

## CTLA-4 and MDR1 polymorphisms increase the risk for ulcerative colitis: A meta-analysis

Jia-Jun Zhao, Di Wang, Hui Yao, Da-Wei Sun, Hong-Yu Li

Jia-Jun Zhao, Di Wang, Hui Yao, Da-Wei Sun, Hong-Yu Li, Department of Gastroenterology, the General Hospital of Shenyang Military Region, Shenyang 110016, Liaoning Province, China

**Author contributions:** Zhao JJ and Wang D performed the majority of experiments; Yao H provided vital reagents and analytical tools and were also involved in editing the manuscript; Sun DW co-ordinated and provided the collection of all the human material in addition to providing financial support for this work; Li HY designed the study and wrote the manuscript.

**Conflict-of-interest statement:** The declaration of the authors reveals that they have not received any financial payments or other benefits from any commercial entity associated with the subject of this article.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** Dr. Hong-Yu Li, Department of Gastroenterology, the General Hospital of Shenyang Military Region, No. 83, Wenhua Road, Shenhe District, Shenyang 110016, Liaoning Province, China. [lihongyu0913@126.com](mailto:lihongyu0913@126.com)  
Telephone: +86-24-28851113  
Fax: +86-24-28851113

Received: January 6, 2015  
Peer-review started: January 7, 2015  
First decision: January 22, 2015  
Revised: February 26, 2015  
Accepted: May 4, 2015  
Article in press: May 4, 2015  
Published online: September 14, 2015

### Abstract

**AIM:** To evaluate the correlations between cytotoxic T lymphocyte-associated antigen-4 (*CTLA-4*) and multi-drug resistance 1 (*MDR1*) genes polymorphisms with ulcerative colitis (UC) risk.

**METHODS:** PubMed, EMBASE, Web of Science, Cochrane Library, CBM databases, Springerlink, Wiley, EBSCO, Ovid, Wanfang database, VIP database, China National Knowledge Infrastructure, and Weipu Journal databases were exhaustively searched using combinations of keywords relating to *CTLA-4*, *MDR1* and UC. The published studies were filtered using our stringent inclusion and exclusion criteria, the quality assessment for each eligible study was conducted using Critical Appraisal Skill Program and the resultant high-quality data from final selected studies were analyzed using Comprehensive Meta-analysis 2.0 (CMA 2.0) software. The correlations between SNPs of *CTLA-4* gene, *MDR1* gene and the risk of UC were evaluated by OR at 95%CI. *Z* test was carried out to evaluate the significance of overall effect values. Cochran's *Q*-statistic and *I*<sup>2</sup> tests were applied to quantify heterogeneity among studies. Funnel plots, classic fail-safe *N* and Egger's linear regression test were inspected for indication of publication bias.

**RESULTS:** A total of 107 studies were initially retrieved and 12 studies were eventually selected for meta-analysis. These 12 case-control studies involved 1860 UC patients and 2663 healthy controls. Our major result revealed that single nucleotide polymorphisms (SNPs) of *CTLA-4* gene rs3087243 G > A and rs231775 G > A may increase the risk of UC (rs3087243 G > A: allele model: OR = 1.365, 95%CI: 1.023-1.822, *P* = 0.035; dominant model: OR = 1.569, 95%CI: 1.269-1.940, *P* < 0.001; rs231775 G > A: allele model: OR = 1.583, 95%CI: = 1.306-1.918, *P* < 0.001; dominant model: OR = 1.805,

95%CI: 1.393-2.340,  $P < 0.001$ ). In addition, based on our result, SNPs of *MDR1* gene rs1045642 C > T might also confer a significant increases for the risk of UC (allele model: OR = 1.389, 95%CI: 1.214-1.590,  $P < 0.001$ ; dominant model: OR = 1.518, 95%CI: 1.222-1.886,  $P < 0.001$ ).

**CONCLUSION:** *CTLA-4* gene rs3087243 G > A and rs231775 G > A, and *MDR1* gene rs1045642 C > T might confer an increase for UC risk.

**Key words:** Ulcerative colitis; Cytotoxic T lymphocyte-associated antigen-4; Multi-drug resistance 1; rs3087243 G > A; rs231775 G > A; rs1045642 C > T; Polymorphism; Meta-analysis

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** To evaluate the correlations between cytotoxic T lymphocyte-associated antigen-4 (*CTLA-4*) and multi-drug resistance 1 (*MDR1*) genes polymorphisms with ulcerative colitis (UC) risk. *CTLA-4* gene rs3087243 G > A and rs231775 G > A, and *MDR1* gene rs1045642 C > T might confer an increases for UC risk.

Zhao JJ, Wang D, Yao H, Sun DW, Li HY. *CTLA-4* and *MDR1* polymorphisms increase the risk for ulcerative colitis: A meta-analysis. *World J Gastroenterol* 2015; 21(34): 10025-10040 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i34/10025.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i34.10025>

## INTRODUCTION

Ulcerative colitis (UC) is known as an idiopathic, chronic inflammatory disease of the large intestine, frequently involving the rectum, and characterized by continuous inflammation and ulceration of intestinal mucosa and submucosa<sup>[1]</sup>. In the United States, UC affects approximately 500000 individuals with an incidence of 8-12 per 100000 populations per year and the incidence has remained relatively constant over the last five decades<sup>[2]</sup>. Crohn's disease (CD) and UC are two forms of inflammatory bowel diseases (IBD), and while CD can impact any segment of the gastrointestinal tract, UC pathology is restricted to the colon<sup>[3]</sup>. The precise etiology of UC remains unknown, but factors such as the host immune system, other genetic factors, and environmental factors, contribute to the occurrence of UC<sup>[4,5]</sup>. Typical symptoms of UC include abdominal cramping, rectal bleeding and persistent bloody diarrhea, and other symptoms such as severe fecal urgency resulting from reduced rectal compliance, irritability, general malaise, incontinence, and weight loss are also common<sup>[6]</sup>. UC is treated in clinics with azathioprine,

mesalamine, glucocorticoids, and anti-tumor necrosis factor agents (infliximab and adalimumab)<sup>[7]</sup>. Recently, single nucleotide polymorphisms (SNPs) of Cytotoxic T lymphocyte-associated antigen-4 (*CTLA-4*) and multi-drug resistance 1 (*MDR1*) genes were found to be associated with the pathogenesis of UC<sup>[8,9]</sup>.

*CTLA-4* plays a crucial role in the immune system inducing immune tolerance and is an essential negative regulator of T cell-mediated immune response<sup>[10]</sup>. *CTLA-4* usually functions as a co-inhibitory molecule interacting with B7.1 (CD80) and B7.2 (CD86) expressed on antigen-presenting cells<sup>[11]</sup>. *CTLA-4* gene encodes a 40-kDa transmembrane *CTLA-4* glycoprotein and the gene is located on chromosome 2q33 in humans<sup>[12]</sup>. *CTLA-4* dampens the signal transduction in T cells in the presence of antigen presenting cells, and downregulation of *CTLA-4* expression is implicated in T cell associated autoimmunity and lymphoproliferative diseases<sup>[13]</sup>. *MDR1*, also called ATP-binding cassette subfamily B member 1 (*ABCB1*), is extremely important in multidrug resistance of cancer cells and therapy effectiveness in several other disorders<sup>[14]</sup>. The *MDR1* gene is located on chromosome 7q21.1 and encodes a glycoprotein of 170 kDa<sup>[15]</sup>. *MDR1* was originally identified as a gene amplified in multiple drug-resistant cells, and its product, P-gp, plays an important role in drug resistance<sup>[16]</sup>. Previous studies have proposed that some SNPs of *CTLA-4* gene, such as rs3087243 G > A and rs231775 G > A, and SNPs of *MDR1* gene rs1045642 C > T may increase the risk of UC<sup>[17,18]</sup>. However, these associations have not been confirmed, and contradictory data exists in different populations<sup>[19,20]</sup>. In order to address this relationship further, we undertook a meta-analysis based approach to evaluate the associations of SNPs of *CTLA-4* and *MDR1* genes with the risk of UC, by pooling all relevant published data.

## MATERIALS AND METHODS

### Search strategy

An extensive literature search for relevant studies was conducted on PubMed, EMBASE, Web of Science, Cochrane Library, CBM databases, Springerlink, Wiley, EBSCO, Ovid, Wanfang database, VIP database, China National Knowledge Infrastructure (CNKI), and Weipu Journal databases from their inception through to October 1<sup>st</sup>, 2014. We used the following keywords and MeSH terms: "costimulatory and inhibitory t-cell receptors" or "CTLA-4 antigen" or "cytotoxic t-lymphocyte-associated antigen 4" or "CD152 antigen" or "cytotoxic t lymphocyte antigen 4" OR "CTLA-4" and ("colitis, ulcerative" or "idiopathic proctocolitis" or "ulcerative colitis" or "Colitis gravis" or "inflammatory bowel disease, ulcerative colitis type"); "genes, MDR" or "ABCB1" or "MDR1" or "multidrug resistance 1" or "ABCB1 protein, human" or "MDR" and ("colitis, ulcerative" or "idiopathic proctocolitis" or

“ulcerative colitis” or “colitis gravis” or “Inflammatory bowel disease, ulcerative colitis type”). All study types with no language restrictions were included in our search. Manual searches were carried on to retrieve other cross-references.

### **Inclusion and exclusion criteria**

Identified articles were reviewed in their entirety and selected if they met the following inclusion criteria: (1) study type: case-control studies; (2) research topic: correlations between SNPs of *CTLA-4* gene, *MDR1* gene and the risk of UC; (3) subject investigated: UC patients in the case group and normal controls in the control group; (4) end indicators: all the included studies provided complete data such as: age, country, ethnicity, language, detection method, SNP site information; and (5) studies were published in Chinese or in English. The exclusion criteria were: (1) unclear diagnostic basis for subjects investigated; (2) animal research; (3) studies with insufficient data; and (4) duplicate publications.

### **Data extraction and quality assessment**

Literature screening was performed by two independent reviewers, based on a predetermined data collection table. Relevant information including first author, published year, country, ethnicity, sample, disease, source of controls, gender, genotype method, gene, SNP were extracted from the eligible literature. Any disagreements in study selection between the two reviewers were resolved through discussion by all authors until consensus was obtained. The quality assessment for each eligible study was conducted using Critical Appraisal Skill Program (CASP) (<http://www.casp-uk.net/#!casp-tools-checklists/c18f8>).

