

# Association between FOXP3 promoter polymorphisms and cancer risk: A meta-analysis

LING-LING JIANG<sup>1</sup> and LI-WEI RUAN<sup>2</sup>

<sup>1</sup>Department of Neurology, Suzhou Municipal Hospital, Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, Jiangsu 215002; <sup>2</sup>Department of General Surgery (Breast and Thyroid Surgery), Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing, Zhejiang 312000, P.R. China

Received February 13, 2014; Accepted September 11, 2014

DOI: 10.3892/ol.2014.2585

**Abstract.** Epidemiological studies have been conducted to investigate the association between the FOXP3 promoter polymorphisms, rs3761549 and rs3761548, and the risk of cancer. However, the results from these studies have been controversial. In order to obtain a more precise conclusion of this association, the present meta-analysis was performed. The odds ratio (OR) and 95% confidence interval (95% CI) values were used to assess any correlations between the data. Overall, the rs3761549 (C>T) and rs3761548 (C>A) polymorphisms of the FOXP3 gene were not associated with the cancer risk in an Asian population. In the subgroup analyses based on cancer type, no significant associations were identified between these two polymorphisms and breast cancer. However, the results altered when the analyses were restricted to hepatocellular carcinoma (HCC) and non-small cell lung cancer (NSCLC) (for rs3761549: TT+CT vs. CC OR, 0.52, 95% CI, 0.38-0.72; TC vs. CC OR, 0.25, 95% CI, 0.16-0.39; T vs. C OR, 0.76, 95% CI, 0.59-0.97. For rs3761548: AA vs. AC+CC OR, 3.20, 95% CI 1.76-5.81; AA+AC vs. CC OR, 2.56, 95% CI, 1.75-3.76; AA vs. CC OR, 4.41, 95% CI, 2.36-8.25; AC vs. CC OR, 2.15, 95% CI, 1.42-3.25; A vs. C OR, 2.32, 95% CI, 1.74-3.10). The present meta-analysis indicates that the FOXP3 rs3761549 (C>T) and rs3761548 (C>A) polymorphisms are not associated with the risk of breast cancer, but with the risk of HCC and NSCLC. Therefore, a study with a larger sample size is required to further evaluate this association.

## Introduction

Cancer is a worldwide public health problem, which results from a complex interaction between environmental and

genetic factors (1). Several polymorphic genes that are directly involved in tumorigenesis have also been proposed to contribute to the individual susceptibility to cancer (2).

The host immune defense has been shown to play a vital role in modulating human carcinogenesis (3). Regulatory T cells aid in keeping the balance between immunity and autotolerance, and are mainly characterized by CD4<sup>+</sup>/FOXP3<sup>+</sup> or CD4<sup>+</sup>/CD25<sup>+</sup>/FOXP3<sup>+</sup> expression. FOXP3 belongs to the forkhead family of transcription factors, and is involved in the regulation, activation and differentiation of T cells (4). In fact, the absence of a functional FOXP3 gene product has been revealed to cause an abnormal production of regulatory T cells (5). In addition, the loss of expression and somatic mutation of the human FOXP3 gene has been identified in human prostate and breast cancers. This suggests that FOXP3 may be a tumor suppressor and that inactivation of the FOXP3 gene may contribute to the development of cancer in humans (6,7).

The FOXP3 gene is positioned at the Xp11.23 locus on the X chromosome and encodes the FOXP3 protein, which is expressed in epithelial cells from various organs, such as the lungs and the thymus (8-11). The promoter polymorphisms in the FOXP3 gene are considered to affect FOXP3 production and activity. The FOXP3 gene rs3761549 (C>T) and rs3761548 (C>A) polymorphisms, located on the promoter region of the FOXP3 gene, are two of the most common single nucleotide polymorphisms. Previous studies have investigated the association between the FOXP3 rs3761549 and rs3761548 polymorphisms and the cancer risk, however, they have yielded conflicting results (12-16). Therefore, the present meta-analysis was performed to evaluate the role of these two polymorphisms and their association with the risk of cancer.

