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Correlation of Asp299Gly and Thr399Ile polymorphisms in toll-like receptor 4 gene with digestive cancer risk: A meta-analysis

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Abstract. The aim of this meta-analysis was to evaluate the correlation between the Asp299Gly and Thr399Ile polymorphisms in the toll-like receptor 4 (TLR4) gene and the risk of digestive cancer. A comprehensive search in PubMed, Web of Science (ISI), the China National Knowledge Infrastructure (CNKI), the Database of Chinese Scientific and Technical Periodicals (VIP) and the China Biology Medical (CBM) literature databases, including all the studies until May 25 2012, was conducted in order to investigate the abovementioned correlation. Statistical analysis was performed using STATA version 10.1. A total of 12 case-control studies were identified comprising 1,877 cancer patients and 3,181 controls for Asp299Gly polymorphism, and 8 case-control studies with 1,062 cancer patients and 1,867 controls for Thr399Ile polymorphism. Following sensitivity analysis and excluding studies that deviated from the Hardy-Weinberg equilibrium (HWE) in the controls, this meta-analysis demonstrated a significant correlation between the G allele of the Asp299Gly polymorphism and increased risk of gastric cancer in dominant [fixed-effect model (FEM): odds ratio (OR), 1.772; 95% confidence interval (CI), 1.340-2.343] and codominant (FEM: OR, 1.761, CI, 1.347-2.301) models. However, no significant correlation was detected for overall digestive and colorectal cancer. Furthermore, following the sensitivity analysis and exclusion of studies deviating from HWE in controls, no significant effect of the T allele of Thr399Ile polymorphism on overall digestive, gastric and colorectal cancer risk was demonstrated. This study suggests that the G allele of the TLR4 Asp299Gly polymorphism might be correlated with an increased risk of gastric cancer. However, this result needs to be further investigated by future studies.

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Introduction

Digestive cancer, including gastric, colorectal, hepatocellular (HC), gallbladder cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma, which has a higher cancer-related mortality compared to any other system in the body (1), has become a major public health issue worldwide. The causes of digestive cancer, including the interaction between inherited and environmental factors, are complicated (2). Host genetic factors may play a vital role in the genesis of digestive cancer (3). The activation of the immune system and inflammation, regulated by specific single nucleotide polymorphisms (SNPs) of common, low-penetrance susceptibility loci (4), reportedly plays an important role in cancer susceptibility and progression (5).

Toll-like receptors (TLRs) are material constituents of the innate immune response by which the host is able to protect itself from microbial danger and other unsafe agents (6). Inherited polymorphisms in TLR genes have been demonstrated to directly affect the risk of infectious diseases, allergy, cardiovascular disease and more significantly cancer (7). Toll-like receptor 4 (TLR4), which constitutes one of the most active members of TLRs, promotes the transcription of genes involved in immune activation (8). Two SNPs, located on chromosome 9, are reportedly associated with certain types of digestive cancer (9-20). One is Asp299Gly (299A>G, D299G, rs4986790), with G instead of the A allele at 896 base pair (bp), causing glycine to replace aspartic acid at the 299 site of the amino acid sequence (TLR4_896A/G). The second SNP is Thr399Ile (399C>T, T399I, rs4986791), with T instead of the C allele at 1,196 bp, causing isoleucine to replace threonine at the 399 site of the amino acid sequence (TLR4_1196C/T) (21). The common, co-segregating missense mutations (Asp299Gly and Thr399Ile), which alter the extracellular structure of this receptor, are correlated with a blunted response to lipopolysaccharide (LPS) *in vivo* and *in vitro* (21,22). Additionally, these mutations are correlated with an increased risk of inflammatory diseases (23,24), due to the fact that these two SNPs cleave the normal structure of the extracellular domain of the TLR4 and are, therefore, estimated to reduce the reaction to ligands through alterations in binding (7).

Although numerous studies have investigated the correlation between the two SNPs and digestive cancer, data are

limited and the results remain controversial. The aim of this meta-analysis, considering the eligible published studies currently available, was therefore to review and quantitatively analyze the results, in order to reach an evidence-based conclusion.