### **Statistical analysis**

The statistical methods of this study were reviewed by Zhou LP from Clinical Laboratory and Department of Clinical Epidemiology, the First Affiliated Hospital of China Medical University, See Supplement S1. Comprehensive Meta-analysis 2.0 software (Biostat Inc., Englewood, New Jersey, United States) was used for data analysis in present meta-analysis. The correlations between SNPs of *CTLA-4* gene, *MDR1* gene and the risk of UC were evaluated by OR at 95%CI. Z test was carried out to evaluate the significance of overall effect values<sup>[21]</sup>. Forest plots were drawn to reflect the comparisons of OR and 95%CI among the study groups. Cochran's Q-statistic ( $P < 0.05$  was considered significant) and  $I^2$  tests were applied to quantify heterogeneity among studies<sup>[22,23]</sup>. In order to calculate the pool ORs, fixed/random effects model were used. When significant heterogeneity was observed ( $P < 0.05$  or  $I^2 > 50\%$ ), a fixed effect model was used, otherwise, the random effect model was employed<sup>[24]</sup>. Univariate and multivariate meta-regression analysis was utilized to identify potential

sources of heterogeneity, and further confirmed by Monte Carlo method<sup>[25,26]</sup>. One-way sensitivity analysis was performed to assess whether the results had significant influences on the overall outcomes by deleting single studies one by one. Funnel plots, classic fail-safe N and Egger's linear regression test were inspected for indication of publication bias, and confirmed the reliability of original analysis results<sup>[27,28]</sup>. A bilateral test was conducted with  $P$  value of less than 0.05 considered to be significant.

## **RESULTS**

### **Baseline characteristics of included studies**

The initial database and manual search retrieved a total of 107 relevant articles. After excluding duplicates ( $n = 8$ ), non-human studies ( $n = 6$ ), letters, reviews ( $n = 8$ ) and studies unrelated to topic ( $n = 15$ ), 70 full-text articles remained. Twelve studies finally met the inclusion criteria after we eliminated studies that were not case-control ( $n = 14$ ), irrelevant to *CTLA-4* ( $n = 22$ ), not irrelevant *MDR1* ( $n = 12$ ), irrelevant to UC ( $n = 6$ ) and insufficient information ( $n = 4$ ). The twelve selected studies<sup>[17,19,20,29-37]</sup>, published between 2003 and 2013, contained a total of 1860 UC patients and 2663 healthy controls. Sample size varied between 195-900 patients. Of the 12 studies, 5 studies were conducted in Asians (2 from Iran, 1 from China and 2 from Japan), 1 study was performed in Africa (Tunisia), and the remaining 6 studies were in Caucasians with one study each in Croatia, Slovenia, Hungary, United Kingdom, Netherlands and Germany. SNP detection methods included polymerase chain reaction with the restriction fragment length polymorphism (PCR-RFLP) and TaqMan assay. The genotype distributions of all included studies conformed to Hardy Weinberg Equilibrium (HWE) ( $P > 0.05$ ) except for *MDR1* rs1045642 C > T in one study. The baseline characteristics of the included studies and CASP for eligible studies are shown in Table 1 and Figure 1, respectively.

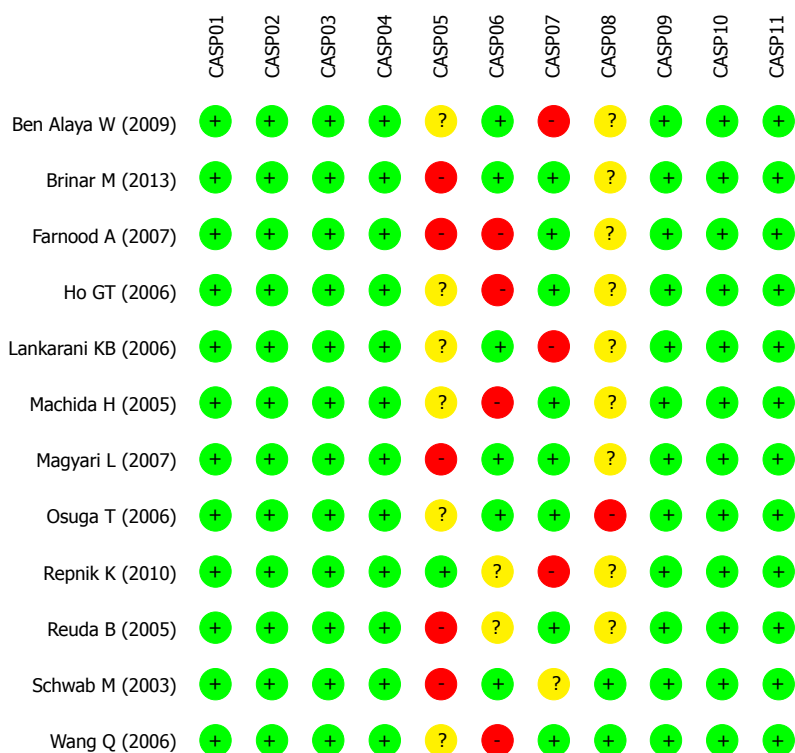
### **Association of CTLA-4 gene rs3087243 G > A and the risk of UC**

The correlations between SNP of *CTLA-4* gene, rs3087243 G > A, and UC risk were reported in 4 studies. A random-effects model was used since there was evidence of heterogeneity under the allele model and a fixed-effects model was used for the absence of heterogeneity under the dominant model ( $P = 0.03$ ;  $P = 0.121$ ). The results of meta-analysis showed that rs3087243 G > A is associated with increased risk of UC (allele model: OR = 1.365, 95%CI: 1.023-1.822,  $P = 0.035$ ; dominant model: OR = 1.569, 95%CI: 1.269-1.940,  $P < 0.001$ ) (Figure 2A and B and Table 2). Subgroup analysis based on ethnicity showed no significant association between rs3087243 G > A and the risk of UC in Asians (allele model: OR = 1.153, 95%CI: 0.860-1.546,  $P = 0.340$ ; dominant model: OR

**Table 1** Baseline characteristics of all eligible studies

First author	Year	Ethnicity	Genotype method	Number		Gender (M/F)		Gene	SNP
				UC	Control	UC	Control		
Brinar <i>et al</i> <sup>[29]</sup>	2013	Caucasian	PCR-RFLP	52/57	-	29.9 (26.0-35.70)	-	MDR1	C3435T
Repnik <i>et al</i> <sup>[35]</sup>	2010	Caucasian	PCR-RFLP	-	119/147	-	37.2	CTLA4	rs3087243 G/A
Ben Alaya <i>et al</i> <sup>[17]</sup>	2009	Africa	PCR-RFLP	18/47	52/48	39.9 (25-74)	32.4 (24-55)	CTLA4	rs231775 G/A
Farnood <i>et al</i> <sup>[30]</sup>	2007	Asian	PCR-RFLP	-	-	-	-	MDR1	C3435T
Magyari <i>et al</i> <sup>[19]</sup>	2007	Caucasian	PCR-RFLP	63/87	49/121	46.1 ± 1.3	57.7 ± 1.3	CTLA4	rs231775 G/A
Lankarani <i>et al</i> <sup>[32]</sup>	2006	Asian	PCR-RFLP	48/52	48/52	31.6 ± 13.4	35.36 ± 11.94	CTLA4	rs231775 G/A
Wang <i>et al</i> <sup>[37]</sup>	2006	Asian	PCR-RFLP	41/38	92/72	44.2 ± 16.2	37.8 ± 14.1	CTLA4	rs3087243 G/A
Osuga <i>et al</i> <sup>[34]</sup>	2006	Asian	PCR-RFLP	37/29	97/76	42.7 ± 14.2	19-76	MDR1	C3435T
Ho <i>et al</i> <sup>[31]</sup>	2006	Caucasian	PCR-RFLP	-	-	-	-	MDR1	C3435T
Rueda <i>et al</i> <sup>[20]</sup>	2005	Caucasian	TaqMan	-	-	-	-	CTLA4	rs3087243 G/A
Machida <i>et al</i> <sup>[33]</sup>	2005	Asian	PCR-RFLP	57/51	125/75	44.0 ± 16.9	32.5 ± 11.1	CTLA4	rs3087243 G/A
Machida <i>et al</i> <sup>[33]</sup>	2005	Asian	PCR-RFLP	57/51	125/75	44.0 ± 16.9	32.5 ± 11.1	CTLA4	rs231775 G/A
Schwab <i>et al</i> <sup>[36]</sup>	2003	Caucasian	TaqMan	86/63	86/63	34 ± 11	-	MDR4	C3435T

PB: Population-based; HB: Hospital-based; PCR-RFLP: Polymerase chain reaction restriction fragment length polymorphism; UC: Ulcerative colitis; MDR1: Multi-drug resistance 1; CTLA4: Cytotoxic T lymphocyte-associated antigen-4; SNP: Single nucleotide polymorphisms.



