Genetics and Molecular Research

Analyzing the association between *XRCC1* c.1804C>A genetic variant and lung cancer susceptibility in the Chinese population

J.J. Jin, H.Q. Wang, H.P. Kuang, B.B. Kang, Y.H. Liu and J. Wang

Department of Respiratory and Critical Medicine,
The First Affiliated Hospital of Zhengzhou University, Zhengzhou,
Henan Province, People's Republic of China

Corresponding author: J. Wang
E-mail: jingwang_12@sina.com

Genet. Mol. Res. 15 (2): gmr.15024661
Received January 4, 2016
Accepted May 2, 2016
Published June 10, 2016
DOI http://dx.doi.org/10.4238/gmr.15024661

ABSTRACT. Lung cancer is the most common cancer occurring worldwide. The human X-ray repair complementing group 1 (*XRCC1*) gene is one of the most important candidate genes that influence the susceptibility to lung cancer. The objective of this study was to analyze the potential association between the c.1804C>A genetic variant of *XRCC1* and lung cancer susceptibility. A total of 703 subjects were recruited for this study. Genotyping of c.1804C>A genetic variant was performed using the created restriction site-polymerase chain reaction. Statistically significant differences in allele frequencies and genotype were found between lung cancer patients and cancer-free controls. The genotype AA was statistically associated with the increased risk of lung cancer when compared to the wild genotype, CC, and the carrier genotype, CA/CC (AA *vs* CC: OR = 2.71, 95%CI = 1.57-4.67, P < 0.001; AA *vs* CA/CC: OR = 2.54, 95%CI = 1.50-4.29, P < 0.001). The allele A likely contributes to the susceptibility to lung cancer (A *vs* C: OR = 1.47, 95%CI = 1.17-1.84, P = 0.001). Our data indicates that the

c.1804C>A genetic variant of *XRCC1* is statistically associated with the susceptibility to lung cancer in the Chinese population.

Key words: Lung cancer; *XRCC1*; Genetic variant; Risk factor; Molecular marker

INTRODUCTION

Lung cancer is a common cancer and the leading cause of cancer-related deaths, worldwide. More than one million people die from this disease each year (Guilbert, 2003; Siegel et al., 2012; Yuan et al., 2013a). It is still associated with poor survival, and the overall five-year survival rate is only 15% in the United States and even lower in China (Parkin et al., 2005; Yin et al., 2009a; Cui et al., 2012). Many factors affect the development of lung carcinogenesis, and the exact mechanism of lung cancer remains poorly understood. It is generally accepted that genetic factors play key roles in the pathogenesis of lung cancer. Previous studies reported that the human X-ray repair complementing group 1 (*XRCC1*) gene is one of the most important candidate genes for influencing susceptibility to lung cancer (Hao et al., 2006; Giachino et al., 2007; López-Cima et al., 2007; Sreeja et al., 2008; Chang et al., 2009; Kalikaki et al., 2009; Wang et al., 2009; Yin et al., 2009a,b; Butkiewicz et al., 2011; Li et al., 2011; Qian et al., 2011; Chen et al., 2012; Cui et al., 2012; Guo et al., 2013). The potential associations between lung cancer and multiple genetic variants of *XRCC1* in different populations have been reported (Hao et al., 2006; Giachino et al., 2007; López-Cima et al., 2007; Improta et al., 2008; Sreeja et al., 2008; Chang et al., 2009; Kalikaki et al., 2009; Yin et al., 2009a,b; Butkiewicz et al., 2011; Qian et al., 2011; Cui et al., 2012; Guo et al., 2013). Several genetic variants, including arginine (Arg) 194 tryptophan (Trp), Arg 280 histidine (His) and Arg399 glutamine (Gln), were significantly associated with the susceptibility to lung cancer (Giachino et al., 2007; López-Cima et al., 2007; Sreeja et al., 2008; Kalikaki et al., 2009; Yin et al., 2009a,b; Butkiewicz et al., 2011; Qian et al., 2011; Guo et al., 2013). However, the potential association between the *XRCC1* c.1804C>A genetic variant and lung cancer susceptibility has not been analyzed. Considering the importance of *XRCC1* genetic variants in the development of lung cancer, we hypothesized that the c.1804C>A genetic variant is associated with lung cancer susceptibility, and we evaluated the influence of this variant on the susceptibility to lung cancer by association analysis.