Materials and methods

Search strategy. In order to evaluate the correlation between the TLR4 gene and digestive cancer susceptibility, several databases, including PubMed, Web of Science (ISI), the China National Knowledge Infrastructure (CNKI), the Database of Chinese Scientific and Technical Periodicals (VIP) and the China Biology Medical literature database (CBM), were searched on May 25, 2012, using the following search terms: 'toll-like receptor 4', and, 'cancer', 'carcinoma', 'tumor', 'malignancy', 'neoplasm', and 'polymorphism'. In addition, we reviewed the reference lists of the identified relevant studies and relevant reviews.

Inclusion criteria. The studies included in this meta-analysis were independently assessed by two investigators using the following inclusion criteria: i) the original study evaluated the relationship between the Asp299Gly (299A>G, D299G, rs4986790, TLR4_896A/G) and Thr399Ile (399C>T, T399I, rs4986791, TLR4_1196C/T) polymorphisms in the TLR4 gene and digestive cancer [defined as cancer of the esophagus, stomach, colorectum, pancreas, gallbladder, liver and gastric MALT lymphoma] risk; ii) in a case-control or cohort study design; iii) provided the genotype frequencies or the data could be calculated in order to determine the odds ratio (OR) with 95% confidence interval (CI). When the studies were duplicated or overlapped, those with the largest number of subjects and the most recently published studies were included in the final analysis.

Data extraction. Two investigators independently extracted and converted the available data from the retrieved studies into a standard format for incorporation into a central database. The information collected from each study was as follows: the name of the first author, year of publication, country, cancer type, genotype frequencies for cases and controls, methods of genotyping, age and gender in cases and controls, and source of control groups (population- or hospital-based controls). Any discrepancies between the two reviewers were settled by discussion and consultation with a third reviewer.

Statistical analysis. The distribution of genotype frequencies in the control groups was assessed with regard to whether or not they deviated from the Hardy-Weinberg equilibrium (HWE) by the Chi-square goodness of fit test. Pooled OR and the corresponding 95% CI were used as measures to estimate the strength of the correlation between the two polymorphisms (Asp299Gly and Thr399Ile) and cancer susceptibility. Dominant (GG+AG vs. AA, TT+TC vs. CC), recessive (GG vs. AA+GA, TT vs. CC+TC) and codominant (G vs. A, T vs. C) models were calculated separately. The heterogeneity between the results of collected studies was evaluated with the I^2 index, which calculates the degree of heterogeneity in the meta-analysis (25). When the heterogeneity was significant (I^2>50%), the random-effect model (REM) was selected to evaluate the results using the DerSimonian and Laird method. By contrast (I^2<50%), the fixed-effect model (FEM) was adopted using the inverse variance method. To explore the potential source of heterogeneity, we performed a stratified analysis (gastric or colorectal cancer) and meta-regression analysis to assess the potentially important covariates across studies. Theoretical consideration and empirical evidence have suggested that specific genetic variants causally associated with common diseases could have small effects (risk ratio mostly <2.0) (26,27), due to the fact that original studies with a relatively limited number of participants might be underpowered to detect the effect. Therefore, for the sensitivity analysis, we excluded the studies with OR>3.0 as a criterion to control the impact of outlier values resulting from low cell counts within each single study on the pooled effect. Influence analysis was conducted, in order to describe how robust the pooled estimator was in order to exclude individual studies. Publication bias was estimated by Begg's funnel plots (28). The software used was STATA version 10.1 (StataCorp LP, College Station, TX, USA). The reported probabilities (P-values) were two-sided and P<0.05 was considered to indicate a statistically significant difference.

Results

Study characteristics. The general characteristics of the available studies are shown in Tables I and II. Due to the fact that there was >1 study in an article (14), there were 12 studies in 10 published articles (9-15,17-19) with 1,877 cases and 3,181 controls for Asp299Gly polymorphism, and 8 studies in 8 published articles (10,13,15-20) with 1,062 cases and 1,867 controls for Thr399Ile polymorphism. The distribution of genotypes in the control groups obeyed the HWE for the obtained studies, with the exception of one study (17) for the Asp299Gly polymorphism.

Quantitative synthesis. The details of pooled ORs for the correlation between TLR4 Asp299Gly and Thr399Ile polymorphisms and digestive cancer risk are summarized in Tables III and IV.

