RESEARCH COMMUNICATION

The Lymphotoxin-α 252 A>G Polymorphism and Breast Cancer: A Meta-analysis

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Abstract

Objective: The aim of this meta-analysis is to evaluate associations between LTA-252 A>G and breast cancer (BC). Methods: Electronic searches of several databases were conducted for all online publications. A total of 7 studies involving 4,625 BC patients and 4,373 controls were identified. Results: This meta-analysis showed no significant association between the LTA-252 A>G polymorphism and BC in overall or Caucasian populations. However, a positive association was found limited to Asian populations. Conclusion: Although there was no significant association found between the LTA-252 A>G polymorphism and BC overall, a positive association was found in Asian populations.

Keywords: Meta-analysis - polymorphism - LTA - breast cancer

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Introduction

Breast cancer (BC) is the third most frequent cancer in the world, which is the largest cause of deaths in the women. Though many studies show that BC onset and progression are multi-step processes resulting from a series of epigenetic, genetic, endocrine and external environmental factors, chronic inflammation was considered to play an important role in BC development. Lymphotoxin-α (LTA, TNF-β) is a cytokine which has a close structural homology and about 30% amino acid sequence identity to TNF-α, which are both recognized by the same widely distributed cellular TNF receptors and have similar effects (Smith et al., 1994). It is produced by diverse kinds of cells including macrophages, NK cells, T and B cells, and tumor cells et al (Anderson et al., 2004) and postulated to play a role in pathogenesis of cancers (Kobayashi et al., 1997; Kohaar et al., 2009). TNF-α and LTA polymorphisms were reported to be associated with cancers (Pooja et al., 2010), which had draw many researchers' attentions to in the breast cancer study. Recently, TNF-α polymorphisms were not found to be associated with the risk of BC in two meta-analysis studies (Fang et al., 2010). However, the results were inconsistent and ambiguous for LTA-252 A>G polymorphism studies (the most investigated site) (Park et al., 2002; Kamali-Sarvestani et al., 2005; Lee et al., 2005; Gaudet et al., 2007; Kohaar et al., 2009; Pooja et al., 2010; Karakus et al., 2011). Because a modest sample size and unified ethnicity of these studies, each of them might not achieve a reliable and stable conclusion, which indicate that a meta-analysis is needed to investigate this issue.

Therefore, we firstly conducted this meta-analysis and combined all current studies to validate whether the LTA-252 A>G polymorphism contribute to BC susceptibility.

Materials and Methods

Publication search

We conducted a comprehensive search by examining several electronic databases (PubMed, EMBase, Cochrane Central Register of Controlled Trials and ISI Web of Science) for all publications on the association between LTA polymorphism and BC through January 2012. The terms were used as follows: breast, cancer/carcinoma/tumor, variant/polymorphism/genotype/SNP, and tumor necrosis factor/LTA/ Lymphotoxin. Two of the authors reviewed all the references of retrieved articles for additional studies. The inclusive studies should meet the following criteria: (1) the outcome should be BC; (2) case-control study; (3) should have BC patient and control groups; and (4) not duplicated studies.

Data extraction

After excluding the studies without controls, this meta-analysis included a total of 16 articles on LTA-252 A>G polymorphism in relation to the risk for BC. Two authors extracted the data independently and in duplicate. Items of author's last name, year of publication, country of origin, ethnicity, genotypes and numbers of cases and

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Table 1. Characteristics of Studies Included in Meta-analysis of LTA 252 A>G Polymorphism and Breast Cancer

Study	Country	Ethnicity	Case	Source of control	Case	Control	Genotyping
Karakus, 2011	Turkey	Caucasian	Age mean: 52.40 (28~82)	Population based Age mean: 47.72 (35~86)	204	204	PCR-RFLP
Guadet, 2007	USA Poland	Caucasian	Age: 20~74	Population based Age: 20~64	3038	2616	Sequencing
Kamali-Sarvestani, 2005	Iran	Asian	Age median: 49.8 (27~85)	Population based Age matched	223	267	PCR-RFLP
Park, 2002	Korea	Asian	Age median: 46 (26~73)	Population based	95	190	PCR-RFLP
Lee, 2005	Korea	Asian	Age mean: 47.9±10.5	Hospital based Age: 46.8±14.0	560	509	Sequencing
Kohaar, 2009	India	Asian	Age mean: 49±9.26	Population based Age: 49.4+12.4	40	150	PCR-RFLP
Pooja, 2011	India	Asian	Age mean: 44.92±13.56	Population based Age: 39±11.30	465	437	Sequencing

