

Meta-analysis of FOXP3 gene rs3761548 and rs2232365 polymorphism and multiple sclerosis susceptibility

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Abstract

Background: Multiple sclerosis (MS) is a common autoimmune disease of the central nervous system (CNS), and is associated with genetic factors. FOXP3 gene polymorphism has been reported as the risk factor for MS, however, previous studies have showed conflicting results. The purpose of this study is to investigate the impact of FOXP3 gene polymorphism on the MS susceptibility through a meta-analysis.

Methods: Pubmed, Embase, library of Cochrane, and Web of Science were used to search the eligible articles up to 1 Oct 2018. The odds ratio (ORs) and its 95 % confidence intervals (CI) were used to evaluate the strength of association. Allele model, homozygote model, heterozygote model, dominant model, and recessive model were used to evaluate the association between FOXP3 gene polymorphism and MS.

Results: A total of 5 studies contained 1276 MS patients and 1447 controls (for rs3761548) and 600 MS patients and 640 controls (for rs2232365) were enrolled in this meta-analysis. The association showed significant differences in allele and dominant model for rs3761548 polymorphism. In addition, a clear tendency to significance was detected in homozygote and recessive model for rs3761548 ($p = 0.052$). Subgroup analysis indicated a significant risk of MS in all genotype models but heterozygotes in Asians.

Conclusion: FOXP3 gene polymorphism rs3761548 was associated with a higher MS risk, especially in Asians. This conclusion needs to be validated in more large samples and multiracial studies.

Keywords: FOXP3 gene, single nucleotide polymorphism, multiple sclerosis, meta-analysis.

Background

Multiple sclerosis (MS) is an immune-related central nervous disease, which manifests as variable symptoms including optic neuritis, fatigue, spasticity, neuro-urological dysfunction, paresthesia and hypesthesia, and headache [1]. In pathophysiology, this disease was characterized by inflammation, demyelination, proliferation of astrocytes, and varying degrees of axonal degeneration [2]. Meanwhile, variant cells and their markers such as T cells, clonal expansion of B cells, and their antibody products were found in MS patients, indicating the involvement of abnormalities of T-cells especially CD4 and CD8 T cells and B-cells response [3, 4]. It was considered that the occurrence and progression of MS may attribute to the virus infection, vitamin deficiency, mitochondrial dysfunction, and oxidative stress [5]. However, up to now, the exact reason for MS has not been determined.

Recently, more and more studies focus on the effect of single nuclei polymorphisms (SNPs) on some autoimmune disease including MS [6]. Previous literatures have reported several genetic variants (IL-6, HLA-DRB1, and STAT4) were correlated with development of MS [2, 7, 8]. The transcription factor forkhead box P3 (FOXP3) which belongs to the fork-winged helix family and encoded by the FOXP3 gene was regarded as an important molecular marker that regulate Treg cell development and function [9]. The prior study demonstrated that CD4⁺CD25⁺Foxp3⁺ Tregs played a crucial role in preventing autoimmunity

and undesirable T cell responses [10]. Moreover, mutation of the FOXP3 gene has been shown to be a risk in unexplained recurrent spontaneous abortions, Grave's disease, and allergic rhinitis [11-13]. Currently, some studies reported two key SNPs (rs3761548 and rs2232365) on FOXP3 gene may be involved in the development of MS [14, 15]. However, recent studies still obtained conflicting results that may lead to inconsistent outcomes.

In this meta-analysis, we collected data from several databases and combined them for analyzing to investigate the overall influence of FOXP3 gene polymorphism on the risk of MS.

Methods

2.1 Study strategy

Pubmed, Embase, Cochrane library, and Web of Science were used for the potential studies searching. (Polymorphism OR "single-nucleotide polymorphism" OR "SNP" OR "mutant" OR "mutation" OR "variant" OR "variation") AND ("FOXP3" OR "transcription factor forkhead box P3" OR "rs3761548" OR "rs2232365") AND ("multiple sclerosis" OR "multiple sclerosis") were used as the key words or Mesh terms for searching. The final search was conducted up to Oct 1, 2018 and the language of searched literatures was not restricted.

2.2 Inclusion and exclusion criteria

The included studies met the following criteria: (1) case-control; (2) the study evaluated the association between FOXP3 gene polymorphism and multiple sclerosis; (3) the study contained sufficient data of genotype frequency. The exclusion criteria were as follows: (1) studies without controls groups; (2) studies with only abstracts and reviews; (3) studies without specific distribution of genotype; (4) studies reported animal models.

2.3 Data extraction

Two independent reviewers (YJ Z and B P) assessed and reviewed all identified studies in terms of inclusion and exclusion criteria. Conflicts were reached to agreement via the discussion with the third authors (HL Y). Authors, date of publication, race, sample size, genotype and allele distribution of cases and controls were all extracted and recorded from the enrolled studies.

2.4 Statistical analysis

This meta-analysis preformed the data analyzing by using Stata version 11.0 (Stata Corp, College Station, TX, USA). The odds ratio (OR) and 95 % confidence interval (CI) were used to assess the relationship between rs3761548 and rs2232356 polymorphism and the risk of multiple sclerosis. The allelic contrast, dominant, recessive, and homozygotes models were all conducted in this study. The evaluation of heterogeneity of studies was using the Q test and was quantified using I^2 . The fixed-effects model was used when $I^2 < 50$ %. In contrast, the random-effects model was applied when $I^2 > 50$ %. The

subgroup analysis was performed stratified by ethnicity. The Begg and Egger's test were used for the assessment of publication bias and the sensitivity analysis was conducted by assessing the change of combined ORs values after elimination of each individual study. A p value equal to or less than 0.05 was considered the threshold for statistical significance.

Results

3.1 Literatures search

Based on the database of Pubmed, Embase, Cochrane library, Web of Science, and other resources, a total of 95 articles were relevant to the search terms. After elimination of duplicated studies and exclusion of improper studies via titles and abstracts, 18 articles remained for full text reviewing. Then, 13 articles were excluded for following reasons (lack of sufficient data of distribution of genotype, animal models, and comment / editorial articles). Ultimately, 5 articles were eligible in quantitative synthesis (current meta-analysis). The specific flow chart was shown in Figure 1.

3.2 Characteristic of eligible studies

For the 5 included studies, 4 studies reported the comparison of rs3761548 polymorphism and 3 studies stated comparison of rs2232365 polymorphism between multiple sclerosis and controls. The analysis of rs3761548 polymorphism contained 1276 MS patients and 1447 controls (Table 1 and Table 2). There was a significant difference in the sex of patients and controls (OR: 0.76, 95 % CI: 0.65~0.90, $P = 0.001$, $I^2 = 34.8\%$, $P_{\text{heterogeneity}} = 0.203$), and no significant difference in age (OR: 0.06, 95 % CI: -0.01~0.14, $P = 0.088$, $I^2 = 12.6\%$, $P_{\text{heterogeneity}} = 0.330$). The analysis of rs2232365 polymorphism contained 600 MS patients and 640 controls. Here, there was no significant difference in both sex (OR: 0.77, 95 % CI: 0.48~1.25, $P = 0.295$, $I^2 = 71.8\%$, $P_{\text{heterogeneity}} = 0.060$) and age between patients and controls (OR: 0.08, 95 % CI: -0.03~0.19, $P = 0.156$, $I^2 = 0\%$, $P_{\text{heterogeneity}} = 0.574$).