## Materials and methods

**Publication search and inclusion criteria.** A comprehensive literature search, using the keywords 'FOXP3', 'polymorphism' and 'tumor or cancer', was performed using the PubMed, EMBASE and Chinese Wanfang databases (last search updated in February 10, 2014). Additional eligible studies were identified by manually searching the reference lists of reviews and original articles. In the event that data

---

*Correspondence to:* Dr Li-Wei Ruan, Department of General Surgery (Breast and Thyroid Surgery), Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, 568 Zhongxing North Road, Shaoxing, Zhejiang 312000, P.R. China  
E-mail: Liwei\_Ruan@126.com

**Key words:** FOXP3, polymorphism, cancer, meta-analysis

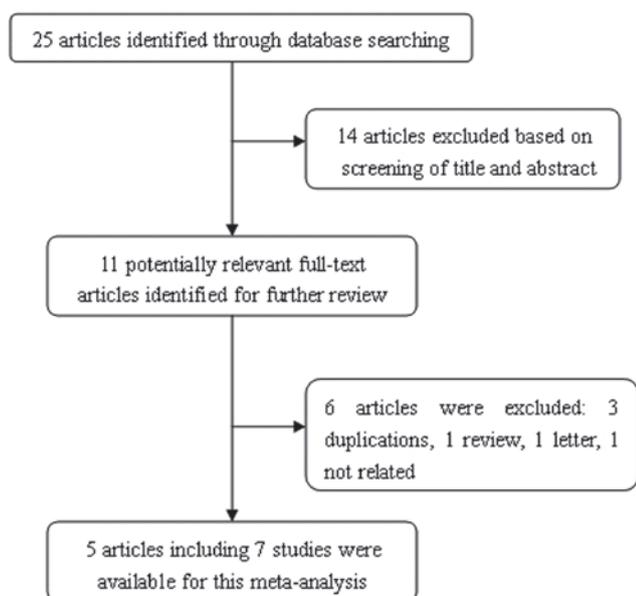


Figure 1. Process of identifying included studies.

were published in more than one article, only studies with the largest sample size were selected for. The selection criteria to identify an eligible study were as follows: i) Investigation of the rs3761549 (C>T) and rs3761548 (C>A) polymorphisms of the FOXP3 gene and cancer risk; ii) the use of a case-control design, based on unrelated individuals; and iii) sufficient genotype distributions for cases and controls, so that an odds ratio (OR) with a 95% confidence interval (CI) could be assessed.

**Data extraction.** The two authors independently reviewed and extracted the required data. Disagreements were resolved through discussion among the authors to achieve a consensus. The following information was recorded for each study: First author, year of publication, country, ethnicity, cancer type and number of genotypes (Table I).

**Statistical analysis.** The OR corresponding to the 95% CI was used to assess the association between the FOXP3 polymorphisms and the risk of cancer. In addition to this comparison among all subjects, a stratified analysis by cancer type was also performed. The statistical heterogeneity among studies was assessed using  $I^2$  statistics and the Q-test (17). In the absence of any obvious heterogeneity, the fixed-effects model (the Mantel-Haenszel method) was applied to estimate the summary OR. Otherwise, the random-effects model (the DerSimonian and Laird method) was used (18,19). Sensitivity analysis was performed to identify the effect that the data from each study had on the pooled OR. Finally, any publication bias was evaluated using a funnel plot. All of the statistical tests were performed using RevMan 5.0 software (The Cochrane Collaboration, Oxford, UK).

## Results

The process of identifying suitable studies is shown in Fig. 1. A total of five studies (12-16), including 3,275 cases and

Table I. Characteristics of studies included in the present meta-analysis.

First author (ref.)	Year	Country	Ethnicity	Cancer type	rs3761549		rs3761548	
					Case (TT/CT/CC)	Control (TT/CT/CC)	Case (AA/AC/CC)	Control (AA/AC/CC)
Chen <i>et al</i> (12)	2013	China	Asian	59/28/301	41/88/233	-	-	
He <i>et al</i> (13)	2013	China	Asian	-	-	37/80/75	18/80/161	
Jahan <i>et al</i> (14)	2013	India	Asian	0/198/4	0/128/2	27/160/15	20/106/4	
Raskin <i>et al</i> (15)	2009	Israel	Asian	-	-	320/722/402	303/763/392	
Zheng <i>et al</i> (16)	2013	China	Asian	32/283/734	34/290/767	38/338/673	30/342/719	