MATERIAL AND METHODS

Study populations

In total, 348 lung cancer patients and 355 cancer-free control patients were recruited from the First Affiliated Hospital of Zhengzhou University (Henan, China) between February 2009 and November 2012. All participants were unrelated Chinese people with Han ethnicity and lived in Zhengzhou city, Henan province of China. Patients with lung cancer were histologically confirmed and diagnosed by doctors. Cancer-free control patients were frequency-matched by sex and age to lung cancer patients. Those with a history of lung cancer or other medical diseases were excluded. Table 1 shows the demographic clinical characteristics, including sex, age, smoking status, pack-years, histology type, and family

history of lung cancer. The protocol of this study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Henan, China). Informed consent was given by each participant.

Table 1. Demographic clinical characteristics of lung cancer patients and cancer-free indiciduals (controls).

Characteristics	Patients (N)	%	Controls (N)		χ^2-value	P value
Number	348	49.50	355	50.50		
Gender (N)					2.5803	0.1082
Male	245	70.40	269	75.77		
Female	103	29.60	86	24.23		
Age (years)					0.0603	0.8061
Mean ± SD	63.67 ± 11.83		60.28 ± 14.55			
<58	185	53.16	192	54.08		
≥58	163	46.84	163	45.92		
Smoking status					0.5584	0.4549
Yes	233	66.95	247	69.58		
No	115	33.05	108	30.42		
Pack-years					0.5004	0.4793
<36	135	38.79	147	41.41		
≥36	213	61.21	208	58.59		
Histology type (N)						
Squamous-cell carcinoma	156	44.83	-			
Adenocarcinoma	133	38.22	-			
Others	59	16.95	-			
Family history of lung cancer (N)						
Yes	51	14.66	-			
No	297	85.34	-			

Genotyping

Venous blood was collected from each subject. Genomic DNA was extracted using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen, Union City, CA). According to the reference DNA and mRNA sequences of human *XRCC1* (GenBank IDs: NC_000019.9 and NM_006297.2), we designed the specific polymerase chain reaction (PCR) primers using Primer Premier 5.0 software (Wu et al., 2005). Table 2 shows the primers sequences, annealing temperature, amplification regions and sizes. Each PCR was carried out in a 20-µL total volume containing 50 ng template DNA, 1X buffer (Tris-HCl 100 mM, pH 8.3; KCl 500 mM), 0.25 µM primers, 2.0 mM $MgCl_2$, 0.25 mM dNTPs (Bioteke Corporation, Beijing, China), and 0.5 U Taq DNA polymerase (Promega, Madison, WI, USA). The PCR cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 32 cycles of 94°C for 30 s, 65.0°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. The genotyping of the c.1804C>A genetic variant was performed using the created restriction site-polymerase chain reaction (CRS-PCR) (Yuan et al., 2013b). In this method, one of the primers contained a nucleotide mismatch, which enables the use of restriction enzymes for discriminating sequence variations (Haliassos et al., 1989; Zhao et al., 2003; Yuan et al., 2012; Yuan et al., 2013b,c). The amplified PCR products (10 µL) were digested with 5 units of *Mae*II (MBI Fermentas, St. Leon-Rot, Germany) at 37°C for 10 h. The digested products were separated using 2.5% agarose gel and visualized under UV light after staining with ethidium bromide. To verify the results of CRS-PCR analyses, 10% of the samples were randomly selected and re-analyzed by DNA sequencing (ABI3730xl DNA Analyzer, Applied Biosystems, Foster City, CA).