3.3 Quantitative synthesis

For rs2232365 polymorphism, we did not find a significant difference in the distribution of genotypes between multiple sclerosis and controls under allele model (OR: 1.22, 95 % CI: 0.68~2.18, $P = 0.503$, $I^2 = 91.5\%$, $P_{\text{heterogeneity}} = 0.000$), homozygote model (OR: 1.40, 95 % CI: 0.52~3.77, $P = 0.507$, $I^2 = 89.5\%$, $P_{\text{heterogeneity}} = 0.000$), heterozygote (OR: 1.37, 95 % CI: 0.67~2.79, $P = 0.383$, $I^2 = 84.1\%$, $P_{\text{heterogeneity}} = 0.002$), dominant model (OR: 1.40, 95 % CI: 0.63~3.09, $P = 0.411$, $I^2 = 89.7\%$, $P_{\text{heterogeneity}} = 0$), and recessive model (OR: 1.15, 95 % CI: 0.61~2.16, $P = 0.663$, $I^2 = 80.9\%$, $P_{\text{heterogeneity}} = 0.005$) (Figure 4 and Figure 5).

For rs3761548 polymorphism, there was a significantly decreased risk of multiple sclerosis under allele model (OR: 0.76, 95 % CI: 0.58~1.00, $P = 0.049$, $I^2 = 80.2\%$, $P_{\text{heterogeneity}} = 0.002$) and dominant model

(OR: 0.77, 95 % CI: 0.64~0.94, $P = 0.008$, $I^2 = 33.8$ %, $P_{\text{heterogeneity}} = 0.209$). No significant association was detected between rs37651548 and MS under the homozygote model (OR: 0.63, 95 % CI: 0.40~1.00, $P = 0.052$, $I^2 = 73.3$ %, $P_{\text{heterogeneity}} = 0.010$), heterozygote model (OR: 0.89, 95 % CI: 0.72~1.10, $P = 0.276$, $I^2 = 0$ %, $P_{\text{heterogeneity}} = 0.878$), and the recessive model (OR: 0.67, 95 % CI: 0.45~1.01, $P = 0.056$, $I^2 = 79.0$ %, $P_{\text{heterogeneity}} = 0.003$) (Figure 2 and Figure 3). Further, subgroup analysis was performed by ethnicity. There were significant differences in all genetic models but heterozygote model in Asians. In contrast, no significant difference was detected in European population under any genetic model (Figure 6 and Figure 7).

3.4 Publication bias

We performed the Begg funnel plot and Egger's test to assess the potential publication bias in the identified studies. The results showed no evidence of publication bias in this meta-analysis (Egger test: $P = 0.733$ under allele model for rs3761548, $P = 0.925$ under dominant model for rs3761548) (Figure 8).

3.5 Sensitive analysis

Sensitive analysis was conducted to assess the influence of one study on the pooled ORs value (allele model and dominant model for rs3761548), and whether the results can be reverted by eliminating the individual study. The result did not alter after deleting each study, indicating the stability of the results of this meta-analysis (Figure 9).

Discussion

As a kind of chronic inflammatory demyelinating disorder of the central nervous system, MS usually occurs in young adults and causes torturous symptoms and complications [16]. Unfortunately, until now, no exact etiology of this disease was found. It was widely believed that MS may be the result of the interaction between genetic and environmental factors together [17]. Lots of potential genetic polymorphisms have also been identified and proved to be associated with the development of MS. A case-control study by Krenek et al. investigated the distribution of the Vitamin D receptor (VDR) gene in MS and revealed five VDR genetic polymorphisms as the risk factors for MS susceptibility [18]. Similar results were also reported between HLA alleles variant and MS [19].

FOXP3 gene was located on the *p* arm of the X chromosome and contained 11 coding exons and 3 non-coding exons in the 5' upstream region in humans [20]. Meanwhile, this gene was mainly expressed in CD4⁺ CD25⁺ thymocytes and CD4⁺ CD25⁺ peripheral T cells, which play a crucial role in immune regulation [21]. Several studies had reported that FOXP3 acted as a key target in the generation and maturation of Treg cells, indicating the mutation of this gene may cause the dysfunction of Treg cells even reversed the process of naïve T cells transfer to Treg cells [22]. Further, recessive X-linked mutations of FOXP3 gene have been identified as a potential reason for immune-dysregulation, which means that even mild alterations of FOXP3 expression may result in common autoimmune diseases [23]. Due to its

X-linked characteristic, the genetic polymorphism of FOXP3 has been regarded as a marked risk factor in breast cancer among different ethnic populations (Chinese, Israeli, and Indians) [24]. Similarly, the balance between Th1 and Th2 cells in the maternal uterus may be broken because of mutation of FOXP3 gene, which can harm the fetus during pregnancy and subsequently result in a re-iterant abortion [25]. Additionally, such disturbance of immune system function and aberrant immune response can affect other organs in body and cause several immune-related disorders including autism, Wilms' tumor, thyroid cancer, recurrent infertility and so on [26-29]. In recent studies, FOXP3 gene polymorphism was correlated with MS. However, these studies were small sample sizes and had low statistical power which may lead to contradictory results. Hence, we performed this meta-analysis by combining the independent studies and estimating the overall effect to overcome the individual limitation and draw more convinced conclusions.

In this meta-analysis, after searching and reviewing 95 potential articles, we finally consolidated a total of 5 eligible studies to seek the variant polymorphism of two representative genes (rs3761548 and rs2232365) in MS susceptibility. To our limited knowledge, this study is the first meta-analysis that investigated the correlation between FOXP3 polymorphism and MS. Our results revealed that the variant allele and dominant model for rs3761548 can significantly decrease the risk of MS compared with the wild genotype. Meanwhile, under homozygote and recessive model, the difference between MS patients and controls also showed a marginal trend toward significance ($p = 0.052$ and $p = 0.056$). Considering the discrepancy of ethnicity between articles, we also performed stratified analysis categorized by race (3 studies from Asia and 1 study from Europe). The outcomes of subgroup analysis showed a significant association between rs3761548 gene polymorphism and MS under all genetic models except for heterozygotes in Asians. Above data supported the hypothesis that FOXP3 gene polymorphism (rs3761548) was associated with MS. On the other hand, no significant trend was identified for the occurrence of MS in each genetic model of rs2232365 gene. Moreover, either the sensitive analysis or the publication bias analysis showed no statistical significance in both the allele and dominant model for rs3761548. Hence, we speculated that rs3761538 polymorphism in FOXP3 gene may have an association with the susceptibility to MS, especially for Asian people.

Although the genetic polymorphism in the promoter region of FOXP3 gene including rs3761548 have been studied for several years, the specific impacts of these SNPs was still unclear. It was widely considered that Tregs acted as an important component in immune system and inflammatory response, which implied that defective Tregs could lead to various autoimmune diseases [10]. At the same time, previous studies found that FOXP3 was an indispensable molecular in the development and function of Treg cells [30]. Therefore, some opinions hypothesized that the mutation of FOXP3 can affect Treg function and thereby lead to immune-related diseases. With modifying the transcription and altering the binding specificity of transcription factors, rs3761548 polymorphism was able to affect the expression of FOXP3 genes [20]. In addition, the allelic alteration of rs3761548 C > A can disturb the binding of E47 and c-Myb on the FOXP3 promoter site, resulting in the defective transcription of FOXP3 [31]. Furthermore, another study suggested that rs3761548 polymorphism was located in the core sequence of the putative binding site for transcription factor, specificity protein 1 (Sp1), indicating the mutation of C to A may

obstruct the interaction between Sp1 and FOXP3 promoter subsequent disturb the expression of FOXP3 gene [32].