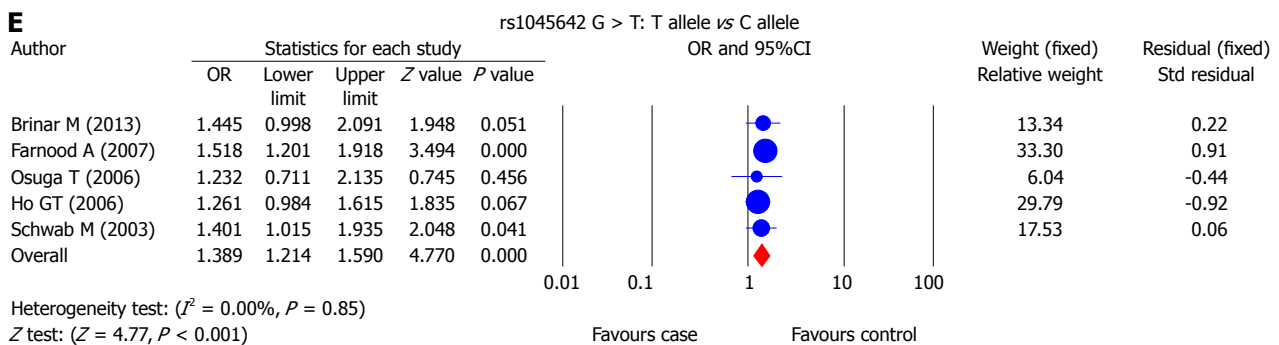
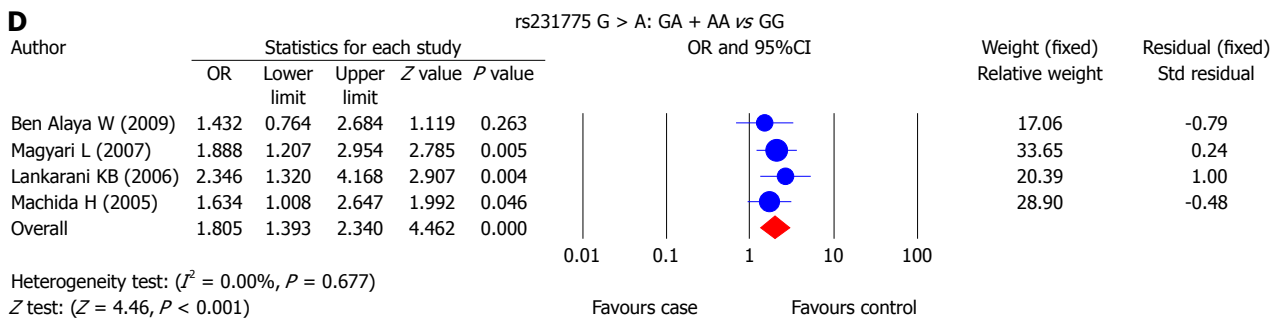
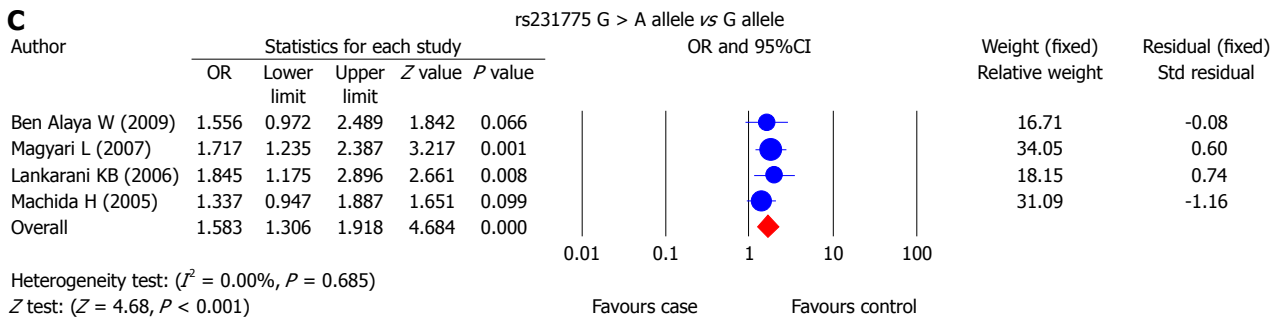
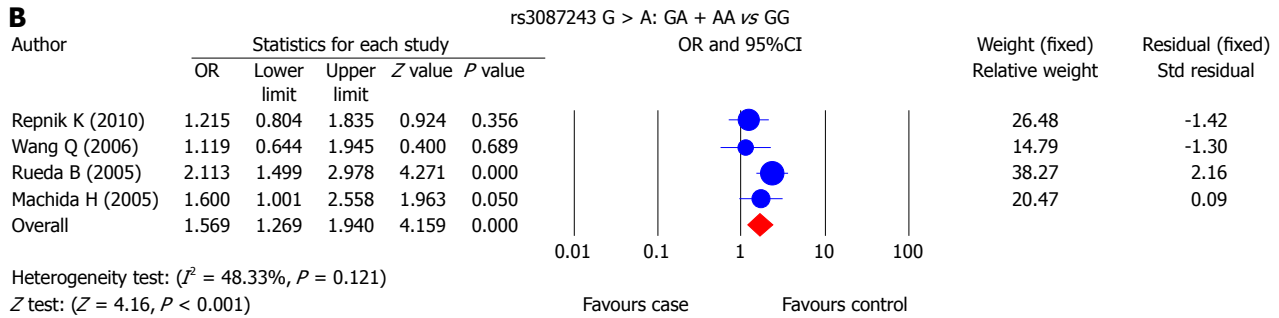
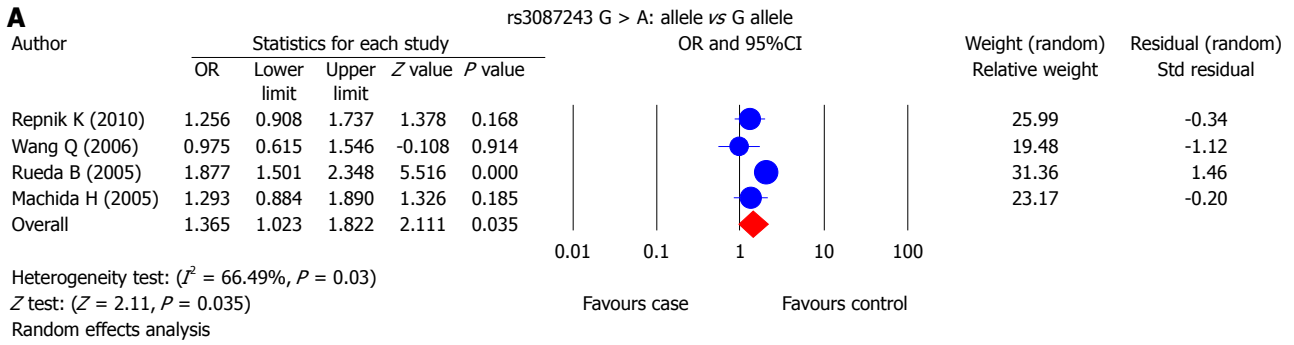
**Figure 1** Methodological quality of included studies was evaluated by Critical Appraisal Skill Program criteria. CASP: Critical Appraisal Skill Program.

= 1.377, 95%CI: 0.963-1.970, *P* = 0.079). However, in Caucasians, rs3087243 G > A is associated with an increased risk of UC (allele model: OR = 1.563, 95%CI: 1.056-2.313, *P* = 0.026; dominant model: OR = 1.685, 95%CI: 1.294-2.194, *P* < 0.001) (Figure 3A and B; Table 2).

**Association of CTLA-4 gene rs231775 G > A and the risk of UC**

The correlation between SNP of CTLA-4 gene rs231775 G > A, and UC risk was reported in 4 studies. The results of the heterogeneity test showed no significant

heterogeneity under the allele model and dominant model, thus the fixed-effects model was applied in this meta-analysis (all *P* > 0.05). Pooled data in this meta-analysis showed that rs231775 G > A is associated with significantly increased risk of UC (allele model: OR = 1.583, 95%CI: 1.306-1.918, *P* < 0.001; dominant model: OR = 1.805, 95%CI: 1.393-2.340, *P* < 0.001) (Figure 2C and D; Table 2). Subgroup analysis based on ethnicity showed no significant association between rs231775 G > A and the risk of UC in Africans (allele model: OR = 1.556, 95%CI: 0.972-2.489, *P* = 0.066; dominant model: OR = 1.432, 95%CI:



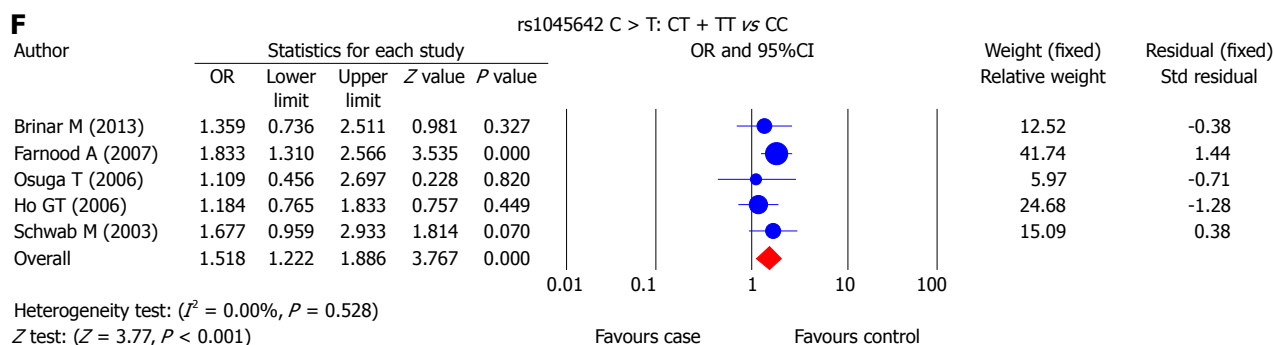
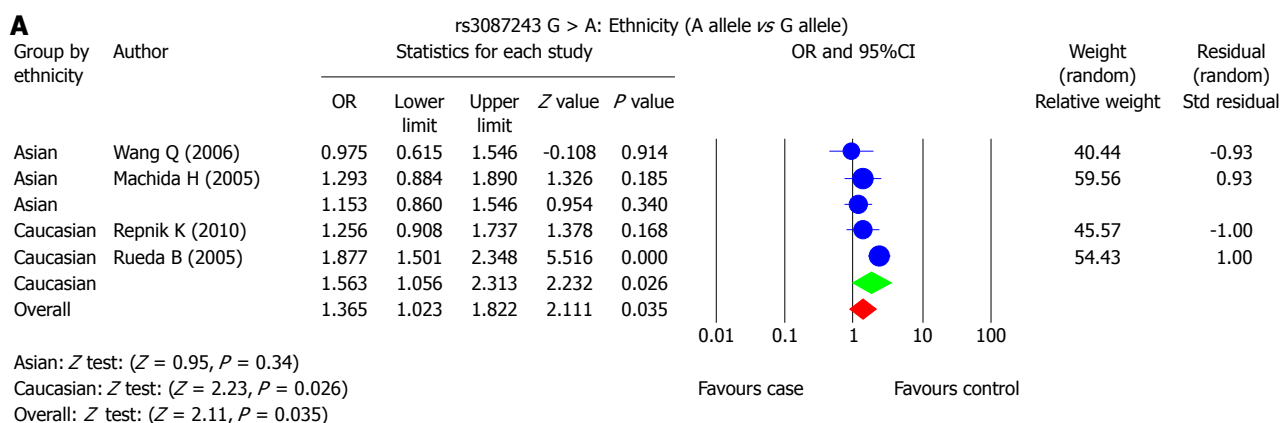


Figure 2 Forest plots for the correlations between single nucleotide polymorphisms of *CTLA-4* and *MDR1* with ulcerative colitis risk.

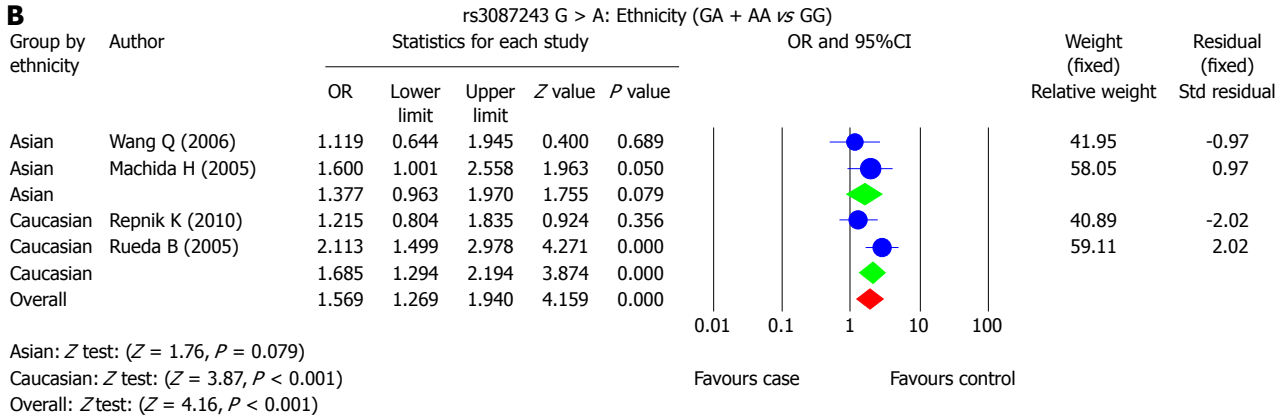
**Table 2** Comparisons of genotype and allele frequencies between the case and the control groups

