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The association of DLG5 polymorphisms with inflammatory bowel disease: a meta-analysis of 25 studies

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Abstract. – OBJECTIVE: The aim of this study was to explore the association of polymorphisms in DLG5 gene (G113A, C4136A and e26) with inflammatory bowel disease (IBD) risk.

MATERIALS AND METHODS: A total of 25 studies involved 26583 subjects were pooled for analysis. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to pool the effect size.

RESULTS: For G113A variant, a significant association was observed with CD risk in children (A *vs*. G: OR = 0.745, 95% CI = 0.569-0.977) and high quality studies (A *vs*. G: OR = 0.913, 95% CI = 0.850-0.981). Additionally, the results of genotype-phenotype analysis suggested G113A variant was associated with colonic involvement in CD. However, in overall population, the results indicated G113A variant was not associated with CD or UC. We also provided evidence that C4136A polymorphism had different effects on CD risk between Europeans (AA *vs*. CC: OR = 3.239, 95% CI = 1.149-9.136) and Asians (AA vs. CC: OR = 0.511, 95% CI = 0.299-0.873). For UC, patients with AA genotype of C4136A variant had a significantly increased UC risk (AA *vs*. CC: OR = 3.877, 95% CI = 1.168-12.867). Finally, no association was detected with G113A or e26 polymorphism in CD or UC patients.

CONCLUSIONS: This meta-analysis indicated G113A variant may be significantly associated with CD risk in children and colonic involvement.

Key Words:
DLG5, Polymosphism, Ulcerative colitis, Crohn's disease.

Introduction

Inflammatory bowel disease (IBD), consisting of Crohn's disease (CD) and ulcerative colitis (UC), is a spectrum of chronic recurring inflammatory disorders affecting the gastrointestinal tract[1]. In the developed world, IBD are common causes of gastrointestinal morbidity; however, the exact etiology of IBD remains unclear[2]. Recent animal models and human studies have demonstrated a genetic contributionto both CD and UC, including NOD2, CARD9 and IL23R[3-5].

Drosophila discs large homolog 5 (DLG5), located on chromosome 10q23, is a member of the membrane-associated guanylate kinase (MAGUK) family which is involved in the formation of cell junctions, maintenance of cell shape, and transduction of intracellular signal[6]. Stoll et al[7] first reported the association between DLG5 polymorphism and IBD susceptibility, including G113A (resulting in the amino acid substitution R30Q), C4136A (resulting in the amino acid substitution P1371Q) and DLG_e26, which was replicated in some studies[8]. However, several other groups failed to confirm these associations[9,10].

Accumulating studies have focused[11,12] on the association between DLG5 polymorphism and IBD, however, the results were inconclusive. A meta-analysis is a statistical procedure for combining results from several studies to overcome the limitation of small sample sizes and inadequate statistical power, and produce a single estimate of the major effect. The purpose of our study was to investigate whether DLG5 (G113A, C4136A, and DLG5_e26) polymorphisms were risk factors to IBD susceptibility (CD and UC, respectively).

Materials and Methods

Identification of Eligible Studies

We performed an exhaustive search in PubMed, Embase and Web of Science databases by the following keywords "drosophila discs large homolog 5" OR "DLG5" and "polymor-

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phism" or "variant" or "SNP" or "rs1248696" or "rs2289310" and "Crohn's disease" or "ulcerative colitis" or "inflammatory bowel disease" or "IBD" or "CD" or "UC". References from the eligible studies were also hand-searched for additional studies. The last retrieval was conducted in April 1, 2014.

Inclusion and Exclusion Criteria

Reports were included if all of the following conditions were met: 1) investigating the association between DLG5 polymorphisms and IBD diseases (Crohn's disease or ulcerative colitis); 2) a case-control study; 3) providing alleles or genotypes distribution in both cases and controls. The exclusion criteria were: 1) studies were considered overlapped with other studies 2) no control population 3) review papers and articles only with an abstract.

Data Collection

Two independent authors extracted the following information from each eligible study: first author, year of publication, ethnicity of subjects, type of diseases, sample sizes, clinical characteristics (age and gender distribution of the subjects), and matching criteria. If the article did not provide sufficient genotype rates, the corresponding author was contacted for the detailed data.

Quality Score Assessment

The quality of each study was independently assessed by two authors, using the quality scoring scale modified from previous meta-analysis of genetic studies[6,13]. The quality score of a given study was determined by the following factors: source of cases, source of controls, genotyping examination, Hardy-Weinberg equilibrium, and total sample size. Total scores ranged from 0 to 12, and a study was considered high quality if score ≥ 8 (Supplementary Table S1).

Statistical Analysis

The odds ratio (OR) and 95% confidence interval (CI) were used to measure the strength of the associations between DLG5 polymorphisms and IBD risk. Two-sided *p* values less than 0.05 were considered statistically significant. The Hardy-Weinberg equilibrium (HWE) in the controls was assessed, and the *p* value less than 0.05 was considered as significant disequilibrium. Stratified analysis was conducted, if feasible, according to sample size, age at diagnosis, ethnicity, source of control and quality score. Sensitivity analysis was conducted to evaluate the stability of the results, in which each study was sequentially removed.

Between-study heterogeneity was assessed by the 2-based Q test and I^2 test. The random-effects

Table I Supplementary. Scale for quality assessment.

