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HLA-G +3142 C>G polymorphism and cancer risk

Evidence from a meta-analysis and trial sequential analysis

You Jiang, MM[a], Wenbo Li, MM[a], Jun Lu, MM[a], Xin Zhao, MM[b], Liang Li, MM[a,*]

Abstract

Background: Human leukocyte antigen-G (HLA-G) plays an important role in the development of human cancers. Several published studies have investigated the relationship between the *HLA-G* +3142 C>G (rs1063320) polymorphism and cancer susceptibility in different populations. However, the results have yet to reach a consensus in different types of cancers. Therefore, we performed a meta-analysis to evaluate the effect of the *HLA-G* +3142 C>G polymorphism on cancer risk.

Methods: A systematic literature search was performed in PubMed, Web of Science, CNKI, VIP, and Wanfang databases to acquire eligible studies up to February 20, 2019. The pooled odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were used to assess the correlation between the *HLA-G* +3142 C>G polymorphism and cancer risk in a fixed-effects or random-effects model. Publication bias assessments, sensitivity analysis and stratified analyses were performed. To reduce the risk of type I error and assess whether the present evidence of the results was adequate and conclusive, trial sequential analysis (TSA) was also performed.

Results: Eight case-control studies comprising 1546 cases and 1595 controls were included in the present meta-analysis. The results revealed that the *HLA-G* +3142 C>G mutation significantly decreased the total cancer risk in recessive comparison model and allelic comparison model. Further stratified analyses showed that the *HLA-G* +3142 C>G mutation significantly decreases the risk of cancer in Asian populations. No similar relationship was found in other subgroups. No publication bias was identified in our present study. Omitting a single study at a time had no significant impact on the pooled OR of the sensitivity analysis assessing the association between the *HLA-G* +3142 C>G polymorphism and cancer risk, which demonstrates the stability of the current meta-analysis. TSA also identified our current findings.

Conclusions: The results of our meta-analysis show that the *HLA-G* +3142 C>G polymorphism plays a protect role in the occurrence of human cancers, particularly in Asian populations. More case-control studies with different types of cancer in various ethnicities are needed to verify the findings.

Abbreviations: CIs = confidence intervals, CNKI = Chinese National Knowledge Infrastructure, CRC = colorectal cancer, HB = hospital-based, HCC = hepatocellular carcinoma, HLA-G = human leukocyte antigen-G, HNSCC = head and neck squamous cell carcinoma, HWE = Hardy–Weinberg Equilibrium, NOS = Newcastle-Ottawa Scale, ORs = odds ratios, PB = population-based, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, SNPs = single nucleotide polymorphisms, TSA = trial sequential analysis.

Keywords: cancer, human leukocyte antigen-G, meta-analysis, polymorphism

1. Introduction

The incidence and mortality of cancer are increasing worldwide, and cancer has been a major human health problem that creates a large economic burden in both developed and undeveloped countries. According to reported statistics, there were approximately 1,688,780 new cancer diagnoses, and 600,920 cases resulting in mortality due to malignant tumors in the United States in the year of 2017.[1] In 2015, there were nearly 4,292,000 new cancer diagnoses and 2,814,000 cancer-related deaths in China.[2] Although the underlying mechanism of carcinogenesis is not completely deciphered, a number of studies have demonstrated that the occurrence of cancer is a complicated process, which includes various environmental factors and genetic susceptibilities.[3] Accumulating evidence has shown that individual genetic susceptibility plays a significant role in the occurrence of a tumor. Moreover, the relationship between polymorphisms and cancer risk has been confirmed for many genes.[4,5] Several lines of evidence have indicated that the progression of a tumor could be related to immunoevasion. Human leukocyte antigen (HLA) may play a critical role in the development and progression of cancer by mediating immune responses.[6]

HLA-G, a non-classical HLA class I molecule, is known for its suppressive function and has 7 different isoforms. Of the 7 isoforms, 4 have membrane-bound forms (HLA-G1 to HLA-G4)

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and 3 have soluble forms (HLA-G5, HLA-G6, and HLA-G7).[7] Differing from the classic HLA class I molecules, HLA-G is characterized by its restricted tissue distribution, low rate of polymorphism, and immunosuppressive properties.[8] The aberrant expression of HLA-G has been considered a mechanism in a wide variety of tumors that helps the tumor cells escape immunosurveillance.[9] HLA-G has been shown to act as a negative regulator of the human immune response by several mechanisms, including the inhibition of the cytotoxic effects of T lymphocytes and natural killer (NK) cells, as well as the prevention of antigen recognition and anti-proliferative responses of CD4+ T cells.[10] Accumulating evidence has shown that HLA-G is highly expressed in a variety of tumor tissues, including breast cancer,[11] cervical cancer,[12] hepatocellular carcinoma (HCC),[13] esophageal carcinoma(EC),[14] thyroid carcinoma,[15] lung cancer,[14] gastric cancer,[14] colorectal cancer(CRC),[14] and renal cell carcinoma.[16] These studies show that HLA-G may play a pivotal role in the occurrence and progression of malignant tumors.

The human *HLA-G* gene, comprised of 8 exons and 7 introns, is located on chromosome 6p21.3. Several published studies have indicated that some polymorphisms of the *HLA-G* gene are related to cancer development.[17] The 14 bp ins/del polymorphism in exon 8 of the 3'UTR of *HLA-G* is the most widely studied. The association between +3142 C>G (also located at the 3′UTR) and cancer risk has been investigated in several studies.[18–25] However, the results of the published articles varied across studies or even were controversial. A single case-control study may not have enough statistical power to evaluate a possible small impact of the polymorphism on cancer, particularly when the study has a relatively small sample size. As far as we know, there is no meta-analysis to evaluate the relationship between *HLA-G* +3142 C>G variant and cancer risk. Therefore, we performed this meta-analysis to explore the precise association of the *HLA-G* +3142 C>G polymorphism with cancer susceptibility.

2. Materials and methods

Ethical approval was not necessary for the present meta-analysis. The review protocol of this study was not preregistered.

2.1. Search strategy

A systematic literature search with no language limitation was performed in PubMed, Web of Science, CNKI, VIP, and Wanfang databases to acquire all eligible studies up to February 20, 2019. The relevant search keywords included: (HLA-G OR 'Human leukocyte antigen-G') AND (mutation OR polymorphism OR genotype OR variation) AND (carcinoma OR cancer OR malignancy OR adenocarcinoma OR neoplasm OR neoplasia OR tumor OR tumor). In addition, other relevant articles were obtained by searching the reference lists of the selected reviews and studies.

