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Original Article

Genetic risk of lung cancer associated with a single nucleotide polymorphism from *EXO1*: a meta analysis

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Abstract: Background/Aim: Several reports have investigated the role of exonuclease 1 (*EXO1*) rs1047840 in lung cancer risk in different ethnic populations. Nevertheless, the results have been controversial. We aimed to assess the possible association between *EXO1* rs1047840 and risk of lung cancer in a meta-analysis. Methods: Human hospital- or population-based studies released before December 16, 2013 were identified by systematic search of the PubMed and Embase databases. Data were extracted in duplicate from each study. An OR and 95% CI (odds ratio and 95% confidence interval) was calculated to evaluate the effects of *EXO1* rs1047840 on lung carcinogenesis. Results: A total of 1,114 lung cancer patients and 1,166 well-matched controls were analyzed in this study. The fixed-effects meta-analysis revealed that carriage of a single A allele, compared to the carriage of single G allele, was associated with 1.18 times increased risk of lung cancer (A vs. G: OR =1.18; 95% CI: 1.03-1.35; $P_{Heterogeneity}$, 0.121). Conclusion: This first meta-analysis demonstrates that the A allele of *EXO1* rs1047840 may confer modulating effects on the risk of lung cancer and could be used as a marker for early detection and primary prevention.

Keywords: Exonuclease 1, polymorphism, lung cancer, risk

Introduction

Frequent exposure to exogenous and endogenous mutagens could induce genome instability and DNA breakage that subsequently initiate various cancers if left unreconstructed. DNA repair systems encompass five major mechanisms known to maintain genomic stability and integrity, including base excision repair (BER), double-strand break repair (DSBR), transcription coupled repair (TCR), nucleotide excision repair (NER), and mismatch repair (MMR). Recent work showed that single-nucleotide polymorphism (SNP) as the most prevalent genetic variation is ideal markers for candidate gene studies to detect SNP-disease associations [1]. The SNPs from more than 150 human DNA repair genes have previously been reported to exert modulating effects on cancer by regulating repair capacity of DNA [2]. Such effects caused by DNA repair abnormality have also been described in multiple breast cancer studies [3-5]. Therefore, identifying the pathogenic role the SNPs in DNA repair pathways play is critical for a clearer understanding of the underlying pathogenesis mechanisms of human diseases.

The MMR pathway has an important role in cell cycle arrest regulation, DNA rehabilitation, and genomic stability maintenance [6]. Deficient mutations may eliminate the function of MMR at the transcriptional level and thereby facilitate the progress of diverse cancers [7-9]. As previously demonstrated, there is a significant association between MMR pathway genes and progression of lung cancer [7, 8]. A well-defined gene in this pathway has been exonuclease 1 (*EXO1*) located on human chromosome 1q42-q43, a highly polymorphic gene consisting of 14 exons and encoding an 846 amino acid protein [10-12]. An association of *EXO1* inactivation with increased likelihood of tumorigenesis and lower survival time has been identified in vivo animal models [13]. An *EXO1* polymorphism at codon 589 (rs1047840) is a non-synonymous SNP that affects EXO1 mRNA expression and has been widely investigated in cancer area, lung cancer area in particular. But the prior reports representing different ethnic popula-

Table 1. The reason for quality assessment

Criteria	Score
♦ Representativeness of cases	
Selected from population or cancer registry	3
Selected from hospital	2
Selected from pathology archives, but with extensive inclusion/exclusion criteria or without clearly defined sampling frame	1
No description	0
♦ Source of controls	
Population-based	3
Blood donors or volunteers	2
Hospital-based (cancer-free patients)	1
No description	0
♦ Pecimens of cases determining genotypes	
White blood cells or normal tissues	1
Tumor tissues or exfoliated cells of tissue	0
♦ Ascertainment of NPC	
Histopathologic confirmation	2
Diagnosis through patient medical record	1
No description	0
♦ Hardy-Weinberg equilibrium in controls	
Hardy-Weinberg equilibrium	1
Hardy-Weinberg disequilibrium	0
♦ Quality control of genotyping methods	
Repetition of partial/total tested samples	1
No description	0
♦ Total sample size	
≥1000	3
400-1000	2
200-400	1
<200	0

tions have presented controversial results [14-16]. It is these disparate results that have promoted us to carry out a meta-analysis to assess the association between *EXO1* rs1047840 and risk of lung cancer.

