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Review Article

A meta-analysis of P1371Q polymorphisms in DLG5 gene with reduced risk of Crohn's disease in European

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Abstract: Background: The DLG5-e26 and P1371Q polymorphisms in the discs large homologue 5 (DLG5) gene may influence the susceptibility to inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC); however, existing results remain inconclusive. Aim: Our aim was to investigate the association between the DLG5 polymorphisms and IBD risk by meta-analysis. Methods: Fourteen studies were extracted from a search of PubMed, Embase, Chinese National Knowledge Infrastructure (CNKI) and Google Scholar databases before December 2015. We estimated the odds ratio (OR) and 95% CI using fixed-effect model or random-effect model. *Results:* The minor A allele at P1371Q decreased risk of CD in European (A vs. C, OR = 0.843, 95% CI = 0.714-0.995, P = 0.044), however, it increased the risk of IBD in North American (1.751 (95% = CI 1.249-2.455), P = 0.001). No significant associations were found between DLG5-e26 and IBD (*del*A *vs*. *ins*A in IBD: 1.053, 95% CI = 0.976-1.136; CD: 1.031, 95% CI = 0.938-1.132; UC: 1.007, 95% CI = 0.832-1.219), and between P1371Q and IBD (A vs. C in IBD: 1.050, 95% CI = 0.930-1.184; CD 0.994, 95% CI = 0.802-1.231; UC: 1.124, 95% CI = 0.962-1.313). *Conclusions*: DLG5-e26 polymorphisms in the DLG5 have no relationship with IBD in either CD or UC, but P1371Q reduces the risk of CD in European, while increases the risk of IBD in North American.

Keywords: Discs large homologue 5, inflammatory bowel disease, Crohn's disease, ulcerative colitis, polymorphism, meta-analysis

Introduction

Inflammatory bowel disease (IBD) is a chronic and relapsing intestinal inflammatory disorder, and includes Crohn's disease (CD) and ulcerative colitis (UC). CD and UC have defined clinical and histopathological characteristics but overlapping symptoms can be observed. UC is characterized by inflammation limited to colorectal mucosal and sub-mucosal layers, while the inflammation in CD can involve any part of the gastrointestinal tract in a discontinuity [1]. Recently, the prevalence rate of IBD has increased, especially in Western countries. The highest prevalence rates of UC and CD in Europe were 505 per 100,000 persons and 322 per 100,000 persons, respectively [2]. Although it is believed that genetics, environmental factors and immunological factors play a part in the pathogenesis of IBD, its aetiology is still not fully understood. Several whole-genome association studies (GWAS) have identified many potential susceptible variants in the following genes: DLG5, NOD2, ATG16L1, IRGM and STAT4 [3-7]. This suggests that genetics may play an important role in the susceptibility of IBD.

The DLG5 gene is located at chromosome 10q23 and comprised of 32 exons [8, 9]. It encodes scaffolding proteins that belong to the Membrane Associated Guanylate Kinases (MAGUKs) and is widely expressed in human tissues, including the small bowel as well as the colon [8]. There are four protein interaction motif (PDZ) domains in the DLG5 gene, including one Scr homology 3 (SH3) domain, a guanylate kinase (GUK) domain, and an N-terminal domain of unknown function (DUF622) [10]. DLG5 interacts with the GUK domain of erythrocyte membraneprotein p55 (MPP1) and forms a heteromeric MAGUK complex at the plasma membrane. It then clusters a variety of intracel-

lular molecules to maintain the structure of epithelial cells and transmits extra cellular signals to the membrane [8, 10, 11]. Therefore, DLG5 plays important roles in maintaining cell shape and polarity [12]. Furthermore, it is located at sites of cell-cell contact as a binding partner of vinexin [13] and takes part in the maintenance of epithelial integrity [7]; loss of cell polarity complexes and adhesion complexes results in a failure to maintenance epithelial cell polarity, inducing epithelial-to-mesenchymal transition (EMT) [14]. Stoll and colleagues [7] found that DLG5 mRNA was expressed in the intestine and the variation of DLG5 was associated with IBD, including CD and UC. Sezaki and colleagues [15] showed that DLG5 could interact with TGF-β type I (TβRI) and TGF-β type II (TβRII) receptors at the plasma membrane and enhance their degradation, and DLG5 was involved in suppressing epithelial-to-mesenchymal transition (EMT) by inhibiting growth factor-β (TGF-β) receptor-dependent signal and transforming TGF-β signalling [16]. In addition, TGF-β signalling dysregulation increased the risk of having CD [15], and TGF-β I knockout mice developed systemic inflammation in the intestine [17]. Thus, it is conceivable that genetic variants in DLG5 might contribute to a disturbance of the epithelial barrier in the gastrointestinal tract and then result in impaired epithelial structure, making it more vulnerable to IBD, CD and UC [7, 9]. DLG5-e26 variant presents insertion or deletion of adenine (insA or delA) in exon 26 [18]. Several research articles showed that DLG5-e26 might have associations with IBD, including CD and UC [18-20]. P1371Q, also known as C4136A [7], presents a C to A transversion in exon 23 of DLG5 gene, resulting in the substitution of proline by glutamine [18]. This substitution probably hinders the scaffolding of DLG5 and alters the normal functions of the intestinal barrier [7], which could then lead to inflammation of the affected area; this association may be observed in different populations [20-22].

The association in different populations between DLG5-e26 and P1371Q with IBD risk has been extensively investigated [23-28]. However, an individual study may not have enough statistical power to find a true association, and some studies have yielded conflicting and inconsistent results. Therefore, to estimate strength, accuracy and characteristics of the relationship between DLG5-e26 and P1371Q and IBD, a meta-analysis was performed.

Materials and methods

Literature search

Systematic computerized searches about DLG5 polymorphisms with IBD (up to December 2015) were performed in the PubMed database without a language limitation. The Chinese National Knowledge Infrastructure (CNKI), Embase and Google Scholar were used to supply other publications that could not be found in PubMed. The following combination of keywords was used to search: ("DLG5" or "Drosophila Discs Large Homologue 5") and ("Crohn's disease" or "Crohn's disease" or "CD" or "inflammatory bowel disease" or "IBD" or "ulcerative colitis" or "UC"). After scanning the titles and abstracts of all related manuscripts, we manually examined reference lists for additionally potential relevant articles. The most complete or recent publications were chosen if more than one study was published with the same content.

Inclusion and exclusion criteria

Two investigators (Zixing Zhou, Shiqi Huang) independently read the titles and abstracts of the publications. The studies were included if they met the following selection criteria: case-control study; DLG5-e26 and P1371Q polymorphisms with risks of IBD or CD or UC; distribution data of allele frequency or genotype; clear diagnosis criteria in IBD, CD and UC; human study; and the reporting of the odds ratio (OR) and 95% confidence interval. We excluded republished articles, overlapping data, studies with inadequate data for pooling, meeting abstracts and reviews.

Data extraction

General information on the included studies was extracted independently by two investigators (Zixing Zhou, Shiqi Huang), including the first author, country and ethnicity, genotyping method and the number of patients and controls. If there was a lack of genotype information, we attempted to contact the corresponding author to obtain the missing information; if we were unsuccessful, the study would be excluded. Any divergence was resolved by the senior investigator (Chunxia Jing).

Assessment of bias risk

The quality of studies was also independently assessed by the same reviewers, based on a bias risk score for genetic association, which was modified on the basis of traditional epidemiologic considerations as well as genetic issues developed by Thakkinstian and colleagues [29]. The score considered five domains, including information bias, confounding bias, selective reporting of outcomes, population stratification and assessment of the Hardy-Weinberg equilibrium (HWE) in the control group. All items were classified with regard to a 'yes', 'no' or 'unclear', on behalf of low risk, high risk or insufficient information, respectively. The dissidences between the two investigators were resolved by a senior reviewer.

