Protective association of TNFSF15 polymorphisms with Crohn’s disease and ulcerative colitis: A meta-analysis

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INTRODUCTION

The inflammatory bowel diseases (IBD), including Crohn's diseases (CD) and ulcerative colitis (UC), are characterized by chronic progressive diseases of the gastrointestinal tract resulting from interactions between host genotypes and the microbiome.1,2 Many environmental and lifestyle factors including diet, environmental carcinogens, and dwelling condition have been reported to be associated with an elevated IBD risk, but IBD are complex diseases in which the susceptibility depends on both genetic predisposition and environmental exposure. Intense and inappropriate mucosal immune responses to constituents of the intestinal microbiota contribute to the genesis of IBD, and these are determined by complex poorly understood genetic factors that confer vulnerability.1,3,4 However, only a minority of

Abstract

Background/Aims: Three extensively investigated polymorphisms (rs3810936, rs7848647, and rs6478108) in tumor necrosis factor super family member 15 (TNFSF15) gene have been implicated in risk for inflammatory bowel disease (IBD). We performed a quantitative synthesis of the evidence to clarify these associations of TNFSF15 polymorphisms with IBD.

Materials and Methods: Data were extracted from PubMed and EMBASE, up to March 15, 2018. Meta-analysis was performed by critically reviewing five studies for rs3810936 polymorphism (2251 cases and 2442 controls), four studies for rs7848647 polymorphism (1503 cases and 1816 controls), and four studies for rs6478108 polymorphism (1502 cases and 1817 controls).

Results: Our analysis suggested that rs3810936 polymorphism was significantly associated with decreased risk of Crohn’s disease (CD) and ulcerative colitis (UC). For rs7848647 polymorphism, significantly protective association between this polymorphism and CD risk was also observed, but not in UC. For rs6478108 polymorphism, we also detected a significantly protective association with CD risk in all genetic model but not in UC.

Conclusions: This meta-analysis suggests that TNFSF15 polymorphisms may contribute to genetic susceptibility of IBD.

Keywords: Inflammatory bowel diseases, meta-analysis, polymorphism, TNFSF15

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those exposed to changes in gut microbiota, that maintain homeostasis of the mucosal surface, eventually develop IBD, suggesting that genetic factors, such as single nucleotide polymorphisms (SNPs), may be crucial in modifying the risk for IBD.[5,6]

The tumor necrosis factor super family member 15 gene (TNFSF15) is a strong candidate IBD gene encoding a novel TNF-like factor. Previous immunological studies have demonstrated that increased TNFSF expression by macrophages, lymphocytes, and plasma cells in intestinal tissue from IBD patients compared to normal controls is further upregulated in inflamed intestine.[7] TNFSF15 binds to specific T-cell receptors and enhances in mucosal CD4+ T cells in synergy with interleukins 12 and 18 (IL12 and 18).[8]

To date, several epidemiologic studies have been performed to elucidate the effect of TNFSF15 polymorphism on IBD risk. TNFSF15 was the first CD-susceptibility gene identified through a genome-wide association screening (GWAS) of 72,738 SNPs in the Japanese population.[9] The association was well replicated in Korean and US populations. The results of previous studies remain inconsistent across these studies due to limitations in individual studies to examine the association between TNFSF15 polymorphisms and the risk of IBD.

MATERIALS AND METHODS

Search strategy
All relevant studies on the association between TNFSF15 polymorphisms and IBD risk published up to March 15, 2018 were identified through literature searches using PubMed, EMBASE, Cochrane Library, and Web of Science, with the following terms and keywords: (“the tumor necrosis factor super family member 15” or “TNFSF15”) and (“polymorphism” or “variation” or “mutation”) and (“inflammatory bowel disease” OR “IBD” or “Crohn’s diseases” or “CD” or “Ulcerative Colitis” or “UC”). The references cited in all studies were also reviewed to identify additional published articles, which were potentially not indexed by the above databases.