Asp299Gly polymorphism. This meta-analysis showed no statistically significant correlation between the G allele and overall digestive cancer susceptibility in the dominant (REM: OR, 1.309, 95% CI, 0.923-1.857), recessive (FEM: OR, 2.355; 95% CI, 0.848-6.641) and codominant (REM: OR, 1.460; 95% CI, 0.951-2.241) models. When studies that deviated from the HWE in the control group were excluded (17), there were still no significant effect of G allele on the risk of overall digestive cancer in the dominant (REM: OR, 1.290; 95% CI, 0.893-1.863), recessive (FEM: OR, 2.522; 95% CI, 0.817-7.783) and codominant (REM: OR, 1.444; 95% CI, 0.917-2.274) models.

Regarding gastric cancer, a statistically significant correlation between G allele and an increased risk of gastric cancer for the dominant (FEM: OR, 1.772; 95% CI, 1.340-2.343) (Fig. 1 and Table III) and codominant (FEM: OR, 1.761; 95% CI, 1.347-2.301) models was detected. However, no statistically significant correlation was observed between the G allele and gastric cancer susceptibility in the recessive

Table I. Characteristics of TLR4 Asp299Gly polymorphism genotype distributions for digestive cancer risk in the studies included in this meta-analysis.

Authors	Year	Country	Cancer type	Genotypes (AA/AG/GG)		Percentage of male patients (case/control)	Mean age (case/control)	Genotyping method	Control source	P-value of HWC	Refs.
				Case	Control						
de Oliveira and Silva	2012	Brazil	Gastric	154/20/0	402/31/0	77/49	62.2/54.7	PCR-RFLP	PB	0.440	(19)
Davoodi and Seow	2011	Iran	Colorectal	58/2/0	50/0/0	Na	Na	PCR-RFLP	PB	1.000	(18)
Yang, *et al*[a]	2011	China	Colorectal	96/4/2	84/2/1	63/60	52.0/50.0	PCR-sequencing	HB	0.035	(17)
Türe-Ozdemir, *et al*[b]	2008	Turkey	GML	38/18/0	39/12/0	54/59	61.0/56.0	PCR-RFLP	HB	0.341	(9)
Santini, *et al*[b]	2008	Italy	Gastric	159/11/1	140/11/0	58/58	60.0/56.0	PCR-RFLP	PB	0.642	(10)
Garza-Gonzalez, *et al*	2007	Mexico	Gastric	72/6/0	239/20/0	64/Na	58.6/57.1	PCR-sequencing	HB	0.518	(15)
Hold, *et al*[c]	2007	Poland	Gastric	258/51/3	387/31/1	Na	Na	PCR-TaqMan	PB	0.651	(14)
Hold, *et al*[c]	2007	USA	Gastric	266/38/3	194/16/1	Na	Na	PCR-TaqMan	PB	0.299	(14)
Hold, *et al*[c]	2007	USA	Esophageal	148/11/0	194/16/1	Na	Na	PCR-TaqMan	PB	0.299	(14)
Boraska Jelavić, *et al*	2006	Croatia	Colorectal	77/10/2	84/4/0	69/Na	61.5/Na	PCR-RFLP	PB	0.827	(13)
Landi, *et al*	2006	Spain	Colorectal	251/31/0	232/37/0	Na	Na	PCR-TaqMan	HB	0.226	(12)
Hellmig, *et al*[b]	2005	Germany	GML	83/4/0	837/114/1	32/34	57.0/56.3	PCR-TaqMan	HB	0.151	(11)

[a]Deviated from HWE; [b]median age was provided; [c]one study with different types of cancer or populations. GML, gastric mucosa-associated lymphoid tissue (MALT) lymphoma; Na, not available in original study; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-TaqMan, PCR-TaqMan probe technique; PCR-sequencing, PCR-sequencing technique; PB, population-based; HB, hospital-based; GML, gastric MALT lymphoma.

Table II. Characteristics of TLR4 Thr399Ile polymorphism genotype distributions for digestive cancer risk in the studies included in this meta-analysis.