Statistical analysis

A comprehensive meta-analysis software (version 2.0) was used for statistical analysis. The Hardy-Weinberg equilibrium (HWE) was calculated in the control groups with Fisher's exact test. If the HWE had no evidence ($P < 0.05$), the article was considered to have disequilibrium. We performed both per-allele and per-genotype approaches to estimate the strength of the association between the polymorphisms of DLG5-e26 and P1371Q and the risks of IBD, including CD and UC.

Per-allele analysis

Assuming that 'D' and 'd' are the risk and non-risk alleles for polymorphism, respectively, then 'DD', 'Dd' and 'dd' are minor homozygous, heterozygous and common homozygous genotype, respectively. 'del/ins' represents a deletion/insertion polymorphism, so 'deldel', 'insdel' and 'insins' are minor homozygous, heterozygous and common homozygous genotypes, respectively [18]. The risk allele frequency in each group was calculated using the reported genotype data, and 95% confidence intervals of the overall prevalence were estimated. The heterogeneity of studies was assessed by the Q test, while the degree of heterogeneity was quantified by the I^2 test [30]. When the inspection result showed $P > 0.10$, a fixed-effect model (the Mantel-Haenszel method) [31] was chosen to pool the data. If not, a random-effect model (the Der Simonian and Laird method) [32] was selected. Furthermore, I^2 was used to quantify the degree of heterogeneity ($I^2 < 25\%$, no heterogeneity; $25\% < I^2 < 50\%$, moderate heterogeneity; $50\% < I^2 < 75\%$, large heterogeneity; and $I^2 > 75\%$ extreme heterogeneity) [33]. The population-attributable risk (PAR) for the risk allele was estimated based on results from the discrete-time model [34, 35]. We performed a sensitivity analysis to assess the stability of the meta-analysis, first omitting one study and then observing the influence of the remaining results on the overall OR.

Per-genotype analysis

We performed the model-free method to assess the genotype effects; thus, DD vs. dd/deldel vs. insins (OR_1) and Dd vs. dd/insdel vs. insins (OR_2) were estimated for each publication [36]. The model of genetic effect, measured by the parameter lambda (λ) (the ratio of $\log OR_2$ to $\log OR_1$), was then estimated by the model-free Bayesian approach. This parameter ranges from 0 to 1, which represents model as follows: $\lambda = 0$, suggesting a recessive (DD vs. Dd + dd/deldel vs. insdel + insins) model; $\lambda = 1$, suggesting a dominant model (DD + Dd vs. dd/deldel + insdel vs. insins); $\lambda = 0.5$, suggesting a co-dominant model (DD vs. dd; Dd vs. dd/deldel vs. insins; insdel vs. insins). If $\lambda > 1$ or $\lambda < 0$, a rare homozygous or heterozygous model is likely. For λ, we used WinBugs 1.4.2 to estimate parameters with vague prior to distributions (i.e., lambda and odds ratio). The publication bias was quantified by Egger's regression intercept ($P < 0.05$ was considered statistically significant) and funnel plot [37].

Results

Characteristics of studies

Ninety-one studies concerning DLG5-e26 were identified in PubMed, CNKI, Embase and Google Scholar databases. There were 76 records after identifying duplicates, and 53 studies were eligible after screening titles and abstracts. Ultimately, a total of 8 studies concerning DLG5-e26 were included (**Figure 1A**), and 13 studies about P1371Q were chosen (**Figure 1B**). HWE was included in all studies except for one [20], so this article was not included for further pooling. The characteristics of the included studies are listed in **Table 1**.

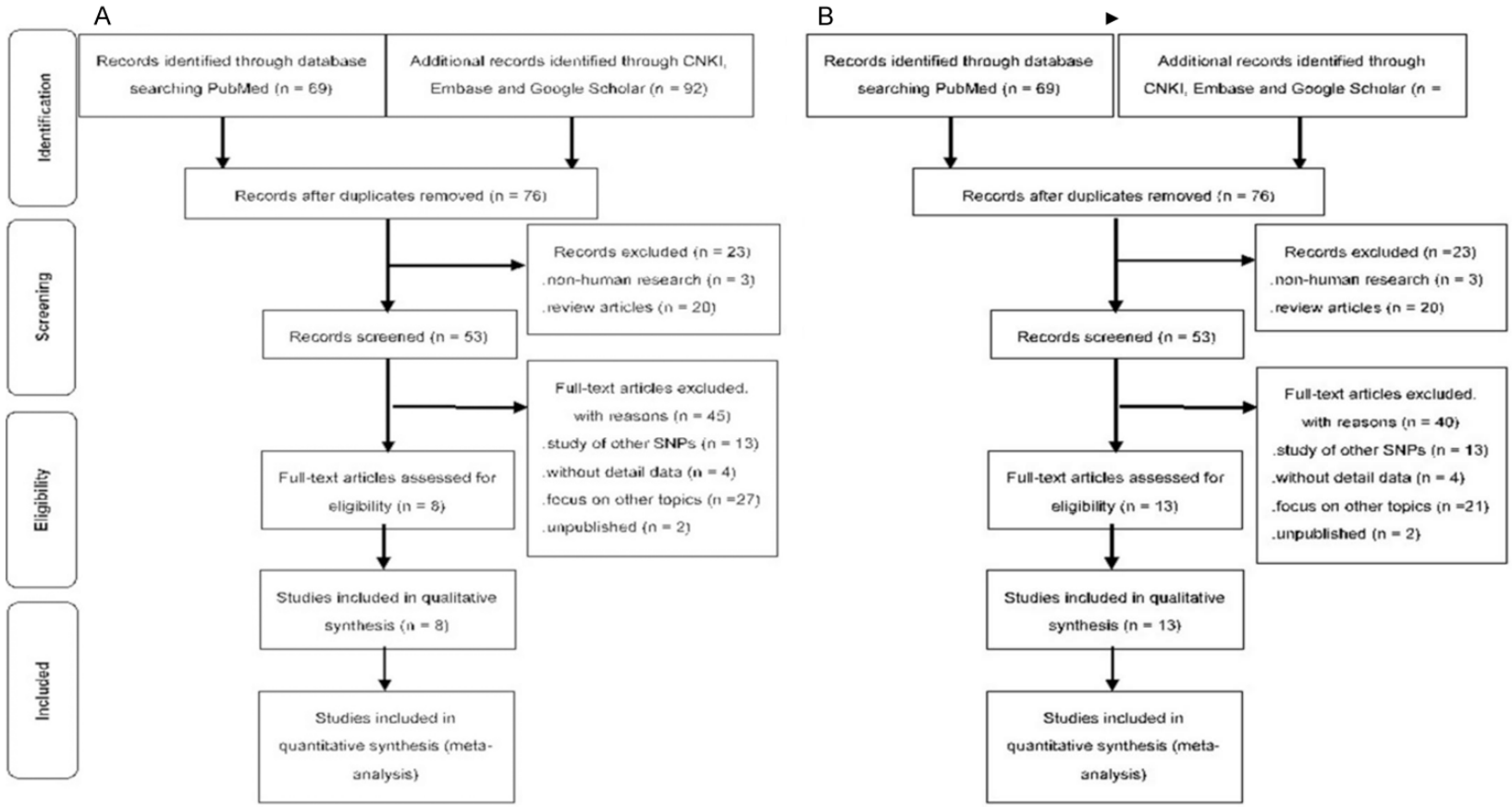

Figure 1. Flowchart for identified studies for DLG5 gene with IBD, CD and UC. A. DLG5-e26; B. P1371Q.