2.2. Inclusion and exclusion criteria

Published articles fulfilling the following criteria were included:

1. articles published in English or Chinese;
2. studies evaluated the correlation between the *HLA-G* +3142 C>G polymorphism and cancer risk;
3. designed as case-control or cohort studies; and
4. contained genotype distribution data for estimating genotype distribution or the overall ORs and 95% CIs.

Exclusion reasons were as following:

1. case reports, not case-control studies, letters, comment articles, reviews or meta-analysis;
2. lacked sufficient data; and
3. duplicated publications or samples.

2.3. Data extraction

Two reviewers (You Jiang and Wen-Bo Li) independently extracted data from the eligible studies based on the inclusion criteria above. Data extracted from all of the eligible studies included the following information: the first author, publication year, country, study population ethnicity, cancer type, sources of controls, genotyping method, number of cases and controls for the +3142 C>G genotypes of *HLA-G*, and results of the Hardy-Weinberg equilibrium (HWE) test in controls. In cases of inconsistent evaluations, all reviewers were consulted to resolve the disagreement to obtain a consensus.

2.4. Methodological quality assessment

According to the Newcastle-Ottawa Scale (NOS), the quality of the included studies was appraised by 2 investigators independently. The score of each study was calculated based on 3 items including selection, comparability, and exposure (maximum score = 9 points). The score of included studies must be higher than 5 (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp).[26] Any discrepancies were settled by all reviewers through discussion.

2.5. Bioinformatics analysis

TargetScan database (http://www.targetscan.org/vert_72/) was used to study the *HLA-G* +3142 C>G polymorphism effect on miRNA binding of 3'UTR of *HLA-G* transcripts. TCGA database (http://cancergenome.nih.gov/) provides researchers with extraordinary amounts of molecular data with cancer information. The cBioPortal (online tool, www.cbioportal.org, based on TCGA database) was used to explore and confirm the correlation of *HLA-G* gene with cancers.

2.6. Statistical analysis

We conducted this meta-analysis based on the checklists and guidelines based on PRISMA.[27] The HWE was evaluated for each study in the control groups using a Chi-square test and every study with a P was less than .05 was considered a significant disequilibrium. ORs with 95% CIs were adopted to assess the strength of the relationship between the *HLA-G* +3142 C>G mutation and cancer risk in the homozygote comparisons (GG vs CC), heterozygote comparisons (CG vs CC), dominant model (GG + CG vs CC), recessive model (GG vs CG + CC), and allelic comparisons (G vs C). Stratified analyses were carried out based on ethnicity (Asian, Caucasian, and Mixed population), type of cancer (publication with only one case-control study was merged as the "other cancers"), and source of controls (hospital-based and population-based). Differences based on a Z test were regarded as statistically significant if the $P < .05$. The heteroge-

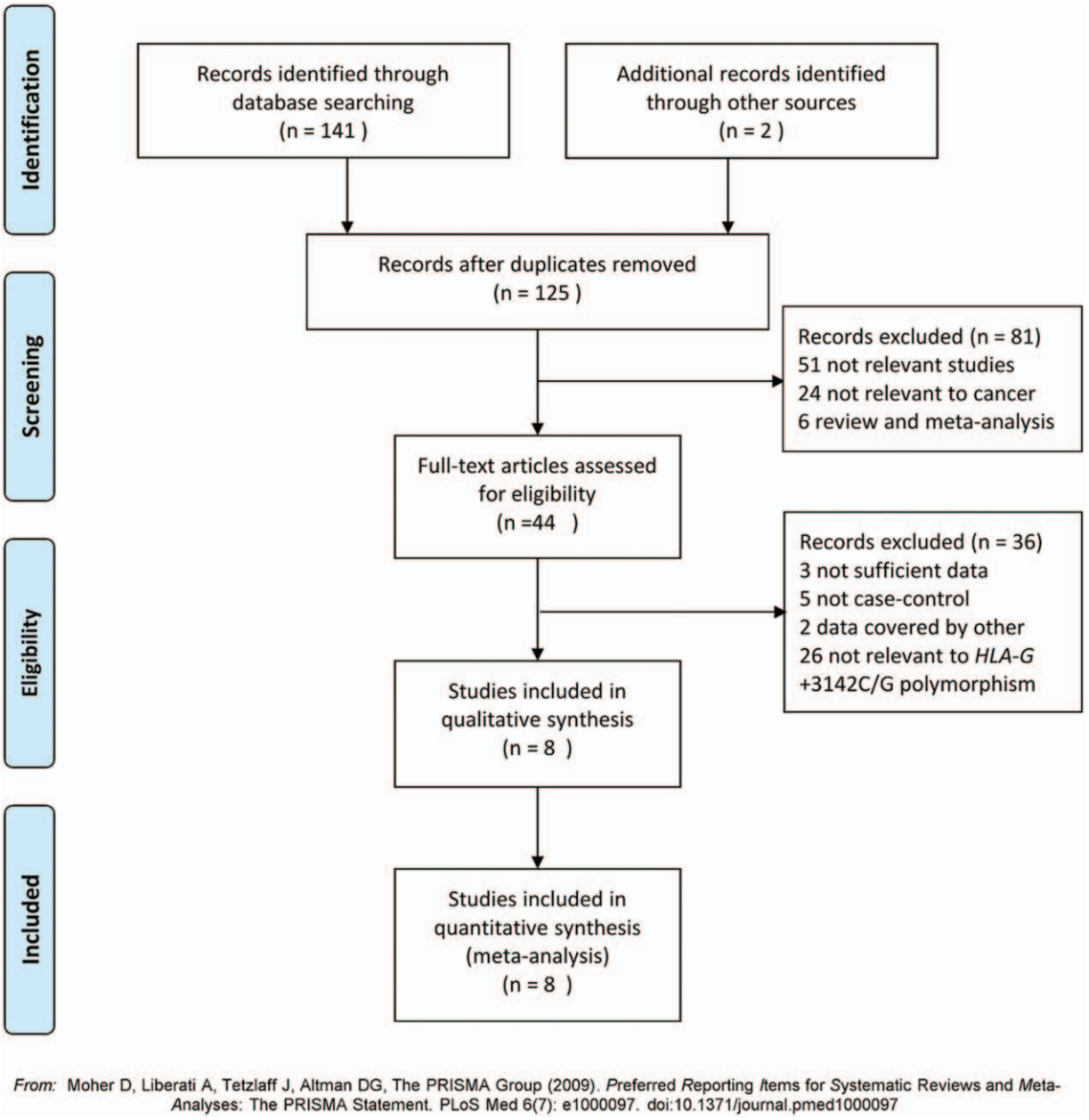

Figure 1. The flow diagram of the included and excluded studies.

neity among each study was measured by Cochran's Q statistic and the I^2 test.[28] A random-effects model was applied to measure the pooled OR when the I^2 value $> 50\%$. Otherwise, a fixed-effects model was adopted according to the heterogeneity.[29] Sensitivity analysis was performed to assess the effect of each study on the pooled OR by removing each publication one by one to examine the stability of the overall results. Begg funnel plot test and Egger test were applied to assess the potential publication bias.[30,31] TSA was described before.[32] All statistical analyses were conducted by STATA 12.0 soft-ware (version 12.0; STATA Corp. College Station, TX). All the tests were 2-sided, and a P value $< .05$ was accepted as statistically significant.