Inclusion criteria
Studies which met the following criteria were included in our meta-analysis: (1) A case–control study evaluating the TNFSF15 polymorphisms; (2) studies with full-text articles; (3) containing enough data for estimating the odds ratios (ORs) with the corresponding 95% confidence interval (CI); (4) no overlapping data.

Data extraction
Information was carefully extracted from all the eligible studies independently by two researchers according to the inclusion criteria listed above and a consensus reached on all the eligibility terms of reference. The following data were collected from each study: first author, publication year, total numbers of cases and controls, numbers of cases and controls for CD and UC, and result of the Hardy-Weinberg equilibrium (HWE) test. We did not define any minimum number of patients for inclusion in our meta-analysis.

Statistical analysis
Odds ratios with a corresponding 95% CI were used as the common measure of assessing the strength of association between TNFSF15 polymorphisms (rs3810936, rs7848647, and rs6478108) and IBD risk for each study. The pooled ORs were calculated in additive model (a allele vs. A allele, a was for the minor allele and A was for the major allele), dominant model (aa + Aa vs. AA), recessive model (aa vs. Aa + AA), and co-dominant model (aa vs. AA, Aa vs. AA).[10] The significance of the pooled ORs was determined by Z-test, and the level of statistical significance was established as $P < 0.05$. The heterogeneity among studies was checked by the Q-test.[11] The $I^2$ statistic, which is a quantitative measure of the proportion of the total variation across studies due to heterogeneity,[12] was also calculated. If the $P$ value for the heterogeneity test was >0.05, the Mantel–Haenszel method-based fixed effects model was used to calculate the pooled OR.[13] Otherwise, the DerSimonian and Laird method-based random effects model was performed.[14] Potential publication bias was evaluated by visual inspection of the Begg funnel plots in which the standard error of log (OR) of each study was plotted against its log (OR). We
also performed Egger's linear regression test ($P < 0.05$ was considered a significant publication bias). All statistical analyses were performed using software programs STATA version 12.0 (Stata, College Station, TX, USA).

**RESULTS**

**Extraction process and study characteristics**

According to our search criterion, 164 articles were retrieved. Among them, the majority were excluded after the first screening based on abstracts or titles, mainly because for reasons of overlapped citations, not relevant to the TNFSF15 polymorphisms and IBD risk, reviews, conference abstracts, or not a related gene polymorphism. Eventually, a total of five case–control studies were selected, including five studies for rs3810936 polymorphism (2251 cases and 2442 controls), four studies for rs7848647 polymorphism (1503 cases and 1816 controls), and four studies for rs6478108...
polymorphism (1502 cases and 1817 controls) [Figure 1]. The characteristics of these included studies and the genotype distribution and allele frequency of TNFSF15 polymorphisms in case- and control-studies are shown in Table 1.

Overall analyses of outcomes

The main results of the meta-analysis are shown in Table 2. Our results revealed that rs3810936 polymorphism was significantly associated with decreased risk of CD (T/T vs. C/C: OR = 0.38, 95% CI = 0.19–0.76, P = 0.000; C/T vs. C/C: OR = 0.57, 95% CI = 0.43–0.74, P = 0.003; recessive model: OR = 0.50, 95% CI = 0.30–0.84, P = 0.000; dominant model: OR = 0.51, 95% CI = 0.36–0.72, P = 0.000; additive model: OR = 0.59, 95% CI = 0.44–0.79, P = 0.000 [Figure 2 and Table 2]); and UC (T/T vs. C/C: OR = 0.53, 95% CI = 0.34–0.81, P = 0.000; C/T vs. C/C: OR = 0.64, 95% CI = 0.51–0.81, P = 0.003; recessive model: OR = 0.54, 95% CI = 0.36–0.83, P = 0.000; additive model: OR = 0.82, 95% CI = 0.69–0.97, P = 0.000 [Figure 3 and Table 2]). For rs7848647 polymorphism, significantly protective association between this polymorphism and CD risk was also observed (T/T vs. C/C: OR = 0.27, 95% CI = 0.15–0.47, P = 0.000; C/T vs. C/C: OR = 0.50, 95% CI = 0.28–0.89, P = 0.003; recessive model: OR = 0.36, 95% CI = 0.27–0.49, P = 0.000; dominant model: OR = 0.44, 95% CI = 0.25–0.80, P = 0.000; additive model: OR = 0.52, 95% CI = 0.36–0.76, P = 0.000 [Figure 4 and Table 2]), but not in UC. For rs6478108 polymorphisms, we also detected significantly protective association with CD risk (C/C vs. T/T: OR = 0.27, 95% CI = 0.15–0.47, P = 0.000; T/C vs. T/T: OR = 0.52, 95% CI = 0.28–0.97, P = 0.003; recessive model: OR = 0.36, 95% CI = 0.27–0.48, P = 0.000; dominant model: OR = 0.46, 95% CI = 0.24–0.86, P = 0.000; additive model: OR = 0.53, 95% CI = 0.36–0.80, P = 0.000 [Figure 5 and Table 2]) in all genetic models but not in UC.

