Discs Large Homolog 5 (DLG5) Gene Polymorphism and Crohn's Disease: A Meta-Analysis of the Published Studies

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Abstract- The real pathophysiology of Crohn's disease is unknown. The higher prevalence of Crohn's disease in Caucasian and Jewish ethnicities, as well as its familial aggregation and higher concordance among monozygotic twins, suggest some roles for genes in its development, clinical progression, and outcome. Recent original studies have indicated DLG5113G/A gene polymorphism as a risk factor for Crohn's disease. Meanwhile, the results of these studies are not consistent. We performed the current meta-analysis to understand whether there is any association between DLG5 gene polymorphism and the risk of Crohn's disease. PubMed was searched to find the case-control studies on DLG5 gene polymorphisms and Crohn's disease. This search compiled 65 articles and based on our criteria. 11 articles were included in this meta-analysis. The association between the DLG5 113G/A polymorphism and the risk of disease was assessed using odds ratio (OR) and 95% confidence interval (95% CI). Heterogeneity was evaluated based on I2 values. Random and fixed-effect models were used when I2>50% and I2≤50%, respectively. Eleven studies with a total of 4648 cases and 5677 controls were pooled. Based on our meta-analysis, DLG5113G/A gene polymorphism both at genotypic and allelic levels were not associated with the risk of Crohn's disease. Pooled data indicated no significant association between DLG5113G/A gene polymorphism and the development of Crohn's disease. In order to achieve a superior conclusion, multicenter studies on larger number of patients are recommended.

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Keywords: Crohn's disease; DLG5 protein; Gene polymorphism

Introduction

Crohn's disease (CD) is a subgroup of inflammatory bowel disease (IBD). Environment, genetics, immune factors, and gastrointestinal (GI) microbiota affect its occurrence and define its clinical course and phenotype. Epidemiological studies have showed that the prevalence of CD is going to be stabilized in Western Europe and North America; however, it continues to increase in South America, Asia and Pacific regions (1). CD can virtually involve all parts of the GI tract. Symptoms of the disease can be subtle and varied due to its various localities. It may present with abdominal pain, diarrhea, loss of appetite, and weight loss (2-4). Significant complications such as colorectal cancer, malnutrition, and opportunistic infections can occur in Crohn's patients either due to chronic inflammation or aggressive medical treatment (3,4).

The pathophysiology of the CD has not been well understood; however, higher prevalence of CD among Caucasian and Jewish ethnicity, familial aggregation of the disease and higher concordance rates among monozygotic compared to dizygotic twins strongly support the genetic aspects of the disease. Based on genome-wide association studies, more than 140 genomic loci have been defined to be involved in the

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pathogenesis and as markers of prognosis of the CD. Recently, SLCO3A1 has been introduced by Wei SC, *et al.,* as a novel CD associated gene polymorphism. These genes participate in different homeostatic mechanisms such as pattern recognition receptors, epithelial barrier homeostasis, molecular mimicry, autophagy, lymphocyte differentiation and apoptosis (5).

Discs large homolog 5 (Dlg5) is a member of the membrane-associated guanylate kinase adaptor family of protein that is involved in the regulation of TGF-β receptor-dependant signals and epithelial to mesenchymal transition (6). Moreover, it is involved in maintaining cell shape and polarity as well as cell to cell contact (7,8).

DLG5 rs1248696 (113G/A) polymorphism results in the amino acid substitution R30Q in the DUF622 domain of the DLG5 gene. In 2004, Stoll *et al.,* described an association between the DLG5 gene polymorphism and IBD which included CD in two large European study samples consisting of the UK and German patients (P=0.001 for allele frequencies in cases versus controls) (9). However, like strongly associated CARD15 variant that was absent in most of the Asian populations, our gene polymorphism was not replicated in similar studies (10,11).

Separate studies have revealed contrasting results when evaluating the association between DLG5 (113G/A) polymorphism and CD (12-23). In this review, we sought to pool data from different studies and perform the current meta-analysis in pursuance of understanding whether there is a statistical association between DLG5 gene polymorphism and CD.

Materials and Methods

Search strategy and study selection

In March 2015, PubMed was searched using the terms shown in Table 1.

Table 1. Search strategy to receive studies on dlg5 in inflammatory bowel disease

Searched terms	Number of articles retrieved
((((dlg5) OR dlg-5) OR "discs large")) AND ((((crohn) OR crohn's) OR "inflammatory bowel disese") OR IBD)	65
(((crohn) OR Crohn's) OR "inflammatory bowel disease") OR IBD	49768
((dlg5) OR dlg-5) OR "discs large"	830

The concluding 65 abstracts were reviewed on DLG5 gene polymorphism and CD. Further analysis was conducted on full-text case-control studies on DLG5 113G/A gene polymorphism and their references. The case-control studies were included if (i) they had evaluated DLG5 113G/A polymorphism in CD (ii) allele and genotype frequencies were reported, (iii) study conducted on adults and (iv) if the publication was in English.

Data extraction

The articles were extracted for genotype and allele frequencies of DLG5 113G/A gene polymorphism, the sample size in patient group versus control, the name of the first author, year of publication, country of origin, ethnicity and genotyping method. The data for the crohn's disease (CD) group were extracted from publications which contained both CD and ulcerative colitis.