3. Results

3.1. Characteristics of eligible studies

Figure 1 demonstrates the flow chart of the study selection process. After a systematic literature search in the databases mentioned above and a manual search in other sources, a total of 143 candidate articles were acquired. Eighteen records were excluded after duplicates. The 81 articles were removed after examining the titles and abstracts of the remaining 125 articles, and 44 articles were left. Among the 81 excluded studies, 51 were obviously irrelevant studies, 24 were not relevant to cancer, and 6 were reviews or meta-analyses. After carefully reviewing the full text of the 44 potential studies, 36 of them were deleted as the

Table 1

Characteristics of eligible case-control studies included in this meta-analysis.

First author	Year	Country	Ethnicity	Cancer Type	Source of controls	Genotyping method	Number (case/control)	HWE	NOS score
Silva et al[18]	2013	Brazil	Mixed	Cervical cancer	HB	PCR	55/50	Yes	7
Zidi et al[19]	2016	Tunisia	Caucasian	Breast cancer	PB	PCR-RFLP	104/83	Yes	8
Yang et al[20]	2014	Taiwan	Asian	Cervical cancer	HB	TaqMan	315/400	Yes	7
Zambra et al[21]	2016	Brazil	Mixed	Prostate cancer	HB	PCR	187/129	Yes	7
Agnihotri et al[22]	2017	India	Asian	HNSCC	PB	PCR-RFLP	383/383	Yes	8
Garziera et al[23]	2016	Italy	Caucasian	CRC	PB	PCR	308/294	Yes	8
Bortolotti et al[24]	2014	Italy	Caucasian	Cervical cancer	HB	PCR	100/100	Yes	7
Figueiredo-Feitosa et al[25]	2017	Brazil	Mixed	Thyroid cancer	PB	PCR	94/156	Yes	8

CRC = colorectal cancer, HB = hospital-based, HNSCC = head and neck squamous cell carcinoma, PB = population-based, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.

following reasons: 3 did not have sufficient data, 5 were not case-control studies, 2 data were covered by other studies, and 26 were not relevant to the *HLA-G* +3142 C>G mutation. Finally, 8 eligible studies were obtained according to the inclusion and exclusion criteria,[18–25] and 1546 cases and 1595 controls were included in the current meta-analysis. The characteristics of the included case-control studies are displayed in Table 1. All studies were published between 2013 and 2017, and all studies were written in English. Among all 8 studies, 2 studies were conducted in Asian populations, 3 in Caucasian populations, and 3 in mixed populations. There were 6 different types of tumors in our study including: breast cancer (n = 1), cervical cancer (n = 3), thyroid cancer (n = 1), prostate cancer (n = 1), CRC (n = 1), and head and neck squamous cell carcinoma (HNSCC) (n = 1). There were 4 population-based studies and 4 hospital-based studies. Five included studies used polymerase chain reaction (PCR) for the genotyping methods; 2 used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP); only 1 used TaqMan. The genotype distributions of controls in all eligible studies did not deviate from the HWE. The distribution of genotypes and allele frequencies of the *HLA-G* +3142 C>G polymorphism in the cases and controls are provided in Table 2. Supplemental Table 1, http://links.lww.com/MD/D39 demonstrated that the included studies were reliable based on methodological quality.

3.2. Meta-analysis results

The relationship between the *HLA-G* +3142 C>G polymorphism and cancer risk was assessed. The results revealed that the *HLA-G* +3142 C>G polymorphism was significantly associated with a decreased cancer risk in the recessive comparison (GG vs CG + CC: OR = 0.71, CI = 0.51–0.99; $P = .041$, Fig. 2, Table 3) and allelic comparison (G vs C: OR = 0.77, CI = 0.60–0.98; $P = .033$, Fig. 3, Table 3). However, no significant association with cancer risk was found in other models: GG vs CC: OR = 0.60, CI = 0.36–1.02; $P = .06$; CG vs CC: OR = 0.82, CI = 0.53–1.25; $P = .34$; and GG + CG vs CC: OR = 0.74, CI = 0.49–1.12; $P = .157$ (Table 3). The random-effects model was used due to the significant heterogeneity of the included studies.

In the stratified analyses shown in Table 3, we explored the association between the *HLA-G* +3142 C>G variation and cancer risk in different ethnicities. The results showed a decreased cancer risk in Asian populations based on all genetic models except for the recessive model (GG vs CC: OR = 0.49, CI = 0.37–0.65, $P = .000$; CG vs CC: OR = 0.45, CI = 0.34–0.60, $P = .000$; GG + CG vs CC: OR = 0.47, CI = 0.37–0.61, $P = .000$; and G vs C: OR = 0.70, CI = 0.61–0.82, $P = .000$). In a stratified analysis based on the cancer types, we found that the *HLA-G* +3142 C>G polymorphism was not associated with cervical cancer risk in any genetic model, and the same results were found in other cancers under 5 genetic models. When stratified according to source of control, neither the hospital-based subgroup nor the population-based subgroup were observed to be related to the risk of cancer in any genetic models.

3.3. Test of heterogeneity

A Q test and I^2 statistic were assessed to evaluate the heterogeneity among the selected studies. High heterogeneity was observed across studies, as well as in some subgroup analyses, as tested by random-effects analysis. Moreover, we

Table 2

***HLA-G* +3142 C>G polymorphism genotype distribution and allele frequency in cases and controls.**

		Genotype(N)								Allele frequency(N)				
		Case				Control				Case		Control		
First author	Year	Total	CC	CG	GG	Total	CC	CG	GG	C	G	C	G	HWE
Silva et al[18]	2013	55	5	28	22	50	10	23	17	38	72	43	57	0.663
Zidi et al[19]	2016	104	61	40	1	83	29	37	10	162	42	95	57	0.737
Yang et al[20]	2014	315	79	123	115	400	61	181	158	281	353	303	497	0.442
Zambra et al[21]	2016	187	51	96	40	129	22	62	45	198	176	106	152	0.935
Agnihotri et al[22]	2017	383	116	119	148	383	59	154	170	351	415	272	494	0.073
Garziera et al[23]	2016	308	51	145	112	294	67	132	95	247	369	266	322	0.108
Bortolotti et al[24]	2014	100	30	55	15	100	21	41	38	115	85	83	117	0.120
Figueiredo-Feitosa et al[25]	2017	94	16	57	21	156	34	77	45	89	99	145	167	0.922

HWE = Hardy–Weinberg Equilibrium.