Table 1. Characteristics of the eligible studies for DLG5-e26 and P1371Q in meta-analysis

Study	Year	Country (ethnicity/ continent)	Method	IBD (DLG5-e26)		CD (DLG5-e26)		UC (DLG5-e26)		IBD (P1371Q)		CD (P1371Q)		UC (P1371Q)	
				Number (case/ control)	HWE	Number (case/ control)	HWE	Number (case/ control)	HWE	Number (case/ control)	HWE	Number (case/ control)	HWE	Number (case/ control)	HWE
Buning [23]	2006	German (C/E)	PCR	389/228	0.43	242/228	0.43	147/228	0.43	339/419	0.29	249/419	0.29	150/419	0.29
		Hungary(C/E)	PCR	266/203	0.49	142/203	0.49	124/203	0.49	268/203	0.64	145/203	0.64	123/203	0.64
Vermeire [21]	2005	Belgium(NA/E)	MALDI-TOF-MS	585/297	0.30	455/297	0.30	115/297	0.30	NA	NA	NA	NA	NA	NA
Lappalainen [44]	2008	Finnish (NA/E)	RFCP/PCR	699/190	0.94	240/190	0.94	459/190	0.96	NA	NA	NA	NA	NA	NA
Tremelling [25]	2006	UK (C/E)	TaqMan	NA	NA	NA	NA	NA	NA	1098/752	0.31	495/752	0.31	507/752	0.31
Browning [45]	2007	New Zealand (C/P)	PCR/TaqMan	NA	NA	NA	NA	NA	NA	790/408	0.51	348/408	0.51	406/408	0.51
Torok [18]	2005	German (C/E)	RFCP	970/972	0.35	615/972	0.35	355/972	0.35	970/972	0.94	615/972	0.94	355/972	0.94
Yamazaki [22]	2004	Japan (A/As)	PCR	NA	NA	NA	NA	NA	NA	NA	NA	477/341	0.58	NA	NA
Weersma[43]	2009	Netherlands(C/E)	TaqMan	NA	NA	NA	NA	NA	NA	2804/1350	0.83	1684/1350	0.83	1120/1350	0.83
Chua [20]	2011	Malaysia(A/As)	RFCP/PCR	NA	NA	80/100	0.02	NA	NA	NA	NA	80/100	0.00	NA	NA
Newman [24]	2006	Canada (C/Na)	MALDI-TOF-MS	NA	NA	NA	NA	NA	NA	387/388	0.71	229/388	0.71	158/388	0.71
Ghyslaine[27]	2012	German (C/E)	PCR	380/218	0.38	232/218	0.38	148/218	0.38	380/218	0.50	232/218	0.50	148/218	0.50
Lin [38]	2011	USA (M/Na)	RFCP/CRFCP/PCR	NA	NA	NA	NA	NA	NA	212/170	0.69	NA	NA	NA	NA
Stoll [7]	2004	German (C/E)	PCR	514/519	0.37	NA	NA	NA	NA	525/516	0.56	NA	NA	NA	NA
Al-Sulaiman [28]	2014	Saudi Arabia (NA/As)	RFCP	NA	NA	NA	NA	NA	NA	77/75	0.11	NA	NA	NA	NA

A, Asians; C, Caucasians; M, mixed; E, Europe; As, Asia; Na, North America; P, Pacific; RFLP, restriction fragment length polymorphism analysis; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; PCR, polymerase chain reaction; NA, not available; IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; HWE, Hardy-Weinberg equilibrium.

Table 2. The risk of bias assessment

Author	Ascertainment of IBD (CD and UC)	Ascertainment of control	Quality control for genotyping	Population stratification	Confounding bias	Selective outcome report	HWE
Vermeire [21]	Yes	Yes	Yes	Yes	No	Yes	No
Buning [23]	Yes	Yes	Unclear	Yes	No	Yes	No
	Yes	Yes	Unclear	Yes	No	Yes	No
Torok [18]	Yes	Yes	Unclear	Yes	No	Yes	No
Lappalainen [44]	Yes	Yes	Unclear	Yes	Yes	Yes	Yes
Chua [20]	Yes	Yes	Unclear	Yes	No	Yes	No
Ghyslaine [27]	Yes	Yes	Unclear	Yes	No	Yes	Yes
Stoll [7]	Yes	Yes	Unclear	Yes	No	Yes	No
Tremelling [25]	Yes	Yes	Yes	Yes	No	Yes	No
Browning [45]	Yes	Yes	Yes	Yes	No	Yes	Yes
Yamazaki [22]	Yes	Yes	Unclear	Yes	Yes	Yes	Yes
Weersma [43]	Yes	Yes	Unclear	Yes	No	Yes	Yes
Newman [24]	Yes	Yes	Yes	Yes	No	Yes	Yes
Lin [38]	Yes	Yes	Unclear	Yes	No	Yes	Yes
Al-Sulaiman [28]	Yes	Yes	Unclear	Yes	No	Yes	No

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; HWE, Hardy-Weinberg equilibrium.

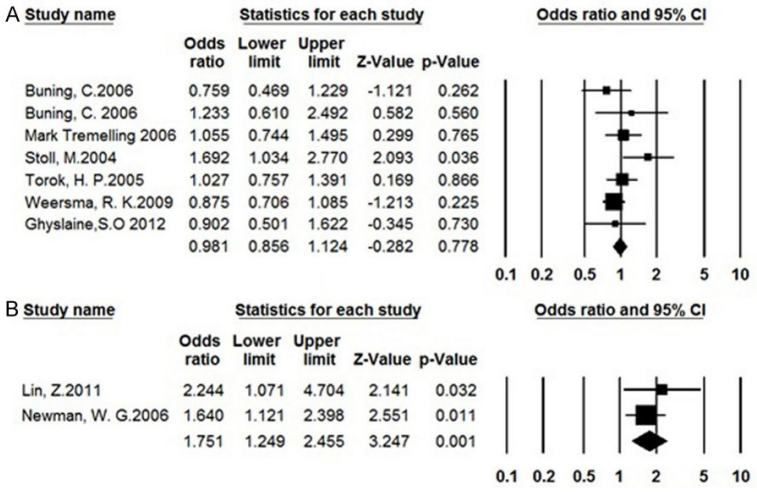

Figure 2. Forest plot of P1371Q polymorphisms with IBD in subgroup analysis (A vs. C). (A) The association between P1371Q and IBD risk in European; (B) The association between P1371Q and IBD risk in North American.

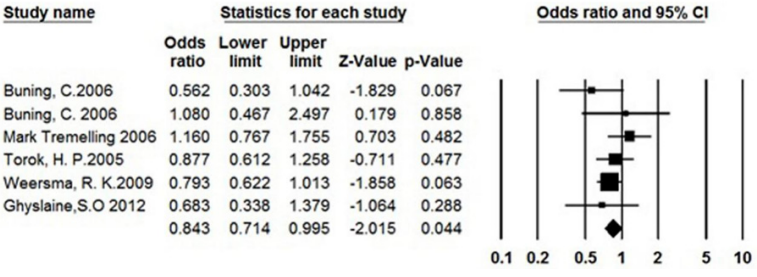

Figure 3. Forest plot of P1371Q polymorphisms with CD in European (A vs. C).

Risk of bias assessment

The criteria for assessing the quality wereclearly described in Table S2. The risk of bias from the quality control for genotyping was the highest, unclear in 10 out of 15 studies (66.7%), followed by not assessing Hardy-Weinberg equilibrium (8/15, 53.3%) and confounding bias (2/15, 13.3%) (**Table 2**).