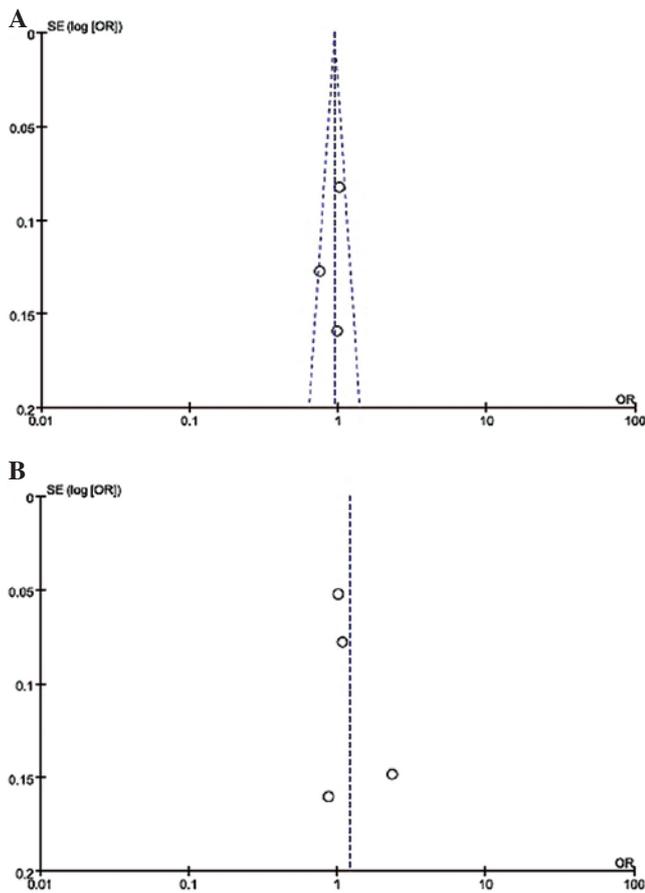


Figure 2. Funnel plot of the meta-analysis data to demonstrate the associations between the FOXP3 promoter polymorphisms and cancer risk. (A) rs3761549 T vs. C and (B) rs3761548 A vs. C. OR, odds ratio; SE, standard error.

3,300 controls, were included in the present meta-analysis. All of the selected studies were based on Asian populations (Table I). The results of the pooled analysis revealed no significant association between the FOXP3 gene polymorphisms and the cancer risk (for rs3761549: TT vs. CT+CC OR, 1.20, 95% CI, 0.87-1.66; TT+CT vs. CC OR, 0.74, 95% CI, 0.41-1.33; TT vs. CC OR, 1.06, 95% CI, 0.76-1.46; TC vs. CC OR, 0.56, 95% CI, 0.17-1.80; T vs. C OR, 0.94, 95% CI, 0.83-1.06. For rs3761548: AA vs. AC+CC OR, 1.37, 95% CI, 0.87-2.16; AA+AC vs. CC OR, 1.18, 95% CI, 0.79-1.78; AA vs. CC OR, 1.36, 95% CI, 0.67-2.77; AC vs. CC OR, 1.11, 95% CI, 0.79-1.58; A vs. C OR, 1.21, 95% CI, 0.90-1.62). Further subgroup analysis was conducted based on cancer type, however, no association between the FOXP3 gene polymorphisms and the risk of breast cancer was revealed (for rs3761549: TT vs. CT+CC OR, 0.98, 95% CI, 0.60-1.60; TT+CT vs. CC OR, 1.01, 95% CI, 0.84-1.22; TT vs. CC OR, 0.98, 95% CI, 0.60-1.61; TC vs. CC OR, 1.02, 95% CI, 0.84-1.23; T vs. C OR, 1.01, 95% CI, 0.87-1.16. For rs3761548: AA vs. AC+CC OR, 1.09, 95% CI, 0.93-1.28; AA+AC vs. CC OR, 1.00, 95% CI, 0.88-1.12; AA vs. CC OR, 1.04, 95% CI, 0.86-1.26; AC vs. CC OR, 0.97, 95% CI, 0.86-1.10; A vs. C OR, 1.02, 95% CI, 0.94-1.11). However, statistical associations were observed with respect to hepatocellular carcinoma

(HCC) and non-small cell lung cancer (NSCLC) (for rs3761549: TT+CT vs. CC OR, 0.52, 95% CI, 0.38-0.72; TC vs. CC OR, 0.25, 95% CI, 0.16-0.39; T vs. C: OR, 0.76, 95% CI, 0.59-0.97. For rs3761548: AA vs. AC+CC OR, 3.20, 95% CI, 1.76-5.81; AA+AC vs. CC OR, 2.56, 95% CI, 1.75-3.76; AA vs. CC OR, 4.41, 95% CI, 2.36-8.25; AC vs. CC OR, 2.15, 95% CI, 1.42-3.25; A vs. C OR, 2.32, 95% CI, 1.74-3.10) (Table II). The funnel plot, which assessed publication bias of the literature, appeared symmetrical in all of the genetic models (Fig. 2).