Limitations

Considering several limitations of this meta-analysis, the results should be interpreted carefully. First, due to the insufficient data, this study did not investigate the influence of the other three SNPs of FOXP3 (rs3761547, rs3761549, and rs2280883) on MS susceptibility. Second, we could not perform the subgroup analysis by gender and stages of MS due to the limited data of included studies. Third, the number of the enrolled studies in this meta-analysis were small due to the limited number of reports about the FOXP3 gene on MS. Fourth, the heterogeneity of rs2232365 was at a high level because of only three identified studies. Last, four of the five eligible studies were from Asians, which may limit our conclusion. Future studies, especially from European and American subjects, should be performed to confirm the effect of FOXP3 gene polymorphism on MS risk.

Conclusions

Based on the limited data, our meta-analysis preliminary indicated that rs3761548 polymorphism was associated with the elevated susceptibility of MS especially among Asian populations. However, more well-designed studies with large sample sizes and multiple ethnicities studies are needed to corroborate our conclusions.

Abbreviations

MS: multiple sclerosis, FOXP3: transcription factor forkhead box P3, OR: odd ratio, CI: confidence intervals

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed in this work are included in the published version.

Competing interests

All authors confirmed no competing interest in this work.

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Authors' contribution

YJ Z and JX Z contributed equally to this work. YJ Z and B P designed the study. JX Z and H L did the literature search, study quality assessment and data extraction. YJ Z and JX Z performed the statistical analysis and drafted the tables and figures. YJ Z wrote the first draft of this analysis, and HL Y helped to finish the final version. All authors approved the conclusions of our study.

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Tables

Table1 - Characteristics of eligible studies for SNPs of rs3761548

Study	Year	Ethnicity	Case	Control	Case			Control		
					AA	AC	CC	AA	AC	CC
Jafarzadeh	2014	Asian	140	140	34	50	56	21	34	85
Eftekharian	2016	Asian	410	446	131	205	74	105	204	137
Gholami	2016	Asian	189	192	49	85	55	48	87	57
Gajdošechová	2017	European	375	426	75	179	121	82	201	143

Table 2 - Characteristics of eligible studies for SNPs of rs2232365

Study	Year	Ethnicity	Case	Control	Case			Control		
					AA	AC	CC	AA	AC	CC
Eftekharian	2016	Asian	410	446	126	191	93	97	211	138
Gholami	2016	Asian	190	194	24	71	95	39	80	75
Mahdavi	2016	Asian	90	90	45	30	15	35	20	35

Figures

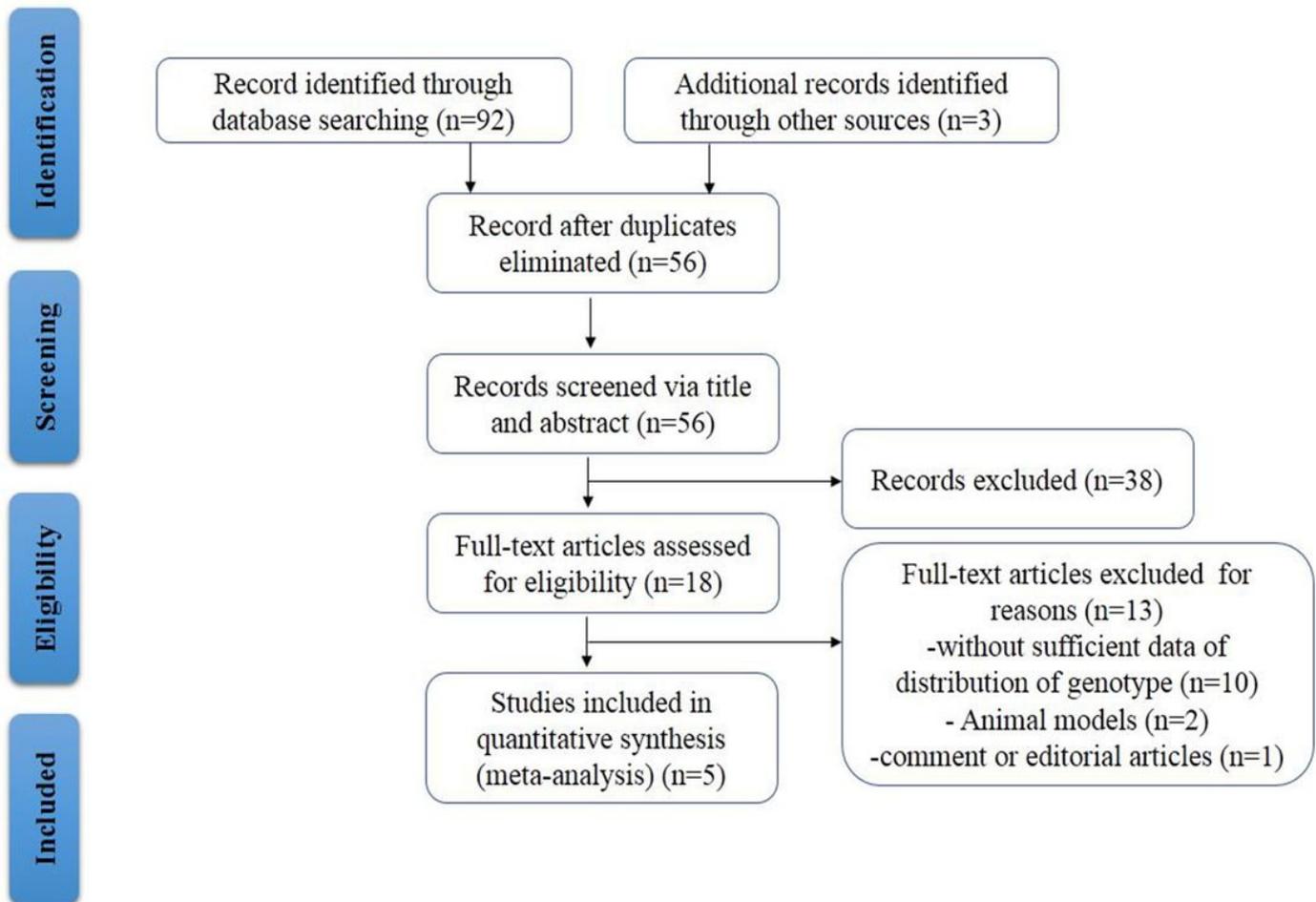


Figure 1

Flow diagram of the selection of literature.