The genetic association between DLG5-e26 and IBD risk

Eight studies, including 3 803 cases and 2 424 controls, assessed the association between DLG5-e26 and IBD, in which there were 2 006 cases and 2 208 controls concerning CD, and 1 348 cases and 2108 controls concerning UC (**Table 1**). The pooled OR of DLG5-e26 (delA vs. insA) for IBD was 1.053 (95% CI: 0.976-1.136, P > 0.05) with moderate heterogeneity (χ^2 = 8.489, P = 0.204, I^2 = 29.319), suggesting that a delA in DLG5-

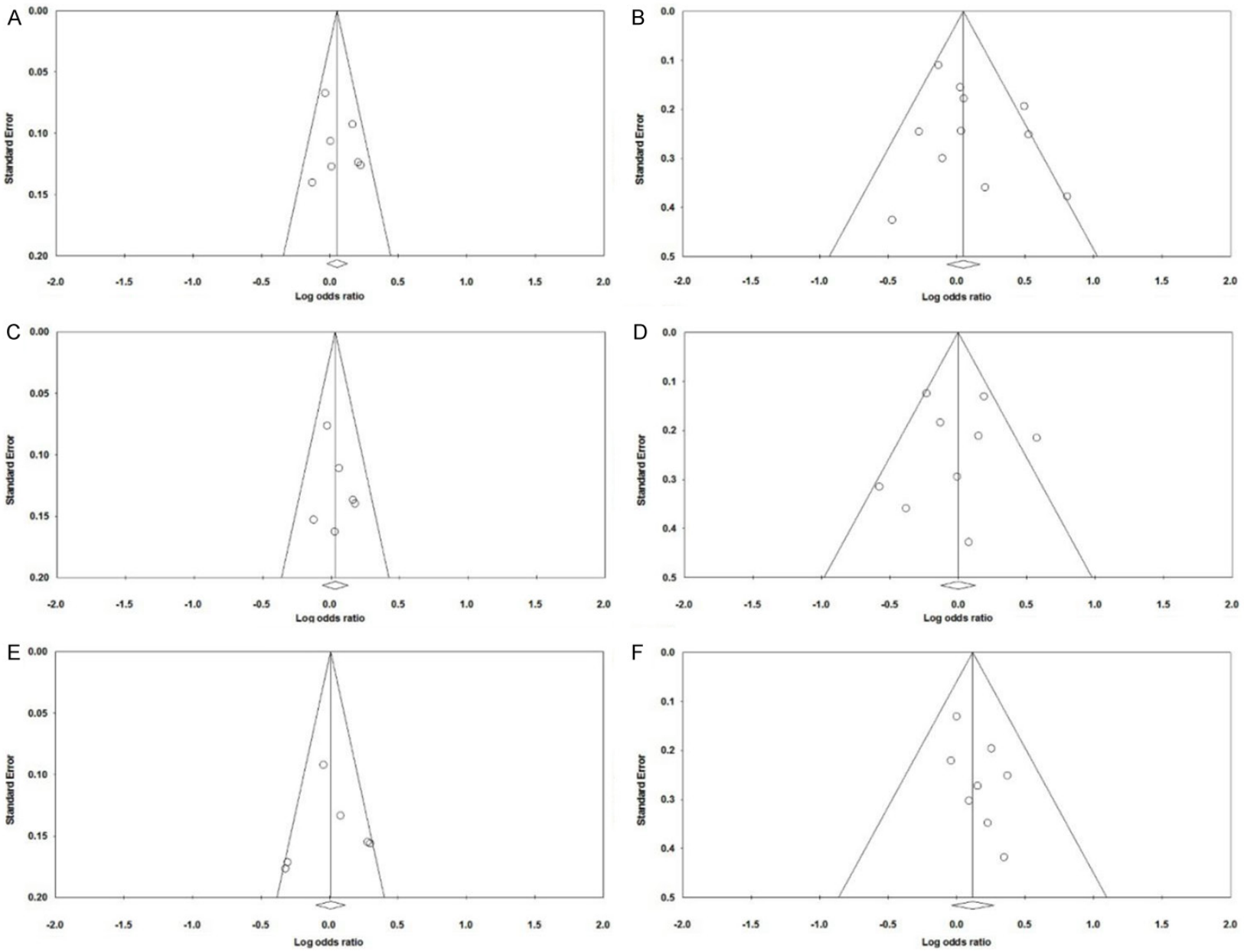
A
B
C
D
E
F
Standard Error
Log odds ratio

Figure 4. Funnel plots for delA vs. insA for DLG5-e26 and funnel plots for A vs. C for P1371Q. A. DLG5-e26 and IBD; C. DLG5-e26 and CD; E. DLG5-e26 and UC; B. P1371Q and IBD; D. P1371Q and CD; F. P1371Q and UC.

e26 did not increase the risk of IBD (Figure S1). The PAR of risk delA was 1.94%.

Both OR_1 (delAdelA vs. insAinsA: $\chi^2 = 5.657$, P = 0.463, $I^2 = 0.000$) and OR_2 (insAdelA vs. insAinsA: $\chi^2 = 7.522$, P = 0.275, $I^2 = 20.236$) were homogenous. The pooled OR_1 and OR_2 were 1.068 (95% CI 0.903-1.262) and 1.029 (95% CI 0.918-1.153), respectively (Figure S3). The $\lambda = 0.630$ (95% CI 0.085-0.983) suggests that a co-dominant effect was most likely, although both genotype effects did not reach statistical significance. No association was detected between DLG5-e26 and CD as well as the overall UC susceptibility (in CD: delA vs. insA, OR = 1.031, 95% CI = 0.938-1.132; delAdelA vs. insAinsA, OR = 1.054, 95% CI = 0.857-1.296; insAdelA vs. insAinsA, OR = 1.045, 95% CI = 0.914-1.195; PAR = 1.1%; in UC: delA vs. insA, OR = 1.007, 95% CI = 0.832-1.219, $I^2 = 64.107$; delAdelA vs. insAinsA, OR = 1.015, 95% CI = 0.796-1.293, $I^2 = 31.928$; insAdelA vs. insAinsA, OR = 1.015, 95% CI = 0.767-1.343, $I^2 = 66.639$; delA, PAR = 0.24%) (Figure S3).

The funnel plot (**Figure 4**) and Egger test did not suggest any publication bias (IBD: SE = 1.85, P = 0.52; CD: SE = 1.33, P = 0.50; UC: SE = 3.16, P = 0.93), and the sensitivity analysis showed that no individual study obviously affected the final conclusion (Table S3).

The genetic association between P1371Q and IBD risk

Thirteen case-control studies, with 7 850 cases and 5 471 controls of IBD, reported the associations between P1371Q and IBD. There were 4 325 cases and 4 763 controls in CD and 2 967 cases and 4 710 controls in UC. The pooled OR of P1371Q on IBD was 1.050 (95% CI 0.930-1.184, P = 0.433), indicating that individuals carrying the A allele in P1371Q had no increased risk of developing IBD than those carrying the C allele (Figure S2). The summary OR_1 and OR_2 for P1371Q were 1.759 (95 % CI: 0.770-3.979) and 1.015 (95% CI: 0.895-1.152), respectively, suggesting that the AA and CA genotypes did not increase the risk of developing IBD compared with the CC genotype (Figure S4 and Table S1). However, when we did subgroups analysis according to continents, the pooled OR of risk allele on IBD in North American was 1.751 (95% CI 1.249-2.455, P = 0.001), suggesting that North American carrying the A allele had increased risk of developing IBD than those with C allele (**Figure 2**). But no association was found in other continents between P1371Q and IBD.

There was also no association between P1371Q and CD (A vs. C, OR = 0.994, 95% CI = 0.802-1.231) or between P1371Q and UC (A vs. C, OR = 1.124, 95% CI = 0.962-1.313). The $\lambda = 0.333$ (95% CI 0.011-0.937) suggested that a co-dominant effect was most likely. In continents subgroups analysis of CD, the European with A allele decreased 15.7% risk of CD than those with C allele (A vs. C, OR = 0.843, 95% CI = 0.714-0.995, P = 0.044), indicating that A allele in P1371Q was the protective factor for CD of European (**Figure 3**). However, no meta-analysis could carry out in other continents because of limited article.

The funnel plot (**Figure 4**) and Egger test did not indicate any publication bias (IBD: SE = 1.08, P = 0.34; CD: SE = 1.40, P = 0.83; UC: SE = 0.61, P = 0.13), and we found no individual study obviously affected the final conclusion from the sensitivity analysis observed in Table S4.