## Discussion

The characterization and identification of genes involved in the genetic predisposition and progression of cancer are critical for clinical practice and basic medical research. FOXP3 is an immunological regulator, and is able to repress oncogenes whilst activating additional tumor suppressor genes (6,20-22). FOXP3-mediated gene regulation follows the histone code of gene activation and suppression and alters histone modifications by binding to gene promoters (23,24). Epidemiological studies suggest that the FOXP3 promoter polymorphisms, rs3761549 and rs376154, are associated with the cancer risk. However, the results from these studies are conflicting. To provide a more detailed overview of the association, five genetic models were used in the current meta-analysis.

To the best of our knowledge, this was the first meta-analysis to provide comprehensive insight into the association between the FOXP3 polymorphisms and the risk of cancer. It was identified that the FOXP3 rs3761549 (C>T) and rs3761548 (C>A) polymorphisms were not associated with the risk of cancer among an Asian population. In addition, subgroup analysis revealed that the FOXP3 gene rs3761549 (C>T) and rs3761548 (C>A) polymorphisms were not associated with the risk of breast cancer. However, the rs3761549 (C>T) and rs3761548 (C>A) polymorphisms were linked with the risk of HCC and NSCLC, respectively. The results therefore indicated that the rs3761549 (C>T) and rs3761548 (C>A) polymorphisms may have a varying effect on carcinogenesis within different organs. However, these findings must be viewed with caution, since studies on HCC and NSCLC are rare. Therefore, the results from the present study may be due to chance.

There were certain limitations of this meta-analysis. Firstly, a relatively small number of studies and subjects were included, which could reduce the statistical power of the analysis. Secondly, the results were based on unadjusted estimates. A more precise analysis could be conducted if individual data were available. Thirdly, all published studies were based on Asian populations. Therefore, the results of this meta-analysis may be applicable to the specified ethnicity alone.

In conclusion, the present study demonstrated that the rs3761549 (C>T) and rs3761548 (C>A) polymorphisms in the promoter region of the FOXP3 gene were not associated with breast cancer, but instead were associated with HCC and NSCLC. Therefore, a future study that consists of a larger sample size is required to further evaluate this association.

Table II. Meta-analysis data of the associations between the FOXP3 promoter polymorphisms and the cancer risk in all genetic models.

A, rs3761549 polymorphism											
Variables	TT vs. CT+CC		TT+CT vs. CC		TT vs. CC		TC vs. CC		T vs. C		
	OR (95% CI)	P-value									
Total	1.20 (0.87-1.66)	0.28	0.74 (0.41-1.33)	0.01	1.06 (0.76-1.46)	0.71	0.56 (0.17-1.80)	0.01	0.94 (0.83-1.06)	0.15	
Breast cancer	0.98 (0.60-1.60)	-	1.01 (0.84-1.22)	0.76	0.98 (0.60-1.61)	-	1.02 (0.84-1.23)	0.75	1.01 (0.87-1.16)	0.92	
HCC	1.40 (0.92-2.15)	-	<b>0.52 (0.38-0.72)</b>	-	1.11 (0.72-1.72)	-	<b>0.25 (0.16-0.39)</b>	-	<b>0.76 (0.59-0.97)</b>	-	
B, rs3761548 polymorphism											
Variables	AA vs. AC+CC		AA+AC vs. CC		AA vs. CC		AC vs. CC		A vs. C		
	OR (95% CI)	P-value									
Total	1.37 (0.87-2.16)	0.01	1.18 (0.79-1.78)	0.01	1.36 (0.67-2.77)	0.01	1.11 (0.79-1.58)	0.01	1.21 (0.90-1.62)	0.01	
Breast cancer	1.09 (0.93-1.28)	0.53	1.00 (0.88-1.12)	0.16	1.04 (0.86-1.26)	0.14	0.97 (0.86-1.10)	0.18	1.02 (0.94-1.11)	0.45	
NSCLC	<b>3.20 (1.76-5.81)</b>	-	<b>2.56 (1.75-3.76)</b>	-	<b>4.41 (2.36-8.25)</b>	-	<b>2.15 (1.42-3.25)</b>	-	<b>2.32 (1.74-3.10)</b>	-	

Bold indicates significant results for the association between FOXP3 promoter polymorphisms and cancer risk. HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; OR, odds ratio; 95% CI, 95% confidence interval; P-value, P-value for heterogeneity.

