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ORIGINAL ARTICLE

Association between HLA-G 14-bp insertion/deletion polymorphism and cancer risk: a meta-analysis

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Summary

Purpose: *A number of studies have investigated the association between human leukocyte antigen-G (HLA-G) 14-bp insertion/deletion polymorphism and cancer risk, but the results remain controversial. In this study we aimed to clarify whether this association really exists.*

Methods: *We carried out a meta-analysis of 8 studies including 1179 cases and 2795 controls from PubMed and Chinese language (CNKI and WanFang) databases to assess the association between the HLA-G 14-bp insertion/deletion polymorphism and cancer risk by pooled odds ratio (OR) and 95% confidence interval (CI).*

Results: *The results showed that the HLA-G 14-bp insertion/deletion polymorphism was not associated with total cancer risk in all genetic models (dominant model: OR=0.90, 95% CI=0.70-1.17; recessive model: OR=0.97, 95% CI=0.67-1.42; insertion/deletion (ID) vs deletion/deletion (DD): OR=0.88, 95% CI=0.66-1.18; insertion/insertion (II) vs DD: OR=0.94, 95% CI=0.62-1.41; insertion (I) vs deletion (D): OR=0.95, 95% CI=0.78-1.16). In the subgroup analysis by ethnicity, no significant association was found between Asians and Caucasians. However, subgroup analysis by cancer type showed that the polymorphism was associated with risk of hepatocellular carcinoma.*

Conclusions: *This meta-analysis suggests that HLA-G 14-bp insertion/deletion polymorphism may not influence the susceptibility of total cancer, but it is related to risk of hepatocellular carcinoma.*

Key words: *cancer risk, HLA-G, meta-analysis, polymorphism*

Introduction

HLA-G is a non-classical MHC class I molecule expressed as 7 isoforms, including 4 membrane-bound (HLA-G1 to HLA-G4) and 3 soluble (HLA-G5 to HLA-G7) forms [1]. HLA-G gene expression is undetectable in almost all healthy cells but becomes detectable in tumor cells [2]. HLA-G exerts inhibitory activity against natural killer (NK) cells, T lymphocytes and antigen-presenting (APCs) cells by binding to inhibitory receptors denoted immunoglobulin-like transcript (ILT)-2, ILT-4 and killer immunoglobulin-like (KIR)2DL4 [1]. Hence, it is attractive to assume that HLA-G expression favors tumor progression by providing tumor cells with a mechanism to evade from surveillance by the host immune system.

Polymorphisms of the HLA-G gene have been shown to influence its expression level, mRNA stability, and the concentration of soluble HLA-G [3,4]. A 14-bp insertion/deletion (Ins/Del) polymorphism in the 3'UTR regions of HLA-G is a functional, extensively studied polymorphism [5]. Several studies have demonstrated the HLA-G 14-bp Ins/Del polymorphism to be associated with risk of human cancers such as cervical cancer, hepatocellular carcinoma, and others [6-10]. However, in other studies, the HLA-G 14-bp insertion/deletion polymorphism was not observed to be associated with cancer risk [11,12]. Given the lack of statistical power of previous studies, we conducted a meta-analysis based on current published evidence to ascertain the relationship between HLA-G 14-bp Ins/Del polymorphism and cancer risk.

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Methods

Search strategy

We searched the following databases to identify pertinent studies that examined the association between HLA-G 14-bp Ins/Del polymorphism and cancer risk: PubMed, Chinese National Knowledge Infrastructure and Chinese WanFang database. All relevant reports were published before November 2013. The search strategy was based on a combination of "Human leukocyte antigen G or HLA-G" and "polymorphism or variant or mutation" and "cancer or carcinoma or neoplasm". In addition, references of retrieved articles were also screened.

Inclusion criteria

In our meta-analysis, the following inclusion criteria were used for study selection:(1) studies that examined the association between cancer incidence and HLA-G 14-bp Ins/Del polymorphism; (2) study designs consisted of either case-control studies or cohort studies; and (3) results were expressed as usable data [e.g., odds ratio (OR), 95% CI or standard deviation, raw data on number of each genotypes]. Duplicated previous research was excluded.

Data extraction

Two independent investigators performed the data extraction. Any disagreement was resolved by discussion. Information included the first author's name, year of publication, ethnicity, cancer type and number of cases and controls. We also recalculated Ins allele frequency and deviation from the Hardy-Weinberg Equilibrium (HWE) in the control group based on the raw genotyping data presented.

