCR products could be further digested while the C form could not. Two fractions 103 and 195 bp were present if the product was digestible G form. The reaction was incubated for 2 h at 37°C. Then, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was categorized as

either (a) G/G homozygote (digested), (b) C/C homozygote (undigested), or (c) C/G heterozygote. As for the *XRCC6* promoter G-31A, the resultant 226 bp PCR products were mixed with 2 U *Mnl I*. The restriction site was located at -31 with a A/G polymorphism, and the A form PCR products could be further digested while the G form could not. Two fractions 80 and 146 bp were present if the product was digestible A form. The reaction was incubated for 2 h at 37°C. Then, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was categorized as either (a) A/A homozygote (digested), (b) G/G homozygote (undigested), or (c) A/G heterozygote. As for the *XRCC6* promoter intron3, the resultant 160 bp PCR products were mixed with 2 U *Msc I*. The restriction site was located at intron3 with a TGG/CCA polymorphism, and the CCA form PCR products could be further digested while the TGG form could not. Two fractions 46 and 114 bp were present if the product was digestible CCA form. The reaction was incubated for 2 h at 37°C. Then, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was categorized as either (a) CCA/CCA homozygote (digested), (b) TGG/TGG homozygote (undigested), or (c) CCA/TGG heterozygote.

Statistical analyses. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *XRCC6* single nucleotide polymorphisms in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *XRCC6* genotypes between cancer cases and controls. Cancer risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. Data was recognized as significant when the statistical *P*-value was less than 0.05.

Results

The characteristics of the lung cancer patients and the healthy controls are listed in Table I. There was no significant difference between both groups in their age, gender, and smoking habits (Table I). The frequencies of the genotypes and alleles of the *XRCC6* promoter T-991C polymorphism in the lung cancer and control groups are summarized in Table II. There were significant differences between both groups in the distribution of genotypic (*P*=3.7E-4) and allelic frequency (*P*=2.7E-5). The odds ratio of the people carrying TT and TC genotypes were 1.98 (95% CI=1.36-2.89) and 2.49 (95% CI=0.95-6.52) respectively, compared to those carrying TT wild-type genotype. The former is significant while the later is not significant. The lack of significance may be due to the limited sample size in those cells. Hence, individuals who carried at least one C-allele (TC and CC) had a 1.98-fold increased odds ratio of developing lung cancer compared to those who carried the T-allele wild type (95% CI=1.43-2.75) (Table II). On the contrary, as for the *Ku70* promoter C-57G (Table III), promoter G-31A (Table IV), and intron3 polymorphisms (Table V), the distributions of these polymorphisms were in Hardy-Weinberg equilibrium but there was no difference between lung cancer and control groups in the distribution of either genotype or allelic frequency at these SNP sites (Table III-V).

Discussion

The present study is the first one to investigate the role of *XRCC6* gene polymorphisms, which has never been reported to be associated with lung cancer risk. Our study revealed that the *XRCC6* promoter T-991C genotype (Table II), not those of C-57G (Table III), G-31A (Table IV) or intron3 (Table V) genotypes, was associated with the risk to lung cancer. The *XRCC6* promoter T-991C genetic variation may not direct result in amino acid coding change, but may possibly influence the expression level of the XRCC6 protein. In previous studies, the *XRCC6* promoter T-991C genotype was found to be associated with oral [Bau DT, Tseng HC, Wang CH, Chiu CF, Hua CH, Wu CN, et al. Oral cancer and genetic polymorphism of DNA double strand break gene Ku70 in Taiwan. Oral Oncol. 2008;44:1047-1051] and gastric cancers [Yang MD, Wang HC, Chang WS, Tsai CW, Bau DT. Genetic polymorphisms of DNA double strand break gene Ku70 and gastric cancer in Taiwan. BC Cancer 2011;11:174]. In addition, this genotype was also found to be associated with two-side pterygium pathology, which are caused by an uncontrolled cell proliferation like that of a tumor [Tsai YY, Bau DT, Chiang CC, Cheng YW, Tseng SH, Tsai FJ. Pterygium and genetic polymorphism of DNA double strand break repair gene Ku70. Mol Vis. 2007;13:1436-1440].

In this study, we have tried our best to conquer some limitations in study design. For instance, to lower the possibility of false-positive or false-negative findings, we have enlarged the sample size of control group and avoided any sub-grouping and adjusting of the

all the cases and the controls recruited in this study were drawn from the same Taiwanese ethnic group and the Taiwanese population has relatively homogenous genetic background [Yang HC, Lin CH, Hsu CL, Hung SI, Wu JY, Pan WH, et al. A comparison of major histocompatibility complex SNPs in Han Chinese residing in Taiwan and Caucasians. J Biomed Sci. 2006;13:489-498], and little population bias can be produced in the sampling process. Therefore, the potential confounding effect of population stratification for genotyping data is not a major concern. Furthermore, the possible selection bias was taken into consideration and reduced to a lowest level by frequency matching on age and gender between the cases and controls. Last, the frequencies of *XRCC6* polymorphisms variant alleles were similar to those reported in the NCBI website in the Asian population studies, for example C allele frequencies of *XRCC6* promoter T-991C are 5.7% in our control group and 4.2~8.9% for Asian populations in NCBI, which also imply that there was no selection bias for the subject's enrolments in terms of various genotypes.

