RESEARCH ARTICLE

No Association of XRCC1 and CLPTM1L Polymorphisms with Non-small Cell Lung Cancer in a Non-Smoking Han Chinese Population

Yan Sun[1], Yong-Jun Zhang[2,3]*, Xiang-Ming Kong[2]

Abstract

Background: This study aimed to explore potential associations between single nucleotide polymorphisms (SNPs) of the x-ray repair cross-complementing group 1 (XRCC1) and cleft lip and palate transmembrane protein 1-like (CLPTM1L) and non-small cell lung cancer (NSCLC) susceptibility in non-smoker Chinese patients. Methods: A total of 200 NSCLC patients and 200 healthy controls with matched age and gender were recruited for genotyping of XRCC1 SNPs (rs2256507 and rs1001581) and CLPTM1L SNPs (rs401681 and rs4975616). Association of these SNPs with NSCLC risk was evaluated by computing the odds ratio (OR) and 95% confidence interval (CI) from multivariate unconditional logistic regression analyses with adjustment for gender and age. Results: The frequencies of genotype and allele in these four loci (rs2256507, rs1001581, rs401681, and rs4975616) were not significantly different between the cases and controls, or between either of the histological subgroups (adenocarcinoma and squamous cell carcinoma) and controls. Conclusions: Although these SNPs are associated with NSCLC risk in patients with a tobacco-smoking habit, this study demonstrated that XRCC1 and CLPTM1L gene SPNs are not linked with NSCLC risk in non-smoking patients, indicating that molecular mechanisms of NSCLC betwee tobacco smokers and non-smokers may be different. Future studies are needed to uncover the underlying molecular mechanisms for NSCLC in non-smokers.

Keywords: CLPTM1L - XRCC1 - single nucleotide polymorphisms - never-smoker - Chinese population

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Introduction

Lung cancer is a leading cause of cancer-related death in the world (Siegel et al., 2013). Tobacco smoke contributes to a great majority of lung cancer cases and tobacco cessation program has significantly reduced the worldwide lung cancer incidence. Nevertheless, global statistical data estimates that 15% of lung cancer cases in men and 50% in women may not be related to tobacco smoke (Parkin et al., 2005). The different risk factors (e.g., tobacco smoking vs. no smoking) may lead to different mechanisms of lung carcinogenesis and tobacco smoke lung cancer is preventable, while lung cancer in non-smoker may have different etiology. If the later is considered as a separate entity, lung cancer in non-smokers would rank as the seventh most common cause of cancer death worldwide (Wang et al., 2010).

Recent genome-wide association studies have shown that polymorphisms of rs2256507 and rs1001581 in x-ray repair cross-complementing group 1 (XRCC1) were significantly associated with the increased risk of non-small cell lung cancer (NSCLC) among Latinos but not among African-Americans (Chang et al., 2009), and the SNPs of Cleft Lip and Palate Transmembrane Protein 1-Like (CLPTM1L) rs401681 (Pande et al., 2011) was associated with decrease in lung cancer risk in Caucasians. Meanwhile, rs401681 (Wang et al., 2008) and rs4975616 (Broderick et al., 2009; Wang et al., 2010)were associated with a significant decrease in lung cancer risk in Europeans. Another study showed a stronger association of rs1001581 heterozygote with the increased NSCLC risk in Korean (Kim et al., 2010). Thus, the present study investigated the polymorphisms of XRCC1 and CLPTM1L for association with NSCLC risk in never-smoking Chinese.

Materials and Methods

Study population

In this study, 200 consecutive NSCLC patients were enrolled in The Department of Surgical and Medical Oncology, Zhejiang Cancer Hospital between May and November 2012. All patients were histopathologically diagnosed as NSCLC. The, study excluded the patients who were diagnosed for any other primary cancer and NSCLC as secondary primary cancer. In addition, 200

*[1]Departments of Chemotherapy, [2]Departments of Integration of Traditional Chinese and Western Medicine, [3]Zhejiang Key Laboratory of Diagnosis and Treatment Technology for Thoracic Oncology, Zhejiang Cancer Hospital, Hangzhou, China *For correspondence: zhangyongjun770323 @163.com*

Table 1. Characteristics of Lung Cancer Cases and Controls

Variable	Controls n (%), (n=200)	Cases n(%), (n=200)
Age (year), median (range)	56.66 (33-80)	57.64(36-77)
Gender		
Female	76 (38.00)	65 (32.50)
Male	124 (62.00)	135 (67.50)
Histology		
Squamous cell carcinoma		55 (27.50)
Adenocarcinoma		145 (72.50)

healthy subjects were also recruited as controls from the same region of The Second Affiliated Hospital of Zhejiang Chinese University. The control subjects didn't have any lung-related diseases and were non smokers according to our study questionnaire. Clinicopathological data from the patients and healthy controls were collected from medical records and study questionnaire, respectively. Written informed consent was received from each subject. The study protocol was approved by the Ethical Review Committee of Zhejiang Cancer Hospital. The characteristics of all study participants were summarized in Table 1.