Statistics

The meta-analysis was performed using STATA version 12.0. OR with 95% CI assessed the strength of association between HLA-G 14-bp Ins/Del polymorphism and cancer risk. The pooled OR with 95% CI were calculated in a co-dominant model (Ins/Ins vs Del/Del, Ins/Del vs Del/Del), a recessive model (Ins/Ins vs Ins/Del + Del/Del), an additive model (Ins vs Del) and a dominant model ((Ins/Ins + Ins/Del vs Del/Del). The goodness-of-fit x^2 test evaluated HWE for control subjects in each study, and p-values for HWE < 0.05 were considered to be deviated from the HWE. Cochran's x^2-based Q-statistic test was applied to assess between-study heterogeneity. The inter-study heterogeneity in terms of degree of association was tested using the Cochran's Q-statistics [13]. If p <0.05, the heterogeneity was considered significant, which was further explored by using I^2 statistics. I^2 is expressed as the percentage of between-study variability that is attributable to genuine variation rather than sample error [14]. If there was heterogeneity among studies, we used a random-effect (RE) model to pool the OR; otherwise, a fixed-effect (FE) model was selected [15].

Sensitivity analysis was conducted by excluding each study, one at a time, and recalculating the OR and 95% CI to assess the effects of each study on the pooled risk of cancer. Then, Funnel plot was performed to assess the publication bias of the literature.

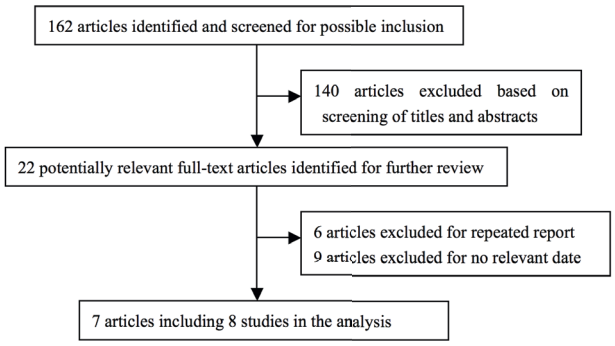

Figure 1. The detailed process of identifying eligible studies.

Results

Characteristics of the included studies

A total of 162 publications from PubMed, CNKI and WanFang databases were reviewed using the predefined specified key words. After a review of titles and abstracts, 8 studies with 1179 cases and 2795 controls were included in this meta-analysis. A flow chart of the study selection procedure is shown in Figure 1. The main characteristics of the included studies are listed in Table 1. The respective studies focused on the following cancer types: 2 cervical cancer [6,8], 2 hepatocellular carcinoma [7,9], 2 esophageal cancer [10], 1 neuroblastoma [11] and 1 papillary thyroid carcinoma [12]. The genotypes distribution in the controls was in agreement with HWE test in all included studies.

Association between the HLA-G 14-bp polymorphism and cancer risk

Results of the meta-analysis are shown in Table 2. Q test showed significant heterogeneity in all of the models. Therefore, RE model was utilized to generate a larger pool of studies with 95% CI. Figure 2 shows that no difference was observed in all genetic models (dominant model: OR=0.90, 95% CI=0.70-1.17; recessive model: OR=0.97, 95% CI=0.67-1.42; ID vs DD: OR=0.88, 95% CI=0.66-1.18; II vs DD: OR=0.94, 95% CI=0.62-1.41; I vs D: OR=0.95, 95% CI=0.78-1.16). However, subgroup

Table 1. Main characteristics of studies involved in this meta-analysis

First author[Ref]	*Year*	*Ethnicity*	*Cancer type*	*Cases/controls*	*Ins allele[a]*	*HWE[b]*
Silva [8]	2012	Caucasian	CC	33/50	0.43	0.11
Dardano [12]	2012	Caucasian	PTC	183/245	0.43	0.15
Ferguson [6]	2011	Caucasian	CC	144/833	0.43	0.43
Jiang [7]	2011	Asian	HCC	318/599	0.29	0.47
Lau [11]	2011	Caucasian	NBL	153/404	0.40	0.94
Teixeira [9]	2013	Caucasian	HCC	109/202	0.44	0.06
Chen [10]	2012	Asian	EC	132/251	0.44	0.56
Chen [10]	2012	Asian	EC	107/211	0.37	0.12

CC: cervical cancer, PTC: papillary thyroid carcinoma, HCC: hepatocellular carcinoma, NBL: neuroblastoma, EC: esophageal cancer. [a]The frequency of the Ins allele of the HLA-G 14-bp polymorphism in controls; [b]Hardy-Weinberg Equilibrium in controls