SNP	rs3087243 G/A			rs231775 G/A			rs1045642 C/T		
	OR	95%CI	P value	OR	95%CI	P value	OR	95%CI	P value
Allele model									
Asians	1.15	0.86-1.55	0.340	1.51	1.15-1.98	0.003	1.47	1.19-1.82	< 0.001
Caucasians	1.56	1.06-2.31	0.026	1.72	1.24-2.39	0.001	1.34	1.13-1.59	0.001
Africas	-	-	-	1.56	0.97-2.49	0.066	-	-	-
Overall	1.37	1.02-1.82	0.035	1.58	1.31-1.92	< 0.001	1.39	1.21-1.59	< 0.001
Dominant model									
Asians	1.38	0.96-1.97	0.079	1.90	1.31-2.75	0.001	1.72	1.26-2.36	0.001
Caucasians	1.69	1.29-2.19	< 0.001	1.89	1.21-2.95	0.005	1.35	1.00-1.83	0.048
Africas	-	-	-	1.43	0.76-2.68	0.263	-	-	-
Overall	1.57	1.27-1.94	< 0.001	1.81	1.39-2.34	< 0.001	1.52	1.22-1.89	< 0.001
Homozygous model									
Overall	2.41	1.67-3.48	< 0.001	2.21	1.45-3.37	< 0.001	1.92	1.44-2.56	< 0.001
Heterozygous model									
Overall	0.62	0.44-0.89	0.008	0.76	0.50-1.15	0.190	0.67	0.53-0.86	0.001
Recessive model									
Overall	1.98	1.42-2.76	< 0.001	1.73	1.16-2.57	0.007	1.62	1.29-2.05	< 0.001

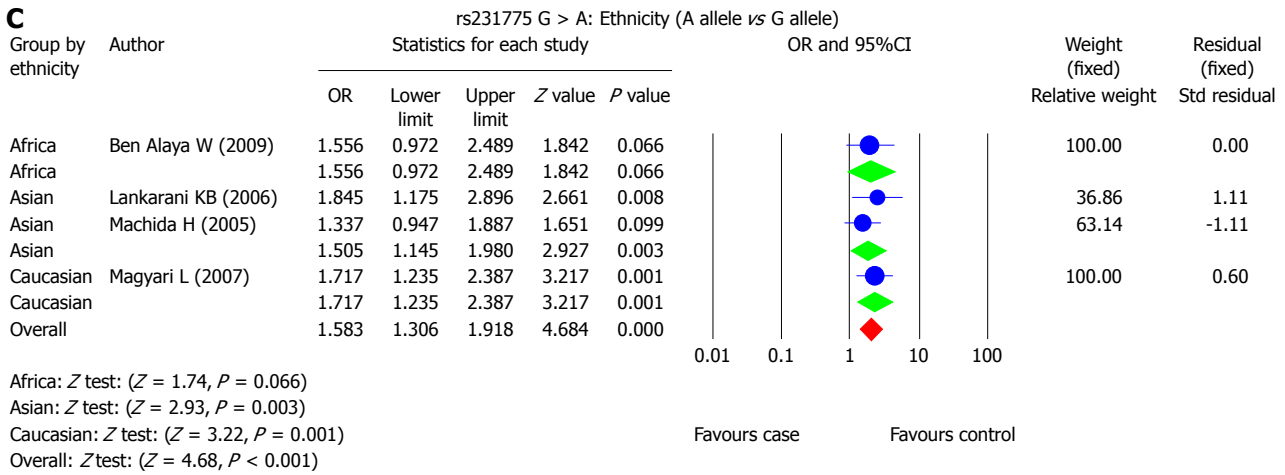
OR: Odds ratio.



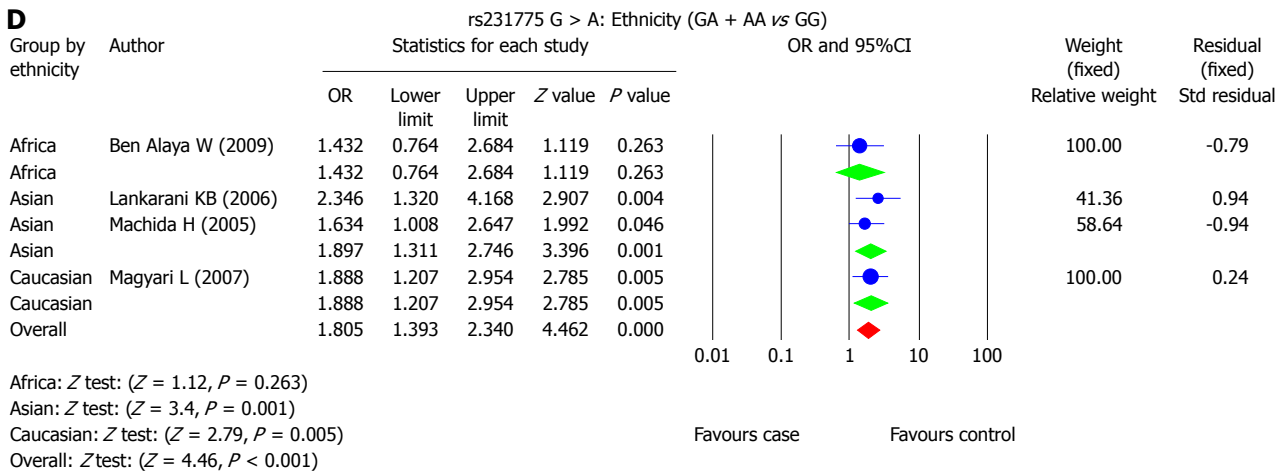
**B**



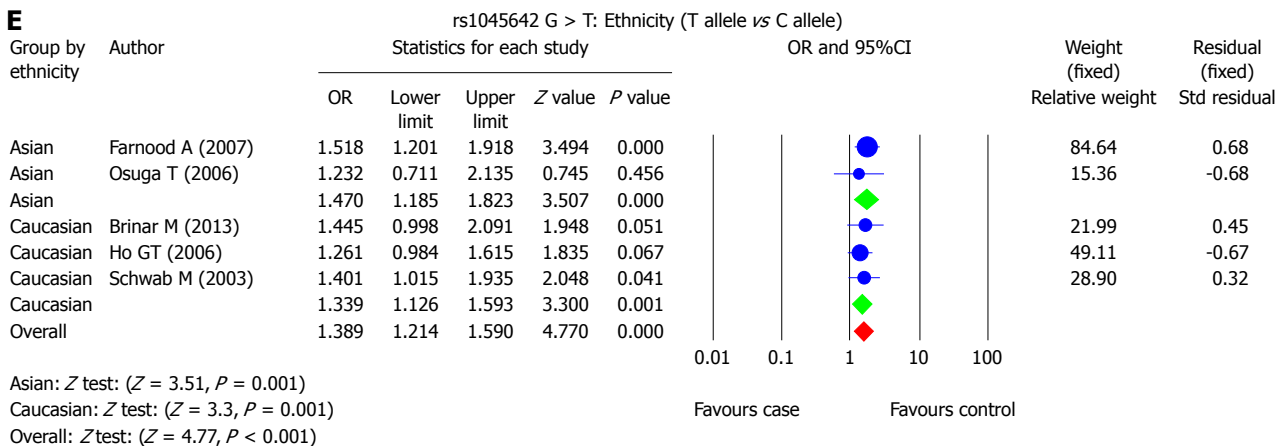
**C**



**D**



**E**



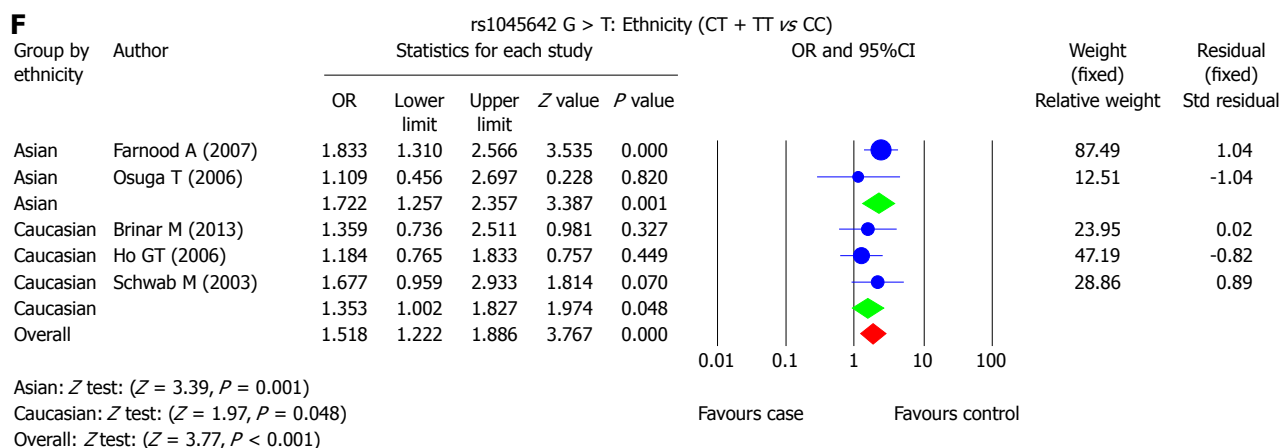
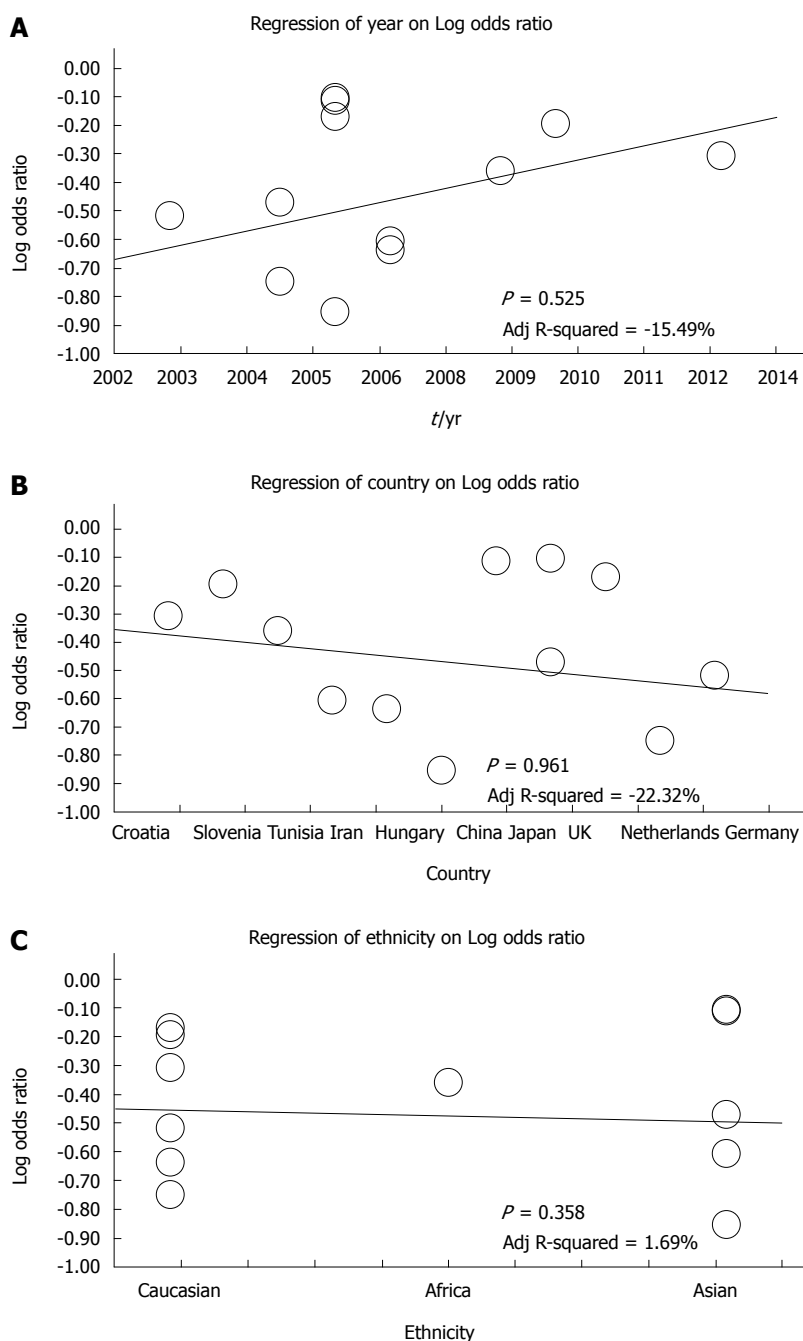


Figure 3 Subgroup analysis for the correlations between single nucleotide polymorphisms of CTLA-4 and MDR1 with ulcerative colitis risk.