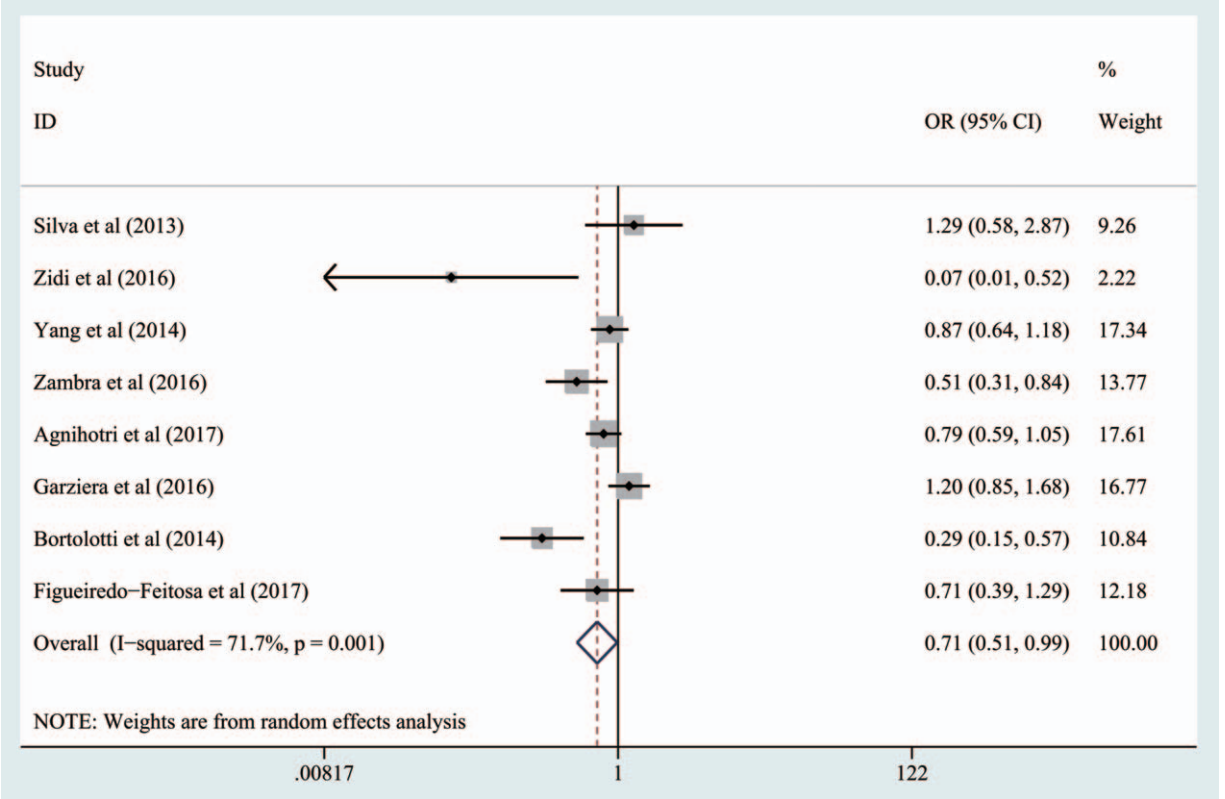

Figure 2. Forest plots of the *HLA-G* +3142 C>G polymorphism and cancer risk in the recessive model.

evaluated the heterogeneity of all genetic models in regard to different ethnicities, cancer types, and the source of the controls. However, the observed heterogeneity could not be completely explained by different ethnicities, types of cancer, or the source of the controls (data not shown).

3.4. Sensitivity analyses

Sensitivity analysis was carried out to examine the influence of each eligible study on the pooled ORs by the sequential removal of each individual study form the analysis. The individual removal procedure did not significantly affect the pooled Ors, indicating the robustness and reliability of our findings (Fig. 4).

3.5. Publication bias

Begg and Egger tests were conducted to explore the potential for publication bias in assessment of the relationship between the *HLA-G* +3142 C>G polymorphism and cancer risk in all genetic models. No asymmetry was observed in the Begg funnel plots, and neither Begg rank correlation nor Egger regression showed publication bias among the studies (Fig. 5, Table 3).

3.6. Trial sequential analysis

Figures 6 and 7 shows the results of the TSA of the association between *HLA-G* +3142 C>G polymorphism and cancer risk. The cumulative z-curve had crossed the traditional boundary, and it further demonstrated the results of our conventional meta-analysis that *HLA-G* +3142 C>G polymorphism was significantly associated with total cancer risk. However, the cumulative z-curve failed to cross the trial monitoring boundary before reaching the required information size and revealed that the cumulative sample size is not sufficient and further relevant case-control studies are necessary.

3.7. Bioinformatics analysis results

The results of bioinformatics analysis are displayed in Figure 8. A network of miRNAs and their target genes are showed in Figure 8A. The result indicated that *HLA-G* variation was closely related to effect on miRNA binding of 3'UTR of *HLA-G* transcripts. The expression level of HLA-G mRNA was different in different types of cancer based on TCGA cancer datasets (Fig. 8B). *HLA-G* somatic mutation frequency showed in TCGA cancer datasets was 0.4% (Fig. 8C). Figure 8D shows the overall survival Kaplan-Meier estimate of cases with or without alterations. It suggested that there is no significant difference in overall survival in the 2 groups.

4. Discussion

Evasion from antitumor immune destruction, well characterized as a distinguishing feature of malignant tumor, had been proved to be of great help to tumorigenesis.[33] HLA-G is an important complex molecule that plays an important role in facilitating tumor escape from immune surveillance by its immunosuppressive function on T and NK cells,[10] and the aberrant expression of HLA-G has been reported to be related to a variety of tumors.[11–16] The expression levels of the HLA-G protein have

Table 3

Meta-analysis results.