## References

1. Gao X, Huang M, Liu L, *et al*: Insertion/deletion polymorphisms in the promoter region of BRM contribute to risk of hepatocellular carcinoma in Chinese populations. *PLoS One* 8: e55169, 2013.
2. Ying H, Wang J and Gao X: CCL5-403, CCR5-59029, and Delta32 polymorphisms and cancer risk: a meta-analysis based on 20,625 subjects. *Tumour Biol* 35: 5895-5904, 2014.
3. Schneeberger A, Koszik F and Stingl G: Immunologic host defense in melanoma: delineation of effector mechanisms involved and of strategies for the augmentation of their efficacy. *J Invest Dermatol* 105 (Suppl 1): S110-S116, 1995.
4. Hori S, Nomura T and Sakaguchi S: Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299: 1057-1061, 2003.
5. Roncador G, Garcia JF, Garcia JF, *et al*: FOXP3, a selective marker for a subset of adult T-cell leukemia/lymphoma. *Leukemia* 19: 2247-2253, 2005.
6. Wang L, Liu R, Li W, *et al*: Somatic single hits inactivate the X-linked tumor suppressor FOXP3 in the prostate. *Cancer Cell* 16: 336-346, 2009.
7. Zuo T, Liu R, Zhang H, *et al*: FOXP3 is a novel transcriptional repressor for the breast cancer oncogene SKP2. *J Clin Invest* 117: 3765-3773, 2007.
8. Bennett CL, Brunkow ME, Ramsdell F, *et al*: A rare polyadenylation signal mutation of the FOXP3 gene (AAUAAA→AAUGAA) leads to the IPEX syndrome. *Immunogenetics* 53: 435-439, 2001.
9. Gupta S, Joshi K, Wig JD and Arora SK: Intratumoral FOXP3 expression in infiltrating breast carcinoma: Its association with clinicopathologic parameters and angiogenesis. *Acta Oncol* 46: 792-797, 2007.
10. Karanikas V, Speletas M, Zamanakou M, *et al*: FOXP3 expression in human cancer cells. *J Transl Med* 6: 19, 2008.
11. Katoh H, Zheng P and Liu Y: Signalling through FoxP3 as an X-linked tumor suppressor. *Int J Biochem Cell Biol* 42: 1784-1787, 2010.
12. Chen Y, Zhang H, Liao W, *et al*: FOXP3 gene polymorphism is associated with hepatitis B-related hepatocellular carcinoma in China. *J Exp Clin Cancer Res* 32: 39, 2013.
13. He YQ, Bo Q, Yong W, Qiu ZX, Li YL and Li WM: FoxP3 genetic variants and risk of non-small cell lung cancer in the Chinese Han population. *Gene* 531: 422-425, 2013.
14. Jahan P, Ramachander VR, Maruthi G, Nalini S, Latha KP and Murthy TS: Foxp3 promoter polymorphism (rs3761548) in breast cancer progression: a study from India. *Tumour Biol* 35: 3785-3791, 2014.
15. Raskin L, Rennert G and Gruber SB: FOXP3 germline polymorphisms are not associated with risk of breast cancer. *Cancer Genet Cytogenet* 190: 40-42, 2009.
16. Zheng J, Deng J, Jiang L, *et al*: Heterozygous genetic variations of FOXP3 in Xp11.23 elevate breast cancer risk in Chinese population via skewed X-chromosome inactivation. *Hum Mutat* 34: 619-628, 2013.
17. Higgins JP and Thompson SG: Quantifying heterogeneity in a meta-analysis. *Stat Med* 21: 1539-1558, 2002.
18. Mantel N and Haenszel W: Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 22: 719-748, 1959.
19. DerSimonian R and Laird N: Meta-analysis in clinical trials. *Control Clin Trials* 7: 177-188, 1986.
20. Zuo T, Wang L, Morrison C, *et al*: FOXP3 is an X-linked breast cancer suppressor gene and an important repressor of the HER-2/ErbB2 oncogene. *Cell* 129: 1275-1286, 2007.
21. Liu R, Wang L, Chen G, *et al*: FOXP3 up-regulates p21 expression by site-specific inhibition of histone deacetylase 2/histone deacetylase 4 association to the locus. *Cancer Res* 69: 2252-2259, 2009.
22. Li W, Wang L, Katoh H, *et al*: Identification of a tumor suppressor relay between the FOXP3 and the Hippo pathways in breast and prostate cancers. *Cancer Res* 71: 2162-2171, 2011.
23. Katoh H, Qin ZS, Liu R, *et al*: FOXP3 orchestrates H4K16 acetylation and H3K4 trimethylation for activation of multiple genes by recruiting MOF and causing displacement of PLU-1. *Mol Cell* 44: 770-784, 2011.
24. Marson A, Kretschmer K, Frampton GM, *et al*: FoxP3 occupancy and regulation of key target genes during T-cell stimulation. *Nature* 445: 931-935, 2007.