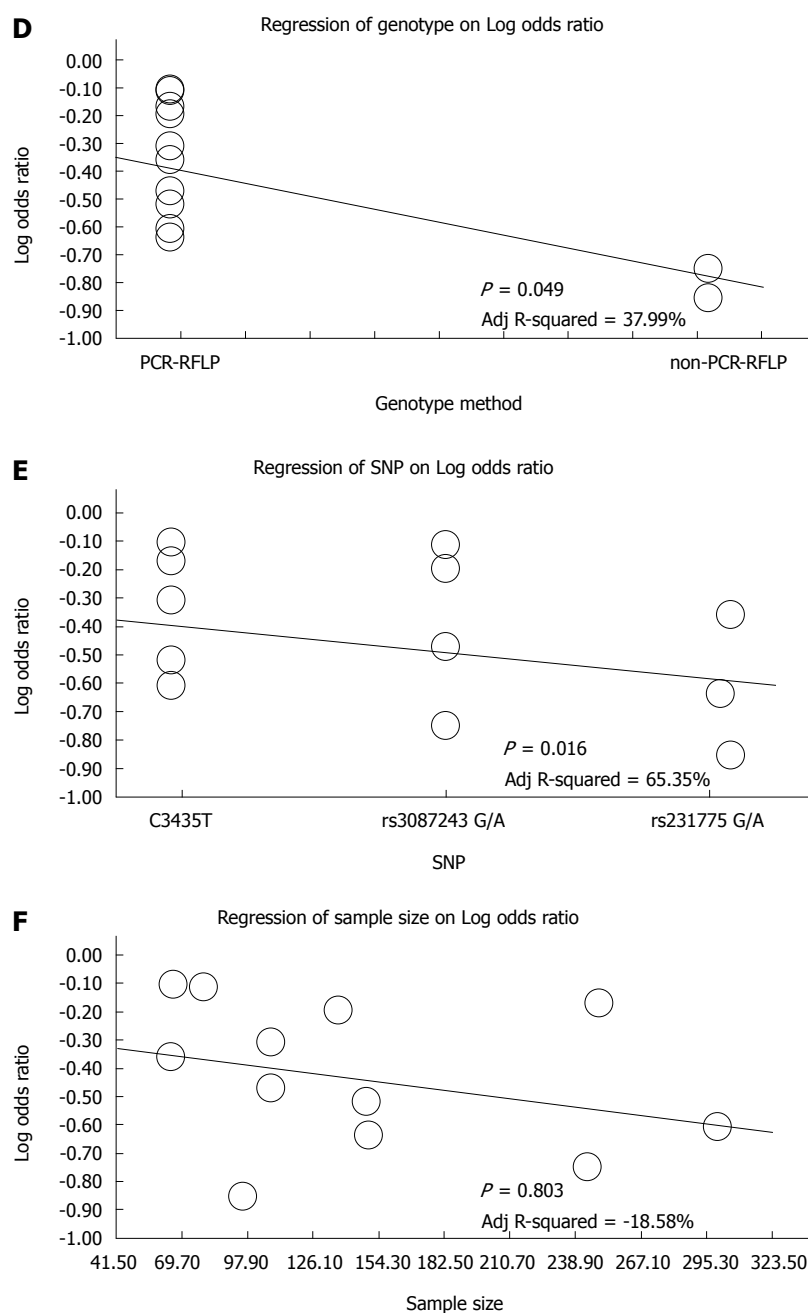
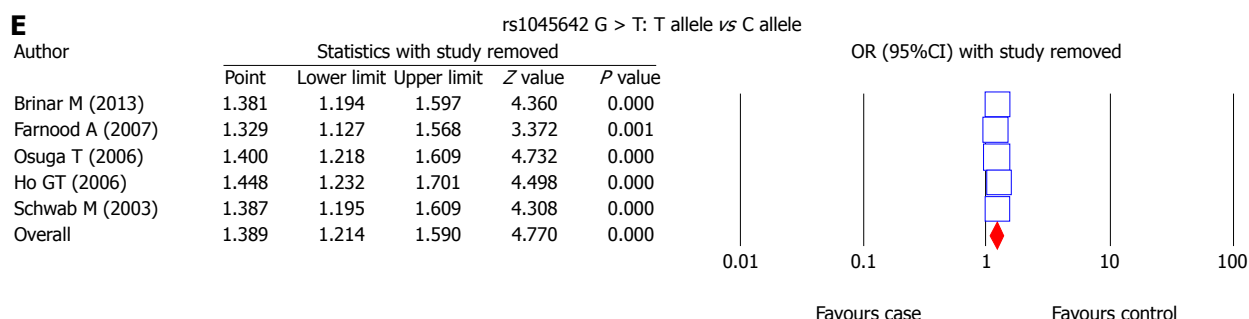
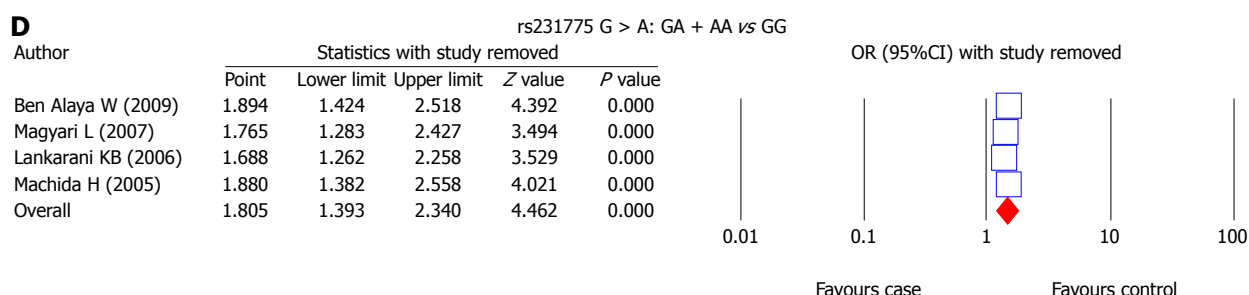
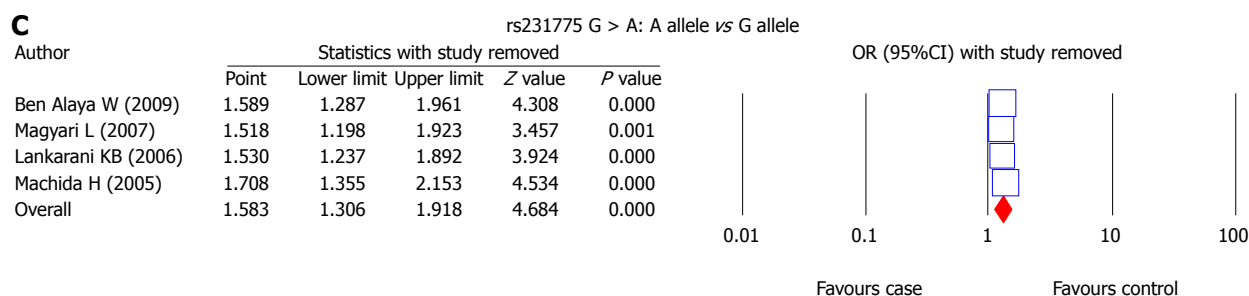
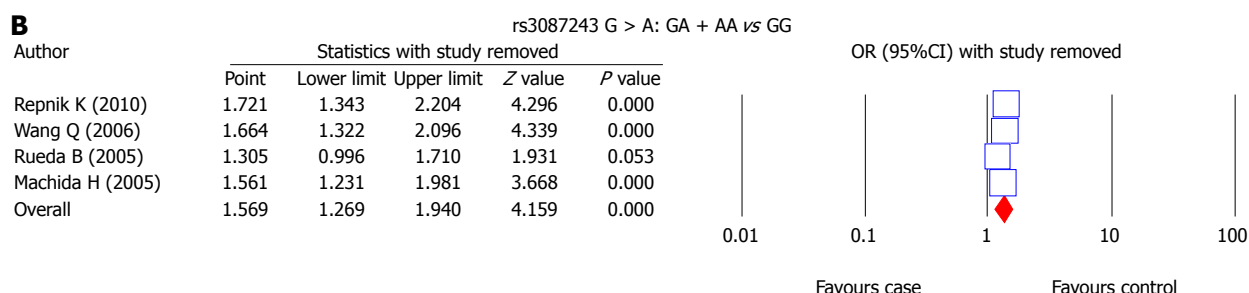
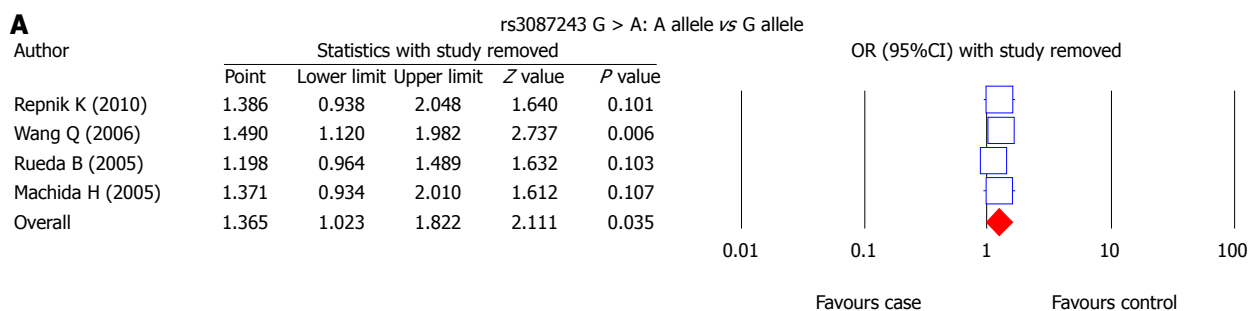


Figure 4 Sensitivity analysis for the correlations between single nucleotide polymorphisms of *CTLA-4* and *MDR1* with ulcerative colitis risk. SNP: Single nucleotide polymorphism.