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

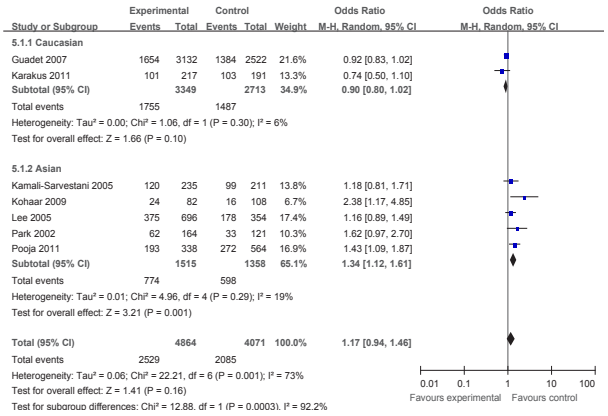

Figure 1. ORs and 95% Confidence Interval (CI) of Breast Cancer for LTA-252 A>G Polymorphism in 7 Studies Using Random-effect Model. GG+GA, GG and GA genotypes of LTA-252 A>G polymorphism; AA, AA genotype of LTA-252 A>G polymorphism; ORs, odds ratios

controls and LTA genotyping method were extracted in each study. The results were compared, disagreements were discussed and consensus was reached.

Statistical analysis

The crude odds ratios (OR) and 95% CI were estimated in a fixed- or random-effects model. If there exists a significant difference of heterogeneity ($P < 0.05$), a random-effects model was selected to pool the data. Otherwise, a fixed-effects model was employed. Heterogeneity among studies was examined with I2 statistic interpreted as the proportion of total variation contributed by between-study variation. Relative influence of each study on pooled estimates was assessed by omitting one study at a time for sensitivity analysis. Funnel plots were employed to evaluate publication bias. Two-sided P-values < 0.05 were considered as statistical significant. All analyses were done using Review manager, version 5.1.

Results

In this article, the associations of LTA-252 A>G polymorphism with BC susceptibility were evaluated using meta-analysis in a wide range of populations. There were a total of 7 studies met the inclusion criteria and included in the study. The detailed characteristics of the studies were shown in Table 1. Totally 4,625 BC patients and 4,373 controls for LTA-252 A>G polymorphism were included in the analyses. Among the studies, there were 2 Caucasus and 5 Asian studies, respectively.

Table 2. Results of Pooled ORs for LTA 252 A>G Polymorphism and Breast Cancer Using Random-Effects Model in the Meta-analysis

Group	N	Participants	OR (95% CI)	P
GG+GA vs AA				
Caucasian	2	6062	0.90(0.80,1.02)	0.1
Asian	5	2873	1.34(1.12,1.61)	0.001
Overall	7	8935	1.17(0.94,1.46)	0.16
GG vs GA+AA				
Caucasian	2	6062	1.40(0.63,3.08)	0.41
Asian	5	1781	1.73(0.70,4.26)	0.23
Overall	7	7843	1.48(0.97,2.27)	0.07
GG vs AA				
Caucasian	2	3374	1.19(0.65,2.16)	0.57
Asian	3	963	1.22(0.88,1.69)	0.17
Overall	5	4337	1.86(0.77,4.51)	0.41
GA vs AA				
Caucasian	2	5401	0.82(0.61,1.10)	0.18
Asian	3	1504	1.86(0.77,4.51)	0.12
Overall	5	6905	0.94(0.86,1.03)	0.82
G vs A				
Caucasian	2	12124	0.96(0.89,1.04)	0.29
Asian	4	3942	1.45(1.03,2.03)	0.03
Overall	6	16066	1.21(0.98,1.49)	0.08

For we found the evidence of heterogeneity among the subgroups and overall studies for LTA-252 A>G polymorphism, a random-effect model was employed in the ORs calculation (Table 2). There was non-significant relationship between LTA-252 A>G polymorphism and BC in overall studies (AA+AG vs GG: OR = 1.17, 95% CI = 0.94 - 1.46, P = 0.16). The ORs (95% CI) were 0.90 (0.80 - 1.02) and 1.34 (1.12 - 1.61) in Caucasian and Asian populations, respectively. Summary ORs (95% CI) for GG vs GA+AA, GG vs AA, GA vs AA genotypes, and G vs A allell were 1.48 (0.97 - 2.27), 1.86 (0.77 - 4.51), 0.94 (0.86 - 1.03) and 1.21 (0.98 - 1.49) in the overall population, respectively. Importantly, the significant associations were limited in GG+GA vs AA and G vs A in Asian populations. The forest plots of the meta-analysis for LTA-252 A>G polymorphism were shown in Figure 1 and 2.