In this study, the genotype distribution of the C allele at *XRC6* promoter T-991C was significantly higher in the lung cancer group (10.7%) than in the control group (5.7%) (Table

II). It was also found that patients carrying heterozygous TC for *XRCC6* promoter T-991C had a 1.98-fold higher risk of lung cancer (Table I). Although the CC genotype did not show any significance, the combination of heterozygous and homozygous (TC or CC) was almost at the same level (OR=2.03) for lung cancer risk (Table I). All these data suggested that the C allele at *XRCC6* promoter T-991C was indeed a novel and important biomarker for lung carcinogenesis. In the future early detection and prediction work, as long as -991C is detected, the carriers were more susceptible to lung cancer, and should prevent themselves from the exposure to some environmental risky factors, such as smoking habit. If the sample size could be enlarged in the future, the further stratification analysis about gene-gene or environment interaction may add more information to the understanding of lung carcinogenesis and etiology.

In conclusion, this is the first report to investigate the association between *XRCC6* gene polymorphisms and lung cancer. Our findings suggested that *XRCC6* promoter T-991C, but not *XRCC6* promoter C-57G, promoter G-31A or intron3 polymorphisms, was associated with higher susceptibility to lung cancer. The *XRCC6* promoter T-991C polymorphism might become a potential biomarker for the lung oncology prediction and this paper may also provide a valuable insight into the lung carcinogenesis.

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References

Table I. Characteristics of lung cancer patients 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%		
Habit							
Cigarette smokers	563	78.6%		293	81.8%		0.23
Non-smokers	153	21.4%		65	18.2%		

[a] Based on chi-square test.

Table II. Distribution of *XRCC6* promoter T-991C genetic and allelic frequencies among nasopharyngeal carcinoma patient and control groups.

XRCC6 T-991C	Controls	%	Patients	%	OR (95% CI)[a]	*P-value*[b]
Genetic frequency						
TT	642	89.7%	290	81.0%	1.00 (ref)	**3.7E-4**
TC	**66**	**9.2%**	**59**	**16.5%**	**1.98 (1.36-2.89)**	
CC	8	1.1%	9	2.5%	2.49 (0.95-6.52)	
Carrier comparison						
TT+TC	708	98.9%	349	97.5%	1.00 (Reference)	NS
CC	8	1.1%	9	2.5%	2.28 (0.87-5.97)	
TT	642	89.7%	290	81.0%	1.00 (Reference)	**0.0001**
TC+CC	**74**	**10.3%**	**68**	**19.0%**	**2.03 (1.42-2.91)**	

Allele frequency						
Allele T	1350	94.3%	639	89.3%	1.00 (Reference)	**2.7E-5**
Allele C	**82**	**5.7%**	**77**	**10.7%**	**1.98 (1.43-2.75)**	

[a] OR: odds ratio, CI: confidence interval; [b] Based on Chi-square test, NS: non-significant.

Table III. Distribution of *XRCC6* promoter C-57G genetic and allelic frequencies among lung cancer patient and control groups.

XRCC6 C-57G	Controls	%	Patients	%	OR (95% CI)[a]	*P-value*[b]
Genetic frequency						
CC	490	68.4%	250	69.8%	1.00 (ref)	NS
CG	213	29.7%	101	28.2%	0.93 (0.70-1.23)	
GG	13	1.8%	7	2.0%	1.05 (0.42-2.68)	
Carrier comparison						
CC+CG	703	98.2%	351	98.0%	1.00 (Reference)	NS
GG	13	1.8%	7	2.0%	1.08 (0.43-2.73)	
CC	490	68.4%	250	69.8%	1.00 (Reference)	NS
CG+GG	226	31.6%	108	30.2%	0.94 (0.71-1.23)	

Allele frequency						
Allele C	1193	83.3%	601	83.9%	1.00 (Reference)	NS
Allele G	239	16.7%	115	16.1%	0.95 (0.75-1.22)	

[a] OR: odds ratio, CI: confidence interval; [b] Based on Chi-square test, NS: non-significant.

Table IV. Distribution of *XRCC6* promoter G-31A genetic and allelic frequencies among lung cancer patient and control groups.

XRCC6 G-31A	Controls	%	Patients	%	OR (95% CI)[a]	*P-value*[b]
Genetic frequency						
GG	574	80.2%	294	82.1%	1.00 (ref)	NS
GA	100	14.0%	46	12.9%	0.90 (0.62-1.31)	
AA	42	5.8%	18	5.0%	0.84 (0.47-1.48)	
Carrier comparison						
GG+GA	674	94.2%	340	95.0%	1.00 (Reference)	NS
AA	42	5.8%	18	5.0%	0.85 (0.48-1.50)	
GG	574	80.2%	294	82.1%	1.00 (Reference)	NS
GA+AA	142	19.8%	64	17.9%	0.88 (0.63-1.22)	

Allele frequency						
Allele G	1248	87.2%	634	88.6%	1.00 (Reference)	NS
Allele A	184	12.8%	82	11.4%	0.88 (0.66-1.16)	

[a] OR: odds ratio, CI: confidence interval; [b] Based on Chi-square test, NS: non-significant.

Table V. Distribution of *XRCC6* intron 3 genetic and allelic frequencies among lung cancer patient and control groups.

XRCC6 intron 3	Controls	%	Patients	%	OR (95% CI)[a]	*P-value*[b]
Genetic frequency						
TGG/TGG	592	82.7%	299	83.5%	1.00 (ref)	NS
TGG/CCA	124	17.3%	59	16.5%	0.94 (0.67-1.32)	
CCA/CCA	0	0%	0	0%		
Allele frequency						
TGG	1308	91.3%	657	91.8%	1.00 (Reference)	NS
CCA	124	8.7%	59	8.2%	0.95 (0.69-1.31)	

[a] OR: odds ratio, CI: confidence interval; [b] Based on Chi-square test, NS: non-significant.

Figure Legend

Fig 1. PCR-based restriction analysis of the promoter T-991C polymorphism of *XRCC6* gene shown on 3% agarose electrophoresis. Marker: 100 bp DNA size ladder marker, T/T: indivisible homozygote, T/C: heterozygote, and C/C: divisible homozygote.

Figure 1