RESEARCH ARTICLE

The *Exonuclease 1* Glu589Lys Gene Polymorphism and Cancer Susceptibility: Evidence Based on a Meta-analysis

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Abstract

 <u>Background</u>: Published studies on the association between the *exonuclease 1* (*EXO1*) Glu589Lys polymorphism and cancer susceptibility have yielded conflicting results. Thus, a meta-analysis of published studies was performed to assess the possible association. <u>Materials and Methods</u>: All eligible case-control studies published up to January 2013 on the association between the *EXO1* Glu589Lys polymorphism and cancer susceptibility were identified by searching PubMed, Web of Science, Science Direct and hand search. Either fixed-effect or random-effect models were used to calculate pooled odds ratios (ORs) with 95% confidence intervals (CIs) using the Comprehensive Meta-Analysis software version 2.2. <u>Results</u>: A total of 4,391 cancer cases and 4,339 controls from 10 studies were included. Overall, no significant association between the *EXO1* Glu589Lys polymorphism and cancer susceptibility was observed in either genetic model. However; in subgroup analyses by cancer type, a significant association between *EXO1* Glu589Lys and lung cancer risk was found (Lys *vs* Glu: OR=1.23, 95%CI=1.07-1.41, $p_{heterogeneity}$=0.05). Further, subgroup analysis by ethnicity indicated that there was a statistically increased cancer risk in Asians (Lys *vs* Glu: OR=1.42, 95%CI=1.30-1.55, $p_{heterogeneity}$=0.07; Lys/Lys *vs* Glu/Glu: OR=1.93, 95%CI=1.20-3.12, $p_{heterogeneity}$=0.01; Lys/Lys+Glu/Lys *vs* Glu/Glu: OR=1.52, 95%CI=1.37-1.68, $p_{heterogeneity}$=0.42; Lys/Lys *vs* Glu/Lys+Glu/Glu: OR=1.68, 95%CI=1.07-2.65, $p_{heterogeneity}$=0.02). However, significant association was absent in Caucasians. <u>Conclusions</u>: This meta-analysis suggests, for the first time, that the *EXO1* Glu589Lys polymorphism is not associated with overall cancer susceptibility, although marginal associations were found for lung cancer and Asian subgroups. Additional well-designed studies with larger sample size focusing on different ethnicities and cancer types are needed to confirm these findings.

Keywords: *EXO1* - polymorphism - *EXO1* Glu589Lys polymorphism - cancer susceptibility - meta-analysis

Introduction

The gene *Exonuclease 1* (*EXO1*; MIM # 606063) is a member of the mismatch repair (MMR) system, and also belongs to the RAD2 nuclease family. It locates at chromosome 1q42-q43, contains one untranslated exon followed by 13 coding exons and encodes an 846 amino acid protein (Bayram et al., 2012). *EXO1* can interact physically with the MMR proteins MSH2 and MLH1 in both yeast and human cells, and with MSH3 in human cells (Bayram et al., 2012). *EXO1* functions in DNA replication, repair, recombination, mutation avoidance and are essential for male and female meiosis (Bayram et al., 2012).

A guanine (G)/adenine (A) common single nucleotide polymorphism (SNP) at first position of codon 589 in exon 13 of *EXO1* gene (dbSNP ID: rs1047840), resulting in the substitution of an glutamic acid (Glu, E) residue (GAG) by lysine (Lys, K) residue (AAG) (also designated *EXO1* Glu589Lys) in the exonic splicing enhancer (ESE), has been suggested to influence the products of *EXO1* mRNA (Jin et al., 2008). To date, a few molecular epidemiological studies have investigated the association between the *EXO1* Glu589Lys polymorphism and the cancer risk including lung cancer (Zienolddiny et al., 2006; Jin et al., 2008; Hsu et al., 2008), glioma (Chang et al., 2008), breast cancer (Wang et al., 2009), gastric cancer (Bau et al., 2009), oral cancer (Tsai et al., 2009), melanoma (Ibarrola-Villava et al., 2011), cervical (Luo et al., 2012), hepatocellular cancer (Bayram et al., 2012). However, no consistent conclusion has been drawn. The frequency of the Lys589 allele of *EXO1* Glu589Lys polymorphism varies in different geographic areas and ethnic populations. Besides, genetic effects of the *EXO1* Glu589Lys polymorphism have been shown to vary form one type of cancer to other. Even at the same cancer type, the results are conflicting (Zienolddiny et al., 2006; Hsu et al., 2008; Jin et al., 2008). As a result, the statistical power of an individual study could be very limited for efficient assessment of the *EXO1* Glu589Lys polymorphism. For