Table 3 Meta-regression analyses of potential source of heterogeneity

Heterogeneity factors	Coefficient	SE	<i>t</i>	<i>P</i> value (adjusted)	95%CI	
					LL	UL
Year	-0.061	0.035	-1.76	0.359	-0.15	0.028
Country	-0.054	0.03	-1.78	0.352	-0.131	0.024
Ethnicity	0.001	0.042	0.03	1	-0.106	0.109
Method	0.203	0.092	2.2	0.219	-0.034	0.44
SNP	0.045	0.057	0.79	0.85	-0.101	0.191
Sample	0	0	0.09	1	0	0

SE: Standard error; LL: Lower limit; UL: Upper limit. SNP: Single nucleotide polymorphism.



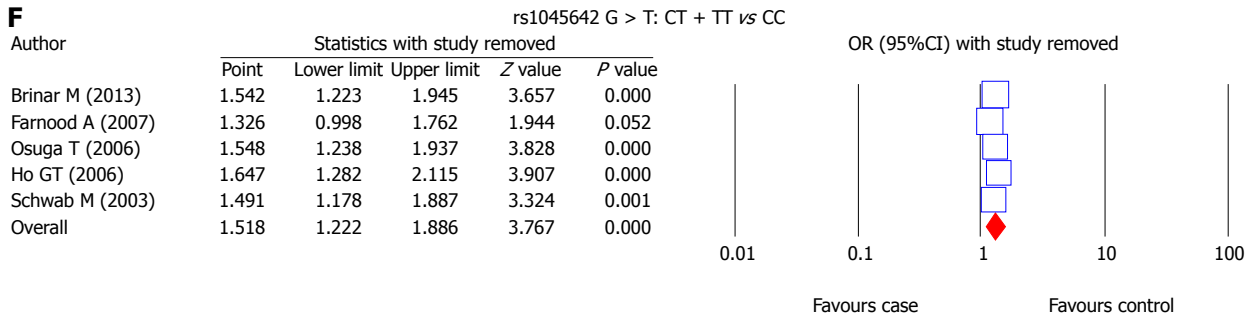
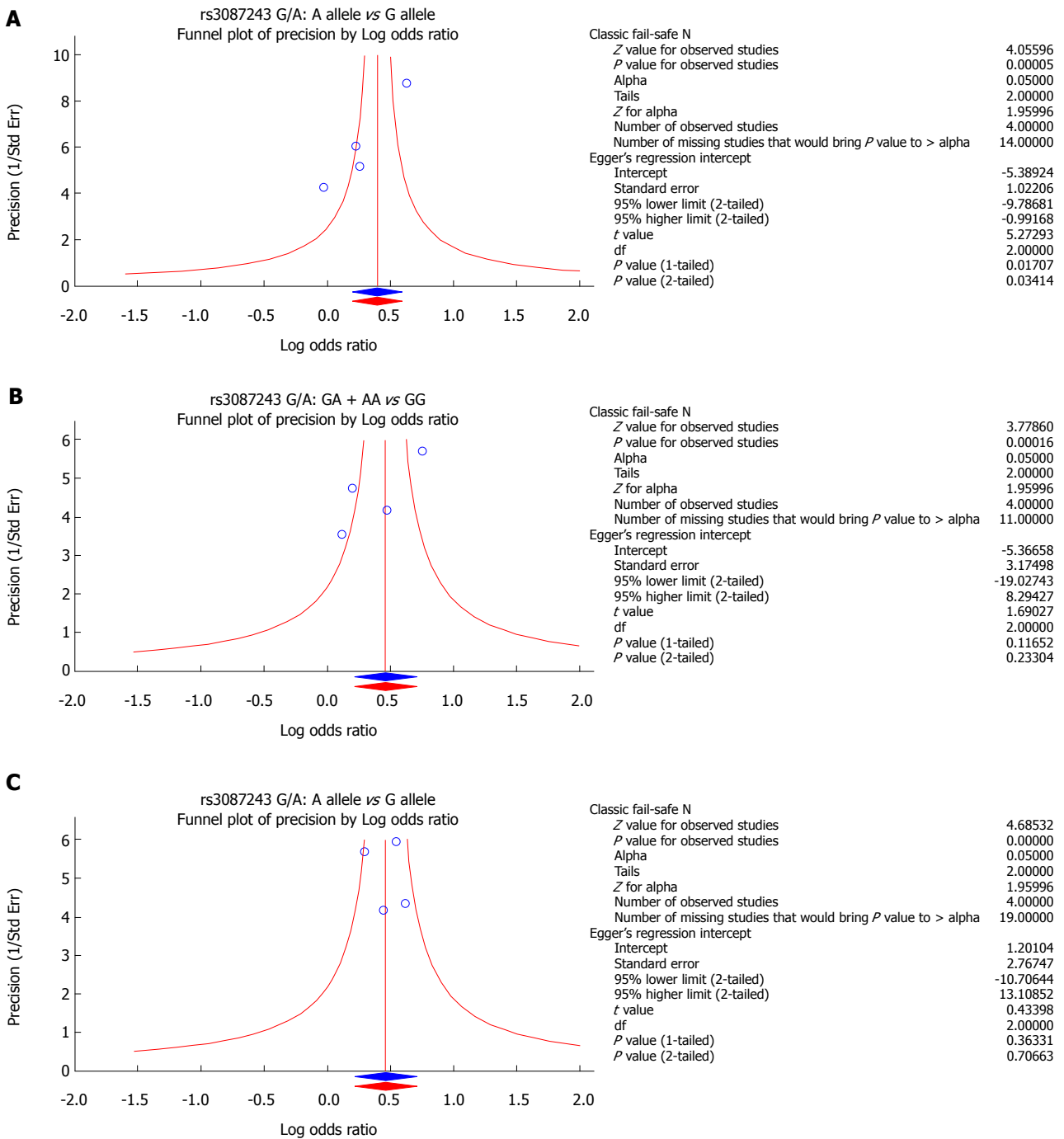


Figure 5 Meta-regression analysis on the correlations between single nucleotide polymorphisms of *CTLA-4* and *MDR1* with ulcerative colitis risk.



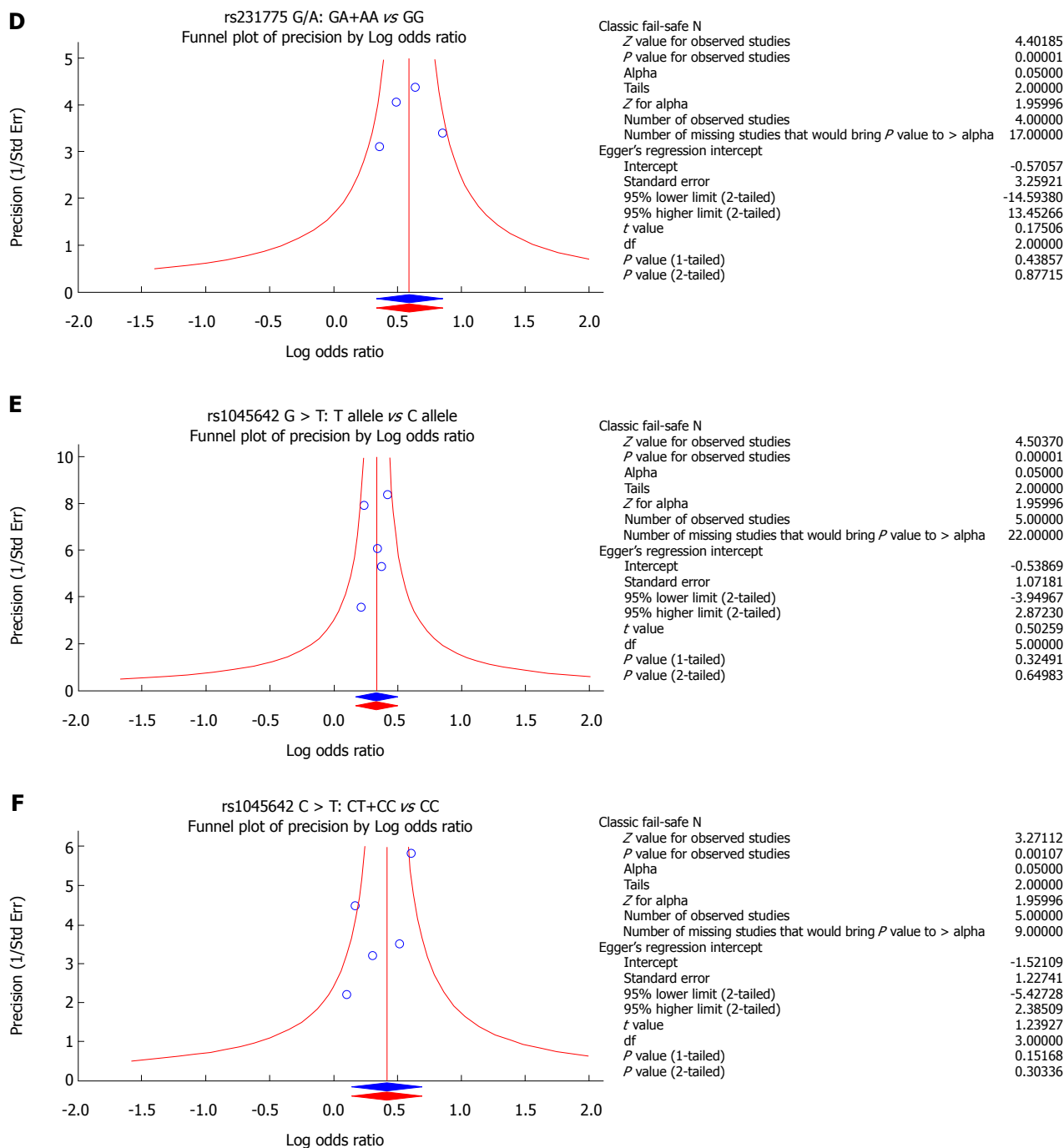


Figure 6 Funnel plot of publication biases on the correlations of single nucleotide polymorphisms of *CTLA-4* and *MDR1* with ulcerative colitis risk.

0.764-2.684,  $P = 0.263$ ), but in both Asians and Caucasians, rs231775 G > A was strongly associated with significant increases in the incidence of UC (Asians: allele model: OR = 1.505, 95%CI: 1.145-1.980,  $P = 0.003$ ; dominant allele: OR = 1.897, 95%CI: 1.311-2.746,  $P = 0.001$ ; Caucasians: allele model: OR = 1.717, 95%CI: 1.235-2.387,  $P = 0.001$ ; dominant model: OR = 1.888, 95%CI: 1.207-2.954,  $P = 0.005$ ) (Figure 3C and D; Table 2).