Genetic model	Category	OR(95%CI)	P	Heterogeneity I^2	Heterogeneity P	Begg test P	Egger test P
Homozygote (GG vs CC)	Overall	0.604 [0.357;1.021]	.060	80.9%	.000	.902	.762
	Asian	0.494 [0.373;0.654]	**.000**	0.0%	.406		
	Mixed	0.894 [0.325;2.458]	.829	75.6%	.017		
	Caucasian	0.348 [0.063;1.915]	.225	90.6%	.000		
	Cervical Ca	0.650 [0.254;1.666]	.370	76.9%	.013		
	Others	0.564 [0.266;1.199]	.137	85.4%	.000		
	HB	0.543 [0.286;1.031]	.062	68.8%	.022		
	PB	0.611 [0.242;1.541]	.297	87.6%	.000		
Heterozygote (CG vs CC)	Overall	0.815 [0.533;1.246]	.344	78.1%	.000	.174	.200
	Asian	0.452 [0.341;0.599]	**.000**	0.4%	.316		
	Mixed	1.081 [0.718;1.627]	.709	63.1%	.067		
	Caucasian	0.913 [0.488;1.711]	.778	71.7%	.029		
	Cervical Ca	0.897 [0.424;1.899]	.777	70.2%	.035		
	Others	0.780 [0.433;1.408]	.410	84.0%	.000		
	HB	0.778 [0.479;1.266]	.313	55.3%	.082		
	PB	0.813 [0.387;1.707]	.584	88.0%	.000		
Dominant (GG + CG vs CC)	Overall	0.740 [0.488;1.123]	.157	80.1%	.000	.108	.368
	Asian	0.472 [0.366;0.610]	**.000**	0.0%	.326		
	Mixed	1.112 [0.481;2.573]	.803	73.4%	.023		
	Caucasian	0.746 [0.329;1.181]	.482	85.1%	.001		
	Cervical Ca	0.777 [0.397;1.520]	.461	67.4%	.046		
	Others	0.716 [0.396;1.296]	.270	86.1%	.000		
	HB	0.668 [0.430;1.038]	.073	52.4%	.098		
	PB	0.766 [0.366;1.599]	.477	89.1%	.000		
Recessive (GG vs CG + CC)	Overall	0.710 [0.512;0.986]	**.041**	71.7%	.001	.386	.123
	Asian	0.827 [0.671;1.020]	.076	0.0%	.640		
	Mixed	0.719 [0.439;1.179]	.191	47.7%	.148		
	Caucasian	0.374 [0.093;1.494]	.164	89.9%	.000		
	Cervical Ca	0.689 [0.321;1.477]	.338	80.6%	.006		
	Others	0.716 [0.471;1.090]	.119	72.1%	.006		
	HB	0.637 [0.370;1.098]	.104	75.8%	.006		
	PB	0.794 [0.498;1.264]	.330	70.9%	.016		
Allelic (G vs C)	Overall	0.765 [0.598;0.979]	**.033**	80.6%	.000	.902	.791
	Asian	0.704 [0.608;0.816]	**.000**	14.2%	.280		
	Mixed	0.909 [0.580;1.425]	.678	73.3%	.023		
	Caucasian	0.668 [0.328;1.360]	.266	91.5%	.000		
	Cervical Ca	0.796 [0.512;1.235]	.309	75.9%	.016		
	Others	0.747 [0.528;1.056]	.098	85.5%	.000		
	HB	0.736 [0.541;1.000]	.050	68.1%	.024		
	PB	0.780 [0.512;1.186]	.245	88.0%	.000		

Ca = cancer, HB = hospital-based, PB = population-based.

been reported to be related to *HLA-G* gene polymorphisms. As reported, the presence of G at position +3142 can increase the affinity for microRNAs (miR-148a, miR- 148b e miR-152), sequentially decreasing the stability and expression of HLA-G mRNA.[8] To date, more and more studies have been attracted to explore the relationship between the *HLA-G* gene polymorphisms and cancer risk. Among *HLA-G* gene polymorphisms, the *HLA-G* +3142 C>G polymorphism is one of the most explored. Up to now, multiple published case-control studies have been performed to investigate the underlying correlation between the *HLA-G* +3142 C>G polymorphism and cancer risk. However, the biological role of the *HLA-G* +3142 C>G mutation in the development of cancer remains controversial. Considering that the results of the published articles were inconsistent or even were contradictory and individual case-control studies may have been underpowered to assess the effect of the polymorphism in the risk of cancer, we conducted the present meta-analysis which included all eligible studies to explore the precise association of the *HLA-G* +3142 C>G polymorphism with cancer susceptibility.

In this meta-analysis, we evaluated the relationship between the *HLA-G* +3142 C>G mutation and cancer susceptibility with all qualified case-control studies including 1546 cases and 1595 controls. By quantitatively analyzing the integrated data, the results of our present meta-analysis revealed evidence that the *HLA-G* +3142 C>G polymorphism decreases the susceptibility of overall cancer. There were several studies that had been performed to evaluate the correlation between the +3142 C>G polymorphism and the susceptibility of different types of cancer. However, paradoxical conclusions have been acquired. Zambra et al[21] carried out a case-control and they found the *HLA-G* +3142 C>G mutation was associated with an elevated risk of prostate cancer. The similar results were confirmed in other types of cancer, including thyroid cancer[25] and cervical cancer.[24] However, there were not a few studies reporting the opposite

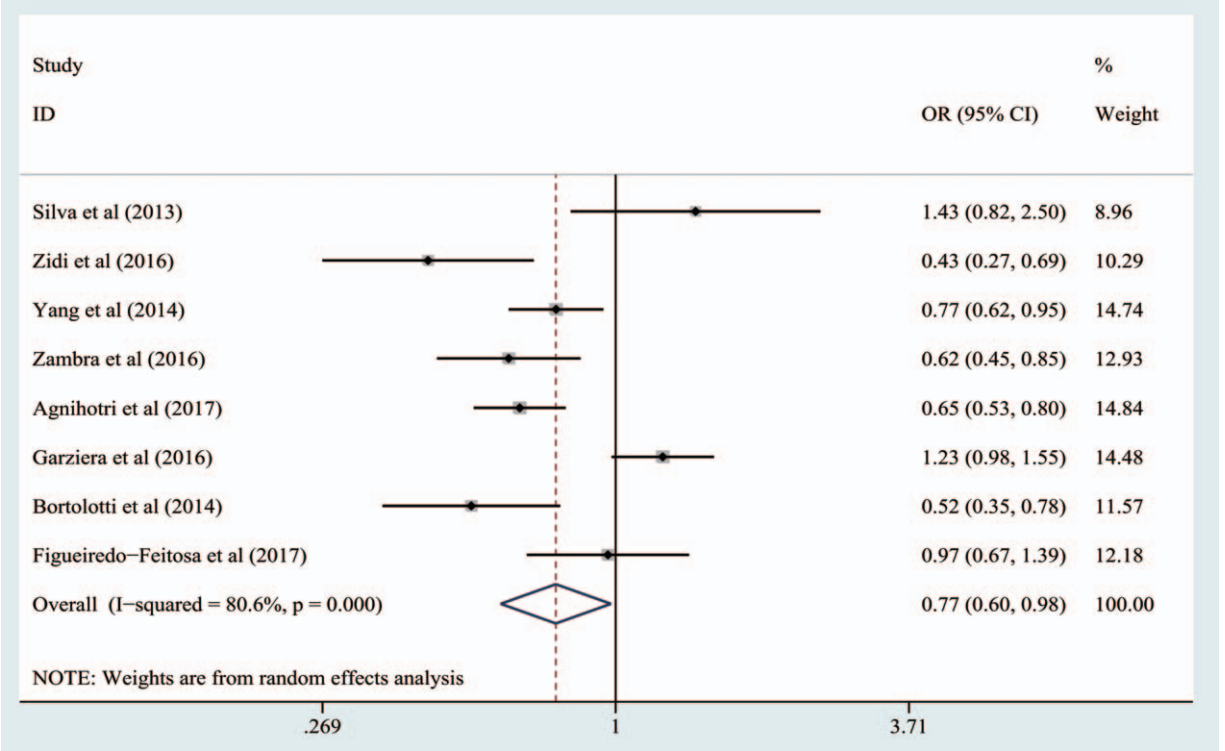

Figure 3. Forest plots of the *HLA-G* +3142 C>G polymorphism and cancer risk in the allelic comparisons.