Authors	Year	Country	Cancer type	Genotypes (CC/CT/TT)		Percentage of male patients (case/control)	Mean age (case/control)	Genotyping method	Control source	P-value of HWC	Refs.
				Case	Control						
de Oliveira and Silva	2012	Brazil	Gastric	165/9/0	421/12/0	77/49	62.2/54.7	PCR-RFLP	PB	0.770	(19)
Agúndez, *et al*	2012	Spain	HC	143/12/0	472/68/3	75/59	66.9/44.1	PCR-TaqMan	HB	0.746	(20)
Davoodi and Seow	2011	Iran	Colorectal	58/2/0	50/0/0	Na	Na	PCR-RFLP	PB	1.000	(18)
Yang, *et al*	2011	China	Colorectal	93/8/1	86/1/0	63/60	52.0/50.0	PCR-sequencing	HB	0.957	(17)
Srivastava, *et al*	2010	India	Gallbladder	195/32/5	232/24/1	35/36	54.1/53.1	PCR-RFLP	PB	0.657	(16)
Santini, *et al*[a]	2008	Italy	Gastric	155/15/1	147/4/0	58/58	60.0/56.0	PCR-RFLP	PB	0.869	(10)
Garza-Gonzalez, *et al*	2007	Mexico	Gastric	77/1/0	246/13/0	64/NP	58.6/57.1	PCR-RFLP	HB	0.679	(15)
Boraska Jelavić, *et al*	2006	Croatia	Colorectal	77/12/0	82/5/0	Na	61.5/Na	PCR-RFLP	PB	0.783	(13)

[a]Median age was provided. HC, hepatocellular; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-TaqMan, PCR-TaqMan probe technique; PCR-sequencing, PCR-sequencing technique; PB, population-based; HB, hospital-based; Na, not available in original study.

(FEM: OR, 2.852; 95% CI, 0.679-11.978) model. No study deviated from the HWE in the control group for gastric cancer.

Concerning the various types of colorectal cancer, no statistically significant correlation with the G allele was found for the dominant (REM: OR, 1.546; 95% CI, 0.639-3.738), recessive (FEM: OR, 2.607; 95% CI, 0.392-17.343) and codominant (REM: OR, 2.790; 95% CI, 0.561-13.885) models. Following exclusion of studies that deviated from the HWE in the control groups (17), there was still no statistically significant correlation in the abovementioned inherited models.

Thr399Ile polymorphism. No statistically significant correlation was found between the T allele and risk of overall digestive cancer in the dominant (REM: OR, 1.681; 95% CI, 0.888-3.181), recessive (FEM: OR, 2.506; 95% CI, 0.629-9.991) and codominant (REM: OR, 1.706; 95% CI, 0.895-3.251) models (Table IV). No study deviated from the HWE in the control group for overall digestive cancer.

With regard to gastric cancer, this meta-analysis showed no significant impact of the T allele on the risk of overall digestive cancer in the dominant (REM: OR, 1.611; 95% CI, 0.496-5.236), recessive (FEM: OR, 2.666; 95% CI, 0.108-65.927) and codominant (REM: OR, 1.626; 95% CI, 0.500-5.286) models. No study deviated from the HWE in the control group for gastric cancer.

Regarding colorectal cancer, the correlation with T allele was significant in the dominant (FEM: OR, 3.372; 95% CI, 1.343-8.466) and codominant (FEM: OR, 3.286; 95% CI, 1.331-8.116) models. No statistically significant correlation was found in the recessive (FEM: OR, 2.586; 95% CI, 0.104-64.299) model. No study deviated from the HWE in the the control group for colorectal cancer.

Sources of heterogeneity. As shown in Tables III and IV, prior to sensitivity analysis, evident heterogeneity among studies in the dominant and codominant models was demonstrated for Asp299Gly and Thr399Ile polymorphisms, following the exclusion of studies deviating from the HWE in the control groups. However, in the recessive model no significant heterogeneity was found for Asp299Gly and Thr399Ile polymorphisms, following the exclusion of studies deviating from the HWE in the control groups.

We performed a univariate meta-regression analysis with the covariates of publication year, continent (including Asia, Europe and America), gender [ratio of males (%) in case/control group), age (ratio of mean age or median age in case/control group), sample size (the sum of cases and controls) and genotype method [including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), PCR-TaqMan probe technique (PCR-TaqMan) and PCR-sequencing technique (PCR-sequencing)]. No covariates were demonstrated to have a significant effect on between-study heterogeneity for Asp299Gly and Thr399Ile polymorphisms.