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these reason, integration of these data sets may ensure improved statistical power to detect any significant effects. As is known, meta-analysis could improve the statistical power and draw reliable conclusion. To date, no meta-analysis has been conducted to investigate the association between Glu589Lys polymorphism of *EXO1* and cancer risk. Therefore, a meta-analysis based on a total of ten independent case-control studies was performed to identify whether there was any evidence of relationship between the *EXO1* Glu589Lys polymorphism and cancer susceptibility.

Materials and Methods

Study identification and selection

Publication search: In this meta-analysis, a comprehensive literature research of the US National Library of Medicine's PubMed database, ISI Web of Knowledge, and Science Direct was conducted using the search terms including "*EXO1*" or "*Exonuclease 1*", "Glu589Lys" or "K589E" or "rs1047840", "polymorphism" or "SNPs", "cancer" or "carcinoma", "tumor" or "neoplasm" and the combined phrases in order to obtain all genetic studies on the relationship of *EXO1* Glu589Lys polymorphism and cancer. Last search was updated on January 17, 2014.

The search was focused on studies that had been conducted in humans. Furthermore, citations in the original studies or reviewed articles on this topic were manually examined to identify additional studies.

Inclusion and exclusion criteria: The following criteria were used to select studies for this meta-analysis (a) published in peer reviewed journals, (b) articles about *EXO1* Glu589Lys polymorphism and risk of cancers, (c) case-control studies comparing cancer cases with healthy or non-cancerous controls (d) articles containing useful allele and genotype frequency. The exclusion criteria were (a) studies with case only (without control population), (b) animal studies, (c) pure cell studies, (d) not concerned with cancer risk, (f) meta-analysis or reviews and (f) duplication of previous publication.

Data extraction: I reviewed and extracted information from all eligible studies independently, according to the inclusion and exclusion criteria listed above. The following characteristics were collected from each study: name of the first author, year of publication, country where the study was conducted, genotyping method for the assessment of *EXO1* Glu589Lys polymorphism, ethnicity, cancer types, source of controls, total number of case and controls with Glu/Glu, Glu/Lys and Lys/Lys genotypes of *EXO1* Glu589Lys polymorphism, and Hardy-Weinberg equilibrium (HWE). Different ethnicities were classified as Caucasian, and Asian. All eligible studies were defined as hospital-based (HB) or population-based (PB) according to the source of controls.

Statistical analysis

Observed genotype frequencies for *EXO1* Glu589Lys polymorphism in controls were examined for deviations form Hardy-Weinberg equilibrium (HWE) using a goodness-of-fit χ^2-test with one degree of freedom and a p<0.05 was considered with a significant selective bias. The strength of the association between *EXO1* Glu589Lys polymorphism and cancer susceptibility was assessed by using crude ORs with 95%CIs. The significance of the summary OR was determined with a Z test and p<0.05 was considered as statistically significant. In this meta-analysis, the following comparisons for *EXO1* Glu589Lys polymorphism were evaluated: allele contrast (Lys *vs* Glu), homozygous model (Lys/Lys *vs* Glu/Glu), heterozygous model (Lys/Lys *vs* Glu/Lys), dominant genetic model (Lys/Lys+Glu/Lys *vs* Glu/Glu) and recessive genetic model (Lys/Lys *vs* Glu/Lys+Glu/Glu). The statistical heterogeneity among each study were estimated by χ^2-based Q-test, and the heterogeneity was considered significant when p<0.05. I also quantified the effect of heterogeneity using the I^2 test (Higgins and Thompson, 2002; Higgins et al., 2003) with the value >50% as a statistically significant heterogeneity. I^2 statistics was used to quantify inter study variability that can be attributed to heterogeneity rather than chance. It ranges between 0% and 100%, where a value of 0% indicates no observed heterogeneity and larger values indicates an increasing degree heterogeneity (I^2=0-25%, no heterogeneity; I^2=25-50%, moderate heterogeneity; I^2=50-75%, large heterogeneity; I^2=75-100%, extreme heterogeneity). A p value greater than 0.05 for the Q test indicates a lack of heterogeneity between studies; so the pooled OR estimate of each study was calculated by fixed-effects model (the Mantel-Haenszel method) (Mantel and Haenszel, 1959). Otherwise, the random-effects model (the DerSimonian-Laird method) was used (DerSimonian and Laird, 1986).