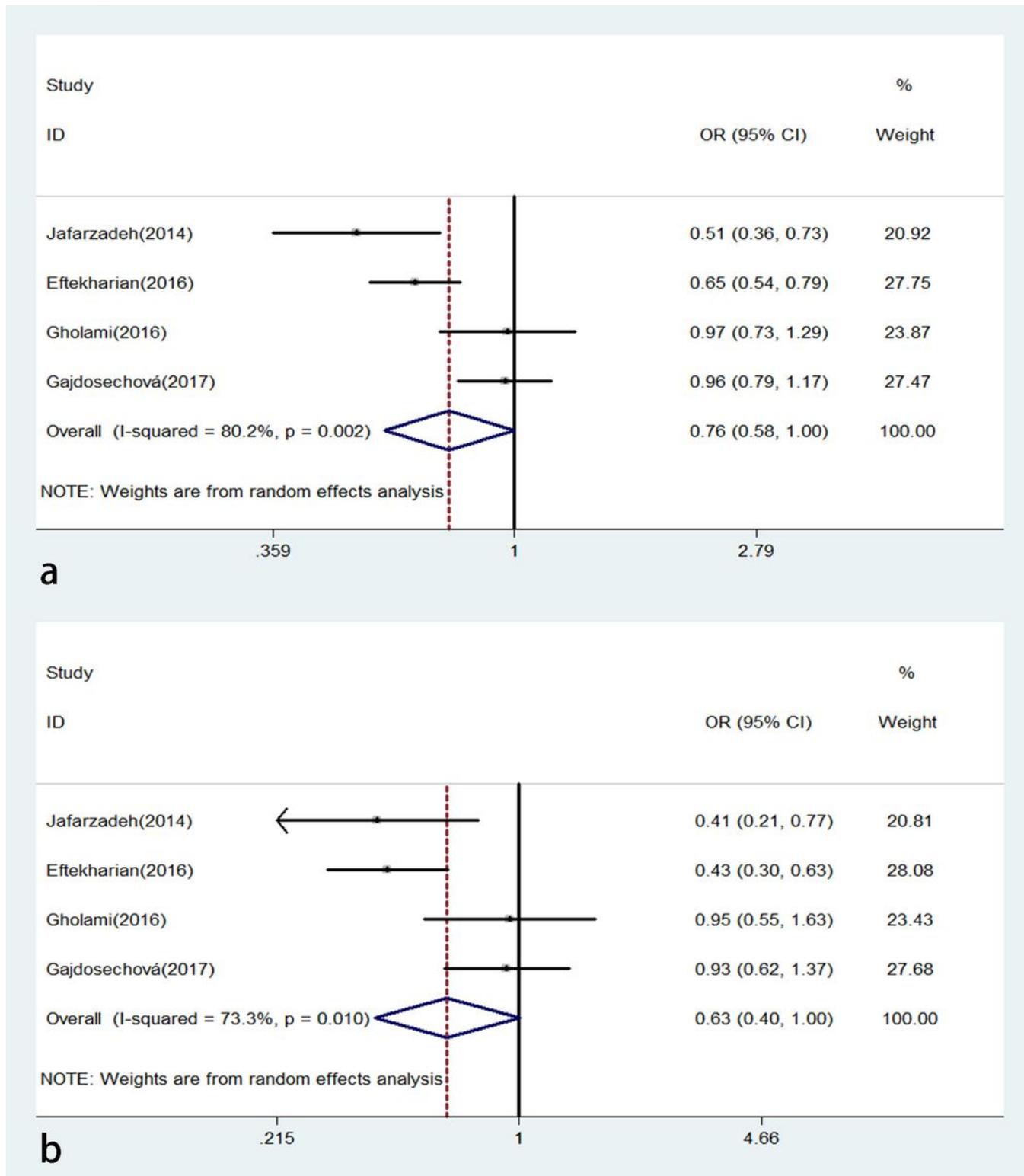


Figure 2

Forest of association between rs3761548 polymorphism of FOXP3 gene and risk of MS under (a) allele model (C / A); (b) homozygote model (CC / AA). OR = odd ratio, CI = confidence interval.

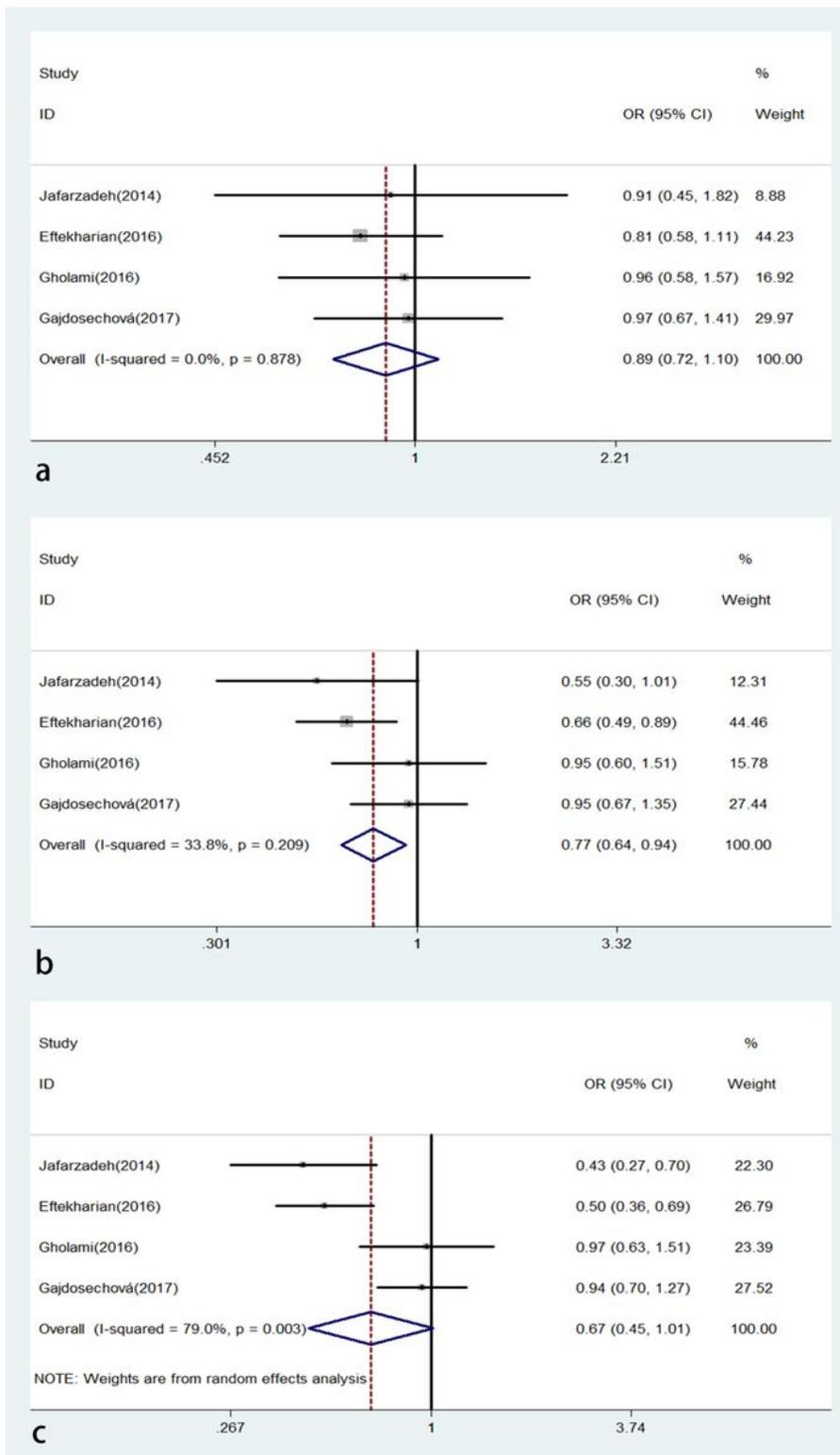


Figure 3

Forest of association between rs3761548 polymorphism of FOXP3 gene and risk of MS under (a) heterozygote model (CA / AA); (b) dominant model (CC + CA / AA); (c) recessive model (CC / CA + AA).