Sensitivity analysis

Asp299Gly polymorphism. Concerning overall digestive cancer, following the exclusion of studies with OR>3.0 (13) (OR, 3.273 in the dominant; 5.057 in the recessive and 4.241 in the codominant model), (18) (OR, 4.316 in the dominant model and OR, 4.241 in the codominant model), (11) (OR, 3.625 in the recessive model) and (14) (OR, 4.058 in the recessive

Table III. Pooled measures of the correlation between TLR4 Asp299Gly polymorphism and digestive cancer.

			Prior to sensitivity analysis				Following sensitivity analysis			
				Pooled OR (95% CI)				Pooled OR (95% CI)		
Disease	Population	Inherited model	No. of cases/controls	FEM	REM	I^2 (%)	No. of cases/controls	FEM	REM	I^2 (%)
Overall	All related studies	Dominant	1,877/3,181	1.373 (1.109-1.700)[a]	1.309 (0.923-1.857)	55.8	1,728/3,043	1.325 (1.066-1.648)[b]	1.217 (0.847-1.748)	59.3
		Recessive	1877/3181	2.355 (0.848-6.641)	2.355 (0.848-6.641)	0	1389/1730	1.562 (0.410-5.948)	1.562 (0.410-5.948)	0
		Codominant	1877/3181	1.463 (1.193-1.794)[a]	1.460 (0.951-2.241)	73.5	1728/3043	1.328 (1.079-1.636)[a]	1.221 (0.866-1.721)	58.7
	Excluded for DHWC	Dominant	1775/3094	1.366 (1.100-1.695)[a]	1.290 (0.893-1.863)	59.6	1626/2956	1.316 (1.056-1.641)[b]	1.190 (0.812-1.744)	63.6
		Recessive	1775/3094	2.522 (0.817-7.783)	2.522 (0.817-7.783)	0	1287/1643	1.497 (0.301-7.450)	1.497 (0.301-7.450)	0
		Codominant	1775/3094	1.456 (1.184-1.790)[a]	1.444 (0.917-2.274)	73.6	1626/2956	1.318 (1.067-1.628)[b]	1.192 (0.828-1.715)	63.0
Gastric cancer[c]	All related studies	Dominant	1042/1473	1.772 (1.340-2.343)[a]	1.685 (1.193-2.382)[a]	29.9	-	-	-	-
		Recessive	1042/1473	2.852 (0.679-11.978)	2.852 (0.679-11.978)	0	730/419	2.254 (0.353-14.378)	2.254 (0.353-14.378)	0
		Codominant	1042/1473	1.761 (1.347-2.301)[a]	1.696 (1.237-2.326)[a]	23.3	-	-	-	-
Colorectal cancer	All related studies	Dominant	533/494	1.062 (0.685-1.648)	1.546 (0.639-3.738)	52.4	384/356	0.850 (0.526-1.373)	0.881 (0.492-1.577)	11.2
		Recessive	533/494	2.607 (0.392-17.343)	2.607 (0.392-17.343)	0	444/414	1.720 (0.153-19.298)	1.720 (0.153-19.298)	-
		Codominant	533/494	1.410 (0.932-2.133)	2.790 (0.561-13.885)	88.0	384/356	0.880 (0.557-1.389)	0.953 (0.491-1.847)	28.0
	Excluded for DHWC	Dominant	431/407	1.007 (0.634-1.598)	1.629 (0.480-5.533)	65.3	282/269	0.774 (0.465-1.289)	0.774 (0.465-1.289)	-
		Recessive	431/407	5.057 (0.239-106.859)	5.057 (0.239-106.859)	-	-	-	-	-
		Codominant	431/407	1.372 (0.883-2.131)	3.420 (0.336-34.857)	91.9	282/269	0.788 (0.481-1.289)	0.788 (0.481-1.289)	-

[a]P<0.01; [b]P<0.05; [c]all the studies for gastric cancer were within the HWE in the controls. OR, odds ratio; CI, confidence interval; dominant model, GG+AG vs. AA; recessive model, GG vs. AG+AA; codominant model, G vs. A; DHWE, deviated from HWE in controls; FEM, fixed-effect model; REM, random-effect model.

Table IV. Pooled measures of the correlation between TLR4 Thr399Ile polymorphism and digestive cancer.