Subgroup analyses were also performed to investigate the effects of confounding factors: cancer types, ethnicities, genotyping methods, study design, and HWE. Sensitivity analysis was performed by sequential omission of each study to assess the stability of the results. Funnel plots, which is the main graphical method of assessing publication bias, were used to assess publication bias by Begg's test (Begg and Mazumdar, 1994) and Egger's test (Egger et al., 1997). An asymmetric plot suggested possible publication bias (p>0.05 suggested no bias).

All statistical analysis for the current meta-analysis was performed by comprehensive meta-analysis version 2.2 software (Biostat, Englewood, New Jersey) (Borenstein et al., 2007). All p values were two-sided. Statistical tests performed in the present analysis were considered significant whenever the corresponding null-hypothesis probability was p<0.05.

Results

Characteristics of eligible studies

After careful retrieve and selection, 10 articles listed in Table 1 were identified according to inclusion and exclusion criteria. The study selection process is shown in Figure 1. A total of 10 case-control studies including 4391 cases and 4339 controls were analyzed in this meta-analysis (Zienolddiny et al., 2006; Chang et al., 2008; Hsu et al., 2008; Jin et al., 2008; Wang et al., 2009; Bau et al., 2009; Tsai et al., 2009; Ibarrola-Villava et al., 2011; Luo et al., 2012; Bayram et al., 2012). The characteristics of

selected studies are summarized in Table 1. Genotype and allele distributions of *EXO1* Glu589Lys polymorphism among cancer cases and controls and p value of HWE in controls were shown in Table 2. The sample size in these case-control studies varied considerably (range 110-1272). All studies were case-control studies, including three lung cancer studies (Zienolddiny et al., 2006; Jin et al., 2008; Hsu et al., 2008), and the others including glioma (Chang et al., 2008), breast cancer (Wang et al., 2009), gastric cancer (Bau et al., 2009), oral cancer (Tsai et al., 2009), melanoma (Ibarrola-Villava et al., 2011), cervical cancer (Luo et al., 2012), hepatocellular carcinoma (Bayram et al., 2012). 6 studies conducted in the Asian population, and 4 in Caucasian population. Population-based controls were carried out in 2 studies, while hospital-based controls were carried in 8 studies (Table 1). The genotyping methods contained the classic polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), TaqMan, and microarray (Table 1). The genotype frequencies of *EXO1* Glu589Lys polymorphism in the control subjects were in Hardy-Weinberg equilibrium except the two studies (Jin et al., 2008; Wang et al., 2009).

Quantitative synthesis

As shown in Table 3, no significant association between *EXO1* Glu589Lys polymorphism and the risk of cancer was observed in any genetic model and allele contrast (Lys *vs* Glu: OR=1.20, 95%CI=0.98-1.46, Z=1.74, p=0.08; Lys/Lys *vs* Glu/Glu: OR=1.39, 95%CI=0.91-2.15, Z=1.52, p=0.13; Lys/Lys *vs* Glu/Lys: OR=1.24, 95%CI=0.92-1.69, Z=1.39, p=0.16; Lys/Lys+Glu/Lys *vs* Glu/Glu: OR=1.18, 95%CI=0.93-1.50, p=0.17; Lys/Lys *vs*

Glu/Lys+Glu/Glu: OR=1.38, 95%CI=0.96-1.97, Z=1.73, p=0.08)