Table 2. CRS-PCR analysis for the c.1804C > A genetic variants in *XRCC1*.

Primer sequences	Annealing temperature (°C)	Amplification fragment (bp)	Region	Restriction enzyme	Genotype (bp)
5'-AATATGAGTGACCGGGTTCAGTTTG-3'	65.0	205	Exon17	*Mae*II	CC: 205
5'-ACGAACGAATGCCAGGGACG-3'					CA: 205,187, 18
					AA: 187,18

PCR = polymerase chain reaction; CRS-PCR = created restriction site-polymerase chain reaction; Underlined nucleotides mark nucleotide mismatches enabling the use of the selected restriction enzymes for discriminating sequence variations.

Statistical analyses

All statistical analyses were assessed using the Statistical Package for Social Sciences software (SPSS, Windows version release 15.0; SPSS Inc.; Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) of the frequencies of genotype/allele, and the differences of demographic clinical characteristics between cases and controls were evaluated using the chi-squared (χ^2) test. The potential association of c.1804C>A genetic variant with lung cancer susceptibility was assessed by unconditional logistic regression model using the odds ratios (ORs) with the 95% confidence intervals (95%CIs). A P value < 0.05 was considered to be statistically significant.

RESULTS

General characteristics

The demographic clinical characteristics of 348 lung cancer patients and 355 cancer-free control patients are shown in Table 1. No statistically significant difference was found between lung cancer patients and cancer-free control patients with regard to sex, age, smoking status and pack-years (P > 0.05).

XRCC1 genetic variant identification

We genotyped the *XRCC1* c.1804C>A genetic variant using CRS-PCR and DNA sequencing. Our DNA sequence analyses indicated that this genetic variant is a non-synonymous mutation, which was caused by C to A mutations in exon 17 of human *XRCC1*. The mutation has resulted into a proline (Pro) to threonine (Thr) amino acid substitution in the polypeptide (p.Pro602Thr, Reference sequences GenBank IDs: NC_000019.9, NM_006297.2 and NP_006288.2). The *Mae*II restriction enzyme was selected to digest the PCR products, and then three genotypes were observed: CC (205 bp), CA (205, 187 and 18 bp) and AA (187 and 18 bp, Table 2). The frequencies of allelic and genotypic are shown in the Table 3. We found that the frequencies of allele C and genotype CC were maximums in the studied populations. Statistically significant differences were detected between the allele frequencies of lung cancer patients (C, 65.95%; A, 34.05%) and cancer-free control patients (C, 73.94%; A, 26.06%; χ^2 = 10.6961, P = 0.0011). The frequencies of different genotypes in lung cancer patients (CC, 46.26%; CA, 39.37%; AA, 14.37%) were statistically significant different from the frequencies in cancer-free control patients (CC, 54.08%; CA, 39.72%; AA, 6.20%; χ^2 = 13.6005, P = 0.0011, Table 4). Results from the chi-squared (χ^2) test indicated that the

distributions of genotypes in the studied subjects were in accordance with the HWE (all P > 0.05, Table 3).

Table 3. Genotypic and allelic frequencies of c.1804C>A genetic variants in *XRCC1* in lung cancer patients and healthy controls.

Groups	Genotypic frequencies (%)			Allelic frequencies (%)		χ^2	P
	CC	CA	AA	C	A		
Case group (N = 348)	161 (46.26)	137 (39.37)	50 (14.37)	459 (65.95)	237 (34.05)	5.3049	0.0705
Control group (N = 355)	192 (54.08)	141 (39.72)	22 (6.20)	525 (73.94)	185 (26.06)	0.3353	0.8456
Total (N = 703)	353 (50.21)	278 (39.55)	72 (10.24)	984 (69.99)	422 (30.01)	2.4233	0.2977
	χ^2 = 13.6005, P = 0.0011			χ^2 = 10.6961, P = 0.0011			