		Prior to sensitivity analysis				Following sensitivity analysis			
			Pooled OR (95% CI)				Pooled OR (95% CI)		
Disease	Inherited model	No. of cases/controls	FEM	REM	I^2 (%)	No. of cases/controls	FEM	REM	I^2 (%)
Overall[a]	Dominant	1062/1867	1.454 (1.048-2.016)[b]	1.681 (0.888-3.181)	63.9	729/1579	1.244 (0.877-1.763)	1.212 (0.606-2.427)	68.3
	Recessive	1062/1867	2.506 (0.629-9.991)	2.506 (0.629-9.991)	0	829/1610	1.420 (0.234-8.620)	1.420 (0.234-8.620)	0
	Codominant	1062/1867	1.485 (1.084-2.035)[b]	1.706 (0.895-3.251)	66.4	729/1579	1.279 (0.915-1.788)	1.217 (0.601-2.462)	71.0
Gastric cancer[a]	Dominant	423/843	1.962 (1.018-3.784)[b]	1.611 (0.496-5.236)	62.1	252/692	1.388 (0.617-3.123)	0.852 (0.119-6.084)	69.2
	Recessive	423/843	2.666 (0.108-65.927)	2.666 (0.108-65.927)	-	-	-	-	-
	Codominant	423/843	1.982 (1.036-3.791)[b]	1.626 (0.500-5.286)	63.0	252/692	1.382 (0.619-3.086)	0.857 (0.124-5.918)	68.5
Colorectal cancer[a]	Dominant	251/224	3.372 (1.343-8.466)[b]	3.372 (1.343-8.466)[b]	0	89/87	2.556 (0.860-7.591)	2.556 (0.860-7.591)	-
	Recessive	251/224	2.586 (0.104-64.299)	2.586 (0.104-64.299)	-	-	-	-	-
	Codominant	251/224	3.286 (1.331-8.116)[b]	3.286 (1.331-8.116)[b]	0	89/87	2.443 (0.842-7.088)	2.443 (0.842-7.088)	-

[a]All the studies were within the HWE in the controls; [b]$P<0.05$, [c]$P<0.01$. OR, odds ratio; CI, confidence interval; FEM, fixed-effect model; REM, random-effect model; dominant model, TT+CT vs. CC; recessive model, TT vs. CT+CC; codominant model, T vs. C.

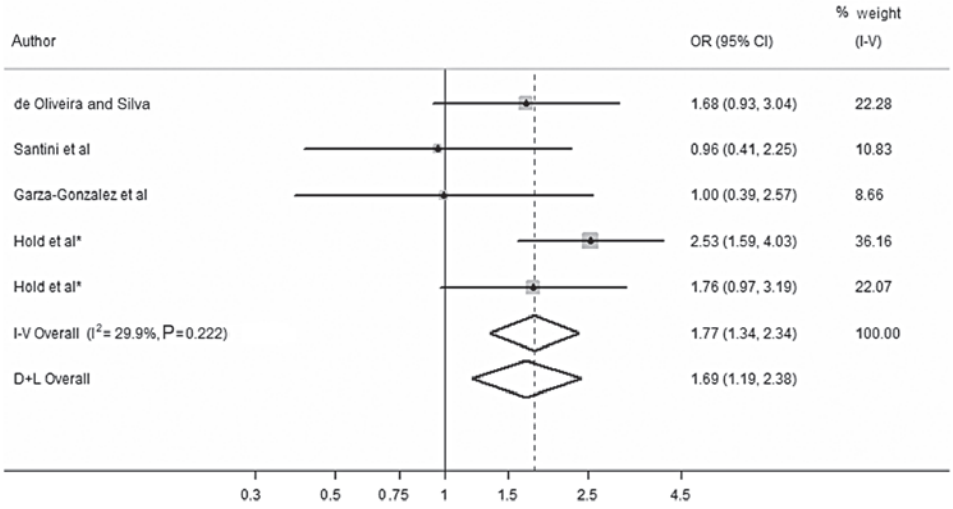

Figure 1. Forest plot of ORs for gastric cancer in the dominant model (GG+AG vs. AA) of TLR4 Asp299Gly polymorphism for studies that obeyed Hardy-Weinberg equilibrium (HWE) in the control group. Open diamond denotes the pooled ORs including fixed- (denoted as I-V) and random- (denoted as D-L) effects. Black squares indicate the OR in each study, with square sizes inversely proportional to the standard error of the OR. Horizontal lines represent 95% CI. *One study with different types of populations.