Considering the influence of disequilibrium, i performed subgroup analysis by HWE. After excluding two studies that is not conforming to HWE, there was no evidence of significant association between *EXO1* Glu589Lys polymorphism and the risk of cancer in any genetic model and allele contrast (Table 3). When i performed subgroup analyses by cancer types, increased cancer risk was found in the allele contrast comparison for lung cancer (Lys *vs* Glu: OR=1.23, 95%CI=1.07-1.41, Z=2.89, p=0.004) (Figure 1). However, no significant associations were found in any of the other genetic models (Table 3). In the subgroup analysis by ethnicity, studies were categorized into two groups: Asians and Caucasians. In Asian population, significant association between the *EXO1* Glu589Lys polymorphism and the increased risk for cancer were observed in the allele contrast (OR=1.42, 95%CI=1.30-1.55, Z=7.80, p<0.001), homozygous (OR=1.93, 95%CI=1.20-3.12, Z=2.70, p=0.007), dominant (OR=1.52, 95%CI=1.37-1.68, Z=7.86, p<0.001), and recessive (OR=1.68, 95%CI=1.07-2.65, Z=2.23, p=0.03) genetic models (Table 3). For the subgroup of Caucasian population, i found no significant association in any genetic model and allele contrast (Table 3). According to the source of controls, significant effects were observed in hospital-based studies (under allele contrast, homozygous and dominant models); while in population-based studies, significant association was observed only in recessive comparison (Table 3). When stratified separately by genotyping, i found that allele contrast, homozygous, dominant and recessive genetic

Table 1. Main Characteristics of Included Studies in the Mmeta-Analysis

Author (year)	Cancer type	Country	Ethnicity	Genotyping	Source	Case N	Control N
Zienolddiny et al. (2006)	Lung	Norway	Caucasian	TaqMan	PB	256	291
Jin et al. (2008)	Lung	China	Asian	Microarray	HB	500	517
Hsu et al. (2008)	Lung	Taiwan	Asian	PCR-RFLP	HB	358	358
Chang et al. (2008)	Glioma	USA	Caucasian	Microarray	PB	112	110
Wang et al. (2009)	Breast	China	Asian	PCR-RFLP	HB	1272	1272
Bau et al. (2009)	Gastric	Taiwan	Asian	PCR-RFLP	HB	179	179
Tsai et al. (2009)	Oral	Taiwan	Asian	PCR-RFLP	HB	680	680
Ibarrola-Villava et al. (2011)	Melanoma	Spain	Caucasian	TaqMan	HB	684	406
Luo et al. (2012)	Cervical	China	Asian	PCR-RFLP	HB	126	278
Bayram et al. (2012)	Hepatocellular	Turkey	Caucasian	PCR-RFLP	HB	224	224

*HB: hospital-based; PB: population-based; PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

Table 2. Distribution of the *EXO1* Glu589Lys Genotypes and Allele Frequencies, and p values of HWE

Author (year)	Distribution of *EXO1* Glu589Lys genotypes						Distribution of *EXO1* Glu589Lys alleles				HWE
	Case (n)			Control (n)			Case (n)		Control (n)		p value
	Glu/Glu	Glu/Lys	Lys/Lys	Glu/Glu	Glu/Lys	Lys/Lys	Glu	Lys	Glu	Lys	
Zienolddiny et al. (2006)	115	106	35	116	145	30	176	336	205	377	0.12
Jin et al. (2008)	304	172	24	355	138	24	220	780	186	848	0.03
Hsu et al. (2008)	214	125	19	251	97	10	163	553	117	599	0.86
Chang et al. (2008)	55	42	15	29	59	22	72	152	103	117	0.42
Wang et al. (2009)	794	421	57	898	341	57	535	2009	455	2137	0.001
Bau et al. (2009)	103	64	12	125	49	5	88	270	59	299	0.94
Tsai et al. (2009)	391	244	45	482	183	15	334	1026	213	1147	0.63
Ibarrola-Villava et al. (2011)	319	282	83	163	175	68	448	920	311	501	0.08
Luo et al. (2012)	73	48	5	196	77	5	58	194	87	469	0.41
Bayram et al. (2012)	95	94	35	99	108	17	164	284	142	306	0.09

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models increased cancer risk in the PCR-RFLP group (Table 3).