Discussion

Our meta-analysis gave no evidence of an association between DLG5-e26 polymorphisms in the DLG5 gene and IBD, including CD and UC. However, we identified the minor allele A in P1371Q increased the IBD risk in North American, but not in European. These inconsistent results might be due to various genetic backgrounds, different environment and limited publications. A allele in P1371Q decreased the risk of CD in European, without any founding in the people in other continents. Lin. Z and colleagues [38] suggested that the risk allele A of P1371Q was associated with IBD in USA, this association was female-specific. P1371Q was complementary to R30Q in the DLG5 gene, with R30Q exhibiting a dominant effect in IBD susceptibility. A previous meta-analysis revealed that R30Q was associated with a small reduction in the risk of CD, but only in women [39]. Interestingly, Purmonen and colleagues [40] suggested that DLG5 was a primary progesterone target gene in human breast cancer

cells. Furthermore, progesterone is a principal component of contraceptives [41]. Therefore, further well-designed association analysis on gender specific differences and continents specific differences is needed to explore P1371Q and the risk of IBD, including CD and UC.

Heterogeneity, divided into genetic heterogeneities of the model and effect, is one of the important potentially elements affecting pooled results [42]. In research on DLG5-e26 polymorphisms in CD on a per-allele analysis, no heterogeneity existed. Moreover, for more precise results, we excluded one article [20] without HWE in the control group when we calculated the summary ORs, and there was moderate heterogeneity in DLG5-e26 with UC. Due to limited information, we speculate that this moderate heterogeneity might have resulted from differences in demographic characters, behaviours or environmental factors that are not easily gained in primary data [24]. The result of P1371Q on CD risk in overall summery OR suffered moderate heterogeneity, although we had already eliminated the study without HWE [20]. When we excluded the studies from Weersma and colleagues [43] and Newman and colleagues [24] during the sensitivity analysis, the I^2 reduced from 55.839 to 47.853 and 31.642, respectively, so we speculated that these two studies may increase the heterogeneity. In the continent specific study of P1371Q on CD of European, no heterogeneity existed. The different genetic backgrounds of geographically diverse regions and ethnicities and distinct environment may be the source of heterogeneity. Newman and colleagues [24] found that P1371Q was associated with IBD, including CD and UC, in the non-Jewish population but not in the Jewish population. Therefore, it may be hypothesized that populations of the same race from geographically diverse regions have different genetic backgrounds, and more geography-specific studies should performed in the future. The summary genetic association was estimated by a genetic model-free approach, which does not assume the underlying genetic mode is known in advance but still takes advantage of the information available on all genotypes.

However, some limitations still exist in our study. Firstly, some sources of control are neither clear nor uniform, which may lead to insufficient estimations. Secondly, all included articles were case-control studies, which might overestimate the genetic association. To avoid confounding bias, the best method is to establish the population-based nested case-control study, although it is difficult to do. In addition, we had a small sample size, with limited studies pooled in P1371Q in subgroup analysis, which limited our assessment. A more accurate association needs to be explored with more data. From our risk of bias assessment, many of the included studies did not meet the criteria for assessing quality, so uncertainty of the gene results of DLG5-e26 and P1371Q was still present. Finally, a lack of original data from the eligible articles limited assessments of the effects of the gene-gene, gene-gender and gene-environment interactions during IBD development. Therefore, our results should be interpreted with caution until further verification of sequencing approaches and a larger meta-analysis has been performed.

In conclusion, this meta-analysis implied that DLG5-e26 polymorphisms are not implicated in susceptibility of IBD, but P1371Q has association with increasing risk of IBD in North American and decreasing risk of CD in European. More race-specific, geography-specific and gene-gender studies are needed to give further clarification on this question, and gene-gene and gene-environment studies should be explored to confirm the aetiology of IBD.

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Disclosure of conflict of interest

None.

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Table S1. Genotype frequencies between P1371Q and IBD (CD and UC) and genotype effects of studies included in meta-analysis

Author	IBD									CD									UC								
	A vs. C			AA vs. CC			CA vs. CC			A vs. C			AA vs. CC			CA vs. CC			A vs. C			AA vs. CC			CA vs. CC		
	OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI	
Buning [23]	0.759	0.469	1.229	NA	NA	NA	0.777	0.477	1.265	0.562	0.303	1.042	NA	NA	NA	0.549	0.293	1.029	1.095	0.605	1.982	NA	NA	NA	1.101	0.598	2.027
Buning [23]	1.233	0.610	2.492	NA	NA	NA	1.243	0.607	2.545	1.080	0.467	2.497	NA	NA	NA	1.083	0.461	2.542	1.415	0.624	3.210	NA	NA	NA	1.435	0.622	3.312
Tremelling [25]	1.055	0.744	1.495	3.432	0.165	71.61	1.004	0.701	1.438	1.160	0.767	1.755	7.651	0.366	159.7	1.049	0.679	1.620	0.960	0.623	1.480	NA	NA	NA	0.958	0.617	1.490
Browning [45]	1.034	0.641	1.669	NA	NA	NA	1.035	0.636	1.684	0.992	0.557	1.766	NA	NA	NA	0.992	0.551	1.783	1.166	0.683	1.989	NA	NA	NA	1.172	0.680	2.020
Torok [18]	1.027	0.757	1.391	2.503	0.484	12.93	0.952	0.689	1.316	0.877	0.612	1.258	1.561	0.219	11.11	0.837	0.572	1.226	1.290	0.877	1.896	4.189	0.697	25.18	1.158	0.761	1.762
Yamazaki [22]	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.207	0.934	1.559	1.187	0.529	2.663	0.930	0.406	2.132	NA	NA	NA	NA	NA	NA	NA	NA	AN
Weersma [43]	0.875	0.706	1.085	0.950	0.237	3.807	0.866	0.691	1.085	0.793	0.622	1.013	0.785	0.158	3.895	0.785	0.608	1.013	1.000	0.773	1.293	1.205	0.243	5.982	0.990	0.756	1.297
Newman [24]	1.640	1.121	2.398	3.247	0.336	31.38	1.635	1.089	2.456	1.774	1.163	2.705	3.717	0.335	41.27	1.776	1.127	2.799	1.449	0.886	2.371	2.591	0.161	41.72	1.439	0.848	2.442
Ghyslaine [27]	0.902	0.501	1.622	NA	NA	NA	0.898	0.492	1.636	0.683	0.338	1.379	NA	NA	NA	0.673	0.329	1.377	1.254	0.634	2.481	NA	NA	NA	1.270	0.630	2.558
Lin [38]	2.244	1.071	4.704	NA	NA	NA	2.335	1.097	4.972	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Al-Sulaiman [28]	0.625	0.271	1.439	0.456	0.040	5.153	0.663	0.250	1.755	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Stoll [7]	1.692	1.034	2.770	11.09	0.612	201.1	1.318	0.779	2.231	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Overall odds ratio	1.050	0.930	1.184	1.759	0.770	3.979	1.015	0.895	1.152	0.994	0.802	1.231	1.346	0.712	2.545	0.906	0.777	1.056	1.124	0.962	1.313	2.165	0.722	6.491	1.000	0.934	1.294

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; OR, odds ratio; CI, Confidence intervals; NA, not available.