Criteria	Score
Source of cases	
Consecutive/randomly selected from population with clearly defined sampling frame	2
Consecutive/randomly selected from population without clearly defined sampling frame	1
Not described	0
Source of controls	
Population-based	3
Blood donors or volunteers	2
Hospital-based	1
Not described	0
Genotyping examination	
3D DNA microarray	2
TaqMan	2
PCR-RFLP	1
Hardy-Weinberg equilibrium	
Hardy-Weinberg equilibrium in control group	2
Hardy-Weinberg disequilibrium in control group	1
Not described	0
Total sample size	
> 1000	3
> 500 but < 1000	2
> 200 but < 500	1
< 200	0

model (DerSimonian and Laird method) was applied when the $p < 0.1$ or $I^2 > 50\%$; otherwise, the fixed-effects model (Mantel-Haenszel method) was used[14,15]. Meta-regression analysis was carried out to assess the sources of heterogeneity, including sample size (more than 500), year of publication, ethnicity, and age at diagnosis. In addition, a Galbraith plot was constructed to visually assess the heterogeneity, spotting outliers as the possible major sources of heterogeneity[16,17].

Publication bias was evaluated by visualizing Begg's funnel plots and Egger's linear regression test, the p values <0.05 from the Egger's test were considered statistically significant[8,19]. All statistical analyses were performed by STATA, version 12, software, (StataCorp LP, College Station, TX, USA).

Results

Characteristics of the Eligible Studies

The combined search retrieved 195 publications, of which 174 were excluded by review of abstract, and 21 were included in this meta-analysis[8-10,20-35]. Three publications reported the results on different subpopulations[21,25,32], one article[10] had both adult and children data, and therefore, we treated them as separate studies. In total, the eligible articles reported on 25 case-control studies involved 9402 CD patients, 6386 UC patients and 10795 controls. The details of the search flow were shown in Figure 1.

A list of characteristics of included studies is provided in Table I. Overall, for CD, 23 studies focused on G113A polymorphism, 10 studied the C4136A polymorphism, and 8 studies exam-

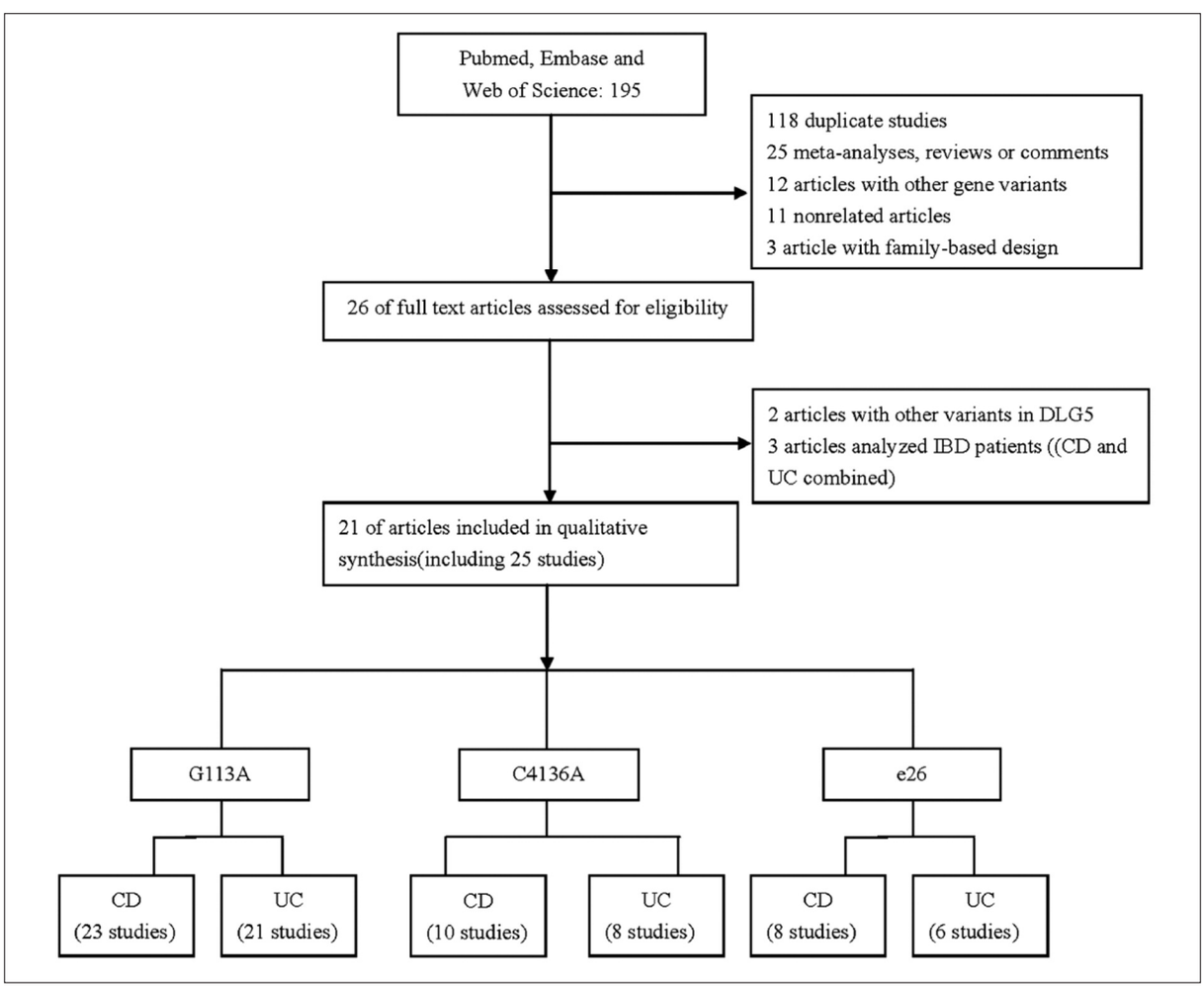

Figure 1. Study selection procedures for a meta-analysis of DLG5 polymorphisms and Crohn's disease and ulcerative colitis, 2004-2011.