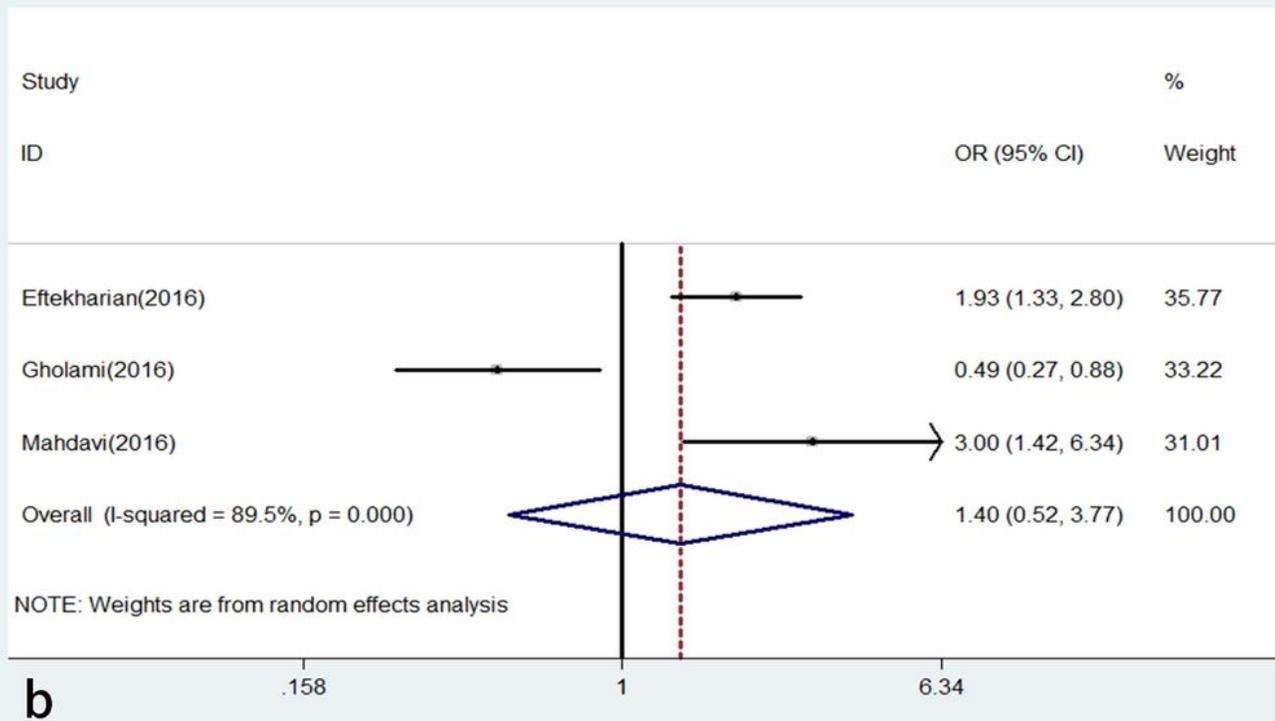
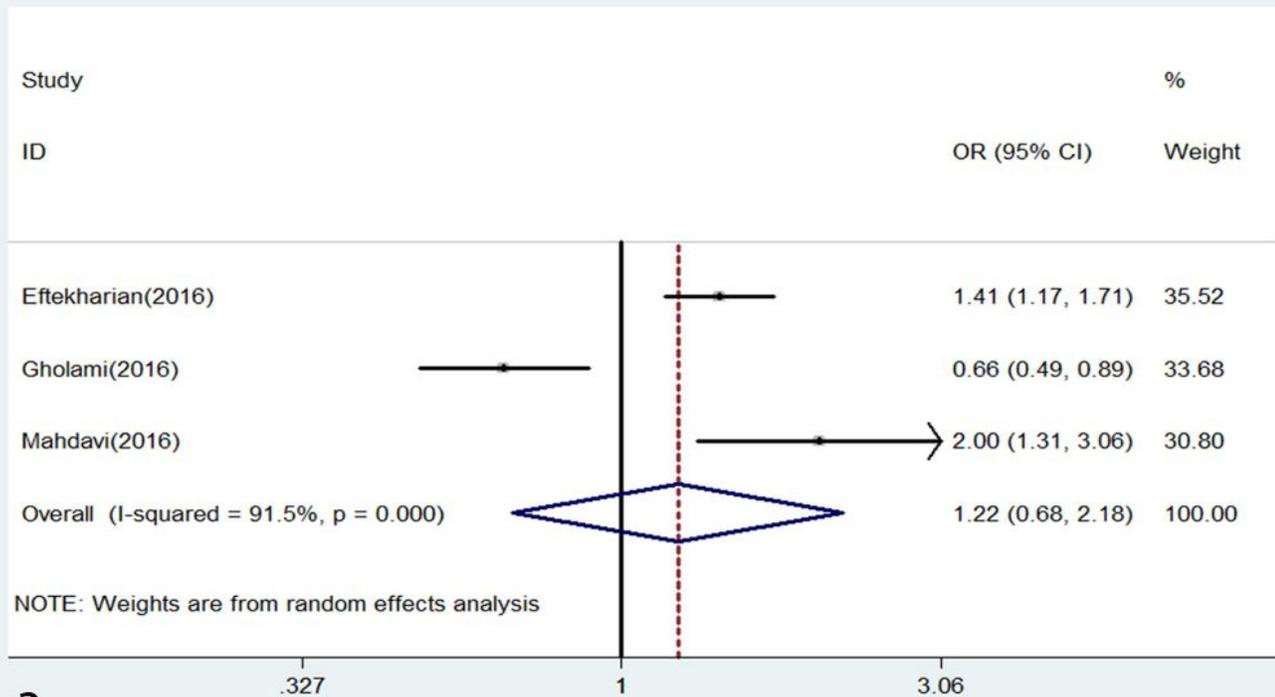


Figure 4

Forest of association between rs2232365 polymorphism of FOXP3 gene and risk of MS under (a) allele model (A / C); (b) homozygote model (AA / CC).