**Association of *MDR1* gene rs1045642 C > T and the risk of UC**

The correlation between SNP of *MDR1* gene, rs1045642 C > T, and UC risk was discussed in 5 studies. A fixed-effects model was used for the absence of heterogeneity under the allele model and the dominant model (all  $P > 0.05$ ). The results of meta-analysis showed that rs1045642 C > T is linked to increased risk of UC (allele model: OR = 1.389, 95%CI:

1.214-1.590,  $P < 0.001$ ; dominant model: OR = 1.518, 95%CI: 1.222-1.886,  $P < 0.001$ ) (Figure 2E and F and Table 2). Subgroup analysis based on ethnicity showed a significant association between rs1045642 C > T and the risk of UC in both Asians and Caucasians (Asians: allele model: OR = 1.470, 95%CI: 1.185-1.823,  $P < 0.001$ ; dominant model: OR = 1.722, 95%CI: 1.257-2.357,  $P = 0.001$ ; Caucasians: allele model: OR = 1.339, 95%CI: 1.126-1.593,  $P = 0.001$ ; dominant model: OR = 1.353, 95%CI: 1.002-1.827,  $P = 0.048$ ) (Figure 3E and F; Table 2).

### Sensitivity analysis and publication bias

The results of sensitivity analysis demonstrated that any single study had no significant effect on pooled ORs of correlations between SNPs of *CTLA-4* gene rs3087243 G > A, rs231775 G > A and *MDR1* gene rs1045642 C > T, and the risk of UC (Figure 4). Univariate meta-regression analysis suggested that publication year, country, ethnicity, sample size, SNP, and detection methods were not the main sources for heterogeneity or the key factors affecting the overall effect values ( $P > 0.05$ ). Multivariate meta-regression analysis further confirmed that the published year, country, ethnicity, sample size, SNP, and detection methods are not the sources of heterogeneity (Figure 5 and Table 3). Funnel plots for rs3087243 G > A under the allele model were asymmetric, suggesting the existence of publication bias. Classic fail-safe N and Egger's linear regression tests further confirmed there was publication bias. However, funnel plots for rs3087243 G > A under the dominant model, rs231775 G > A and rs1045642 C > T both under the allele model and dominant model were symmetrical, revealing no significant publication bias. Classic fail-safe N and Egger's linear regression tests further confirmed there was no publication bias (Figure 6).

## DISCUSSION

UC is a non-specific chronic inflammatory disorder which, together with CD, is known as IBD, and it is characterized by diffuse mucosal inflammation confined to the colon<sup>[8]</sup>. Evidence has revealed that genetic factors and immune dysregulation may be two main important components in the etiology and pathogenesis of UC<sup>[38]</sup>. Recent genome-wide association studies (GWAS) have discovered multiple genes and loci for UC risk factors; for example, GWAS meta-analyses have established more than 30 loci in CD, and several of these loci have also been found to be correlated to UC<sup>[39]</sup>. The GWAS that evaluated the correlation between UC and the variants of the *CTLA-4* gene and *MDR1* genes produced contradictory or inconclusive results; we found that associations have been found in some, but not all populations<sup>[40]</sup>. Therefore, in order to investigate the correlations between SNPs of the *CTLA-4* gene, *MDR1* gene and

the risk of UC, a meta-analysis was conducted.

Our meta-analysis demonstrated that *CTLA-4* gene polymorphisms, rs3087243 G > A and rs231775 G > A, are closely associated with the increased risk of UC. *CTLA-4* is primarily expressed in activated T cells, and it also plays an inhibitory role in the regulation of self-tolerance and T-cell functions, suggesting that *CTLA-4*, by virtue of its influence on T-cell regulation, may potentially influence UC disease susceptibility<sup>[17]</sup>. *CTLA-4* is described as a vital downregulator of T-cell activation resulting in peripheral tolerance, and is also a negative regulator of T/B, T-cell activation and T/monocyte-macrophage cognate interaction. Thus, *CTLA-4* has been recognized as a good candidate gene for the susceptibility to UC<sup>[41]</sup>. In this context, downregulation of *CTLA-4* may contribute to an exaggerated T cell response, along with a persistent inflammation response in the gastrointestinal mucosa, possibly initiating the development of UC<sup>[42]</sup>. Data from a previous study showed that the *CTLA-4* gene is involved in the pathogenesis of IBD and UC among Slovenian patients and that CT60 (rs3087243 G > A) polymorphism is an important target regulating *CTLA-4* gene expression<sup>[35]</sup>.

Our meta-analysis also revealed a significant association between *MDR1* gene polymorphism, rs1045642 C > T, and the risk of UC. As an energy-dependent efflux pump, *MDR1* (P-gp) plays a crucial role in the bioavailability and cytotoxicity of a large number of drugs, substances, and xenobiotics including sugars, glycans, ions, proteins, phospholipids, antibiotics, corticosteroids, anticancer drugs, immunosuppressants, calcium-channel blocker agents and anti- (human immunodeficiency virus) HIV protease inhibitors<sup>[43]</sup>. UC patients frequently exhibit reduced P-gp expression levels, and *MDR1* mRNA expression might also be reduced in the colonic tissue of UC patients. G2677T/A (Ala893Ser/Thr, rs2032582) and C3435T (Ile1145Ile, rs1045642) are two common polymorphisms (variants) in *MDR1* gene, and correlate with the function and activity of P-gp<sup>[44]</sup>. Data from a previous study revealed a 2-fold increased OR for the development of UC in patients with the *MDR1* rs1045642 C > T genotype, supporting the notion that P-gp expression plays an essential role in defense against intestinal bacteria and low P-gp expression, as a result of an rs1045642 C > T genotype, contributes to the development of UC<sup>[34]</sup>. Based on our results and previous studies supporting our conclusions, we propose that individuals with the rs1045642 C > T have a reduced intestinal barrier function, and thus are at a significantly higher risk for developing UC.

To further explore the effect of other influential factors like ethnicity on the correlation between SNPs of *CTLA-4* gene, *MDR1* gene and the risk of UC, a subsequent subgroup meta-analysis was conducted. Subgroup analysis based on ethnicity showed that in Asians, there was no significant association between

rs3087243 G > A and the risk of UC. However, in Caucasians, rs3087243 G > A may increase the risk of UC. In Africans, no significant association was found between rs231775 G > A and the risk of UC. But both in Asians and Caucasians, rs231775 G > A was likely to increase the incidence of UC. Furthermore, both in Asians and Caucasians, there was a significant association between rs1045642 C > T and the risk of UC. Our overall results are consistent with previous studies, which suggested a significant association of SNPs of the *CTLA-4* gene and the *MDR1* gene with the pathogenesis of UC.

Limitations of this meta-analysis need to be addressed. First, the sample size is relatively small. Second, all eligible studies were written in English and Chinese indexed by the selected databases. It is possible that published studies in other languages or unpublished studies could be missed, which might bias the results. Third, the genotyping methods were not uniform and might increase the deviation of outcomes. Therefore, more studies with larger sample sizes are still needed to provide a more accurate statistical analysis.

This meta-analysis provides strong evidence that SNPs of *CTLA-4* gene rs3087243 G > A and rs231775 G > A, and *MDR1* gene polymorphism rs1045642 C > T significantly increase the risk of UC, and the polymorphisms can be used as important biological indicators for early diagnosis of UC.

## COMMENTS

### Background

Ulcerative colitis (UC) is known as an idiopathic, chronic inflammatory disease of the large intestine, frequently involving the rectum, and characterized by continuous inflammation and ulceration of intestinal mucosa and submucosa.

### Research frontiers

Recently, single nucleotide polymorphisms (SNPs) of Cytotoxic T lymphocyte-associated antigen-4 (*CTLA-4*) and multi-drug resistance 1 (*MDR1*) genes were found to be associated with the pathogenesis of UC.

### Innovations and breakthroughs

The authors undertook a meta-analysis based approach to evaluate the associations of SNPs of *CTLA-4* and *MDR1* genes with the risk of UC, by pooling all relevant published data.

### Applications

This meta-analysis provides strong evidence that SNPs of *CTLA-4* gene rs3087243 G > A and rs231775 G > A, and *MDR1* gene polymorphism rs1045642 C > T significantly increase the risk of UC, and the polymorphisms can be used as important biological indicators for early diagnosis of UC

### Peer-review

The authors address, with their meta-analysis, the risk of ulcerative colitis in the presence of genetic polymorphisms of *CTLA* and *MDR1*.

## REFERENCES

- 1 Reinisch W, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, Panaccione R, Fedorak RN, Tighe MB, Huang

- B, Kampman W, Lazar A, Thakkar R. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011; **60**: 780-787 [PMID: 21209123 DOI: 10.1136/gut.2010.221127]
- 2 Kornbluth A, Sachar DB. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2010; **105**: 501-523; quiz 524 [PMID: 20068560 DOI: 10.1038/ajg.2009.727]
- 3 Krishnan K, Arnone B, Buchman A. Intestinal growth factors: potential use in the treatment of inflammatory bowel disease and their role in mucosal healing. *Inflamm Bowel Dis* 2011; **17**: 410-422 [PMID: 20848489 DOI: 10.1002/ibd.21316]
- 4 Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature* 2011; **474**: 298-306 [PMID: 21677746 DOI: 10.1038/nature10208]
- 5 Salim SY, Söderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflamm Bowel Dis* 2011; **17**: 362-381 [PMID: 20725949 DOI: 10.1002/ibd.21403]
- 6 Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, Marano CW, Strauss R, Oddens BJ, Feagan BG, Hanauer SB, Lichtenstein GR, Present D, Sands BE, Sandborn WJ. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology* 2011; **141**: 1194-1201 [PMID: 21723220 DOI: 10.1053/j.gastro.2011.06.054]
- 7 Sandborn WJ, Ghosh S, Panes J, Vranic I, Su C, Rousell S, Niezychowski W. Tofacitinib, an oral Janus kinase inhibitor, in active ulcerative colitis. *N Engl J Med* 2012; **367**: 616-624 [PMID: 22894574 DOI: 10.1056/NEJMoa1112168]
- 8 Jiang T, Ge LQ, Chen ZT, Li C, Zhou F, Luo Y, Xia B. Effect of cytotoxic T lymphocyte-associated molecule 4 1661 gene polymorphism on its expression and transcription in ulcerative colitis. *J Dig Dis* 2010; **11**: 369-375 [PMID: 21091900 DOI: 10.1111/j.1751-2980.2010.00462.x]
- 9 Herrlinger KR, Koc H, Winter S, Teml A, Stange EF, Fellermann K, Fritz P, Schwab M, Schaeffeler E. ABCB1 single-nucleotide polymorphisms determine tacrolimus response in patients with ulcerative colitis. *Clin Pharmacol Ther* 2011; **89**: 422-428 [PMID: 21289623 DOI: 10.1038/clpt.2010.348]
- 10 Gao JW, Guo YF, Fan Y, Qiu JX, Bao ED, Liu Y, Qin Y, Zhang F. Polymorphisms in cytotoxic T lymphocyte associated antigen-4 influence the rate of acute rejection after renal transplantation in 167 Chinese recipients. *Transpl Immunol* 2012; **26**: 207-211 [PMID: 22418270 DOI: 10.1016/j.trim.2012.02.005]
- 11 Li M, Zheng H, Li T, Gao P, Zhang XL, Liu DW. Cytotoxic T-lymphocyte associated antigen-4 gene polymorphisms and primary biliary cirrhosis: a systematic review. *J Gastroenterol Hepatol* 2012; **27**: 1159-1166 [PMID: 22414241 DOI: 10.1111/j.1440-1746.2012.07118.x]
- 12 Wells AD, Walsh MC, Bluestone JA, Turka LA. Signaling through CD28 and CTLA-4 controls two distinct forms of T cell anergy. *J Clin Invest* 2001; **108**: 895-903 [PMID: 11560959 DOI: 10.1172/JCI13220]
- 13 Tang ST, Tang HQ, Zhang Q, Wang CJ, Wang YM, Peng WJ. Association of cytotoxic T-lymphocyte associated antigen 4 gene polymorphism with type 1 diabetes mellitus: a meta-analysis. *Gene* 2012; **508**: 165-187 [PMID: 22964358 DOI: 10.1016/j.gene.2012.07.044]
- 14 Ni LN, Li JY, Miao KR, Qiao C, Zhang SJ, Qiu HR, Qian SX. Multidrug resistance gene (*MDR1*) polymorphisms correlate with imatinib response in chronic myeloid leukemia. *Med Oncol* 2011; **28**: 265-269 [PMID: 20204543 DOI: 10.1007/s12032-010-9456-9]
- 15 Bodor M, Kelly EJ, Ho RJ. Characterization of the human *MDR1* gene. *AAPS J* 2005; **7**: E1-E5 [PMID: 16146331 DOI: 10.1208/aapsj070101]
- 16 Takakura Y, Hinoi T, Oue N, Sasada T, Kawaguchi Y, Okajima M, Akyol A, Fearon ER, Yasui W, Ohdan H. CDX2 regulates multidrug resistance 1 gene expression in malignant intestinal epithelium. *Cancer Res* 2010; **70**: 6767-6778 [PMID: 20699370 DOI: 10.1158/0008-5472.CAN-09-4701]