Table I. Main characteristics of studies involved in DLG5 polymorphisms and Crohn's disease or ulcerative colitis risk, 2004-2011.

Author, year of publication	Ethnicity	Cases					Controls				
		DLG5 variant	Phenotype studied	Number	Males (%)	Age or Age at diagnosis	Number	Males (%)	Age	Matching	Score
Browning, 2007	European	G113A C4136A	CD and UC separately	CD: 389 UC: 406	CD: 36.0 UC: 48.0	nr	416	nr	nr	nr	11
Buning, 2006	European	G113A C4136A e26	CD and UC separately	CD: 250 UC: 150	CD: 62.0 UC: 52.7	CD: 30.3 ± 11.4 at diagnosis UC: 37.0 ± 14.5 at diagnosis	422	nr	nr	nr	8
Buning, 2006	European	G113A C4136A e26	CD and UC separately	CD: 144 UC: 122	CD: 45.1	28.8 ± 11.7 at diagnosis	205	nr	nr	nr	8
Cucchiara, 2007	European	G113A	CD and UC separately	CD: 200	CD: 57.5	CD: 21 ± 8 and 12 ± 4 at diagnosis	347	53.0	32 (20-45)		9
				UC: 186	UC: 39.2	UC: 19 ± 9 and 11 ± 5 at diagnosis					
Dema, 2010	European	G113A	CD and UC separately	CD: 411 UC: 447	CD: 48.0 UC: 59.0	nr	846	47.0	nr	nr	8
Gaj, 2008	European	G113A	CD	CD: 60	CD: 46.7	39 (22-78)	139	53.2	32 (21-66)	nr	7
Lin, 2009	European	G113A	CD and UC separately	CD: 116 UC: 97	nr nr	nr	170	nr	nr	nr	5
Medici, 2006	European Norway	G113A	CD and UC separately	CD: 151 UC: 325	IBD: 51.8	nr	236	nr	nr	Age and sex	10
Medici, 2006	European German	G113A	CD and UC separately	CD: 309 UC: 153	nr	nr	540	nr	nr	Age and sex	10
Noble, 2005	European	G113A e26	CD and UC separately	CD: 374 UC: 305	CD: 48.4 UC: 56.1	CD:27.8 (20.9-40.4) at diagnosis UC: 34 (25-50) at diagnosis	294	48.6	39 (27-52)	nr	9
Pearce, 2007	European	G113A	CD and UC separately	CD: 630 UC: 518	nr nr	nr nr	749	nr	nr	nr	11
de Ridder, 2007	European	G113A	CD and UC separately	CD: 386 UC: 226	IBD: 43.0	IBD:35.3 (19-69) at diagnosis	289	nr	nr	nr	7
de Ridder, 2007	European	G113A	CD and UC separately	CD: 72 UC: 31	IBD:49.0	IBD:12 (0.5-18) at diagnosis	289	nr	nr	nr	7
Torok, 2005	European	G113A C4136A e26	CD and UC separately	CD: 625 UC: 363	nr nr	CD: 27.8 (7-71) nr	1012	nr	nr	nr	9
Tremelling, 2006	European	G113A C4136A	CD and UC separately	CD: 496 UC: 512	CD: 33.0 UC: 53.3	CD: 43 and 26 at diagnosis UC: 54 and 36 at diagnosis	760	43.4	60	nr	10
Vermeire, 2005	European	G113A e26	CD and UC separately	CD: 472 UC: 120	IBD: 42.1	IBD: 45.1 ± 13.8 and 31 ± 12 at diagnosis	305	45.6	41.1 ± 17		8

Table continued

Table I *(Continued)*. Main characteristics of studies involved in DLG5 polymorphisms and Crohn's disease or ulcerative colitis risk, 2004-2011.

Author, year of publication	Ethnicity	Cases					Controls				
		DLG5 variant	Phenotype studied	Number	Males (%)	Age or Age at diagnosis	Number	Males (%)	Age	Matching	Score
Biank, 2007	European	G113A	CD	CD: 281	CD: 58.4	CD: 12.1 at diagnosis	479	nr	nr	nr	9
Weersma,2009	European	G133A C4136A	CD and UC separately	CD: 1684 UC: 1120	CD: 35.5 UC: 53.7	nr nr	1350	nr	nr	nr	10
Lappalainen, 2008	European	G113A e26	CD and UC separately	CD: 240 UC: 459	nr	nr	312	nr	45.0	Sex	10
Newman, 2006	European	G113A C4136A	CD and UC separately	CD: 296 UC: 136	nr nr	CD: 20.4 at diagnosis	447	nr	31	nr	11
Newman, 2006	European	G113A C4136A	CD and UC separately	CD:106 UC:43	nr nr	UC: 18.5 at diagnosis	90	nr	31	nr	11
Torok, 2009	European	G113A C4136A e26	CD and UC separately	CD: 606 UC: 350	CD: 43.6 UC: 47.8	CD: 40.1 ± 13.0 and 27.8 ± 11.5 at diagnosis UC: 42.4 ± 13.7 and 32.0 ± 12.0 at diagnosis	792	nr	nr	nr	10
Lakatos, 2006	European	G133A	CD and UC separately	CD: 639 UC: 134	CD: 48.3 UC: 47.0	CD: 36.8 ± 12.7 and 28.4 ± 11.4 at diagnosis UC: 44.1 ± 15.1 and 33.9 ± 14.1 at diagnosis	150	48.0	37.6 ± 10.3	Age and sex	7
Chua, 2011	Asian	C4136A e26	CD	CD: 80	nr	nr	100	nr	nr	nr	4
Yamazaki, 2004	Asian	C4136A e26	CD	CD: 484	nr	nr	345	nr	nr	nr	7

CD: Crohn's disease; UC: ulcerative colitis; nr: not report. Quality scores ranged from 0 points (worst) to 12 points (best).

ined e26 variant. The interaction between DLG5 polymorphisms and CARD15 (NOD2) was reported in 7 studies. Of the studies which examined the risk of UC, 21 reports focused on the G113A polymorphism, 8 focused on C4136A variant, and 6 investigated e26 polymorphism. The proportion in patients of males ranged from 33.0%-62.0% in controls. The distribution of the genotypes in control group of all studies was in agreement with the HWE except for two[27,35]. Therefore, a sensitivity analysis was carried out by excluding these studies from the analysis.