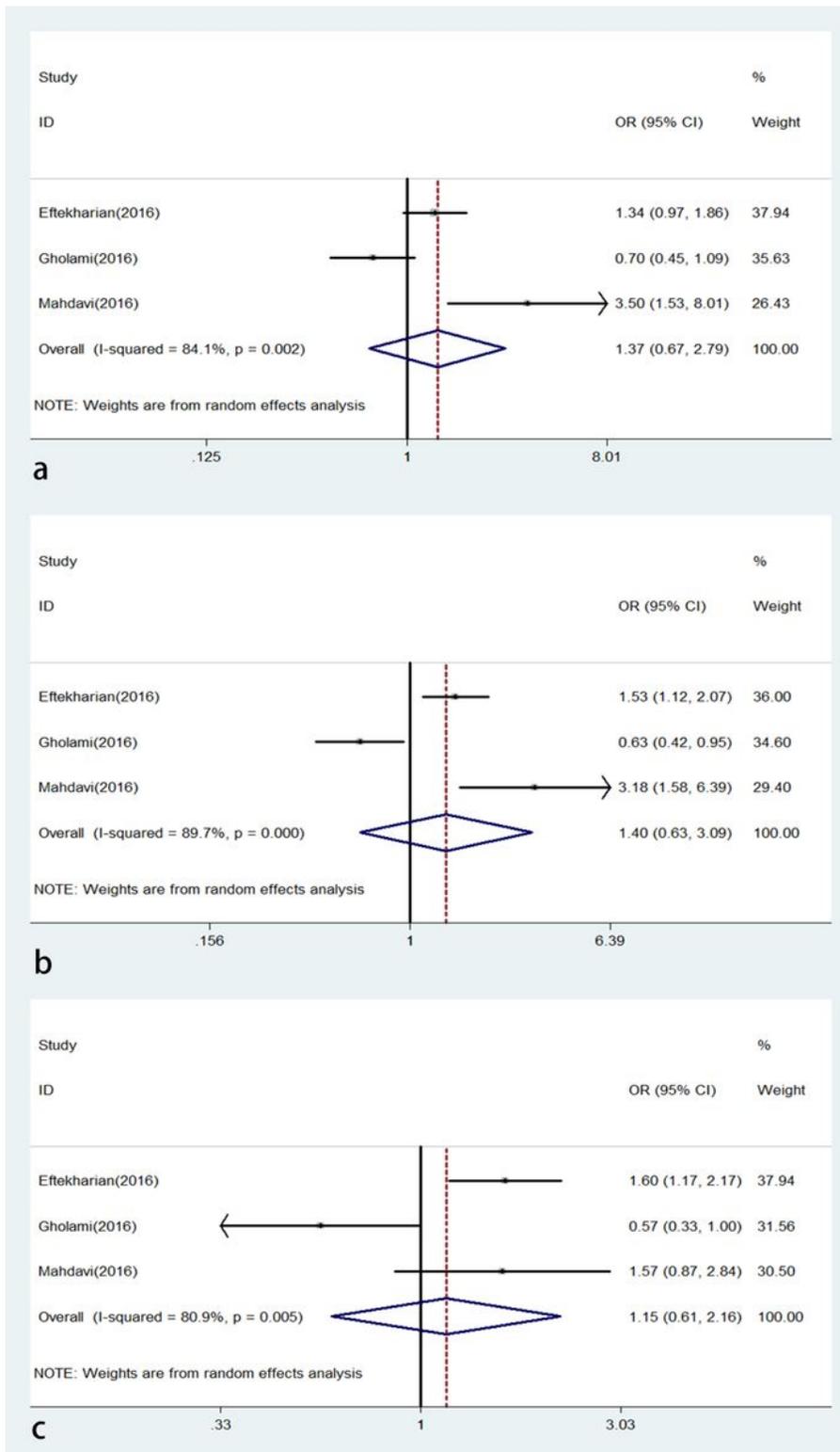
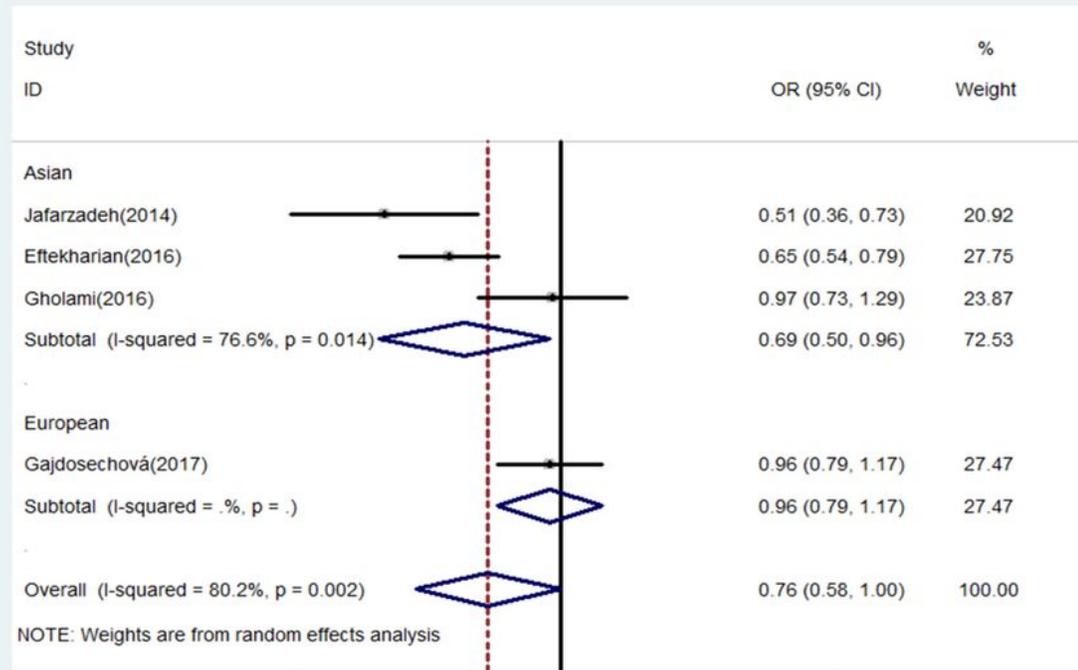
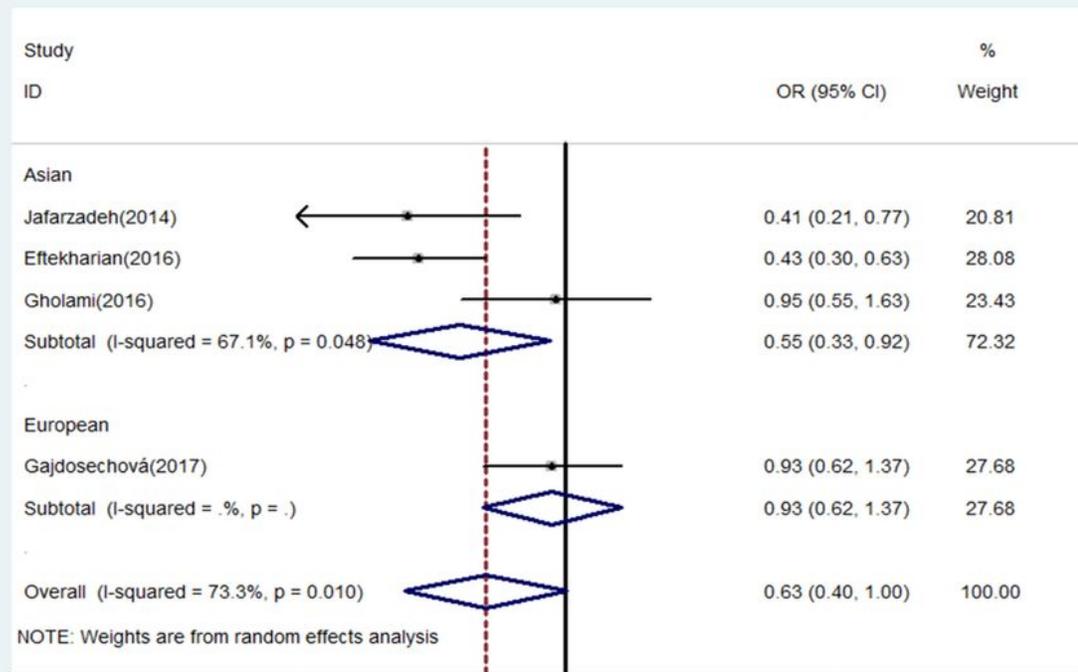


Figure 5

Forest of association between rs2232365 polymorphism of FOXP3 gene and risk of MS under (a) heterozygote model (AC / CC); (b) dominant model (AA + AC / CC); (c) recessive model (AA / AC + CC).