Test of heterogeneity

The heterogeneity was reckoned between each of the studies using the χ^2-based Q-test. Significant heterogeneity existed in all genetic model and allele contrast of the *EXO1* Glu589Lys polymorphism (Table 3). However, the heterogeneity decreased markedly after stratification, especially in the subgroups of lung cancer (Lys *vs* Glu: $p_{heterogeneity}$=0.05; Lys/Lys *vs* Glu/Glu: $p_{heterogeneity}$=0.36;

Lys/Lys *vs* Glu/Lys: $p_{heterogeneity}$=0.23; Lys/Lys *vs* Glu/Lys+Glu/Glu: $p_{heterogeneity}$=0.44). When patients were stratified based on ethnicity, heterogeneity disappeared in the Asian (Lys *vs* Glu: $p_{heterogeneity}$=0.07; Lys/Lys *vs* Glu/Lys: $p_{heterogeneity}$=0.07; Lys/Lys+Glu/Lys *vs* Glu/Glu: $p_{heterogeneity}$=0.42).

Sensitivity analysis

Sensitivity analysis was conducted to evaluate the stability of the meta-analysis. Sensitivity analysis was carried out after sequential removal of each eligible study. When i investigated the *EXO1* Glu589Lys polymorphism and cancer susceptibility, the results suggested that the all ORs were not influenced excessively by omitting any single study (data not shown). Hence, results of the sensitivity analysis suggested that the data of this meta-analysis are relatively stable and credible.

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias. The shape of funnel plots did not reveal any evidence of obvious asymmetry in the overall meta-analysis. Then, the Egger's test was used to provide to statistical evidence of funnel plot symmetry. No publication bias was detected for *EXO1* Glu589Lys polymorphism (allele contrast: p=0.86 for Begg's test, p=0.94 for Egger's test; homozygous model: p=0.28 for Begg's test, p=0.18 for Egger's test; heterozygous model: p=0.21 for Begg's test, p=0.08 for Egger's test; dominant
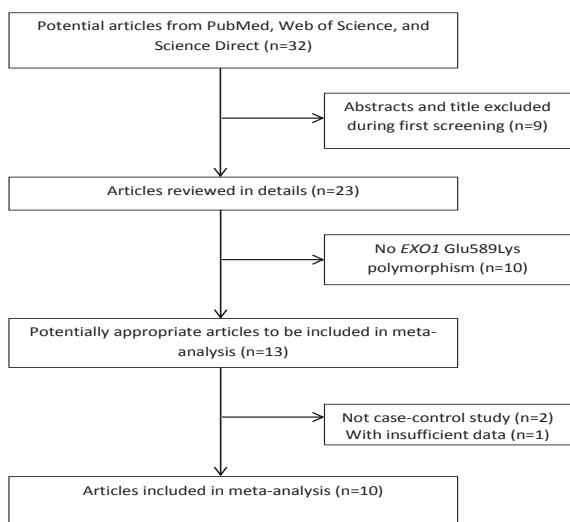

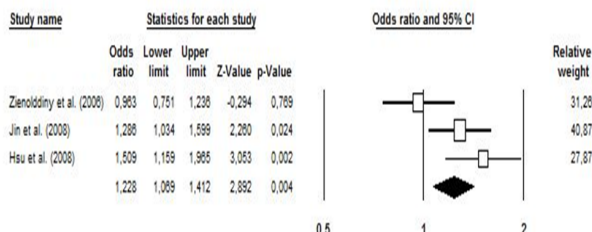**Figure 1. Flow Diagram of Inclusion/Exclusion of the Individual Articles**

Figure 2. Forest Plot of ORs with a Random Effect Model for Association *EXO1* Glu589Lys Polymorphism and Overall Lung Cancer Risk Under Allele Model (Lys *vs* Glu)
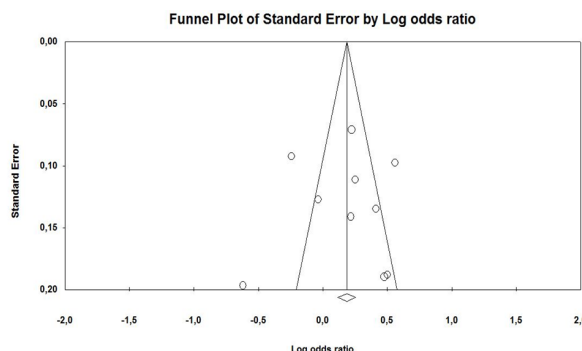

Figure 3. Funnel Plot Analysis to Detect Publication Bias for the Allele (Lys *vs* Glu) Model

Table 3. Results of Meta-Analysis for *EXO1* Glu589Lys Polymorphism and Cancer Risk