Table S2. Risk of bias assessment for genetic association studies of IBD (CD and UC) of studies included in the meta-analysis

Domain and item	Low risk of bias
Information bias	
Ascertainment of IBD (CD and UC)	
Clearly described objective criteria of diagnosis of IBD (CD and UC)	Yes
Not clearly described	No
Did not mention	Unclear
Ascertainment of controls	
Controls were non-IBD and without family history	Yes
Mentioned the sources of controls	Yes
Not described	No
Ascertainment of genotyping examination	
Genotyping done under “blind” conditions of case specimens and control specimens	Yes
Genotyping of cases and controls was performed together	Yes
Genotyping error rate 5%	Yes
Quality control procedure (e.g., reanalysis of random specimens, by using different genotyping methods for analysis, analysis if replicate sample)	Yes
Unblind	No
Genotyping error rate > 5%	No
Did not mention what was done	Unclear
Confounding bias	
Population stratification	
No difference in ethnic origin between cases and controls	Yes
Use of controls who were not related to cases with clearly identification	Yes
Use of some controls who came from the same family	No
No report of what was done	Unclear
Other confounding bias	
Controls for confounding variables (e.g., age, gender, or BMI) in analysis	Yes
Not controlled for confounding variables	No
Not mentioned	Unclear
Selective reporting (for replication studies)	
Reported results of all polymorphisms mentioned in the objectives, no significant or not	Yes
Reported results of only significant polymorphisms	No
HWE	
HWE in the control group	Yes
HWD in the control group	No
HWE not checked or mentioned	No

IBD, inflammatory bowel disease; CD, Crohn’s disease; UC, ulcerative colitis; BMI, body mass index; HWE, Hardy-Weinberg equilibrium; HWD, Hardy-Weinberg disequilibrium.

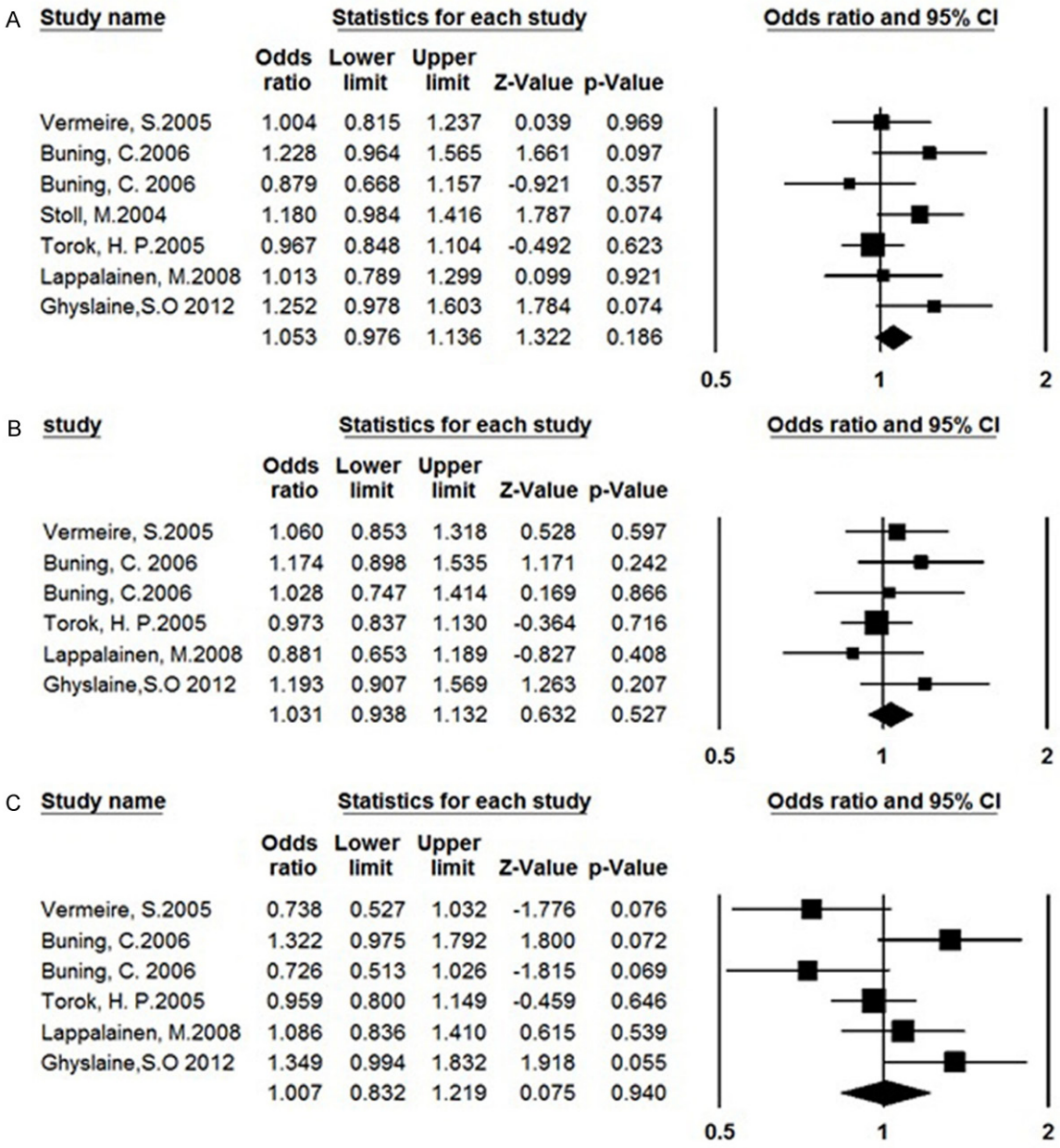

Figure S1. Forest plot of DLG5-e26 polymorphisms with IBD, CD and UC (delA vs. insA). A. The association between DLG5-e26 and IBD risk; B. The association between DLG5-e26 and CD risk; C. The association between DLG5-e26 and UC risk.