Crohon's Disease

Overall, no significant association was observed in any genetic models when all studies were pooled (Figure 2 and Table II). In the subgroup analysis stratified by age at diagnosis, children with A allele had a significantly decreased CD risk (A *vs.* G: OR=0.745, 95% CI = 0.569-0.977, $p = 0.033$), but which was not observed in adult subgroup (A *vs.* G: OR = 0.944, 95% CI = 0.858-1.038, $p = 0.232$). When stratified by sample size, no statistical association was found in either large sample (> 500) subgroup or small sample (< 500) subgroups. When pooling studies published before 2007, individuals with A allele had a reduced CD risk (A *vs.* G: OR = 0.891, 95% CI = 0.802-0.990, $p = 0.032$), which was not observed in the subgroup after or during 2007. Additionally, a significantly decreased CD risk was observed in high quality studies (A *vs.* G: OR = 0.913, 95% CI = 0.850-0.981, $p = 0.013$). For C4136A polymorphism, no association was found with CD risk when all 10 reports were pooled (Table III). In the stratified analysis by ethnicity, a significantly increased risk of CD was observed in Europeans (AA *vs.* CC: OR = 3.239, 95% CI = 1.149-9.136, $p = 0.026$), conversely, Asians with AA genotype had a reduced CD susceptibility (AA *vs.* CC: OR = 0.511, 95% CI = 0.299-0.873, $p = 0.014$) However, no significant correlation between DLG5_e26 variant and CD susceptibility was found in overall or subgroup analysis (Table IV).

Ulcerative Colitis

The association results of DLG5 polymorphisms and UC susceptibility were presented in Table II, III and IV. No association with UC risk was observed in G113A polymorphisms in any genetic models or in the subgroup analysis by age at diagnosis, quality scores, data of publication and sample size (Table II). For C4136A polymorphism, individuals with AA genotype had a significantly increased UC risk compared with individuals with CC genotype. (OR = 3.877, 95% CI = 1.168-12.867, $p = 0.027$) (Table III). However, for e26 polymorphism, no association was detected in any genetic models. (Table IV).

Test of Heterogeneity

Significant between-study heterogeneity existed in most comparisons of DLG5 polymorphisms and CD when all papers were pooled. Thus, we performed meta-regression to identify the source of heterogeneity. The results demonstrated that publication year, age at diagnosis, or sample size (subjects more than 500) did not contribute to the heterogeneity. However, for the association between DLG_e26 polymorphism and CD risk, the year of publication can explain χ^2-values (100.00%, $p = 0.030$ and 100.00%, $p = 0.032$ in heterozygote comparison and dominant model, respectively) In addition, Galbraith plots indicated that three studies[20,23,25] were the potential source of heterogeneity in analysis of G113A and CD, after excluding which, the effect estimate became significant and the heterogeneity was removed (A *vs.* G: OR = 0.874, 95% CI = 0.813-0.939, $p < 0.001$; $p_h = 0.692$).

Sensitivity analyses indicated that the pooled ORs were stable by omitting one study at a time in both CD and UC (data not shown). In addition, when excluding two studies derived from HWE, the corresponding pooled ORs were not materially altered. Cumulative meta-analysis for the G113A polymorphism was conducted by publication date, the results of which suggested that the association was still not significant with accumulation of more data over time (data not shown).

Publication Bias

Begg's funnel plots and Egger's test were performed to examine publication bias. For all 3 SNPs, no evidence of obvious asymmetry was observed in the funnel plots (Figure 3). Moreover, Egger's test did not show any publication bias of G113A polymorphism (A *vs.* G: $p = 0.782$ and $p = 0.261$ in CD and UC, respectively), C4136A polymorphism (A *vs.* C: $p = 0.846$ and $p = 0.176$ in CD and UC, respectively), or e26 polymorphism (delA *vs.* insA: $p = 0.219$ and $p = 0.439$ in CD and UC, respectively).