Heterogeneity and sensitivity analyses

The results of heterogeneity test indicated that there was no significant heterogeneity for all polymorphisms of UC across studies [Table 2]. However, we found heterogeneity for CD [Table 2]. Although one study deviated from HWE for the rs3810936 polymorphism, one study deviated from HWE for the rs7848647 polymorphism and two studies deviated from HWE for the rs6478108 polymorphism, the corresponding pooled ORs were not altered by including or not including these studies [Table 2]. Additionally, we also evaluated the influence of each individual study on the pooled ORs by sequential omission of individual studies. The results showed that the pooled ORs of these three polymorphisms were not materially altered by the contribution of any individual study, suggesting that the results of this meta-analysis are credible (data also not shown).

Publication bias

Both Begg’s funnel plot and Egger’s test were performed to assess the publication bias of literature. All these three genetic polymorphisms showed consistent results, indicating no evidence of publication bias in the meta-analysis. If we take rs3810936 polymorphism as an example, the shapes of the funnel plot did not indicate any evidence of obvious asymmetry in these five models [Figure 6], and the Egger’s test suggested the absence of publication bias (P = 0.354 for T/T vs. C/C, P = 0.128 for C/T vs. C/C, P = 0.496 for recessive model, P = 0.107 for dominant model, and P = 0.216 for additive model).

DISCUSSION

TNFSF15, a member of the tumor necrosis factor super family, plays an important role in activation and proliferation of T cells. An increased expression level of TNFSF15 in intestinal lamina propria cells correlated with the degree of intestinal inflammation in CD and

Table 1: Characteristics of studies included in the meta-analysis and their genotype distributions of TNFSF15 polymorphisms

| Polymorphism | First author | Year   | Case (CD/UC) | Control | CD        | UC        | MAF |
|--------------|--------------|--------|--------------|---------|-----------|-----------|-----|
|             |              |        |              |         | A/A       | A/a       |     |
| rs3810936   | Nakagome     | 2017   | 223/164      | 412     | 139       | 72        | 12  |
|              | Lee          | 2015   | 108/165      | 599     | 52        | 46        | 10  |
|              | Baskaran     | 2014   | 302/325      | 430     | 152       | 125       | 25  |
|              | Yang         | 2008   | 380/378      | 378     | 181       | 164       | 35  |
|              | Tremelling   | 2008   | 749/623      |         | 371       | 311       | 67  |
| rs7848647   | Nakagome     | 2017   | 223/164      | 412     | 153       | 60        | 10  |
|              | Lee          | 2015   | 108/165      | 599     | 54        | 46        | 8   |
|              | Baskaran     | 2014   | 304/324      | 431     | 176       | 117       | 11  |
|              | Yang         | 2008   | 380/378      | 378     | 208       | 141       | 31  |
| rs6478108   | Nakagome     | 2017   | 223/164      | 412     | 146       | 67        | 10  |
|              | Lee          | 2015   | 108/165      | 599     | 54        | 46        | 8   |
|              | Baskaran     | 2014   | 303/324      | 429     | 166       | 125       | 12  |
|              | Yang         | 2008   | 380/377      | 377     | 205       | 143       | 32  |