Table 2. Meta-analysis of the associations between the HLA-G 14-bp polymorphism and cancer risk in all genetic models

Variables	*Dominant model*		*Recessive model*		*II vs DD*		*ID vs DD*		*I vs D*	
	OR (95 % CI)	P_{het}	*OR (95 % CI)*	P_{het}	*OR (95 % CI)*	P_{het}	*OR (95 % CI)*	P_{het}	*OR (95 % CI)*	P_{het}
Total	0.90 (0.70-1.17)	<0.05	0.97 (0.67-1.42)	<0.05	0.94 (0.62-1.41)	<0.05	0.88 (0.66-1.18)	<0.05	0.95 (0.78-1.16)	<0.05
Cancer type										
CC	1.33 (0.39-4.49)	<0.05	1.68 (0.99-2.86)	0.25	1.47 (0.97-2.22)	0.56	1.20 (0.21-6.86)	<0.05	1.19 (0.95-1.51)	0.44
HCC	0.70 (0.55-0.89)	0.71	0.60 (0.40-0.91)	0.98	0.53 (0.34-0.81)	0.89	0.75 (0.59-0.97)	0.86	0.73 (0.60-0.88)	0.70
EC	0.93 (0.44-1.97)	<0.05	0.84 (0.24-2.91)	<0.05	0.82 (0.17-3.93)	<0.05	0.88 (0.66-1.18)	0.12	0.93 (0.48-1.79)	<0.05
Other cancer	1.05 (0.53-2.10)	<0.05	1.15 (0.82-1.61)	0.65	1.18 (0.81-1.72)	0.39	1.01 (0.44-2.34)	<0.05	1.06 (0.81-1.39)	0.16
Ethnicity										
Asian	0.83 (0.56-1.24)	<0.05	0.75 (0.37-1.53)	<0.05	0.71 (0.30-1.71)	<0.05	0.85 (0.63-1.15)	0.20	0.85 (0.59-1.23)	<0.05
Caucasian	0.97 (0.66-1.43)	<0.05	1.15 (0.77-1.72)	<0.05	1.11 (0.75-1.63)	0.09	0.93 (0.56-1.53)	<0.05	1.03 (0.82-1.28)	<0.05

P_{het}: *P* value for heterogeneity. CC: cervical cancer, HCC: hepatocellular carcinoma, EC: esophageal cancer

analysis by cancer type showed that the polymorphism was associated with susceptibility of hepatocellular carcinoma. In the subgroup analysis by ethnicity, no significant association was found between Asians and Caucasians.

Tests for sensitivity and publication bias

No single study was found to change the pooled OR qualitatively by sensitivity analysis, indicating that this meta-analysis is stable. Funnel plot was performed to assess the publication bias of the literature. Symmetrical funnel plots were obtained in the polymorphism tested in all of the models (Figure 3).

Discussion

Characterization and identification of genes involved in the genetic predisposition or progression of cancer are critical for both clinical practice and basic research.

HLA-G is a non-classical class I major histocompatibility complex molecule. HLA-G has been suggested as a potential candidate biomarker for influencing the etiology and clinical outcome of several diseases including cancer because of the immunosuppressive properties of the HLA-G protein [16]. The 14-bp ins/del polymorphism of HLA-G was first reported in 1993 [17] and was associated with the stability and splicing pattern of HLA-G mRNA, which could affect HLA-G protein expression [4,18].

To the best of our knowledge till now, this was the first meta-analysis providing comprehensive insights into the association of HLA-G 14-bp Ins/Del polymorphism with risk of cancer. The current meta-analysis explored the role of HLA-G 14-bp Ins/Del polymorphism in the susceptibility of cancer among 7 articles including 8 studies with 1179 cancer cases and 2795 non-cancer controls. Our results showed that there was no association