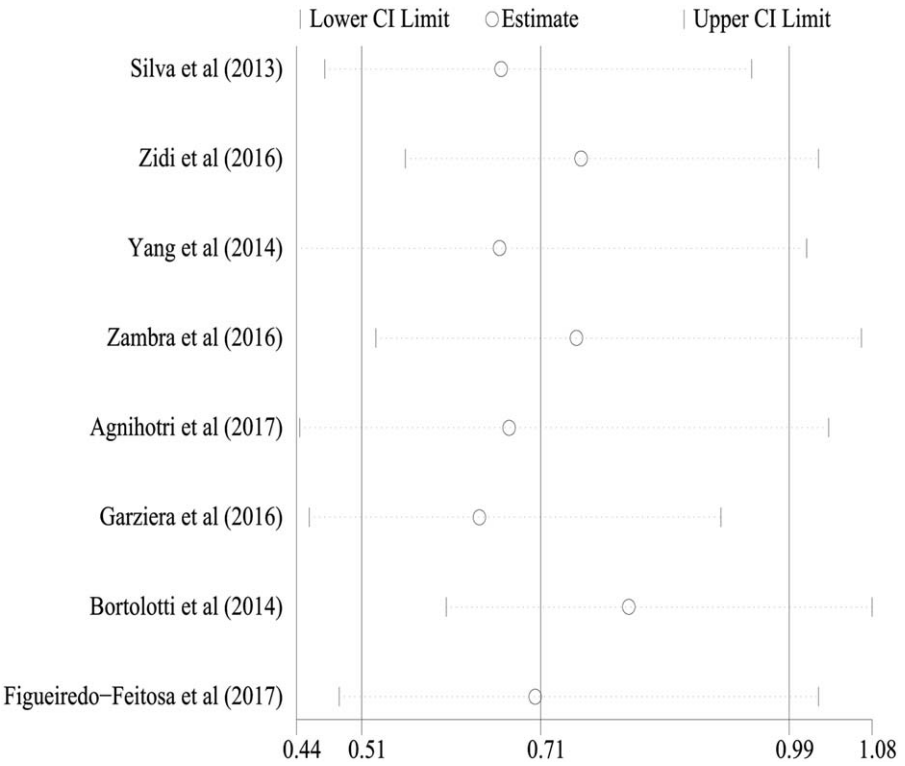

Figure 4. Sensitivity analysis of the *HLA-G* +3142 C>G polymorphism and cancer risk in the recessive model.

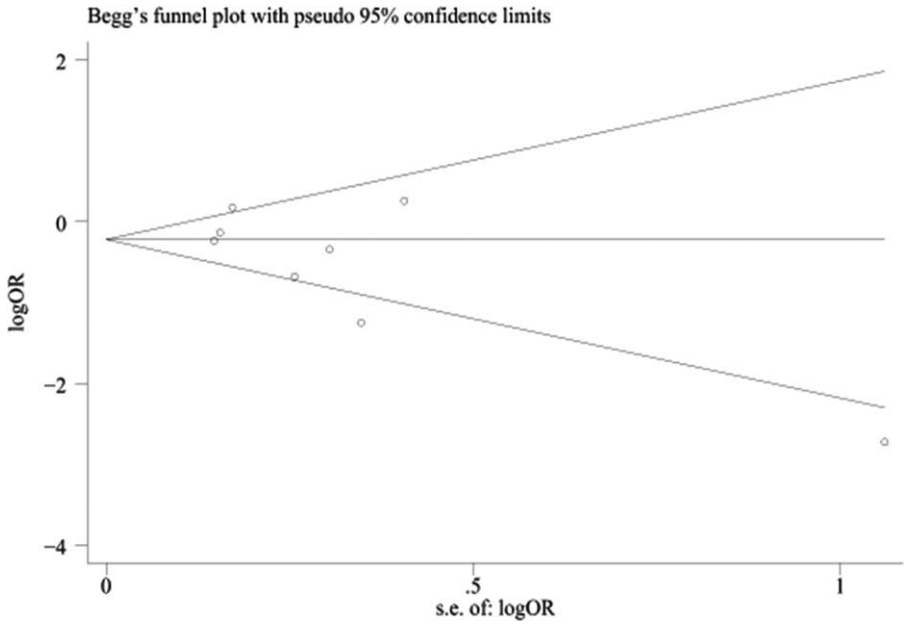

Figure 5. Funnel plot assessing evidence of publication bias in the recessive model.

result that the *HLA-G* +3142 C>G polymorphism could decreased the risk of some types of cancer. Additionally, some experiments showed that the *HLA-G* +3142 C>G polymorphism did not seem to play a role in cancer susceptibility. Even the results of the studies on the correlation between the *HLA-G* +3142 C>G polymorphism and the same types of cancer were inconsistent. For example, the study conducted by Bortolotti et al[24] demonstrated that individuals with the *HLA-G* +3142