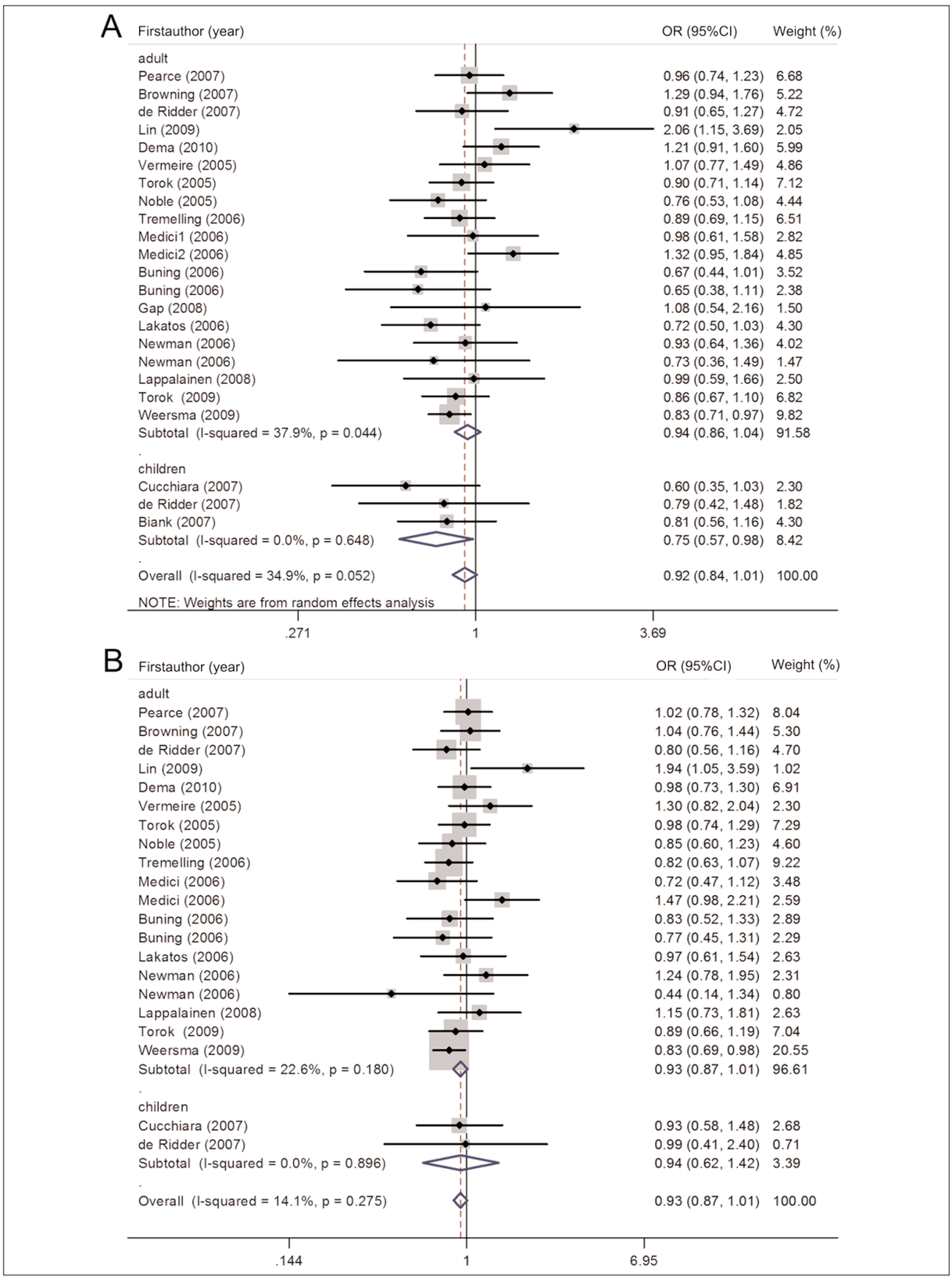

Figure 2. Results from random-effect meta-analysis of studies of the DLG5 G113A polymorphism with Crohn's disease ***(A)*** or ulcerative colitis ***(B)***, 2005-2010. (A *vs*. G) CI, confidence interval (bars); OR, odds ratio.

Table II. Pooled analysis for the associations between the DLG5 G113A polymorphism and the risk of Crohn's disease and ulcerative colitis, 2005-2010.