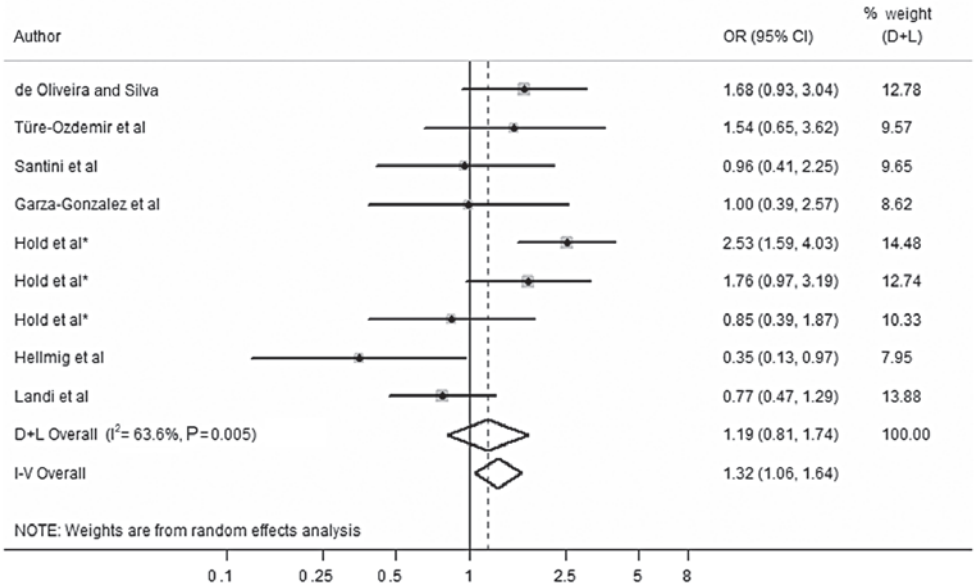

Figure 2. Forest plot of ORs for digestive cancer in the dominant model (GG+AG vs. AA) of TLR4 Asp299Gly polymorphism for studies that obeyed Hardy-Weinberg equilibrium (HWE) in the control group. Open diamond denotes the pooled ORs including fixed- (denoted as I-V) and random- (denoted as D-L) effects. Black squares indicate the OR in each study, with square sizes inversely proportional to the standard error of the OR. Horizontal lines represent 95% CI. *One study with different types of cancer or populations.

model), the risk effect of G allele in the dominant, recessive and codominant models was still not significant (Fig. 2). For gastric cancer, following the exclusion of studies with OR>3.0 (14) (OR, 4.058 in the recessive model), no significant correlation was found in the G allele in the recessive model. For colorectal cancer, following the exclusion of studies with OR>3.0 (13) (OR, 3.273 in the dominant, 5.057 in the recessive and 4.241 in the codominant model) and (18) (OR, 4.316 in the dominant model and OR, 4.241 in the codominant model), the analysis did not show any significant effect of G allele in the dominant, recessive and codominant models (Table III).

Thr399Ile polymorphism. Regarding overall digestive cancer, following the exclusion of studies with OR>3.0 (18) (OR, 4.316 in the dominant and 4.241 in the codominant model), (10) (OR, 3.794 in the dominant and 3.897 in the codominant model), (16) (OR, 5.639 in the recessive model) and (17) (OR, 8.323 in the dominant and 8.918 in the codominant model), no significant correlation was found in the dominant, recessive and codominant models. Following the exclusion of studies with OR>3.0, for gastric cancer (10) (OR, 3.794 in the dominant model and 3.897 in the codominant model) and colorectal cancer (18) (OR, 4.316 in the dominant model and 4.241 in the codominant model) and (17) (OR, 8.323 in the dominant model and 8.918 in the codominant model), no significant correlation to the T allele was indicated in dominant and codominant models (Table IV).

Influence analysis. Following the exclusion of studies deviating from HWE in controls and sensitivity analysis, no individual study was found to have an impact on the pooled effect in the dominant, recessive and codominant models for either the Asp299Gly or Thr399Ile polymorphisms.

Publication bias evaluation. Following the exclusion of studies deviating from HWE in controls and sensitivity analysis, no significant publication bias was detected in any of the above-mentioned inherited models for Asp299Gly and Thr399Ile polymorphisms (data not shown).