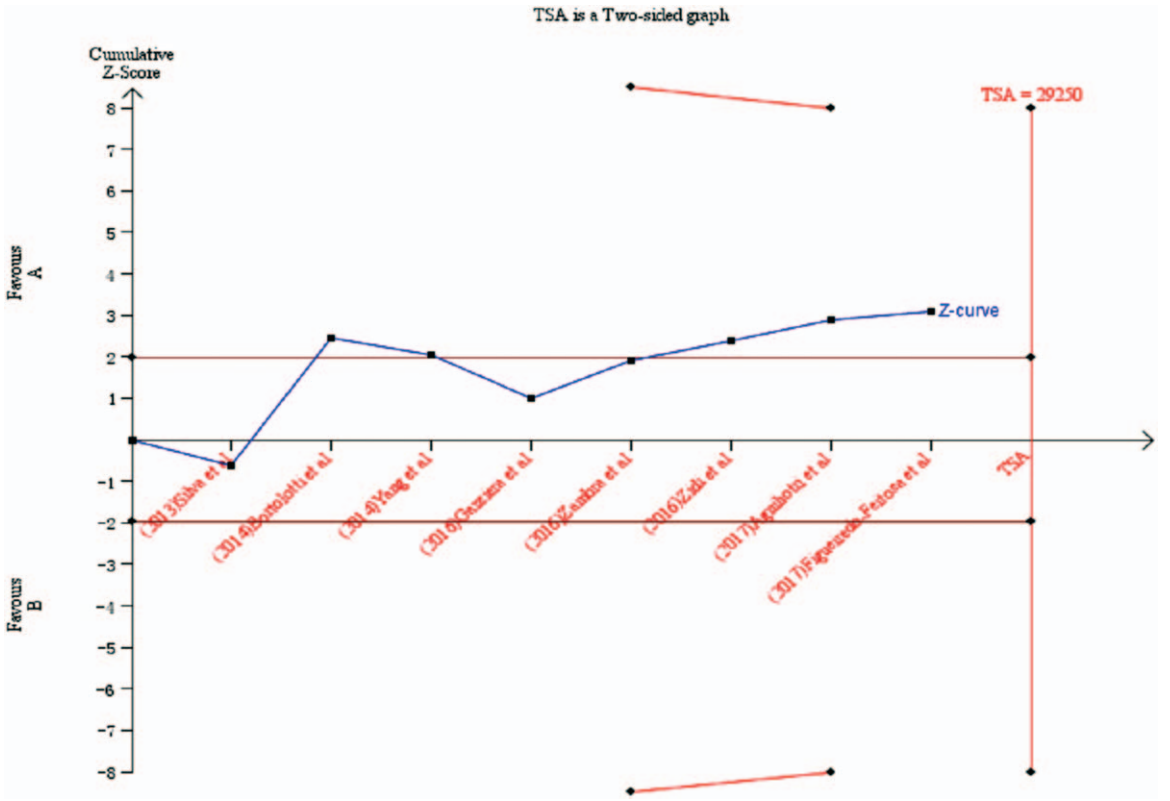

Figure 6. Trial sequential analysis for the *HLA-G* +3142 C>G polymorphism and cancer risk in the recessive model.

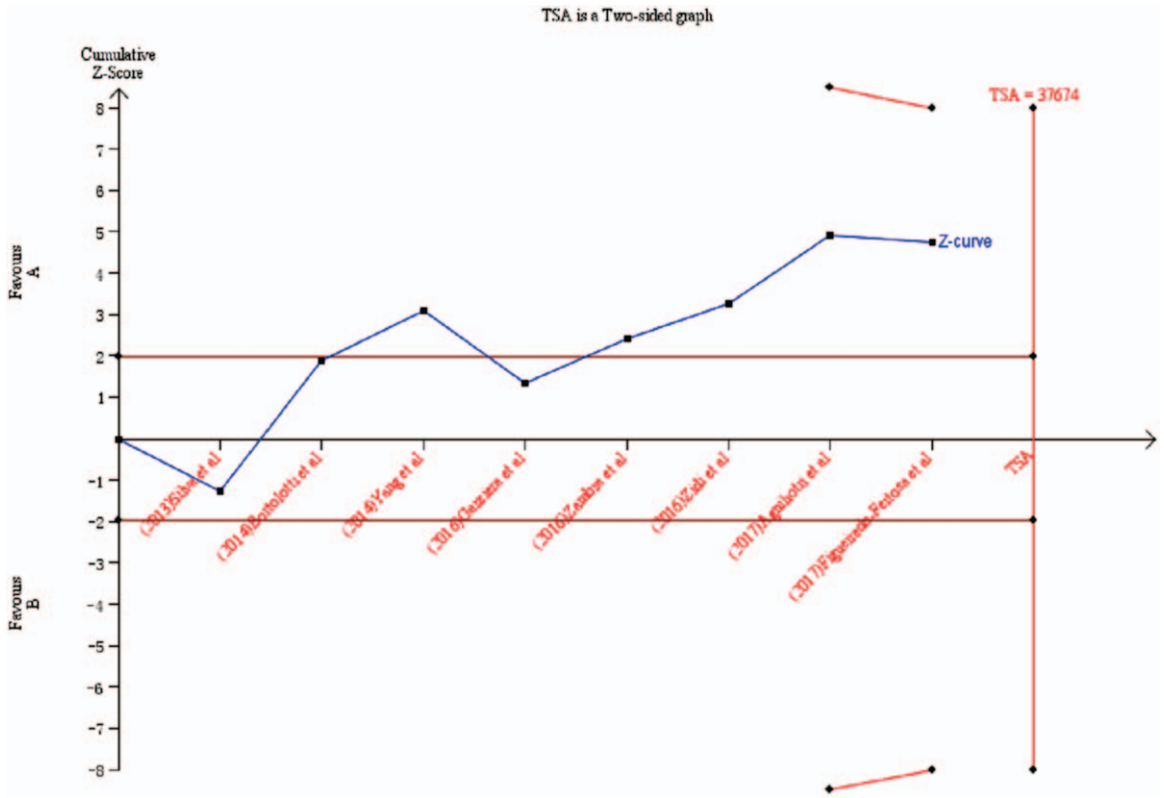

Figure 7. Trial sequential analysis for the *HLA-G* +3142 C>G polymorphism and cancer risk in the allelic comparisons.

C>G polymorphism had significantly increased risk for the occurrence of cervical cancer, and Silva et al[18] showed that there was no relationship between the *HLA-G* +3142 C>G mutation and cervical cancer susceptibility; however, Yang et al[20] indicated that this variation may be a protective factor in cervical cancer. To solve this controversy and obtain a more accurate conclusion, we carried out present meta-analysis. Our results demonstrated significant relationship between the *HLA-G* +3142 C>G polymorphism and decreased overall cancer risk. The informatics analysis indicated that *HLA-G* variation was closely related to effect on miRNA binding of 3'UTR of *HLA-G* transcripts; the expression level of HLA-G mRNA was different in different types of cancer, but the different value levels of mRNA expression might have no association with survival rate of overall cancer.