DNA extraction and genotyping

Blood samples from all 400 subjects were collected and subjected to DNA extraction and genotyping of XRCC1 rs2256507 and rs1001581 and CLPTM1L rs401681 and rs4975616. SNPs were analyzed by Shanghai Benegene Biotechnologies Co., Ltd. (Shanghai, China). The linkage disequilibrium (LD) was calculated according to the data from H apMap population JPT+CHB, and r2 was 0.21 and 0.56 in XRCC1 (rs2256507 and rs1001581) and CLPTM1L (rs401681 and rs4975616), respectively. Briefly, genomic DNA was isolated from 1 ml of whole blood using a genomic DNA isolation kit (Axygen Biosciences, Union City, CA, USA). Genotype of these SNPs was analyzed by using MassARRAY compact analyzer based on the chip-based matrix-assisted laser desorption ionization time-of-flight mass spectrometry platform (Sequenom, San Diego, CA, USA) (Carbonnelle et al., 2011). Multiplex reaction was designed using Assay Designer software (version 3.0, Sequenom, San Diego, CA, USA) and processed by following standard protocols for iPLEX chemistry. Primers were designed and synthesized (Sangon Biotech, Shanghai, China) (Table 2).

Table 2. Oligonucleotide Sequence Used for Genotyping

Genes	SNP	Sequences
XRCC1	rs1001581	5'-ACGTTGGATGTCTGGAGAGGTCAGGGAAG-3'
		5'-ACGTTGGATGGCAGAAACTCACAAAATAGTC-3'
		5'-GCATAGTCACTGAATGAATGCATA-3'
XRCC1	rs2256507	5'-ACGTTGGATGCCTTCGCTATCTAAACCCAG-3'
		5'-ACGTTGGATGTAAGACGGCCGCTGAGTTAG-3'
		5'-CTGAGTTAGTTGTGGAGAC-3'
CLPTM1L	rs401681	5'-ACGTTGGATGGCCAGAAAGCTGCTTCACAC-3'
		5'-ACGTTGGATGATGCATAGTGGGCAGAAAAC-3'
		5'-ATCCAGACAACTTCAGAGTC-3'
CLPTM1L	rs4975616	5'-ACGTTGGATGACAGTCTGACTTCTGCCCTG-3'
		5'-ACGTTGGATGGATCTGCATGAGGCTCAGTC-3'
		5'-GGCTCAGTCCTCTCTT-3'

Table 3. HWE Pearson's P in Controls

Genes	rs	HWE Pearson's *P*
XRCC1	rs1001581	0.96
XRCC1	rs2256507	0.39
CLPTM1L	rs401681	0.22
CLPTM1L	rs4975616	0.07

Statistical analysis

Calculation of allele and genotype frequency and Hardy–Weinberg equilibrium (HWE) test were performed by using Microsoft Excel macro PHARE version 2.1. Comparison of the allele and 3 genotypes of dominant model in cases versus controls were carried out using Pearson's chi-squared tests. Odds ratios (ORs) and their 95% confidence intervals (95% CI) were obtained with Plink software. P value of lesser than 0.05 was considered statistically significant.

Results

In this study, we first genotyped all the 400 subjects (200 NSCLC patients with 145 lung adenocarcinoma and 55 squamous cell carcinoma and 200 healthy controls) for polymorphisms of rs2256507, rs1001581, rs401681 and rs4975616. And, there was no evidence of deviation from Hardy-Weinberg equilibrium in each gene (Table 3). The alleles were "T" and "C" in loci of rs2256507 and rs1001581 in XRCC1 and rs401681 in CLPTM1L. The alleles were "A" and "G" in locus of rs4975616 in CLPTM1L. The genetic types were "C/C", "T/T" and "C/T" in rs2256507, rs1001581 and rs401681, and "A/A", "G/G" and "A/G" in rs4975616. There was no statistical difference between these 4 markers (rs2256507,