- 17 **Ben Alaya W**, Sfar I, Aouadi H, Jendoubi S, Najjar T, Filali A, Gorgi Y, Ben Abdallah T, Mouelhi L, Matri S, Ayed K. Association between CTLA-4 gene promoter (49 A/G) in exon 1 polymorphisms and inflammatory bowel disease in the Tunisian population. *Saudi J Gastroenterol* 2009; **15**: 29-34 [PMID: 19568552 DOI: 10.4103/1319-3767.43285]
- 18 **Zintzaras E**. Is there evidence to claim or deny association between variants of the multidrug resistance gene (MDR1 or ABCB1) and inflammatory bowel disease? *Inflamm Bowel Dis* 2012; **18**: 562-572 [PMID: 21887726 DOI: 10.1002/ibd.21728]
- 19 **Magyari L**, Faragó B, Bene J, Horvatovich K, Lakner L, Varga M, Figler M, Gasztonyi B, Mózsik G, Melegh B. No association of the cytotoxic T-lymphocyte associated gene CTLA4 +49A/G polymorphisms with Crohn's disease and ulcerative colitis in Hungarian population samples. *World J Gastroenterol* 2007; **13**: 2205-2208 [PMID: 17465502 DOI: 10.3748/wjg.v13.i15.2205]
- 20 **Rueda B**, Zhernakova A, López-Nevot MA, Gomez-Garcia M, Ortega E, Piñero A, Corroero F, Brieva JA, Nieto A, Koeleman BP, Martin J. CTLA4/CT60 polymorphism is not relevant in susceptibility to autoimmune inflammatory intestinal disorders. *Hum Immunol* 2005; **66**: 321-325 [PMID: 15784471 DOI: 10.1016/j.humimm.2004.11.005]
- 21 **Chen H**, Manning AK, Dupuis J. A method of moments estimator for random effect multivariate meta-analysis. *Biometrics* 2012; **68**: 1278-1284 [PMID: 22551393 DOI: 10.1111/j.1541-0420.2012.01761.x]
- 22 **Jackson D**, White IR, Riley RD. Quantifying the impact of between-study heterogeneity in multivariate meta-analyses. *Stat Med* 2012; **31**: 3805-3820 [PMID: 22763950 DOI: 10.1002/sim.5453]
- 23 **Peters JL**, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of two methods to detect publication bias in meta-analysis. *JAMA* 2006; **295**: 676-680 [PMID: 16467236 DOI: 10.1001/jama.295.6.676]
- 24 **Zintzaras E**, Ioannidis JP. Heterogeneity testing in meta-analysis of genome searches. *Genet Epidemiol* 2005; **28**: 123-137 [PMID: 15593093 DOI: 10.1002/gepi.20048]
- 25 **Huizenga HM**, Visser I, Dolan CV. Testing overall and moderator effects in random effects meta-regression. *Br J Math Stat Psychol* 2011; **64**: 1-19 [PMID: 21506942 DOI: 10.1348/000711010X522687]
- 26 **Ferrenberg AM**, Swendsen RH. New Monte Carlo technique for studying phase transitions. *Phys Rev Lett* 1988; **61**: 2635-2638 [PMID: 10039183]
- 27 **Wikstrom EA**, Naik S, Lodha N, Cauraugh JH. Balance capabilities after lateral ankle trauma and intervention: a meta-analysis. *Med Sci Sports Exerc* 2009; **41**: 1287-1295 [PMID: 19461536 DOI: 10.1249/MSS.0b013e318196cbc6]
- 28 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563]
- 29 **Brinar M**, Cukovic-Cavka S, Bozina N, Ravic KG, Markos P, Ladic A, Cota M, Krznicaric Z, Vucelic B. MDR1 polymorphisms are associated with inflammatory bowel disease in a cohort of Croatian IBD patients. *BMC Gastroenterol* 2013; **13**: 57 [PMID: 23537364 DOI: 10.1186/1471-230x-13-57]
- 30 **Farnood A**, Naderi N, Moghaddam SJ, Noorinayer B, Firouzi F, Aghazadeh R, daryani NE, Zali MR. The frequency of C3435T MDR1 gene polymorphism in Iranian patients with ulcerative colitis. *Int J Colorectal Dis* 2007; **22**: 999-1003 [PMID: 17242936 DOI: 10.1007/s00384-007-0270-6]
- 31 **Ho GT**, Soranzo N, Nimmo ER, Tenesa A, Goldstein DB, Satsangi J. ABCB1/MDR1 gene determines susceptibility and phenotype in ulcerative colitis: discrimination of critical variants using a gene-wide haplotype tagging approach. *Hum Mol Genet* 2006; **15**: 797-805 [PMID: 16434479 DOI: 10.1093/hmg/ddi494]
- 32 **Lankarani KB**, Karbasi A, Kalantari T, Yarmohammadi H, Saberi-Firoozi M, Alizadeh-Naeni M, Taghavi AR, Fattahi MR, Ghaderi A. Analysis of cytotoxic T lymphocyte associated antigen 4 gene polymorphisms in patients with ulcerative colitis. *J Gastroenterol Hepatol* 2006; **21**: 449-453 [PMID: 16509873 DOI: 10.1111/j.1440-1746.2005.03956.x]
- 33 **Machida H**, Tsukamoto K, Wen CY, Narumi Y, Shikuwa S, Isomoto H, Takeshima F, Mizuta Y, Niikawa N, Murata I, Kohno S. Association of polymorphic alleles of CTLA4 with inflammatory bowel disease in the Japanese. *World J Gastroenterol* 2005; **11**: 4188-4193 [PMID: 16015687]
- 34 **Osuga T**, Sakaeda T, Nakamura T, Yamada T, Koyama T, Tamura T, Aoyama N, Okamura N, Kasuga M, Okumura K. MDR1 C3435T polymorphism is predictive of later onset of ulcerative colitis in Japanese. *Biol Pharm Bull* 2006; **29**: 324-329 [PMID: 16462040]
- 35 **Repnik K**, Potocnik U. CTLA4 CT60 single-nucleotide polymorphism is associated with Slovenian inflammatory bowel disease patients and regulates expression of CTLA4 isoforms. *DNA Cell Biol* 2010; **29**: 603-610 [PMID: 20491567 DOI: 10.1089/dna.2010.1021]
- 36 **Schwab M**, Schaeffeler E, Marx C, Fromm MF, Kaskas B, Metzler J, Stange E, Herfarth H, Schoelmerich J, Gregor M, Walker S, Cascorbi I, Roots I, Brinkmann U, Zanger UM, Eichelbaum M. Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. *Gastroenterology* 2003; **124**: 26-33 [PMID: 12512026 DOI: 10.1053/gast.2003.50010]
- 37 **Wang Q**, Xia B. Association between the cytotoxic T lymphocyte associated antigen 4/CT60 gene polymorphism and ulcerative colitis in the Chinese. *Zhonghua Weichangbingxue Yu Ganbingxue Zazhi* 2006; **15**: 158-160 [DOI: 10.3969/j.issn.1006-5709.2006.02.019]
- 38 **Vermeire S**, Rutgeerts P. Current status of genetics research in inflammatory bowel disease. *Genes Immun* 2005; **6**: 637-645 [PMID: 16107869 DOI: 10.1038/sj.gene.6364257]
- 39 **Rioux JD**, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW, Shugart YY, Griffiths AM, Targan SR, Ippoliti AF, Bernard EJ, Mei L, Nicolae DL, Regueiro M, Schumm LP, Steinhardt AH, Rotter JI, Duerr RH, Cho JH, Daly MJ, Brant SR. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; **39**: 596-604 [PMID: 17435756 DOI: 10.1038/ng2032]
- 40 **Barrett JC**, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhardt AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossom A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008; **40**: 955-962 [PMID: 18587394 DOI: 10.1038/ng.175]
- 41 **Chen Z**, Brant SR, Li C, Shrestha UK, Jiang T, Zhou F, Jiang Y, Shi X, Zhao Y, Li J, Xia B. CTLA4 -1661A/G and 3'UTR long repeat polymorphisms are associated with ulcerative colitis and influence CTLA4 mRNA and protein expression. *Genes Immun* 2010; **11**: 573-583 [PMID: 20445568 DOI: 10.1038/gene.2010.16]
- 42 **Berman D**, Parker SM, Siegel J, Chasalow SD, Weber J, Galbraith S, Targan SR, Wang HL. Blockade of cytotoxic T-lymphocyte antigen-4 by ipilimumab results in dysregulation of gastrointestinal immunity in patients with advanced melanoma. *Cancer Immunol* 2010; **10**: 11 [PMID: 21090563]
- 43 **Yang QF**, Chen BL, Zhang QS, Zhu ZH, Hu B, He Y, Gao X, Wang YM, Hu PJ, Chen MH, Zeng ZR. Contribution of MDR1 gene polymorphisms on IBD predisposition and response to glucocorticoids in IBD in a Chinese population. *J Dig Dis* 2015; **16**: 22-30 [PMID: 25346426 DOI: 10.1111/1751-2980.12205]
- 44 **Krupoves A**, Mack D, Seidman E, Deslandres C, Amre D.

Associations between variants in the ABCB1 (MDR1) gene and corticosteroid dependence in children with Crohn's disease.

*Inflamm Bowel Dis* 2011; **17**: 2308-2317 [PMID: 21987299 DOI: 10.1002/ibd.21608]

**P- Reviewer:** Kapischke M, Karatapanis S, Pessi T  
**S- Editor:** Qi Y **L- Editor:** O'Neill M **E- Editor:** Liu XM







Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgooffice@wjgnet.com](mailto:bpgooffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



ISSN 1007-9327



9 771007 932045