XRCC1 genetic variant associated with lung cancer risk

The potential association of the *XRCC1* c.1804C>A genetic variant with lung cancer risk is summarize in Table 4. We found that there were statistically significant risk of lung cancer in the homozygote phenotype comparison (AA versus *vs* CC: OR = 2.71, 95%CI = 1.57-4.67, χ^2 = 13.56, P < 0.001), dominant model (AA/CA *vs* CC: OR = 1.37, 95%CI = 1.02-1.84, χ^2 = 4.29, P = 0.038), recessive model (AA *vs* CA/CC: OR = 2.54, 95%CI = 1.50-4.29, χ^2 = 12.74, P < 0.001) and allele contrast (A *vs* C: OR = 1.47, 95%CI = 1.17-1.84, χ^2 = 10.69, P = 0.001, Table 4).

Table 4. The association between the *XRCC1* c.1804C>A genetic variants and risk of lung cancer.

Comparisons	Test of association		
	OR (95%CI)	χ^2-value	P value
Homozygote comparison (AA *vs* CC)	2.71(1.57-4.67)	13.56	0.000
Heterozygote comparison (CA *vs* CC)	1.16 (0.851.59)	0.84	0.359
Dominant model (AA/CA *vs* CC)	1.37(1.02-1.84)	4.29	0.038
Recessive model (AA *vs* CA/CC)	2.54 (1.50-4.29)	12.74	0.000
Allele contrast (A *vs* C)	1.47 (1.17-1.84)	10.69	0.001

OR = odds ratio; CI = confidence interval; *vs* = versus.

DISCUSSION

Lung cancer is global health problem that causes high incidence and high mortality rate. There is increasing evidence that lung cancer is a polygenic and common, malignant solid cancer resulting from complex interactions between environmental and genetic factors. The genetic factors include candidate genes that might play roles functions in the susceptibility to lung cancer. The *XRCC1* gene is an important candidate gene for influencing lung cancer susceptibility (Hao et al., 2006; Giachino et al., 2007; López-Cima et al., 2007; Sreeja et al., 2008; Chang et al., 2009; Kalikaki et al., 2009; Wang et al., 2009; Yin et al., 2009a,b; Butkiewicz et al., 2011; Li et al., 2011; Qian et al., 2011; Chen et al., 2012; Cui et al., 2012; Guo et al., 2013). In the present study, the influence of the *XRCC1* c.1804C>A genetic variant on the susceptibility to lung cancer in Chinese Han population was evaluated by association analysis in 348 lung cancer patients and 355 cancer-free control patients. We found statistically significant differences in the frequencies of allele and genotype between lung cancer patients and cancer-free control patients (Table 3). Compared to the wild genotype, CC, and the heterozygous genotype, CA/CC, the genotype, AA, was significantly associated with the

increased risk of lung cancer (Table 4). Our data suggested that the allele C contributes to reduced lung cancer susceptibility, and the genotype CC is associated with a protection factor from lung cancer (Table 4). Previously published studies, which reported the influence of several genetic variants in *XRCC1* (such as Arg194Trp, Arg280His and Arg399Gln) on the risk of lung cancer, are consistent with our findings that genetic variants in *XRCC1* genetically impact lung cancer (Giachino et al., 2007; López-Cima et al., 2007; Sreeja et al., 2008; Chang et al., 2009; Kalikaki et al., 2009; Yin et al., 2009a,b; Butkiewicz et al., 2011; Qian et al., 2011; Chen et al., 2012; Guo et al., 2013). These genetic variants likely affect the function of XRCC1 in base excision DNA repair pathway, which is significantly associated with lung cancer risk (Hao et al., 2006; Giachino et al., 2007; López-Cima et al., 2007; Sreeja et al., 2008; Kalikaki et al., 2009; Yin et al., 2009a,b; Butkiewicz et al., 2011; Qian et al., 2011). The c.1804C>A genetic variant may be linked to these known non-synonymous genetic variants, and may play similar functions in the development of lung cancer. Our findings demonstrate that this genetic variant may be used as a molecular marker for evaluating the risk of lung cancer. Future functional investigations on larger and different ethnic populations are necessary to confirm these findings and to clarify the underlying molecular mechanisms and pathophysiology in lung cancer.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank the patients who participated in the study, and the laboratory technicians for their most valuable efforts.