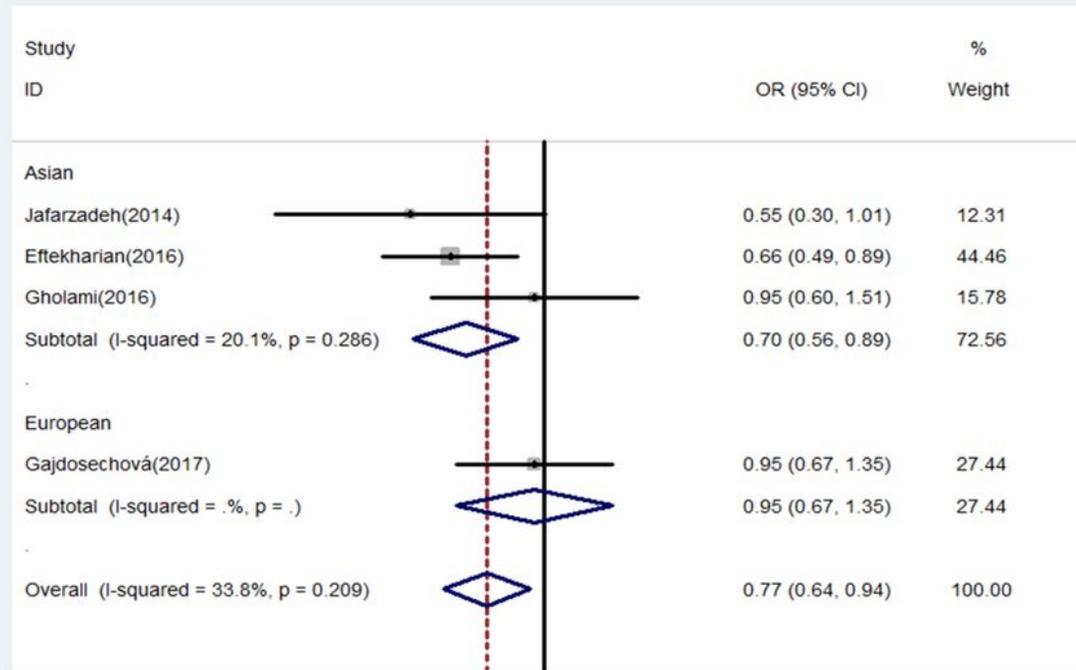
a



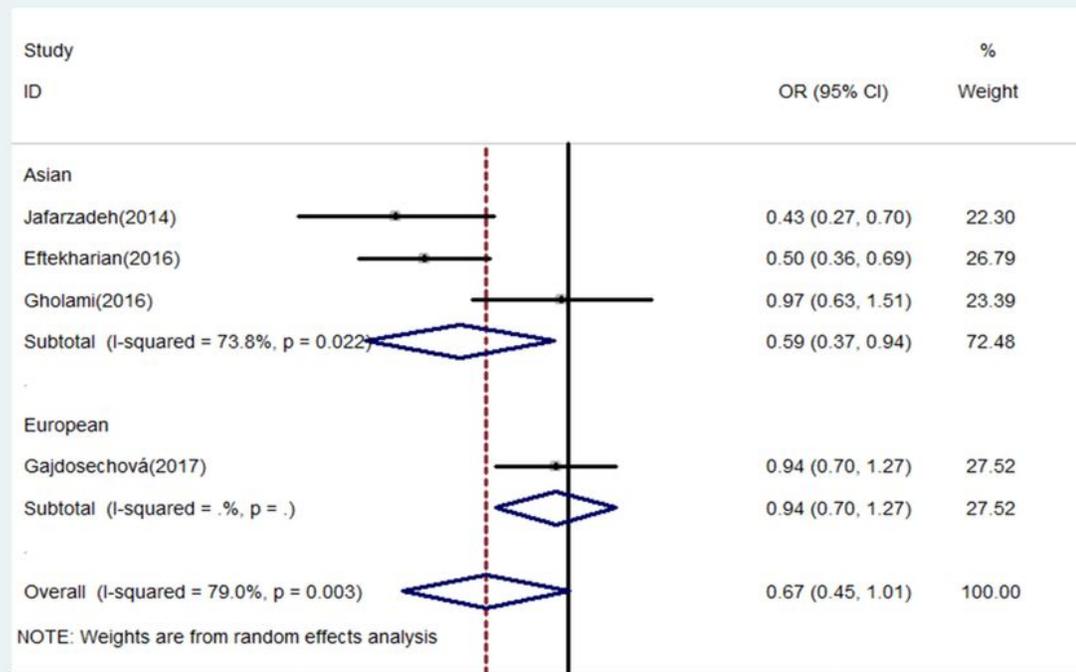
b

Figure 6

Forest of association between rs3761548 polymorphism of FOXP3 gene and risk of MS for subgroup analysis by ethnicity under (a) allele model (C / A); (b) homozygote model (CC / AA).



a



b

Figure 7

Forest of association between rs3761548 polymorphism of FOXP3 gene and risk of MS for subgroup analysis by ethnicity under (a) dominant model (CC + CA / AA); (b) recessive model (CC / CA + AA).