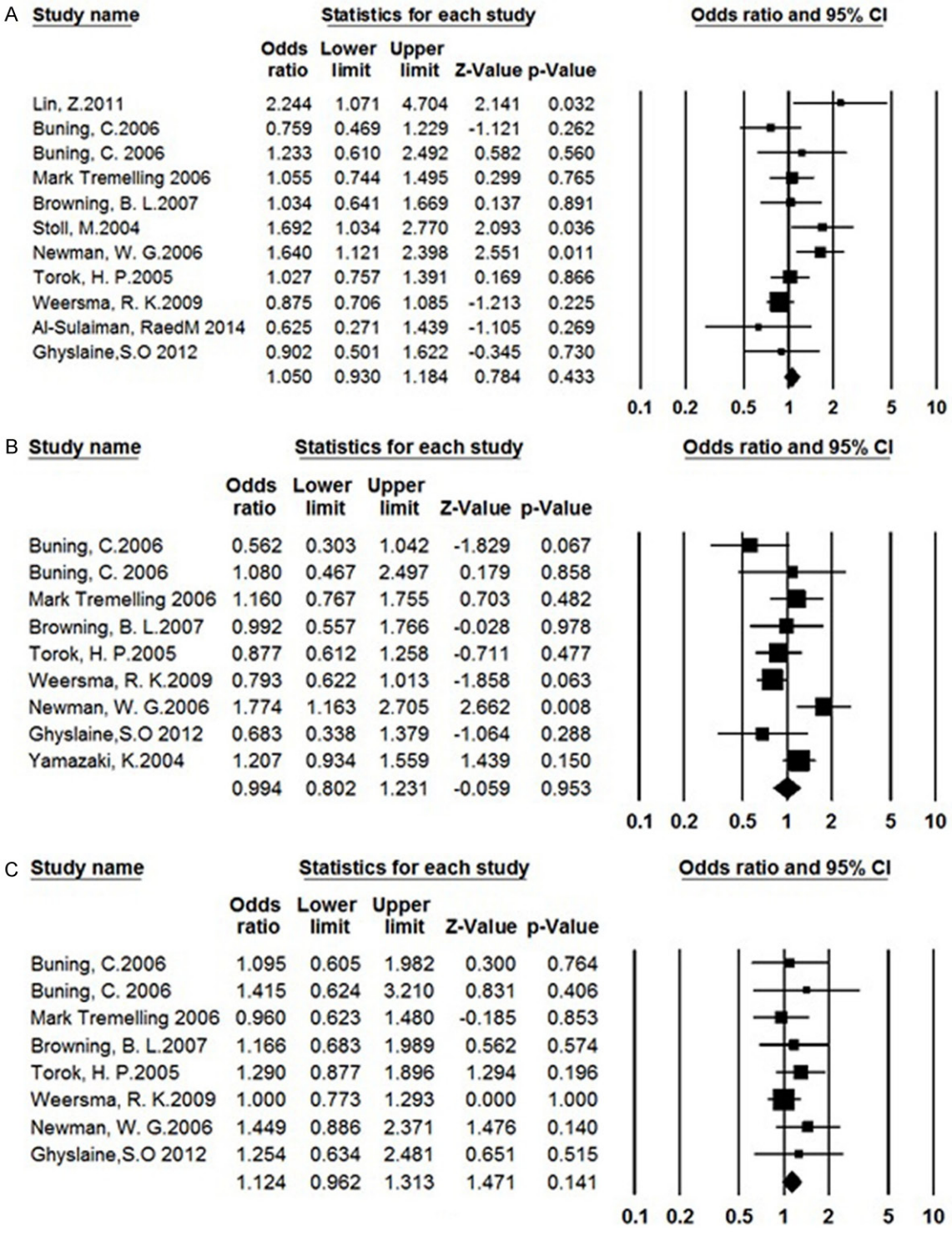

Figure S2. Forest plot of P1371Q gene with IBD, CD and UC (A vs. C). A. The association between P1371Q and IBD risk; B. The association between P1371Q and CD risk; C. The association between P1371Q and UC risk.

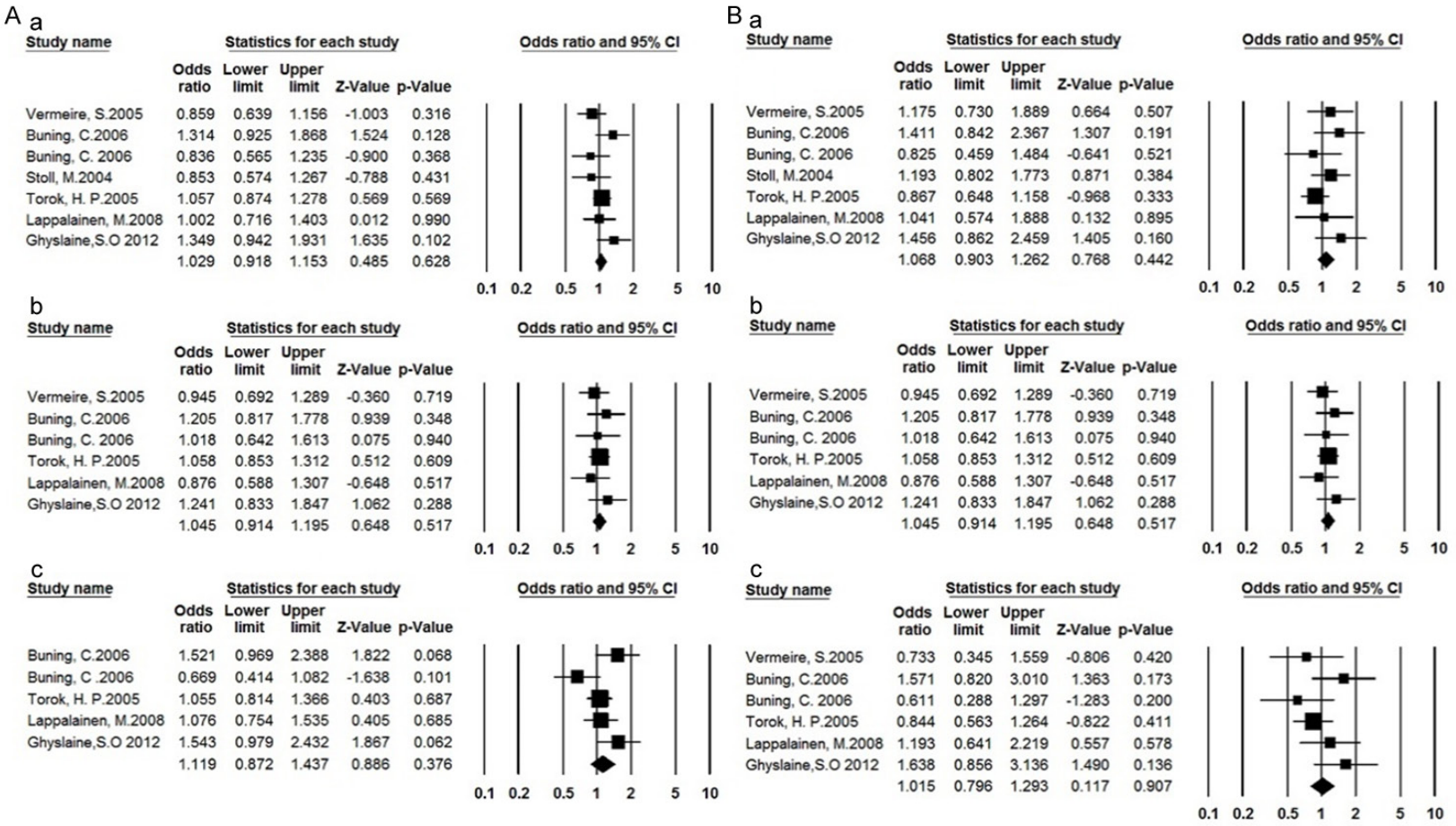

Figure S3. Forest plot of DLG5-e26 gene with IBD, CD and UC, A. (insAdelA vs. insAinsA); B. (delAdelA vs. insAinsA). a. The association between DLG5-e26 and IBD risk; b. The association between DLG5-e26 and CD risk; c. The association between DLG5-e26 and UC risk.

Table S3. The results of sensitivity analysis

SNP	Excluded study	Pooled OR	95% CI	P	I^2 (%)	*P* value for I^2
DLG5-e26 of UC	Vermeire [21]	1.062	0.875-1.289	0.541	60.766	0.037
	Buning [23]	1.063	0.878-1.287	0.532	60.254	0.039
		0.958	0.784-1.170	0.671	61.709	0.034
	Torok [18]	1.017	0.790-1.310	0.893	70.247	0.009
	Lappalainen [44]	0.990	0.782-1.252	0.932	70.516	0.009
	Ghyslaine [27]	0.955	0.786-1.160	0.642	59.846	0.041
P1371Q of CD	Buning [23]	0.988	0.786-1.241	0.915	61.285	0.012
		1.042	0.854-1.286	0.697	52.210	0.041
	Tremelling [25]	0.970	0.763-1.235	0.807	60.118	0.014
	Browning [45]	0.992	0.784-1.255	0.946	61.358	0.011
	Torok [18]	1.010	0.790-1.292	0.934	60.127	0.014
	Weersma [43]	1.090	0.937-1.269	0.265	47.853	0.062
	Newman [24]	0.940	0.821-1.077	0.373	31.642	0.175
	Ghyslaine [27]	1.020	0.816-1.276	0.862	58.724	0.018
	Yamazaki [22]	0.955	0.749-1.217	0.708	54.169	0.033

CD, Crohn's disease; UC, ulcerative colitis; OR, odds ratio; CI, Confidence intervals.