REFERENCES

Butkiewicz D, Rusin M, Sikora B, Lach A, et al. (2011). An association between DNA repair gene polymorphisms and survival in patients with resected non-small cell lung cancer. *Mol. Biol. Rep.* 38: 5231-5241. http://dx.doi.org/10.1007/s11033-010-0674-1

Chang JS, Wrensch MR, Hansen HM, Sison JD, et al. (2009). Base excision repair genes and risk of lung cancer among San Francisco Bay Area Latinos and African-Americans. *Carcinogenesis* 30: 78-87. http://dx.doi.org/10.1093/carcin/bgn261

Chen J, Zhao QW, Shi GM and Wang LR (2012). XRCC1 Arg399Gln and clinical outcome of platinum-based treatment for advanced non-small cell lung cancer: a meta-analysis in 17 studies. *J. Zhejiang Univ. Sci. B* 13: 875-883. http://dx.doi.org/10.1631/jzus.B1200083

Cui Z, Yin Z, Li X, Wu W, et al. (2012). Association between polymorphisms in XRCC1 gene and clinical outcomes of patients with lung cancer: a meta-analysis. *BMC Cancer* 12: 71. http://dx.doi.org/10.1186/1471-2407-12-71

Giachino DF, Ghio P, Regazzoni S, Mandrile G, et al. (2007). Prospective assessment of XPD Lys751Gln and XRCC1 Arg399Gln single nucleotide polymorphisms in lung cancer. *Clin. Cancer Res.* 13: 2876-2881. http://dx.doi.org/10.1158/1078-0432.CCR-06-2543

Guilbert JJ (2003). The world health report 2002 - reducing risks, promoting healthy life. *Educ. Health (Abingdon)* 16: 230.

Guo S, Li X, Gao M, Li Y, et al. (2013). The relationship between XRCC1 and XRCC3 gene polymorphisms and lung cancer risk in northeastern Chinese. *PLoS One* 8: e56213. http://dx.doi.org/10.1371/journal.pone.0056213

Haliassos A, Chomel JC, Tesson L, Baudis M, et al. (1989). Modification of enzymatically amplified DNA for the detection of point mutations. *Nucleic Acids Res.* 17: 3606. http://dx.doi.org/10.1093/nar/17.9.3606

Hao B, Miao X, Li Y, Zhang X, et al. (2006). A novel T-77C polymorphism in DNA repair gene XRCC1 contributes to diminished promoter activity and increased risk of non-small cell lung cancer. *Oncogene* 25: 3613-3620. http://dx.doi.org/10.1038/sj.onc.1209355

Improta G, Sgambato A, Bianchino G, Zupa A, et al. (2008). Polymorphisms of the DNA repair genes XRCC1 and XRCC3 and risk of lung and colorectal cancer: a case-control study in a Southern Italian population. *Anticancer Res.* 28 (5B): 2941-2946.

Kalikaki A, Kanaki M, Vassalou H, Souglakos J, et al. (2009). DNA repair gene polymorphisms predict favorable clinical outcome in advanced non-small-cell lung cancer. *Clin. Lung Cancer* 10: 118-123. http://dx.doi.org/10.3816/CLC.2009.n.015

Li Y, Huang XE, Jin GF, Shen HB, et al. (2011). Lack of any relationship between chemotherapy toxicity in non-small cell lung cancer cases and polymorphisms in XRCC1 codon 399 or XPD codon 751. *Asian Pac. J. Cancer Prev.* 12: 739-742.