Table 3. Sensitivity Analysis with Each Study Omitted in Random-effects Model (OR, 95% CI)

Study	GG+GA vs AA	GG vs GA+AA	GG vs AA	GA vs AA	G vs A
Guadet	1.25(0.98,1.59)	1.84(0.88,3.83)	1.85(0.94,3.66)	1.02(0.77,1.35)	1.32(0.99,1.74)
Karakus	1.26(0.99,1.60)	1.37(0.88,2.14)	1.44(0.88,2.34)	1.03(0.87,1.22)	1.28(0.98,1.64)
Kamali-Sarvestani	1.18(0.92,1.52)	1.57(0.95,2.59)	1.56(0.92,2.63)	0.95(0.76,1.18)	1.23(0.96,1.58)
Kohaar	1.11(0.90,1.36)	-	-	-	1.10(0.93,1.31)
Lee	1.19(0.91,1.56)	1.85(0.91,3.77)	1.80(0.86,3.74)	0.92(0.76,1.12)	1.29(0.96,1.73)
Park	1.13(0.90,1.41)	1.07(0.88,1.32)	1.03(0.89,1.26)	0.96(0.78,1.19)	1.10(0.92,1.32)
Pooja	1.12(0.898,1.41)	-	-	-	-
None	1.17(0.94,1.46)	1.48(0.97,2.27)	1.49(0.96,2.32)	0.98(0.81,1.18)	1.21(0.98,1.49)

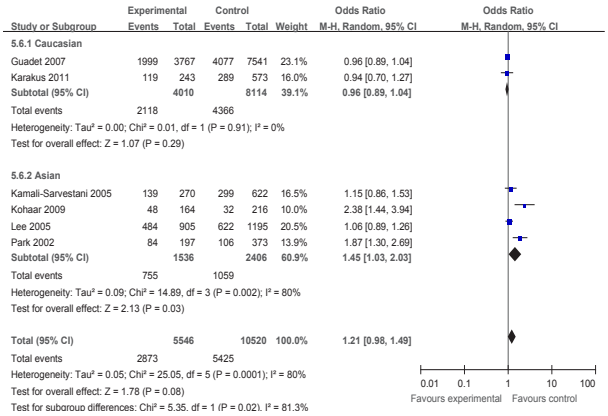

Figure 2. ORs and 95% Confidence Interval (CI) of Breast Cancer for LTA-252 Polymorphism in 6 Studies Using Random-effect Model. A, A alle of LTA-252 A>G polymorphism; G, G alle of LTA-252 A>G polymorphism; ORs, odds ratios

Sub-grouped analyses were also conducted with studies stratified by hospital-based control populations and genotyping methods in the overall populations. However, after sub-grouping the studies by hospital-based controls and genotyping methods, the overall heterogeneity still exist and no significant associations were found.

To further strengthen the confidence for the results, we conducted a sensitivity analysis. This analysis confirmed the stability of the null association between LTA-252 A>G polymorphism and BC (Table 3). Exclusion of individual studies did not modify the estimates much, with pooled ORs ranging from 0.92 to 1.26 without significant influence. However, the positive association in Asian population still remains significant (data not shown). The shape of the funnel plots was symmetrical, suggesting there was no evidence of publication bias among the studies (Figure 3 A and B).