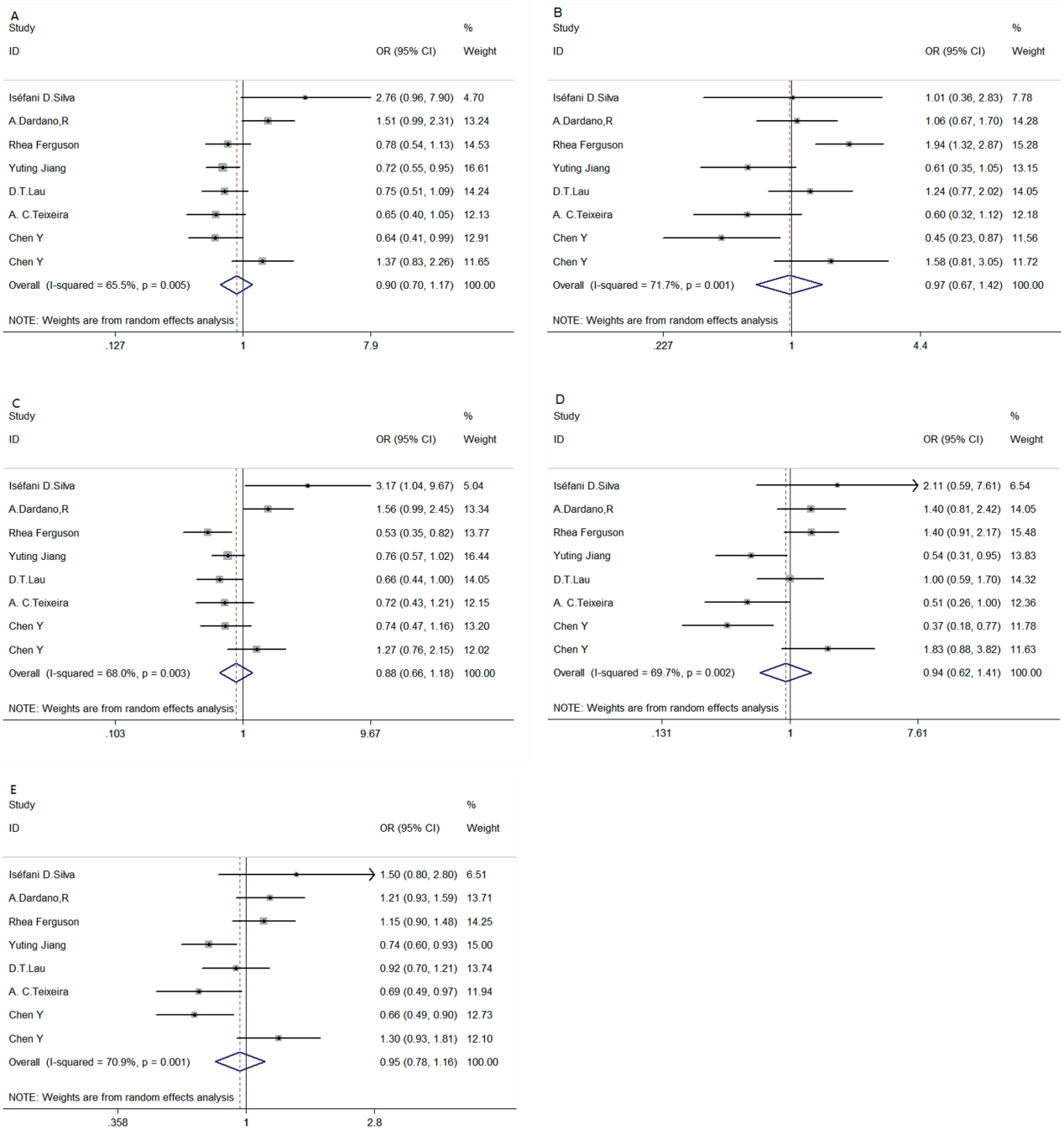

Figure 2. Forest plot of cancer and HLA-G 14-bp polymorphism. The size of the black square corresponding to each study is proportional to the sample size, and the center of each square represents of the OR. Horizontal line shows the corresponding 95% CI of the OR. Pooled OR is represented by diamonds and was obtained using random effect model (A: dominant model; B: recessive model; C: ID vs DD; D: II vs DD; E: I vs D). For abbreviations see text.

between the HLA-G 14-bp polymorphism and cancer risk in all of the models. In the subgroup analysis by ethnicity, no significant association was observed between Asians and Caucasians. However, subgroup analysis by cancer type showed that the polymorphism was associated with risk of hepatocellular carcinoma. This meta-analysis' results did not support the genetic association between HLA-G 14-bp Ins/Del polymorphism and susceptibility to total cancer, but the polymorphism may be associated with risk of hepatocellular carcinoma.