Methods

Literature search and eligibility

The eligible studies were systematically identified by search of the PubMed and Embase databases. The keywords included lung cancer, lung carcinoma, exonuclease 1, *EXO1*, polymorphism, polymorphisms, genotypes and variants. After having retrieved all relevant papers concerning the association of *EXO1* rs1047840 with risk of lung cancer, we screened the references of each paper to identify the additional datasets. Literature search were undertaken without language or minimal sample size restriction. Any hospital- or population-based study was considered eligible for the meta-analysis if: 1) a case-control study evaluated the association between *EXO1* rs1047840 and risk of lung cancer, 2) genotype information provided in the research article must be sufficient for estimation of an OR and 95% CI (odds ratio and 95% confidence interval).

However, the case reports, editorials, narrative reviews, case-case or case-only studies and abstracts with incomplete genetic data were definitely excluded from the analysis. When a prior study was extended by a subsequent study addressing the same subject, we selected the larger study where more participants were included.

Data abstraction

Two independent investigators extracted the following data in duplicate by using a standardized form: last name of the first author, publication journal and year, country in which the study was conducted, ethnicity of each population, source of controls, study design, total cases and controls, Hardy-Weinberg equilibrium

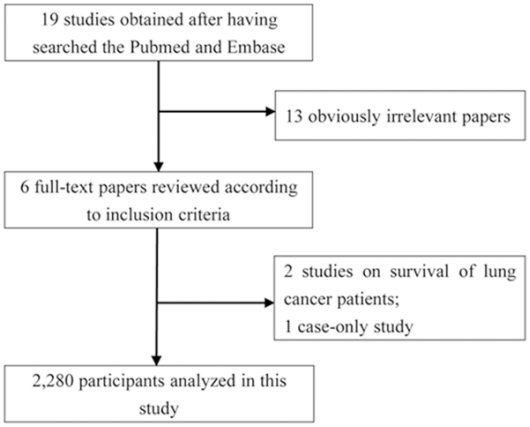

Figure 1. Flow diagram of included studies for this meta-analysis.

(HWE) when available, genotyping methods and genotype distribution. A senior investigator was invited in case of inconsistent evaluations.

Quality assessment

Table 1 shows the reason for quality assessment.

Statistical analysis

HWE was examined for all control populations by a chi-square test to check whether there was selection bias in this analysis. The possible association of *EXO1* rs1047840 with lung cancer was evaluated by calculating ORs and corresponding 95% CIs, which were combined with both the random-effects model derived from the DerSimonian-Laird method and the fixed-effects model derived from the Mantel-Haenszel method. Heterogeneity across the studies was measured by using a X^2-based Q-test, and a *P* value less than .05 was judged as the significance level. In addition, to quantify the proportion of heterogeneity, we also used the I^2 statistic that takes values from 0 to 100%, with higher proportion indicating larger between-study heterogeneity (0-25% corresponds to low heterogeneity, 25%-50% corresponds to moderate heterogeneity, 50%-100% corresponds to large heterogeneity) [17]. Evaluation of publication bias was determined by Begg's funnel plot and Egger's linear regression test [18]. Statistical data were analyzed using the STATA software. P<0.05 was considered statistically significant. The leave-one-out sensitivity analyses including or excluding the study not in HWE were performed to check the stability of pooled results.

Results

Study characteristics

As displayed in **Figure 1**, a total of 19 papers that matched the search terms were identified through computer-based searches. We first reviewed the titles and/or abstracts and excluded 13 obviously irrelevant papers. We downloaded the remaining papers and reviewed their full-texts, excluding 3 papers because of genome-wide association study or base and clinical study on survival of lung cancer patients [19, 20], and case-only study [21]. Finally, three studies satisfying the inclusion criteria were included in the analysis [14-16]. The case-control studies, comprising two Asian studies and one Caucasian study, were published in English language. There were one hospital-based study and two population-based studies, with one showing significant deviation from HWE. In addition, two types of genotyping assays were employed, with the Caucasian study using the Taqman assay and Asian studies using the classical PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). The control subjects were matched to cases by several characteristics, including age and gender (**Table 2**).

Quantitative analysis

Evaluation of the association between *EXO1* rs1047840 and risk of lung cancer was undertaken in 1,114 lung cancer patients and 1,166 well-matched controls. Overall, the pooled ORs and 95% CIs calculated with the random-effects model did not show any significant association of rs1047840 genotypes with risk of developing lung cancer, as shown in **Table 3**. Nevertheless, the fixed-effects meta-analysis revealed 1.18 times increased risk of lung cancer among individuals carrying a single A allele (A vs. G: OR =1.18; 95% CI: 1.03-1.35; $P_{Heterogeneity}$, 0.121), as illustrated in **Figure 2**. In addition, it was interesting that the increase in risk of lung cancer was notably higher in case of absence of heterogeneity (AA vs. GG: OR =1.30; 95% CI: 0.92-1.85; $P_{Heterogeneity}$, 0.385; AA vs. AG + GG: OR =1.31; 95% CI: 0.93-1.84; $P_{Heterogeneity}$, 0.468), even though the association failed to reach the significance level.