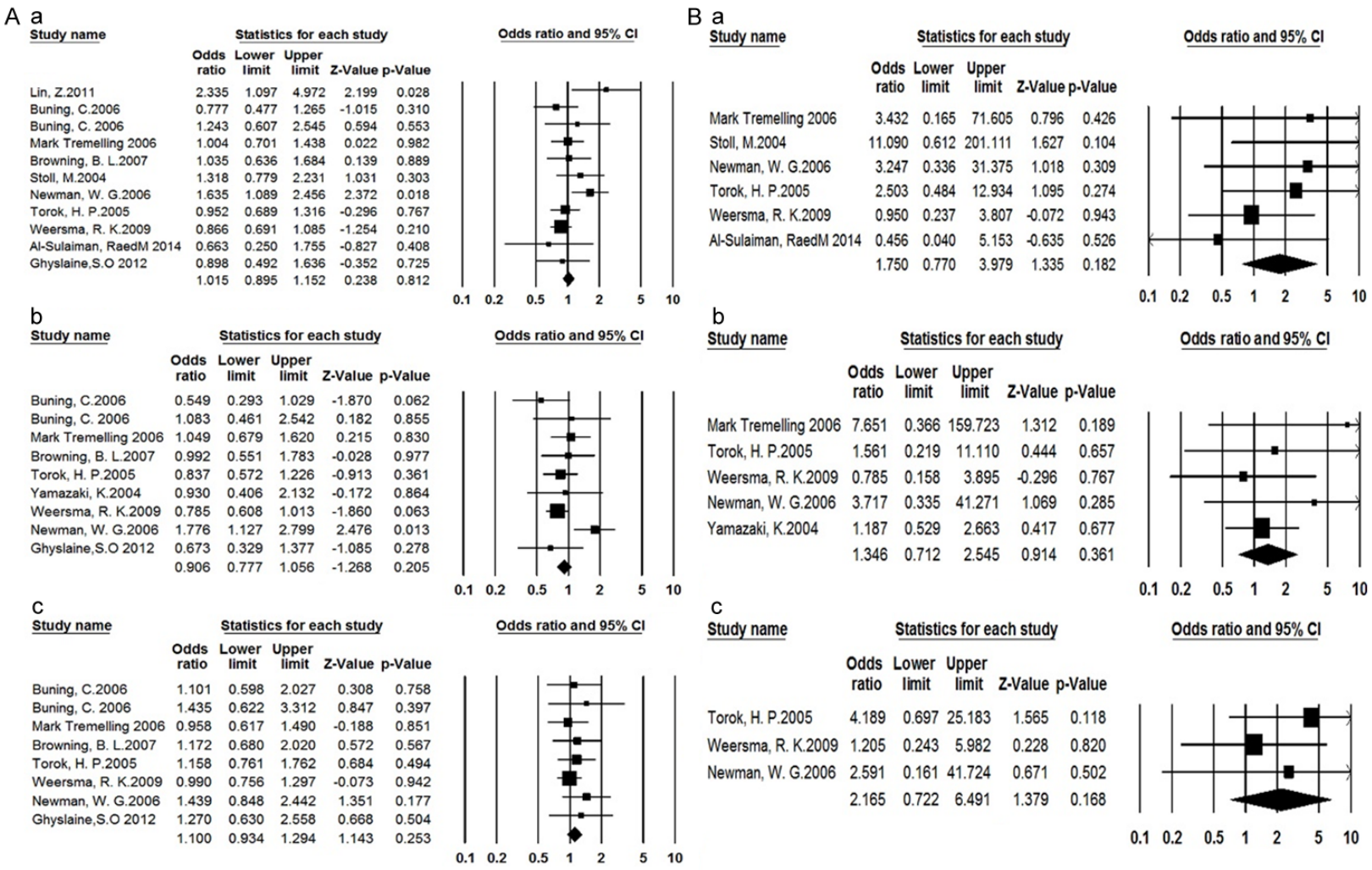

Figure S4. Forest plot of P1371Q gene with IBD, CD and UC, A (CA vs. CC); B (AA vs. CC). a. The association between P1371Q and IBD risk; b. The association between P1371Q and CD risk; c. The association between P1371Q and UC risk.

Table S4. MOOSE checklist: P1371Q polymorphisms in DLG5 gene decrease the risk of Crohn's disease in European: a meta-analysis

Criteria	Brief description of how the criteria were handled in the review
Reporting of background	
√ Problem definition	The epidemiology of inflammatory bowel disease (IBD), Crohn's disease (CD) as well as ulcerative colitis (UC). The inconsistent results of genetic risk with IBD, CD and UC in different population based publications
√ Hypothesis statement	We propose there are significant associations between gene polymorphisms DLG5-e26 and P1371Q and IBD, including CD and UC
√ Description of study outcomes	The pool odds ratio (OR) and 95% confidence interval
√ Type of exposure	Genetic markers
√ Type of study designs used	The population based genetic epidemiological observational studies of IBD, including CD and UC
√ Study population	The worldwide population is considered our analysis
Reporting of search strategy should include	
√ Qualifications of searchers	Two investigators (Z.H) independently browsed all titles and abstracts of the identified articles
√ Search strategy, including time period included in the synthesis and keywords	Time period: from inception of PubMed, Embase, Chinese National Knowledge Infrastructure (CNKI) and Google Scholar, December 2015. Search strategy: ('DLG5' or 'Drosophila Discs Large Homologue 5') and ('Crohn's disease' or 'crohns disease' or 'CD' or 'inflammatory bowel disease' or 'IBD' or 'ulcerative colitis' or 'UC')
√ Databases and registries searched	PubMed, Embase, CNKI and Google Scholar
√ Search software used, name and version, including special features	PubMed was accessed from the National Library of Medicine (free), CNKI was available on the website of Jinan University Library, Embase was purchased in Internet, Google Scholar was free from Internet
√ Use of hand searching	We searched bibliographies of retrieved papers and those of previous reviewers on the subject were examined for further relevant studies
√ List of citations located and those excluded, including justifications	Details of the literature search process are outlined in the flow chart. The citation list for excluded studies is available upon request
√ Method of addressing articles published in languages other than English	We hadno restriction on language
√ Method ofhandling abstracts and unpublished studies	We included proceedings papers and assessed them for eligibility according to our inclusion and exclusion criteria. Unpublished studies were excluded in our analysis
√ Description of any contact with authors	It is applicable; we contact the authors when we needed
Reporting of methods should include	
√ Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria are described in the Methods section
√ Rationale for the selection and coding of data	Data extracted from each of the studies were relevant to the population characteristics name of first author, year of publication, region of study population, genotype method, the number of cases and controls, the risk allele frequency in cases and controls, the genotype of cases and controls and the Hard-Weinberg Equilibrium (HWE)
√ Assessment of confounding	Detailed inclusion is described in the Methods section
√ Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Sensitivity analyses by several quality indicators such as study size, study objects' ethnic, and another influent factors in the Methods section
√ Assessment of heterogeneity	Heterogeneity of the studies was explored with I^2 statistic that provides the relative amount of variance of the summary effect due to the between-study heterogeneity,detailed inclusion is described in the Methods section
√ Description of statistical methods in sufficient detail to be replicated	Description of methods of meta-analyses, sensitivity analyses and assessment of publication bias are detailed in the methods. We performedfixed effects and random effects in meta-analysis with comprehensive meta-analysis software (verson.12)
√ Provision of appropriate tables and graphics	**Tables 1**, **2**, **Figures 1-4**, Tables S1, S2, S3, S4, Figures S1, S2
Reporting of results should include	
√ Graph summarizing individual study estimates and overall estimate	**Figure 1**
√ Table giving descriptive information for each study included	**Tables 1**, **2**

√ Results of sensitivity testing	Table S3
√ Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates, I^2 values and results of sensitivity analyses
Reporting of discussion should include	
√ Quantitative assessment of bias	The funnel plot and Egger's regression.
√ Justification for exclusion	All studies were excluded based on the pre-defined inclusion criteria in methods section.
√ Assessment of quality of included studies	Brief discussion included in Methods section
Reporting of conclusions should include	
√ Consideration of alternative explanations for observed results	Discussed in the context of the results.
√ Generalization of the conclusions	Discussed in the context of the results.
√ Guidelines for future research	Discussed in the context of the results
√ Disclosure of funding source	No separate funding was necessary for the undertaking of this meta-analysis.