Variables	A *vs.* G		AA *vs.* GG		AG *vs.* GG		AA+AG *vs.* GG		AA *vs.* AG+GG	
	OR (95% CI)	p^b	OR (95% CI)	p^b	OR (95% CI)	p^b	OR (95% CI)	p^b	OR (95% CI)	p^b
Crohn's disease										
Overall (Europeans)	0.924 (0.845-1.012)	0.052	1.010 (0.723-1.411)	0.639	0.936 (0.810-1.081)	0.006	0.941 (0.819-1.080)	0.008	1.028 (0.736-1.435)	0.640
All in HWE	0.956 (0.837-1.092)	0.014	1.128 (0.782-1.628)	0.728	0.943 (0.853-1.043)	0.004	0.941 (0.808-1.095)	0.005	1.151 (0.798-1.659)	0.738
High quality studies	**0.913 (0.850-0.981)**	0.115	1.063 (0.737-1.535)	0.419	0.924 (0.790-1.082)	0.015	0.950 (0.859-1.051)	0.023	1.077 (0.747-1.533)	0.412
Age at diagnosis										
Children	**0.745 (0.569-0.977)**	0.648	0.619 (0.102-3.775)	0.627	0.676 (0.435-1.051)	0.259	0.664 (0.430-1.025)	0.362	0.649 (0.107-3.936)	0.605
Adults	0.944 (0.858-1.038)	0.044	1.030 (0.723-1.450)	0.522	0.959 (0.827-1.112)	0.007	0.965 (0.838-1.113)	0.010	1.047 (0.745-1.472)	0.523
Sample size										
> 500	0.918 (0.839-1.005)	0.092	0.989 (0.699-1.400)	0.509	0.926 (0.801-1.070)	0.026	0.930 (0.808-1.070)	0.027	1.005 (0.711-1.422)	0.524
< 500	0.972 (0.694-1.362)	0.085	1.322 (0.384-4.546)	0.539	1.005 (0.620-1.630)	0.018	1.013 (0.642-1.597)	0.028	1.375 (0.404-4.684)	0.511
Date of publication										
After or during 2007	0.965 (0.842-1.107)	0.034	1.403 (0.840-2.343)	0.589	1.026 (0.835-1.261)	0.094	1.042 (0.843-1.287)	0.063	1.405 (0.842-2.346)	0.624
Before 2007	**0.891 (0.802-0.990)**	0.262	0.791 (0.507-1.236)	0.669	0.870 (0.711-1.065)	0.011	0.871 (0.727-1.043)	0.037	0.817 (0.525-1.273)	0.612
Ulcerative colitis										
Overall (Europeans)	0.935 (0.866-1.009)	0.275	0.797 (0.533-1.192)	0.829	0.979 (0.884-1.085)	0.186	0.968 (0.876-1.070)	0.231	0.804 (0.538-1.202)	0.807
All in HWE	0.959 (0.869-1.058)	0.268	0.845 (0.547-1.307)	0.818	0.969 (0.870-1.080)	0.153	0.962 (0.865-1.070)	0.185	0.857 (0.555-1.323)	0.799
High quality studies	0.929 (0.858-1.006)	0.378	0.781 (0.502-1.216)	0.695	0.975 (0.874-1.089)	0.288	0.964 (0.865-1.073)	0.356	0.785 (0.505-1.221)	0.670
Age at diagnosis										
Children	0.939 (0.620-1.420)	0.896	1.475 (0.283-7.684)	0.618	0.879 (0.556-1.391)	0.743	0.904 (0.578-1.413)	0.914	1.520 (0.292-7.916)	0.600
Adults	0.935 (0.865-1.010)	0.180	0.770 (0.508-1.166)	0.767	0.985 (0.887-1.094)	0.114	0.972 (0.877-1.077)	0.139	0.776 (0.513-1.174)	0.747
Sample size										
> 500	0.930 (0.856-1.009)	0.513	0.758 (0.488-1.175)	0.665	0.974 (0.869-1.091)	0.433	0.960 (0.859-1.073)	0.495	0.765 (0.494-1.185)	0.644
< 500	0.980 (0.730-1.316)	0.092	1.059 (0.382-2.933)	0.721	1.023 (0.720-1.453)	0.062	1.208 (0.733-1.441)	0.072	1.067 (0.387-2.945)	0.698
Date of publication										
After or during 2007	0.934 (0.846-1.030)	0.352	1.056 (0.589-1.895)	0.823	0.922 (0.852-1.156)	0.331	0.996 (0.859-1.156)	0.350	1.069 (0.597-1.917)	0.816
Before 2007	0.937 (0.831-1.056)	0.207	0.625 (0.355-1.101)	0.637	0.969 (0.844-1.112)	0.122	0.946 (0.826-1.083)	0.164	0.631 (0.359-1.108)	0.611

Abbreviations: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; [b]A random-effects model was used when the *p* value for heterogeneity was less than 0.10; otherwise, a fixed-effects model was used.

Table III. Pooled analysis for the associations between the DLG5 C4136A polymorphism and the risk of Crohn's disease and Ulcerative colitis, 2004-2011.

Variables	A *vs.* C		AA *vs.* CC		AC *vs.* CC		AA+AC *vs.* CC		AA *vs.* AC+CC	
	OR (95% CI)	p^b	OR (95% CI)	p^b	OR (95% CI)	p^b	OR (95% CI)	p^b	OR (95% CI)	p^b
Crohn's disease										
Overall	0.866 (0.701-1.069)	0.003	1.150 (0.423-3.124)	0.016	0.871 (0.689-1.099)	0.098	0.863 (0.651-1.143)	0.009	1.168 (0.493-2.770)	0.049
All in HWE	0.982 (0.762-1.265)	0.036	1.421 (0.760-2.660)	0.207	0.899 (0.758-1.067)	0.178	0.948 (0.740-1.214)	0.074	1.464 (0.784-2.734)	0.266
High quality studies	0.950 (0.761-1.186)	0.038	3.239 (1.149-9.136)	0.648	0.956 (0.779-1.175)	0.173	0.995 (0.740-1.338)	0.084	**3.151 (1.117-8.885)**	0.665
Ethnicity										
Europeans	0.991 (0.763-1.286)	0.052	**3.239 (1.149-9.136)**	0.648	0.956 (0.779-1.175)	0.173	0.995 (0.740-1.338)	0.084	**3.151 (1.117-8.885)**	0.665
Asians	0.624 (0.341-1.143)	0.017	0.511 (0.299-0.873)	0.104	**0.722 (0.545-0.956)**	0.186	0.588 (0.301-1.149)	0.044	0.602 (0.359-1.011)	0.193
Ulcerative colitis										
Overall (Europeans)	1.079 (0.927-1.255)	0.871	**3.877 (1.168-12.867)**	0.906	1.104 (0.886-1.378)	0.971	1.143 (0.920-1.421)	0.954	**3.840 (1.158-12.735)**	0.910

Abbreviations: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; [b]A random-effects model was used when the *p* value for heterogeneity was less than 0.10; otherwise, a fixed-effects model was used.

Table IV. Pooled analysis for the associations between the DLG5_e26 polymorphism and the risk of Crohn's disease and ulcerative colitis, 2004-2011.