Study Groups	N	Cases/ Controls	Allele (Lys *vs* Glu) OR (95%CI)	P[a]	Homozygous (Lys/Lys *vs* Glu/Glu) OR (95%CI)	P[a]	Heterozygous (Lys/Lys *vs* Glu/Lys) OR (95%CI)	P[a]	Dominant (Lys/Lys+Glu/Lys *vs* Glu/Glu) OR (95%CI)	P[a]	Recessive (Lys/Lys *vs* Glu/Lys+Glu/Glu) OR (95%CI)	P[a]
Total	10	4391/4339	1.20 (0.98-1.46)	<0.001	1.39 (0.91-2.15)	<0.001	1.24 (0.92-1.69)	0.01	1.18 (0.93-1.50)	<0.001	1.38 (0.96-1.97)	<0.001
HWE[b]												
YES	8	2619/2526	1.18 (0.89-1.56)	<0.001	1.50 (0.83-2.73)	<0.001	1.45 (1.00-2.09)	0.02	1.12 (0.80-1.57)	<0.001	1.53 (0.94-2.50)	<0.001
NO	2	1772/1813	1.26 (1.12-1.42)*	<0.001	1.14 (0.83-1.57)	<0.001	0.81 (0.58-1.12)	<0.001	1.37 (1.20-1.58)*c	0.8	1.02 (0.75-1.40)c	0.96
Cancer type												
Lung	3	1114/1166	1.23 (1.07-1.41)*c	0.05	1.34 (0.94-1.92)c	0.36	1.23 (0.85-1.77)c	0.23	1.27 (1.07-1.51)	0.01	1.33 (0.94-1.88)c	0.44
Etnicity												
Asian	6	3115/3308	1.42 (1.30-1.55)*c	0.07	1.93 (1.20-3.12)*	0.01	1.11 (0.86-1.44)c	0.07	1.52 (1.37-1.68)*c	0.42	1.68 (1.07-2.65)*	0.02
Caucasian	4	1276/1031	0.86 (0.65-1.14)	0.003	0.88 (0.45-1.70)	0.001	1.26 (0.73-2.17)	0.01	0.75 (0.54-1.03)	0.02	1.07 (0.60-1.90)	0.002
Study Design												
HB	8	4023/3938	1.33 (1.09-1.63)*	<0.001	1.67 (1.03-2.71)*	<0.001	1.25 (0.86-1.81)	0.007	1.37 (1.11-1.68)*	<0.001	1.53 (0.99-2.36)	<0.001
PB	2	368/401	0.73 (0.42-1.30)	0.01	0.67 (0.21-2.15)	0.02	1.34 (0.86-2.10)c	0.3	0.57 (0.26-1.22)	0.02	1.96 (1.24-3.08)*	0.03
Genotyping												
PCR-RFLP	6	2839/3015	1.42 (1.30-1.56)*c	0.08	2.17 (1.36-3.45)*	0.02	1.56 (0.98-2.49)	0.03	1.49 (1.34-1.66)*c	0.17	1.96 (1.24-3.08)*	0.03
TaqMan	2	940/697	0.84 (0.73-0.98)*c	0.19	0.76 (0.59-1.04)c	0.06	1.07 (0.52-2.21)	0.03	0.78 (0.64-0.96)*c	0.8	0.95 (0.48-1.87)	0.03
Microarray	2	612/627	0.84 (0.36-1.98)	<0.001	0.67 (0.21-2.12)	0.02	0.85 (0.53-1.38)c	0.72	0.74 (0.20-2.74)	<0.001	0.85 (0.54-1.33)c	0.27

*OR with statistical significance; [a]p value of X^2-based Q test for heterogeneity; [b]Conforming to Hardy-Weinberg equilibrium in controls; [c]OR estimates for fixed effects model; N: number of studies included; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; PB: population-based; HB: hospital-based.

model: p=0.60 for Begg's test, p=0.37 for Egger's test; recessive model: p=0.21 for Begg's test, p=0.08 for Egger's test) (Figure 3).