Table 4. Allele Distribution in Non-smoking Chinese with NSCLC and Controls

Gene	Controls N=200	NSCLC[b] N=200				ADC[b] N=145				SCC[b] N=55			
allele	n (%)	n (%)	*p* value	OR	95% CI	n (%)	*p* value	OR	95% CI	n (%)	*p* value	OR	95% CI
XRCC1 rs1001581													
T	154(38.50)	159(39.75)				113(39.00)				46(41.80)			
C	246(61.50)	241(60.25)	0.72	1.05	0.79-1.40	177(61.00)	0.9	1.02	0.75-1.39	64(58.20)	0.53	1.15	0.75-1.76
XRCC1 rs2256507													
T	101(25.30)	112(28.00)				77(26.60)				35(31.80)			
C	299(74.80)	288(72.00)	0.38	1.15	0.84-1.58	213(73.40)	0.7	1.07	0.76-1.51	75(68.20)	0.17	1.38	0.87-2.19
CLPTM1L rs401681													
T	124(31.00)	120(30.00)				88(30.30)				32(29.10)			
C	276(69.00)	280(70.00)	0.76	0.95	0.71-1.29	202(69.70)	0.85	0.97	0.70-1.35	78(70.90)	0.7	0.91	0.58-1.45
CLPTM1L rs4975616													
A	342(85.50)	347(86.80)				251(86.60)				96(87.30)			
G	58(14.50)	53(13.20)	0.61	1.11	0.74-1.66	39(13.40)	0.69	1.09	0.71-1.69	14(12.70)	0.64	1.163	0.62-2.18

NSCLC, Non-small cell lung cancer; ADC, adenocarcinoma; SCC, squamous cell carcinoma; [b]compared with controls

Table 5. Genotypes in Lung Cancer Cases and Controls and Their Association with Risk of Lung Cancer

Gene	Controls N=200	NSCLC[b] N=200				ADC[b] N=145				SCC[b] N=55			
allele	n (%)	n (%)	*p* value	OR	95% CI	n (%)	*p* value	OR	95% CI	n (%)	*p* value	OR	95% CI
XRCC1rs1001581													
C/C	76(38.00)	72(36.00)				52(35.90)				20(36.40)			
T/T	30(15.00)	31(15.50)				20(13.80)				11(20.00)			
C/T	94(47.00)	97(48.50)	0.92			73(50.30)	0.83			24(43.60)	0.67		
TT+CT	124(62.00)	128(64.00)	0.68	0.92	0.61-1.38	93(64.10)	0.68	0.91	0.59-1.42	35(63.60)	0.82	0.93	0.50-1.73
XRCC1rs2256507													
C/C	109(54.50)	103(51.50)				78(53.80)				25(45.50)			
T/T	10(5.00)	15(7.50)				10(6.90)				5(9.10)			
C/T	81(40.50)	82(41.00)	0.56			57(39.30)	0.76			25(45.50)	0.34		
TT+CT	91(45.50)	97(48.50)	0.55	0.89	0.60-1.31	67(46.20)	0.9	0.97	0.63-1.49	30(54.50)	0.23	0.7	0.38-1.27
CLPTM1Lrs401681													
C/C	95(47.50)	93(46.50)				66(45.50)				27(49.10)			
T/T	19(9.50)	13(6.50)				9(6.20)				4(7.30)			
C/T	86(43.00)	94(47.00)	0.47			70(48.30)	0.43			24(43.60)	0.88		
TT+CT	105(52.50)	107(53.50)	0.84	0.96	0.65-1.42	79(54.50)	0.76	0.92	0.60-1.42	28(50.90)	0.83	1.07	0.59-1.94
CLPTM1Lrs4975616													
A/A	149(74.50)	152(76.00)				109(75.20)				43(78.20)			
G/G	7(3.50)	5(2.50)				3(2.10)				2(3.60)			
A/G	44(22.00)	43(21.50)	0.83			33(22.80)	0.73			10(18.20)	0.83		
AG+GG	51(25.50)	48(24.00)	0.73	1.08	0.69-1.71	36(24.80)	0.89	1.04	0.63-1.70	12(21.80)	0.58	1.23	0.60-2.51

NSCLC, Non-small cell lung cancer; ADC, adenocarcinoma; SCC, squamous cell carcinoma; [b]compared with controls

rs1001581, rs401681 and rs4975616) in allele or genotype frequencies between cases and controls or between controls and subgroups of NSCLC (i.e., adenocarcinoma and squamous cell carcinoma of the lung; Table 4 and 5).