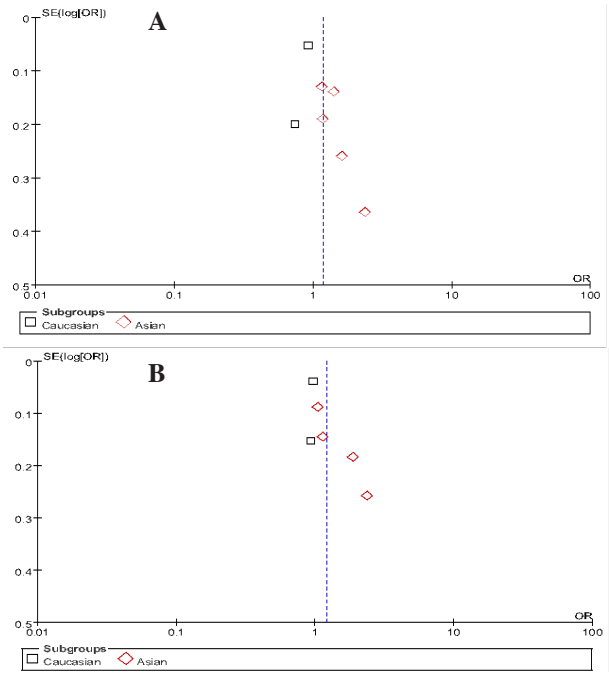

Figure 3. Funnel Plots Analysis to Detect Publication Bias for LTA-252 A>G (GG+GA vs AA) (A) and (G vs A) (B) polymorphism. Each point represents an independent study for the indicated association

Discussion

Inflammation plays an important role in the pathogenesis of cancer. LTA is a crucial proinflammatory cytokine, which has multiple functions in immune system as TNF-α. The LTA +252G allele has been reported to increased LTA production by phytohemagglutinin-activated mononuclear cells in vitro and have higher secretary capacity and circulatory concentrations of TNF-α (Messer et al., 1991; Pociot et al., 1993; Kohaar et al., 2009).

As the investigations on LTA-252 A>G polymorphism and BC studies have been published, however, the results were still unclear. Because the limitations of their small sample size and unified ethnicity, we conducted this meta-analysis to achieve a more reliable and comprehensive conclusion to provide further insights regarding this debated subject.

With totally 4,625 BC patients and 4,373 controls for LTA-252 A>G polymorphism included, this meta-analysis found no significant association between LTA-252 A>G polymorphism and BC susceptibility in the overall population. However, after the analyses stratified by ethnicity, we found LTA-252 A>G polymorphism was positively associated with BC in Asian populations.

Because there was significant heterogeneity among overall populations, we selected random-effects model. As a sensitivity analysis was performed by removing one study for each time and re-running the model to determine the effect on the overall estimate, the estimates did not change significantly, which strongly supported the findings in this meta-analysis (Table 3). Heterogeneity, however, still existed in Asian subgroups when each study was excluded in the sensitivity analysis. When sub-grouping the studies with hospital-based controls and genotyping methods, heterogeneity did not disappeared in the overall population. Above results indicated that variability in frequency of these LTA-252 A>G polymorphisms between the Asian and Caucasian populations may be the source

of heterogeneity. No publication bias was shown, also strengthening our results.

Although the non-significant association between LTA-252 A>G polymorphism and BC in Caucasian population was stable, there are only two Caucasian studies in this meta-analysis, which suggested that this conclusion should be interpreted with caution. As more studies published, the positive results may be found significant in the future.

Additionally, there are two points should be concerned for the association between LTA-252 A>G polymorphism and BC susceptibility in the overall population. On one hand, cancer is a multi-factorial disease with complex interactions of environmental and genetic factors (Shih et al., 2006). Environmental factors may play a key role in cancer development, for example physical activity, food selection, infection and carcinogens exposure. Thus, not considering these important factors may affect the detection of the LTA-252 A>G polymorphism's independent role in cancer development. On the other hand, because the interactions of cytokines are complex and their variants may interfere with each others' bio-functions. Only investigating single variant could cover the true association of LTA-252 A>G polymorphism and cancer. Therefore, other variants as risk factors should be investigated as covariants to determine each variant true effect.

This meta-analysis firstly combined all publications available from the case-control studies and significantly increased the statistical power. However, the numbers of the studies were still small. When more studies are available, an updated meta-analysis should be necessary for a more reliable evaluation on their associations. We cannot neglect those potentially critical factors above and should still be cautious for current results.

In conclusion, this meta-analysis detected non-significant association between LTA-252 A>G polymorphism and BC in overall population; however, LTA-252 A>G polymorphism was positively associated with BC in Asian population. Because case-control studies cannot provide a causal association and the number of studies in Caucasian population is small, well-designed cohort studies are needed for further investigations in different ethnic populations.

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