MAF: Minor allele frequency; A: the major allele; a: the minor allele.
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UC patients. This was accompanied by cytokine-induced interferon-gamma production by CCR9+ mucosal and gut-homing T cells, resulting in generation of enhanced Th1 responses and mucosal inflammation. TNFSF15 encodes a ligand for the receptor of TNFRSF25 and a decoy receptor of TNFRSF21/DR6 and has been shown to respond to a bacterial infection and to activate the nuclear factor-κB pathway. Although epidemiological studies investigate the association of TNFSF15 polymorphisms with CD and UC risk, the individual studies might have been underpowered to detect the overall effect of polymorphisms on the susceptibility to CD and UC, and our meta-analysis enhances the statistical power.

To the best of authors’ knowledge, this is the first meta-analysis undertaken so far of the largest and most comprehensive assessment of the relationship

Figure 3: Forest plots of ORs with 95% CI for TNFSF15 rs3810936 polymorphism and the risk of UC (fixed effects). The center of each square represents the OR, the area of the square is the number of sample and thus the weight used in the meta-analysis, and the horizontal line indicates the 95% CI. (1) Recessive model. (2) Dominant model. (3) T/T vs. C/C. (4) C/T vs. C/C. (5) Additive model
between the TNFSF15 polymorphisms and the risk of CD and UC. Overall, our results suggest that rs3810936, rs7848647, and rs6478108 polymorphisms in TNFSF15 gene were associated with decreased risk of CD in all genetic models, which was consistent with the conclusion of individual studies involving these three polymorphisms. However we detected that only rs3810936 polymorphism might have a protective association of UC.

There was considerable heterogeneity for all polymorphisms of CD across studies but not in UC. The source of heterogeneity might have come from different genetic backgrounds, population stratification, and selection bias. The GWAS studies on SNP in IBD are highly specialized and may likely never go beyond the design of case–control studies. The number of published studies was not sufficiently large for stratified analysis in different ethnicities. In this meta-analysis, we performed sensitivity
analyses to check the robustness of our conclusion and the corresponding pooled ORs were not changed. In addition, we comprehensively assessed the publication bias using several means including the Begg’s and Egger’s tests as well as funnel plot tests, indicating no publication bias for these three genetic polymorphisms. In view of this, we are strongly convinced that the methods are appropriate and well described and the results or data of our meta-analysis, in essence, are sound and reliable.

When interpreting the results of the current study, some limitations should be addressed. First, the number of subjects and studies included in the meta-analysis were small to reveal the associations with IBD and lack the original data for the included studies, thus limiting further evaluation of the association between IBD risk and other risk factors, such as age, gender, environment factors, and other variables, which might have caused serious confounding bias. Second, we did
not estimate the potential interactions among gene–gene, gene–environment, or even various polymorphisms loci of the same gene, which may alter the risk of IBD. Although the analysis of haplotype can increase the power to detect disease associations, our study was limited by analyzing a single SNP site owing to only one study focusing on TNFSF15 haplotype. Third, some inevitable publication bias might exist in the results because only published studies were retrieved, although the funnel plot and Egger's test indicated no remarkable publication bias.

CONCLUSION

In summary, this meta-analysis provides evidence that rs3810936, rs7848647, and rs6478108 polymorphisms may contribute to protective factor of CD. Nevertheless, large-scale, well-designed, and population-based studies are necessary to confirm these findings.
needed to investigate the combined effects of these variants within TNFSF gene and IBD, which may eventually lead to better comprehensive understanding of their possible roles in the pathogenesis of IBD.

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Conflicts of interest
There are no conflicts of interest.

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