Discussion

Cancer has become one of the major public health problems in the world. However, cancer is a multifactorial disease and the precise etiology is still not exactly understood. SNP is the most common form of human genetic variation, and may contribute to susceptibility to cancer. It is therefore proper to investigate gene polymorphisms involved in human cancers. Many molecular epidemiological studies have been performed to evaluate the association between *EXO1* Glu589Lys polymorphism and cancer risk. However, the results were generally inconsistent (Zienolddiny et al., 2006; Jin et al., 2008; Hsu et al., 2008; Chang et al., 2008; Bau et al., 2009; Tsai et al., 2009; Wang et al., 2009; Ibarrola-Villava et al., 2011; Luo et al., 2012; Bayram et al., 2012). These inconsistent results are possibly due to a small effect of the *EXO1* Glu589Lys polymorphism on cancer risk or the relatively low statistical power of the published studies. So, this meta-analysis was needed to show a quantitative approach for combining the different results. Meta-analysis is a statistic method with great statistical power and has been widely performed to epidemiological research, especially for evaluating genetic polymorphisms in cancer susceptibility. It is superior to single study potentially via augmenting sample size, improving statistical power, and subsequently drawing a more reliable conclusion (Qin et al., 2013).

To the best of my knowledge, this is the first meta-analysis of the association between the *EXO1* Glu589Lys polymorphism and cancer susceptibility. The present meta-analysis is based on 10 case-control studies including 4391 cancer cases and 4339. In the current meta-analysis, the *EXO1* Glu589Lys polymorphism was not associated with a significantly increased cancer risk in all genetic models. However, significant association was observed in lung cancer subgroup. The discrepant results may be explained by the concept that different types of cancer may have different mechanisms of carcinogenesis. The discrepancy could also be interpreted partially by the influence of gene-environment interaction in multistep process of carcinogenesis. Another reason may be the limited sample size. Furthermore, in the subgroup analysis by ethnicity, significant association between the *EXO1* Glu589Lys polymorphism and cancer risk was observed in Asians. However, significant association was absent in Caucasians. In my meta-analysis, i also observed inconsistent results between hospital-based studies and population-based studies. Stratified analysis by the study design indicated that studies recruiting controls form hospital population are more included to acquire significant results in allele contrast, homozygous dominant and recessive genetic models. Different cancer risks were also found in the studies using different genotyping methods. I discovered that the association was significant among studies utilizing PCR-RFLP assay, but not for studies with TaqMan and microarray genotyping assays.

Because TaqMan and microarray genotyping assays are more precise than the PCR-RFLP assay, and a limited number of studies were included in the TaqMan and microarray genotyping assays, this results might reflect selection bias, and should be interpreted with caution.

Attention must be paid to the relatively large heterogeneity in my results. However, when stratified by cancer type, the subgroup of lung cancer failed to exhibit heterogeneity, suggesting that different cancer type might be a potential source of heterogeneity. Similarly, after stratifying by ethnicity, heterogeneity was absent in Asian population, suggesting that ethnicity could partly explain the heterogeneity. As these, when stratified by study design, my results showed that the heterogeneity was significantly reduced in subgroup of population-based study design. Therefore, it may be presumed that the heterogeneity exists mainly owing to differences of cancer types, ethnicity and study design.

Some limitations of this meta-analysis should be considered in interpreting the results. First, the number of some published studies was not sufficiently large, and some studies of small size may not have enough statistical power to explore the real association. Additionally, in the some subgroup analyses, the number of cases and controls was relatively small, where there was not enough statistical power to explore the true association. Second, my results were based on unadjusted estimates. In order to provide a more precise estimation on the basis of adjustment for confounders, well-designed studies are warranted by taking potential confounders such as smoking status, drinking status and environmental factors into account. Third, interactions of gene-gene or SNP-SNP or the possibility of linkage disequilibrium between polymorphisms or gene-environment that might have influence on gene-disease association were failed to address due to lack of relevant data. Fourth, because only published and English articles were included in the meta-analysis, publication and potential English language biases might have occurred, even though it was not determined by the use of statistical tests.

In conclusion, my meta-analysis suggested that *EXO1* Glu589Lys polymorphism is not associated with an increased cancer risk. Further stratification by cancer type, study design and genotyping method also identified a significant association of this polymorphism with cancer risk, especially in lung cancer, Asian population, hospital-based study design and the PCR-RFLP genotyping method groups. In the future, large-scale case-control studies are necessary to validate the risk and to investigate the potential gene-gene, and gene-environment interactions between *EXO1* Glu589Lys polymorphism and cancer susceptibility.

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