We found heterogeneity for the polymorphism among these studies that may result from various factors, such as differences in the number of cases and controls, genotyping method and

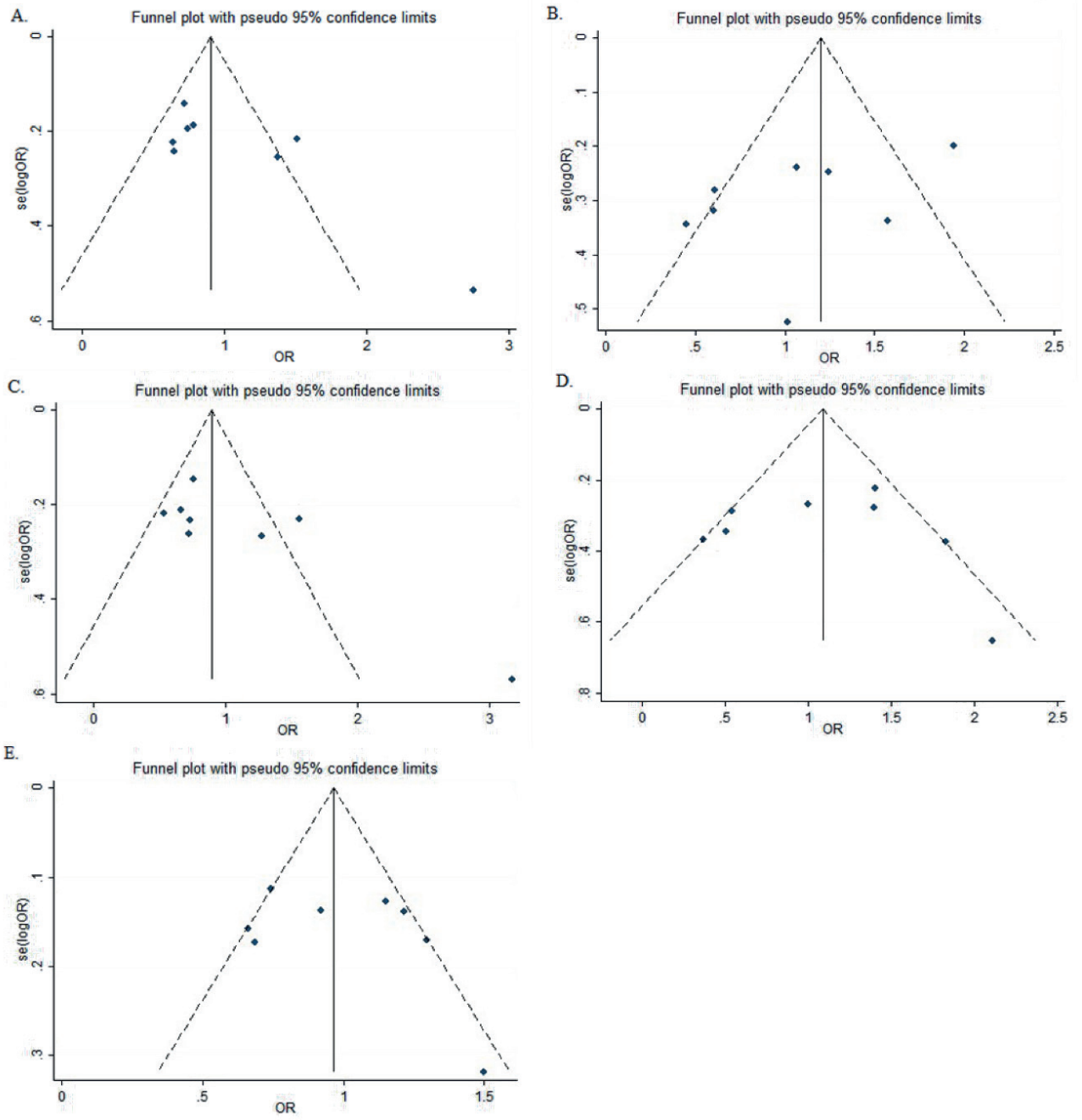

Figure 3. Funnel plot analysis to examine publication bias (A: dominant model; B: recessive model; C: ID vs DD; D: II vs DD; E: I vs D). For abbreviations see text.

study design. In addition, limitations encountered in this meta-analysis should be considered as these results are interpreted. First, the number of the included studies was not large enough. So the analysis precluded the ability to identify other confounding factors by the subgroup analysis. Second, lacking detailed analysis about sex, age, smoking, drinking and so on, these potential factors might influence our results. Third, because only published studies were included in our meta-analysis, some unpublished data had to be ignored in the analysis. Therefore, potential publication bias may be existed in our results, although the statistical data did not reflect it.

In summary, our findings suggested that the HLA-G 14-bp Ins/Del polymorphism could not influence the risk of cancer. However, some results of this present meta-analysis are limited by the small number of studies; thus, additional larger well-designed studies are required.

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