Variables	d *vs.* i		dd *vs.* ii		di *vs.* ii		dd+di *vs.* ii		dd *vs.* di+ii	
	OR (95% CI)	p^b	OR (95% CI)	p^b	OR (95% CI)	p^b	OR (95% CI)	p^b	OR (95% CI)	p^b
Crohn's disease										
Overall	1.022 (0.914-1.142)	0.085	1.048 (0.849-1.294)	0.515	0.954 (0.747-1.219)	0.012	1.019 (0.899-1.155)	0.015	1.041 (0.852-1.270)	0.683
All in HWE	1.050 (0.953-1.156)	0.668	1.076 (0.867-1.334)	0.592	1.068 (0.933-1.223)	0.861	1.071 (0.942-1.218)	0.827	1.043 (0.851-1.279)	0.543
High quality studies	1.042 (0.958-1.134)	0.551	1.043 (0.828-1.314)	0.516	1.046 (0.898-1.218)	0.814	1.045 (0.905-1.207)	0.816	1.018 (0.820-1.264)	0.445
Ethnicity										
Europeans	1.042 (0.958-1.134)	0.551	1.043 (0.828-1.314)	0.516	1.046 (0.898-1.218)	0.814	1.045 (0.905-1.207)	0.816	1.018 (0.820-1.264)	0.445
Asians	0.815 (0.389-1.706)	0.004	1.073 (0.636-1.810)	0.162	0.623 (0.174-2.230)	0.001	0.659 (0.198-2.190)	0.001	1.177 (0.703-1.972)	0.672
Ulcerative colitis										
Overall (Europeans)	0.992 (0.843-1.168)	0.032	0.886 (0.666-1.180)	0.239	0.903 (0.612-1.331)	0.011	0.895 (0.616-1.301)	0.009	0.895 (0.683-1.175)	0.627

Abbreviations: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; [b]A random-effects model was used when the *p* value for heterogeneity was less than 0.10; otherwise, a fixed-effects model was used.

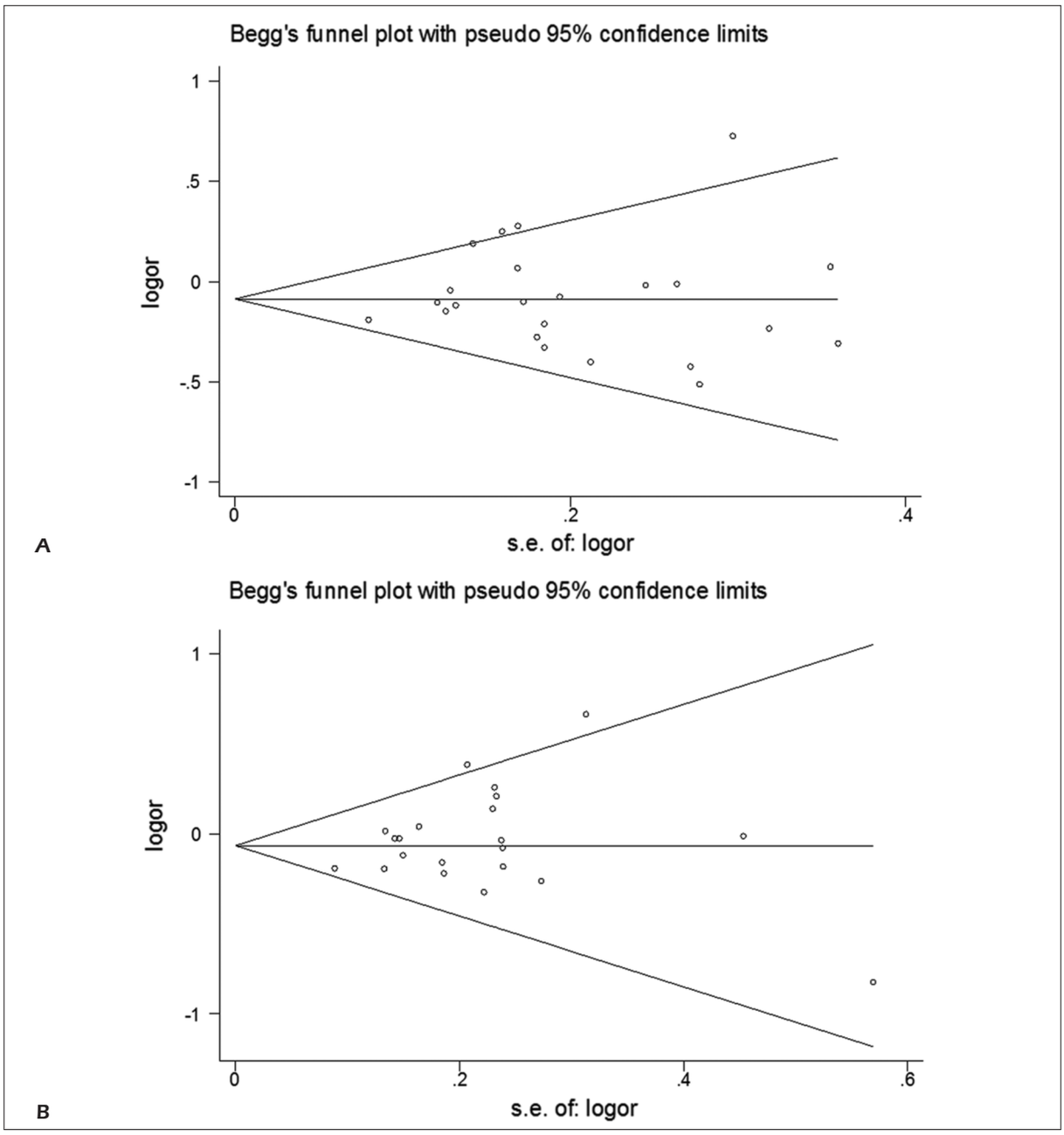

Figure 3. Begg's funnel plot for publication bias test of DLG5 G113A polymorphism and CD ***(A)*** or UC ***(B)***. (A *vs*. G) Each point represents a separate study for the indicated association. Log (OR): natural logarithm of OR.