Discussion

In this meta-analysis, we assessed the correlation between TLR4 gene polymorphisms (Asp299Gly and Thr399Ile) and digestive cancer susceptibility. As a result, a significant correlation was found between TLR4 gene and the risk of gastric cancer.

Epidemiological studies have demonstrated that chronic inflammation is important in the development of digestive cancer (29). A variety of chronic inflammatory statuses, e.g., Barrett's esophagus, ulcerative colitis and chronic gastritis induced by *Helicobacter pylori* infection, significantly increase the risk of developing digestive cancer (29,30). Considering the correlation between inflammation and carcinogenesis, investigators have begun to shed light on the role of TLRs and innate immune responses in inflammation-associated carcinogenesis in the gastrointestinal tract (31-34). TLR4, which constitutes one of the most active members of the TLRs family, performs important immune and non-immune functions in the human intestinal tract (35). From a theoretical point of view, the activation of TLR4 may irritate the immune response that protects the organism against tumors, produce a pro-inflammatory environment and, thus, may promote carcinogenesis (20). The possible effect of the two non-synonymous SNPs at rs4986790 (A299G) and rs4986791 (T399I) in the TLR4 gene on cancer risk has received more attention (6), as the minor A allele in Asp299Gly and T allele in Thr399Ile are associated with reduced activation of nuclear factor-κB (NF-κB) and pro-inflammatory cytokine expression (36,37).

Recently, studies on the correlation of SNPs (Asp299Gly and Thr399Ile) in TLR4 gene with the risk of digestive cancer, including gastric, colorectal, gallbladder, hepatocellular, esophageal cancer and gastric MALT lymphoma, have conducted investigations on different ethnicities (9-20). However, the outcomes were inconclusive and the small sample size of each study was underpowered to confirm the correlation. Thus, a larger-scale meta-analysis including all the available studies was required to evaluate the correlation between TLR4 gene and digestive cancer susceptibility. This meta-analysis, of 12 published studies (9-20), with 12 studies for Asp299Gly polymorphism and 8 studies for Thr399Ile polymorphism (10,13,15-20), constitutes a greater probability to reach an evidence-based conclusion.

According to a previously published study (38), between-study heterogeneity is common in the meta-analyses of genetic association studies. In this meta-analysis, significant between-study heterogeneity in dominant and codominant models was also demonstrated, regarding overall digestive and gastric cancer. A series of uncertain factors that differ among studies may explain between-study heterogeneity, e.g., study quality, different sources of population, characteristics of the sample, non-comparable measures of genotyping, variation of the covariate and deviation from HWE in certain studies. In order to investigate the conceivable substantial causes of between-study heterogeneity, meta-regression was performed. Following the exclusion of the studies deviating from HWE in controls, this meta-analysis did not detect any of the above-mentioned covariates as a substantial contributor to between-study heterogeneity.

In addition, it is noteworthy to consider the outlier values of OR that could cause significant effects by chance. Theoretical and empirical evidence has suggested that specific genetic variants causally associated with common diseases have limited effects (risk ratios mostly <2.0) (26,27). A relatively small sample size and possible genotyping errors might also have an impact on this effect. Besides, large effect estimates could be induced by unsteady effect estimations due to low cell counts within each study. Therefore, we conducted a sensitivity analysis, excluding studies with OR>3.0. However, the correlation of the G allele of Asp299Gly and Thr399Ile polymorphisms in the TLR4 gene with digestive, gastric and colorectal cancer risk, was not significant.

There are significant different incidences of Asp299Gly and Thr399Ile polymorphisms between the continents (39). Although we did not find any significant evidence that different continents were responsible for the heterogeneity, we could not exclude continent as a potential contributor to between-study heterogeneity. Moreover, additional factors that were not analyzed in this study should not be considered, e.g., cigarette smoking, alcohol intake, amount of exercise, dietary history and occupation characteristics, since they potentially affect cancer progression.

In this meta-analysis, no significant publication bias in the above-mentioned inherited models was identified, a fact which may result from the limited number of studies included in this meta-analysis. In summary, this meta-analysis has demonstrated that the G allele of Asp299Gly polymorphism in the TLR4 gene is able to increase the risk of gastric cancer. However, due to the fact that potential biases and confounders could not be completely excluded, further large-scale, well-designed, comprehensive studies on various ethnicities, with more detailed individual data, need to be performed in order for these results to be further validated.

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