López-Cima MF, González-Arriaga P, García-Castro L, Pascual T, et al. (2007). Polymorphisms in XPC, XPD, XRCC1, and XRCC3 DNA repair genes and lung cancer risk in a population of northern Spain. *BMC Cancer* 7: 162. http://dx.doi.org/10.1186/1471-2407-7-162

Parkin DM, Bray F, Ferlay J and Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J. Clin.* 55: 74-108. http://dx.doi.org/10.3322/canjclin.55.2.74

Qian B, Zhang H, Zhang L, Zhou X, et al. (2011). Association of genetic polymorphisms in DNA repair pathway genes with non-small cell lung cancer risk. *Lung Cancer* 73: 138-146. http://dx.doi.org/10.1016/j.lungcan.2010.11.018

Siegel R, Naishadham D and Jemal A (2012). Cancer statistics, 2012. *CA Cancer J. Clin.* 62: 10-29. http://dx.doi.org/10.3322/caac.20138

Sreeja L, Syamala VS, Syamala V, Hariharan S, et al. (2008). Prognostic importance of DNA repair gene polymorphisms of XRCC1 Arg399Gln and XPD Lys751Gln in lung cancer patients from India. *J. Cancer Res. Clin. Oncol.* 134: 645-652. http://dx.doi.org/10.1007/s00432-007-0328-4

Wang Y, Yang H, Li H, Li L, et al. (2009). Association between X-ray repair cross complementing group 1 codon 399 and 194 polymorphisms and lung cancer risk: a meta-analysis. *Cancer Lett.* 285: 134-140. http://dx.doi.org/10.1016/j.canlet.2009.05.005

Wu SH, Lu C, Dong L, Zhou GP, et al. (2005). Lipoxin A4 inhibits TNF-alpha-induced production of interleukins and proliferation of rat mesangial cells. *Kidney Int.* 68: 35-46. http://dx.doi.org/10.1111/j.1523-1755.2005.00379.x

Yin J, Vogel U, Ma Y, Qi R, et al. (2009a). Association of DNA repair gene XRCC1 and lung cancer susceptibility among nonsmoking Chinese women. *Cancer Genet. Cytogenet.* 188: 26-31. http://dx.doi.org/10.1016/j.cancergencyto.2008.08.013

Yin Z, Zhou B, He Q, Li M, et al. (2009b). Association between polymorphisms in DNA repair genes and survival of non-smoking female patients with lung adenocarcinoma. *BMC Cancer* 9: 439. http://dx.doi.org/10.1186/1471-2407-9-439

Yuan Z, Zeng X, Yang D, Wang W, et al. (2013a). Effects of common polymorphism rs11614913 in Hsa-miR-196a2 on lung cancer risk. *PLoS One* 8: e61047. http://dx.doi.org/10.1371/journal.pone.0061047

Yuan Z, Li J, Li J, Gao X, et al. (2013b). SNPs identification and its correlation analysis with milk somatic cell score in bovine MBL1 gene. *Mol. Biol. Rep.* 40: 7-12. http://dx.doi.org/10.1007/s11033-012-1934-z

Yuan Z, Li J, Li J, Gao X, et al. (2013c). Effects of DGAT1 gene on meat and carcass fatness quality in Chinese commercial cattle. *Mol. Biol. Rep.* 40: 1947-1954. http://dx.doi.org/10.1007/s11033-012-2251-2

Yuan Z, Li J, Li J, Zhang L, et al. (2012). Investigation on BRCA1 SNPs and its effects on mastitis in Chinese commercial cattle. *Gene* 505: 190-194. http://dx.doi.org/10.1016/j.gene.2012.05.010

Zhao CJ, Li N and Deng XM (2003). [The establishment of method for identifying SNP genotype by CRS-PCR]. *Yi Chuan* 25: 327-329.