Gene-gene Interaction in Crohon's Disease

For DLG5 G113A variant, there was no difference in allele frequencies of the mutant allele (A allele) between the CARD15-positive CD cases (17.45% ± 5.39%) and CARD15-negative CD cases (15.85% ± 3.25%; $p = 0.261$). Moreover, we did not find a significant increase in frequency of the rare delA allele of DLG5_e26 variant in CD patients carrying the risk-associated CARD15 alleles (57.90% ± 5.06%) compared to CD patients who did not (57.94% ± 1.23%; $p = 0.134$).

Genotype-Phenotype Interaction in Crohon's Disease

In CD, the carriage of the 113A variant allele was significant associated with colonic localiza-

tion (OR = 0.675, 95% CI = 0.470-0.969, p=0.033) when they were compared to controls, which was not observed in patients with ileal localization (OR = 0.659, 95% CI = 0.255-1.706, p = 0.390), ileo-colonic localization (OR = 0.765, 95% CI = 0.530-1.105, p = 0.153), or upper G-I tract (OR = 0.485, 95% CI = 0.168-1.402, p = 0.182).

Discussion

DLG5 protein is located at cell-cell junctions and is thought to be involved in the maintenance of epithelial integrity[36]. DLG5 involves in intracellular signal transduction and cell-cell contact by binding to vinexin, it may be hypothesized that DLG5 affects inflammatory responses. In addition, silico analysis suggested that the DLG5 G113A (R30Q) variant might impair DLG5 scaffolding function. Stoll et al[7] initially found evidence for association of 3 DLG5 variants (G113A, C4136A and DLG_e26) with IBD risk. However, the results of subsequent studies have been conflicting. Here, we performed a meta-analysis of eligible case-control or cohort studies to address the association between DLG5 variants and IBD risk.

The present meta-analysis indicated that no significant association was between G113A polymorphism and risk of CD or UC in overall population. However, in the subgroup analysis, a significantly decreased CD risk was observed in children with A allele (OR = 0.745, p = 0.033), indicating that ages may influence the association between R30Q and disease susceptibility. In pediatric patients, due to a lower influence of environmental risk factor, IBD might offer a better opportunity to understand the genetic factors involving pathology of the diseases than in adults. However, this result should be interpreted cautiously, because only three studies involving 512 cases and 1119 controls were available for pediatric patients. In high quality studies, DLG5 G113A variant was significantly associated with CD risk (A *vs.* G: OR = 0.913, 95% CI = 0.850-0.981, p = 0.013). Significant heterogeneity existed in most genetic models for G113A variant and CD risk, however, meta-regression did not found any sources which contribute to the heterogeneity. The results of Galbraith plots demonstrated that three studies were the potential source of heterogeneity in analysis of G113A and CD, and the effect estimate became significant and the heterogeneity was removed after excluding these studies (A *vs.* G: OR = 0.874, 95% CI = 0.813-0.939, $p < 0.001$; $p_h = 0.692$). There was no statistical association observed of G113A polymorphism with susceptibility of UC in the stratified analysis by ethnicity, age, sample size or date of publication. In addition, no heterogeneity was observed in major models of G113A variant and UC risk.

For C4136A polymorphism, significant association was found in CD and UC in European population (AA *vs.* CC: OR = 3.239, 95%CI= 1.149-9.136, p = 0.026 for CD, and OR = 3.877, 95% CI = 1.168-12.867, p = 0.027 for UC). Interestingly, in Asian subgroup, individuals with AA genotype had a reduced CD susceptibility compared with whom with CC genotype (OR = 0.511, 95% CI = 0.299-0.873, p = 0.014). These results suggested that the relative contribution of DLG5 C4136A polymorphism may vary across different populations. No heterogeneity was observed in any genetic models of C4136A variants and IBD (CD or UC) risk. Additionally, no significant association between DLG5_e26 variant and CD or UC risk was found in overall or subgroup analysis.

The first and most consistently replicated CD-associated mutations were identified in the CARD15 gene on chromosome 16 (IBD1)[37]. Previous studies[24,34] reported that a significantly increased frequency of the G113A variant in CD patients carrying the mutant CARD15 alleles, however, which could not be replicated by several subsequent researches. In the present pooled analysis, we did not find any evidence of epistasis between DLG5 G113A or DLG5_e26 variants and carriage of the 3 common NOD2 variants in CD patients.

Recently, the association of genetic variants with location of CD has been widely reported[38,39], especially of polymorphisms in CARD15 gene, which was shown to be correlated with ileal site. In our study, the results indicated that the persons carrying of A allele of G113A variant had a statistically decreased risk of CD with colonic localization (OR = 0.675, 95% CI = 0.470-0.969, p = 0.033), which was not observed in CD with other localizations, including ileal localization, ileo-colonic localization, and upper G-I tract, suggesting an association between DLG5 G113A variant and the presence of colonic disease.

Some limitations of this meta-analysis should be addressed. First, lack of the original data

might reduce the power of analysis of gene-gene interaction and genotype-phenotype interaction. Second, only reports published in the English and Chinese language were included in this meta-analysis, which may introduce language bias. Third, since only published studies were retrieved in the meta-analysis, publication bias may have occurred, although funnel plot and Egger's test did not show it. Finally, clinical assessment of patients with CD is known to vary between different centres and doctors, and such differences also could have affected phenotypes in our study, which might reduce the power of the analysis to detect genotype-phenotype association.

Conclusions

Our meta-analysis indicated DLG5 G113A variant was significantly associated with CD risk in children. We also provided evidence that the C4136A polymorphism could have different effects on CD risk between Europeans and Asians. In addition, for CD, the results of genotype-phenotype analysis also suggested G113A variant was associated with colonic involvement. These new insights warrant further studies to validate possible ethnic difference in the risk, particularly in C4136A polymorphism. Moreover, gene-gene and gene-phenotype interactions should also be examined in the future.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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