Table 2. Characteristics of studies included in the meta-analysis

First author	Population	Country	Source of controls	Study design	No. (Cases/controls)	HWE	Genotyping methods
Jin GF [14]	Asian	China (Jiangsu)	Population	Case-control	500/517	0.03	PCR-RFLP
Hsu NY [15]	Asian	China (Taiwan)	Hospital	Case-control	358/358	0.86	PCR-RFLP
Zienolddiny S [16]	Caucasian	Norway (Oslo)	Population	Case-control	256/291	0.11	Taqman

PCR-RFLP-polymerase chain reaction-restriction fragment length polymorphism.

Table 3. ORs and heterogeneity results of the meta-analysis

Genetic model	Fixed-effects			Random-effects		
	OR (95% CI)	P_{OR}	$P_{Heterogeneity}/I^2$	OR (95% CI)	P_{OR}	$P_{Heterogeneity}/I^2$
All						
AA vs. GG	1.30 (0.92, 1.85)	0.13	0.38/0.0	1.30 (0.91, 1.85)	0.14	0.38/0.0
AG vs. GG	1.16 (0.98, 1.36)	0.08	0.08/0.59	1.15 (0.88, 1.49)	0.30	0.08/0.59
A vs. G	1.18 (1.03, 1.35)	0.01	0.12/0.52	1.18 (0.97, 1.44)	0.10	0.12/0.52
AA + AG vs. GG	1.16 (0.99, 1.35)	0.05	0.12/0.51	1.16 (0.92, 1.45)	0.20	0.12/0.51
AA vs. AG + GG	1.31 (0.93, 1.84)	0.12	0.468/0.0	1.30 (0.92, 1.84)	0.13	0.468/0.0

OR-odds ratio, CI-confidence interval.