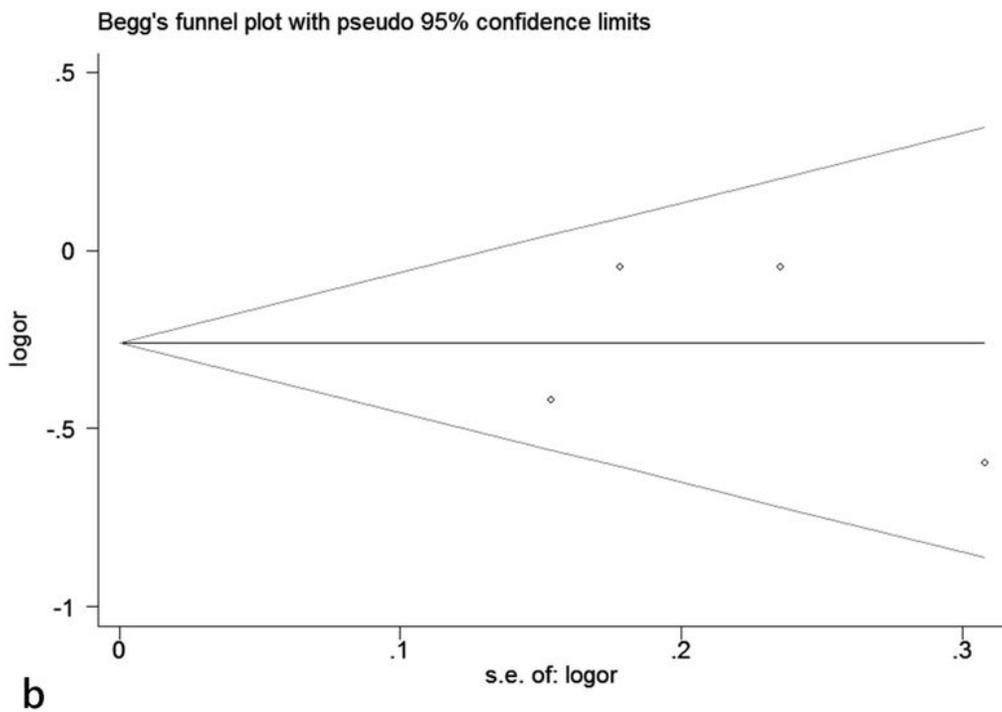
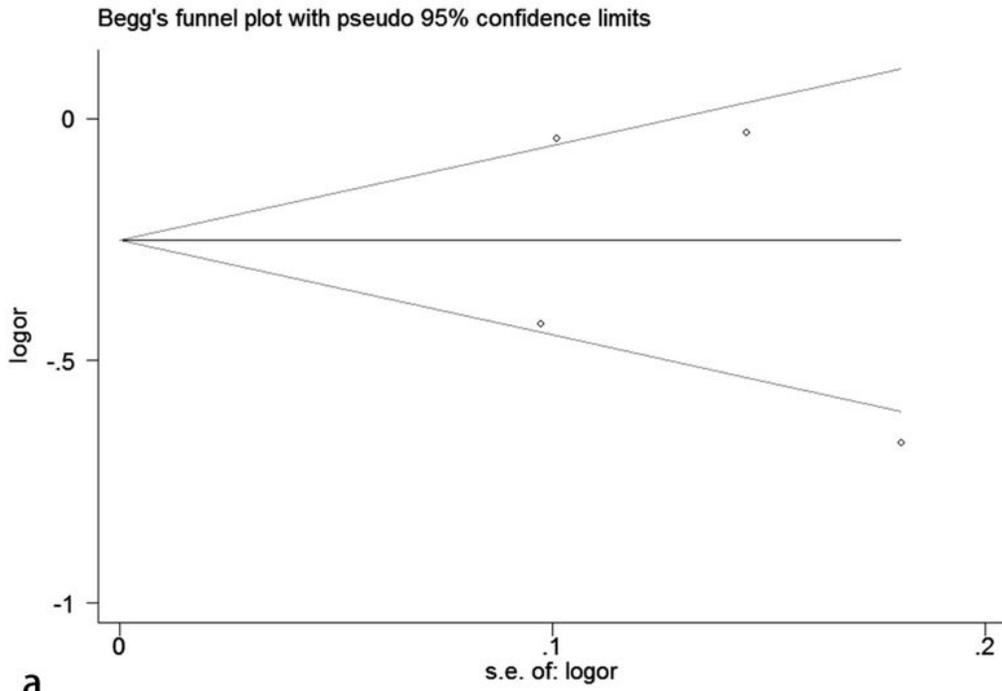
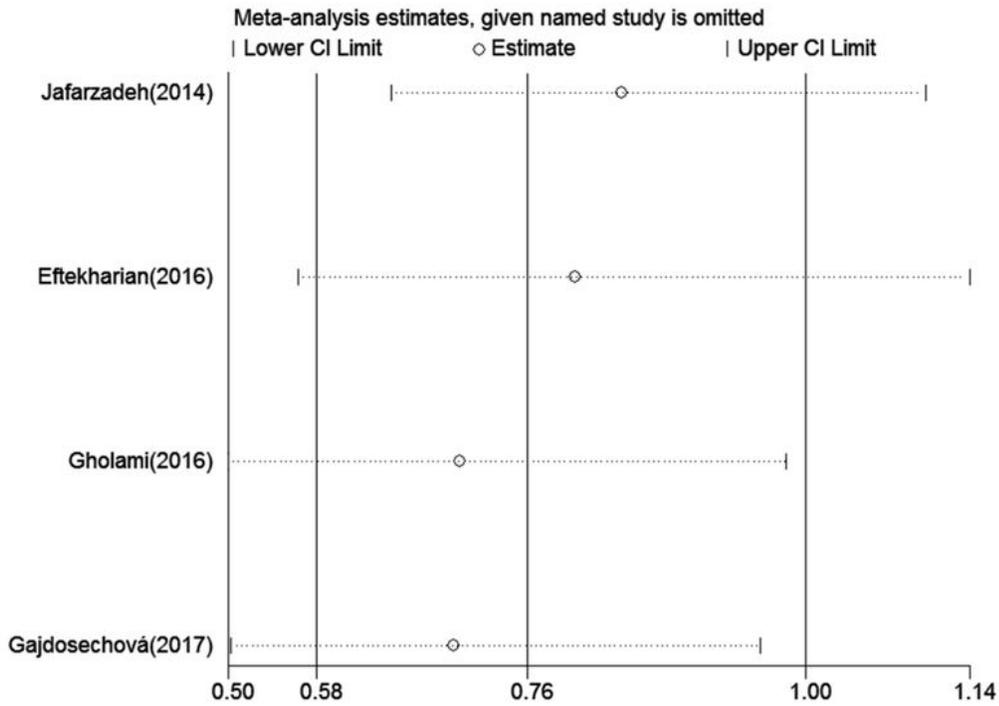
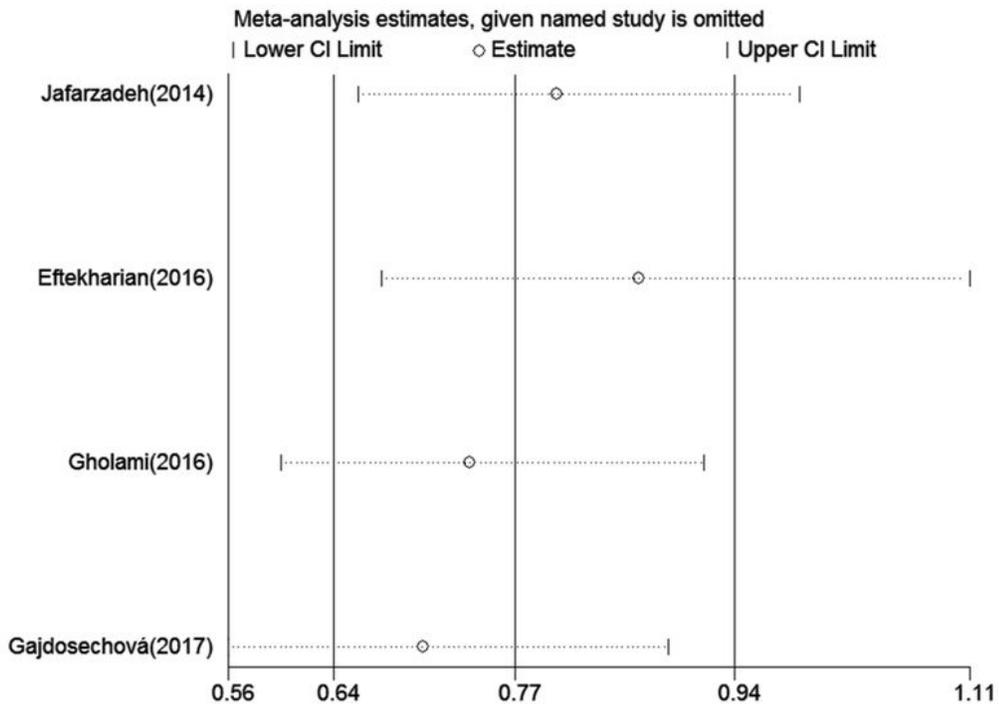


Figure 8

Sensitive analysis of association between rs3761548 polymorphism of FOXP3 gene and risk of MS under (a) allele model (C / A); (b) homozygote model (CC / AA).



a



b

Figure 9

Begg funnel plot for the potential missing studies under (a) dominant model (CC + CA / AA); (b) recessive model (CC / CA + AA).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement1.doc](#)