Significant heterogeneity among the studies was shown in our results; therefore, we performed stratified analyses in terms of ethnicity, types of cancer, and sources of controls. In the subgroup analysis based on ethnicity, an obviously decreased cancer risk was demonstrated in Asian populations but not in Mixed or Caucasian populations. This discrepancy in cancer risk may be explained by geographic climate, daily lifestyle, ethnic diversity, dietary habits, differences in alleles and genotypes in various ethnic populations, and so on. However, this result should be illustrated prudently and need further confirmation by more trials, as only 2 case-control studies included in the Asian subgroup. It has been reported that different types of cancer have different inherent heterogeneity in the occurrence and development of tumor.[34] However, in our present meta-analysis, when we assessed the relationship between the *HLA-G* +3142 C>G and risk of different types of tumors, no differences were found between different types of tumors in the stratified analysis by cancer type. This may be because the case-control studies included in our meta-analysis are too few. Our meta-analysis included 6 types of tumors, while there was only one case-control study for each type of tumor other than the cervical cancer. More case-control studies in different types of cancer are needed to evaluate the real relationship between the *HLA-G* +3142 C>G and risk of different types of tumors.

Although we tried our best efforts to assess the association between the *HLA-G* +3142 C>G variant and the risk of cancer, several limitations which may impact the objectivity of the findings still exist and must be taken into account. First, only unadjusted estimates were used to assess the strength of the relationship between the *HLA-G* 14+3142 C>G variant and cancer risk. Because of the lack of more original data such as life habit, exposing factors, interactions between gene-gene, gene-environment interactions and even different variant loci in the same gene factors, a further precise adjustment analysis could not be conducted by confounding factors. Second, there may be a selection bias existing in our study, since only published case-control studies written in Chinese or English were included in our meta-analysis. Some potential eligible studies may be not acquired, because they were not detected, published, or because they were written in other languages. Third, the total sample sizes of our meta-analysis were small, and the sample sizes of the stratified analysis were extremely small. There were not enough appropriate studies, weakening the statistical power to investigate the real relationship between the *HLA-G* +3142 C>G polymorphism and cancer risk. The result of TSA also

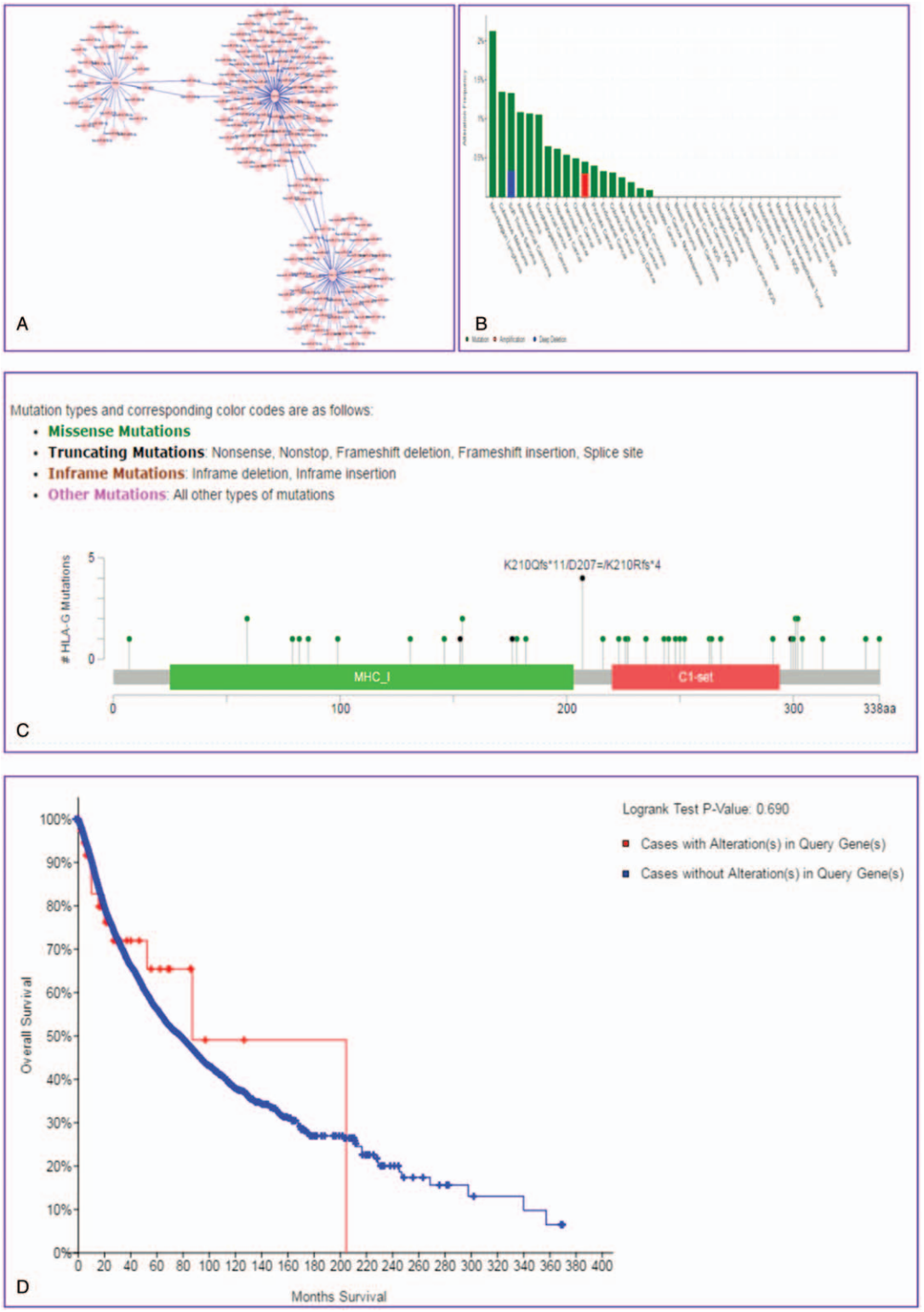

Figure 8. The results of bioinformatics analysis. (A) A network of miRNAs and their target genes. (B) The *HLA-G* mRNA expression in different types of cancer based on TCGA cancer datasets. (C) *HLA-G* somatic mutation in TCGA data. (D) The overall survival of *HLA-G* alteration compared with nonalteration.

demonstrated that the cumulative sample size is not sufficient. Fourth, because of the high heterogeneity existing in our present meta-analysis, the reliability of the findings may be weakened. Despite the application of the random-effects model in our meta-analysis, the findings on the relationship between the *HLA-G* +3142 C>G variant and overall cancer susceptibility should be illuminated cautiously. Larger sample sizes and well-designed case-control experiments with various types of cancer in diverse

ethnicities are needed to further verify the relationship between the *HLA-G* +3142 C>G polymorphism and cancer risk.

To sum up, the pooled results of our meta-analysis demonstrated that the *HLA-G* +3142 C>G polymorphism may be associated with decreased cancer susceptibility, especially in the Asian populations. The results allowed us to hypothesize that the *HLA-G* +3142 C>G mutation may play a protect role in development of cancer. Larger sample sizes and well-designed case-control experiments with various types of cancer in different ethnicities are needed to further verify our findings.

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