Discussion

In the current study, four different SNPs of XRCC1 and CLPTM1L for association with Chinese NSCLC risk of non-smokers was analyzed. The data showed that there is no association between SNPs and NSCLC risk in never smokers and this is the first study in a Chinese population to show this patten. However, the present data were markedly discordant with that of previously published studies (Chang et al., 2009; Kim et al., 2010; Wang et al., 2010). The reason for this discrepancy is unknown, but ethnic background of such patient population may play a role in it. In other words, the essentiality of racial diversities may account for the candidate genes for association with NSCLC in non-smokers. In addition, the present study didn't collect the influence of second hand smoke or cooking smoke data, which may also account for its discrepancy. However, since minor allele frequency (MAF) of SNPs varies significantly between populations, association based on these SNPs will be particularly sensitive to ethnic variability. For example, the highest MAF in the present study was 0.39 in rs1001581 SNP. Indeed, the HapMap database showed a great variability in MAF of SNP rs1001581 among different populations(http://hapmap.ncbi.nlm.nih.gov/cgi-perl/snp_details_phase3?name=rs1001581 &source=hapmap-3r2_B36&tmpl=snp_details_phase3), while the HapMap data showed that the MAF of rs1001581 SNP in European population has low minor allele frequencies (i.e., 0.39) and Africans with a higher MAF (i.e., 0.44), while Asians with intermediate MAF rate (i.e., 0.41–0.43) among their ethnic populations.

Moreover, the present study subjects with non-smoking status were based the self-reported data and defined with ten packs or less tobacco smoked lifetime in an individual, although the definition for non-smokers varies considerably in the literature (LARC Working Group, 2004). In addition, population stratification is a concern in all the associated studies as a source of bias since the frequency of genotypes for many polymorphic variants differ markedly among different ethnic groups. So in the present study, the study population was narrowed to Chinese Han subjects lived in China.

In addition, alterations in genomic DNA can be caused by the exposure to different environmental and endogenous carcinogens or agents. Most of these alterations, if not repaired, may result in cell death, genetic instability, mutagenesis or cancer. Thus, DNA repair mechanisms are very important for maintaining genome integrity and preventing carcinogenesis. Base excision repair (BER) pathway is the key mechanism involved in repairing small base lesions in DNA that are the result of oxidation and alkylation damage (Hoeijmakers, 2001; Mahimkar et al., 2012), and was closely associated with lung cancer development (Qian and Massion, 2008). SNPs in susceptible genes have been increasingly emphasized on the grounds that XRCC1 is considered a crucial scaffold protein closely associated with the base excision repair pathway (Campalans et al., 2005). It serves as a scaffold protein in both single-strand break repair and base excision repair activities (Lindahl et al., 1999). The amount of XRCC1 transcription has shown a significant association with cisplatin resistance (Weaver et al., 2005) and sensitivity to 5-FU treatment (Huang et al., 2011) among NSCLC cells. XRCC1 protein can bind to platinum-containing DNA duplexes (Zhu and Lippard, 2009) and contributed to repair of platinum-induced DNA damage. Previous studies reported that XRCC1 Arg399Gln polymorphism was associated with the clinical outcome of chemotherapy in lung cancer patients (Horgan et al., 2011; Wu et al., 2012), although our current data didn't show XRCC1 gene (rs2256507 and rs1001581) SNPs to be significantly associated with NSCLC risk in never smokers. These data demonstrated that the effects of XRCC1 SNPs on NSCLC risk and treatment outcome may

be dependent on ethnicity of patients. Thus, a large scale and well controlled study is needed to clarify associations of the single polymorphisms, gene-gene interactions and gene-environment interactions with NSCLC in Chinese population.

Again, previous studies showed that CLPTM1L was mapped to chromosome 5p15.33 and was a lung cancer susceptibility gene (Yamamoto et al., 2001; James et al., 2012). CLPTM1L rs401681 polymorphism was associated with risk of various cancers (Wang et al., 2008; McKay et al., 2008; Rafnar et al., 2009; Zienolddiny et al., 2009), though there were some inconsistent data. For example, a previous study reported that the CLPTM1L T genotypes were not associated with any risk of lung cancer in 341 cases and 431 controls in a Caucasian populations (Zienolddiny et al., 2009), whereas another study of 2396 lung cancer cases and 3001 controls did find CLPTM1L T allele to be associated with a significant decrease risk of lung cancer (Wang et al., 2008). However, in a recent GWAS study of 20726 cancer patients and 13465 controls reported that the CLPTM1L C genotypes were associated with a significant increase in the risk of lung cancer (Rafnar et al., 2009). In contrast, CLPTM1L over expression protected lung tumor cells from genotoxic stress induced apoptosis, through the regulation of Bcl-xL and this anti-apoptosis function made CLPTM1L susceptibility to lung tumorigenesis and resistance to chemotherapy (James et al., 2012). The effect of the CLPTM1L variants may be through enhanced metabolic activation of the reactive metabolites and/or enhanced formation and persistence of DNA adducts (Zienolddinay et al., 2009). In the present study, we found that CLPTM1L rs401681 and rs4975616 polymorphisms were similar in magnitude between the cases and controls, although genome wide association studies showed that the 5p15.33 locus accounts for the greatest contribution to lung cancer risk in humans (McKay et al., 2008; Liu et al., 2010).

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