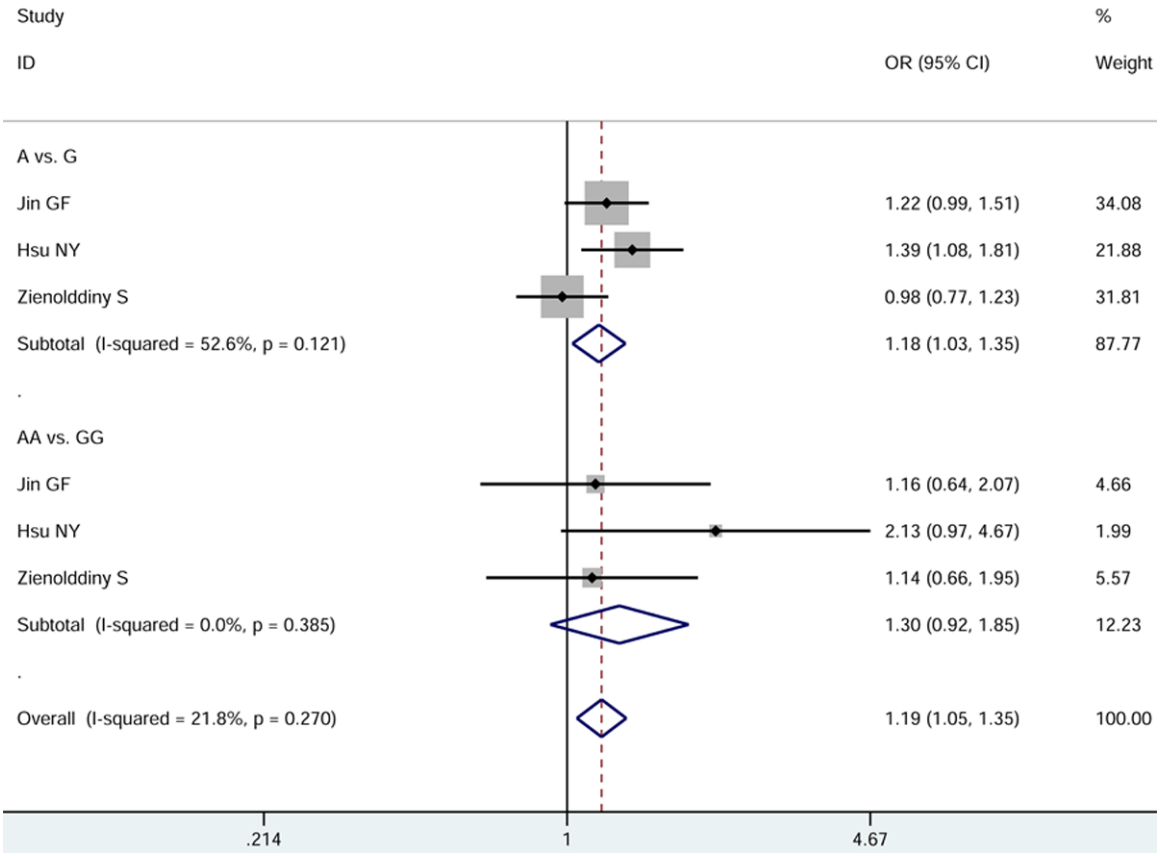

Figure 2. Forest plot of overall risk of lung cancer associated with *EXO1* rs1047840 (A vs. G) by the fixed effects model. For each study, the estimates of OR and its 95% CI were plotted with a box and a horizontal line. The symbol filled diamond indicates pooled OR and its 95% CI.

We checked the stability of combined results by excluding the study with HWE deviation. The recalculated ORs were not significantly different from those calculated based on all studies,

suggesting the findings of this analysis were stable and credible.

Both the funnel plots and the Egger's test showed that there was no obvious publication bias in the meta-analysis.

Discussion

With the increasingly higher rate of lung cancer occurrence and mortality, widespread attention has been diverted to the complex molecular mechanism that underlies the host susceptibility of this cancer. An increasing body of evidence from candidate gene studies, one of the primary approaches extensively used to identify the susceptibility locus of human disease, supported that etiology of lung cancer is clearly associated with predisposing genes [22, 23]. A number of recent genome-wide association studies also lend support to the hypothesis of strong genetic base in lung carcinogenesis [24-26]. These reports point to the importance of genetic constitution in the progress of lung cancer.

Thus it is believable that identification of the susceptibility loci is important for early detection and prevention of the invasive cancer.

The first candidate gene study associating *EXO1* rs1047840 with lung cancer risk was conducted in a Caucasian population, with the authors demonstrating no linkage of genotypes at rs1047840 with lung cancer [16]. Later, several larger studies followed up, attempting to determine the association. Hsu et al. investigated the effects of rs1047840 genotypes and alleles on lung cancer occurrence, and identified a significantly increased risk of lung cancer among Chinese subjects [15]. The significant association was replicated in a most recent study also representing Chinese ethnicity [14]. Although there are multiple possible reasons for the inconsistent results, the most plausible reason may relate to the relatively small sample size and different ethnic populations. In addition, the positive association identified in both Chinese studies makes us think that *EXO1* rs1047840 may predispose to lung cancer in an ethnic-specific manner or Asians may be more susceptible to lung cancer compared with Caucasian. However, since the statistical power may be insufficient due to the limited sample, we cannot exclude the possibility of false negative or positive associations.

In an attempt to derive a more precise estimation of the effects *EXO1* rs1047840 confers on lung cancer, we decided to perform a meta-analysis. The results of our analysis seemed to support a possible association between *EXO1* rs1047840 and lung cancer. This finding is in line with that of a previous gastric cancer study, in which the authors provided evidence in support of increased risk of gastric cancer in relation to the A allele and stated that *EXO1* rs1047840 is a potentially useful marker for anticancer intervention and primary prevention [27]. The results of this analysis are also consistent with a hepatocellular carcinoma study by Bayram et al., who demonstrated as high as 3.14-fold increased risk of nonviral-related HCC associated with the AA genotype [28]. Although cancer at various sites shared certain similarities in etiology, there may be some specific risk factors for each cancer type. Therefore, the exact role of *EXO1* rs1047840 in lung cancer etiology merits further investigation.

The findings of this analysis will be more understandable when the following points are considered. First, we previously hypothesized that people of Caucasian ancestry may be less susceptible to lung cancer compared to those of Asian ancestry. We are not able to test the plausibility of the hypothesis due to the limited data provided in the original articles included in the analysis. Second, Hsu et al. found 1.72-fold and 1.04-fold higher risk of lung cancer in association with AG and AA genotype among smokers and non-smokers respectively [15], and this indicates that the *EXO1* rs1047840 A allele in conjunction with cigarette smoking may accelerate the progress of lung cancer. Third, some results are heterogeneous and they should be interpreted with caution.

In conclusion, *EXO1* rs1047840 polymorphism was significantly associated with lung cancer. Despite the evidence shown in precious work, we for the first time, to the best of our knowledge, examined the possible association in a meta-analysis. Further research is required to establish the linkage of *EXO1* rs1047840 with lung cancer in various populations.

Disclosure of conflict of interest

None.

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