Statistical analysis

The strength of the association between the DLG5 113G/A polymorphism and risk of CD was assessed using odds ratio (OR) and 95% confidence interval (95% CI). The significance of the pooled OR was determined using the Z-test and P<0.05 was considered statistically significant. Review Manager Version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used to analyze the data and the results were presented as forest plots.

Heterogeneity was assessed based on I2 values, where I2 > 50% indicated inconsistency and heterogeneity and warranted using the random-effect model. When I2 was equal or less than 50%, the fixed effects model was used (24).

Results

Overall 11 studies were included in this meta-analysis. A total of 4648 cases and 5677 controls were analyzed. The studies were conducted in distinct locations: Four studies were conducted in Germany, 1 in

Table 2. The characteristics of original studies included in the meta-analysis of *DLG5* rs1248696 (113G/A) polymorphism in Crohn's disease

Author	Year	Country	Cases	Controls	Genotyping method	*P* value	
Torok HP, *et al.*,[12]	2005	Germany	615	972	RFLP-PCR	NS	Interaction with CARD15 risk mutations not significant
Medici V, *et al.*, [13]	2006	Norway	138	226	TaqMan technology	NS	negative correlation with stricture (*P*=0.035)
Tremelling M, *et al.*, [14]	2006	UK	494	756	--	NS	--
Pearce AV, *et al.*, [15]	2007	UK	630	749	PCR	NS	--
Buning C, *et al.*, [15]	2006	Germany, Hungary	394(250,144)	627(422,205)	--	NS	--
Lakatos PL, *et al.*, [17]	2006	Germany	639	150	RFLP-PCR	NS	Associated with steroid resistant
Dema B, *et al.*,[18]	2010	Spain	411	846	--	NS	--
Stoll, *et al.*, [9]	2004	Germany	525	515	--	*P*=0.001	--
Noble CL, *et al.*, [19]	2005	Scotland	356	256	Taqman system	NS	--
Lin Z, *et al.*, [20]	2009	USA	58	170	RFLP-PCR and cRFLP	*P*=0.006	--
Browning B, *et al.*, [21]	2007	New Zealand	388	410	Taqman system	0.114	No gender specific association
Weersma RK, *et al*.,[22]	2009	The Netherlands	1684	1350	TaqMan system	*P*=0.08	--
Daly MJ, *et al.*, [23]	2005	Canada, Italy, UK	249 353	207 493	Automated sequencing	*P*=0.003 NS	--

Norway, 1 in Spain, 1 in Scotland, 1 in the USA, 1 in New Zealand and 2 in the UK (Tables 2,3).

Genotype and allele frequencies

From the collected studies, two of them showed an association between the allele and CD (Stoll *et al*., *P*=0.001 and Lin *et al*., *P*=0.006); however, the meta-analysis showed no statistically significant association.

Genotype and allele frequencies did not differ significantly between the CD group and the controls (Figures 1-5), suggesting based on our meta-analysis that DLG5 113G/A polymorphism is not a risk factor for CD.

Table 3. Review of some other *DLG5* gene SNPs studied in Crohn's disease patient

Author	Year	Country	Case	Control	Genotyping method	*P*.value
Chua KH, *et al*[33]	2011	Malaysia	80	100	PCR-RFLP	*DLG5_e26* *p*=0.0087 *DLG5* (4136 C/A) *p*=0.00026
Lin Z, *et al* [34]	2011	USA	212(IBD)	170	RFLP-PCR and cRFLP	P1371Q(rs2289310) *P*= 0.0246
Browning B, *et al* [21]	2007	New Zealand	384 385	408 415	Taqman system	P1371Q(rs2289310) *P*=0.705 rs2289311 *p*=0.077
Weersma RK, *et al* [22]	2009	The Netherlands	1684	1350	Taqman system	rs2289310 *p*=0.024 rs2289311 *p*=0.79 rs2165047 $2.90*10^{-3}$

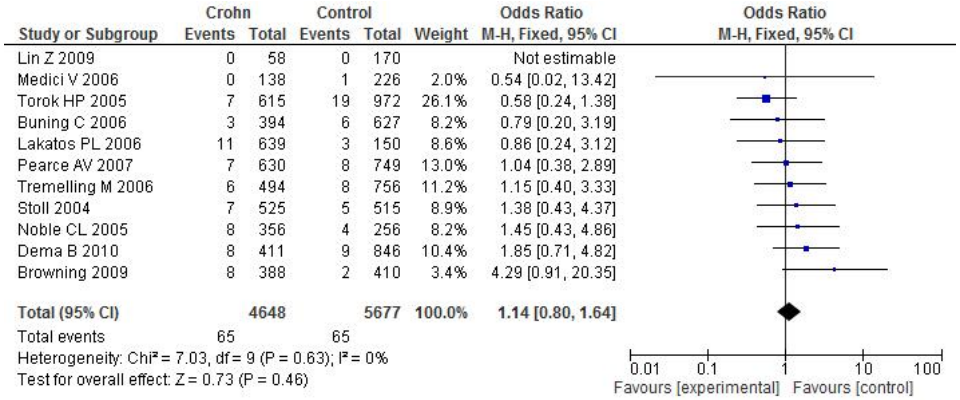

Figure 1. The meta-analysis of DLG5 113G/A gene polymorphism genotypes in Crohn's disease: there is no association between AA genotype and Crohn's disease. Events show the number of positive cases for the mentioned genotypes under the total number of cases in IBS or healthy control groups. Weight shows how much each study contributes to the pooled estimated odds ratio. M-H, Mantel-Haenszel; CI, confidence interval

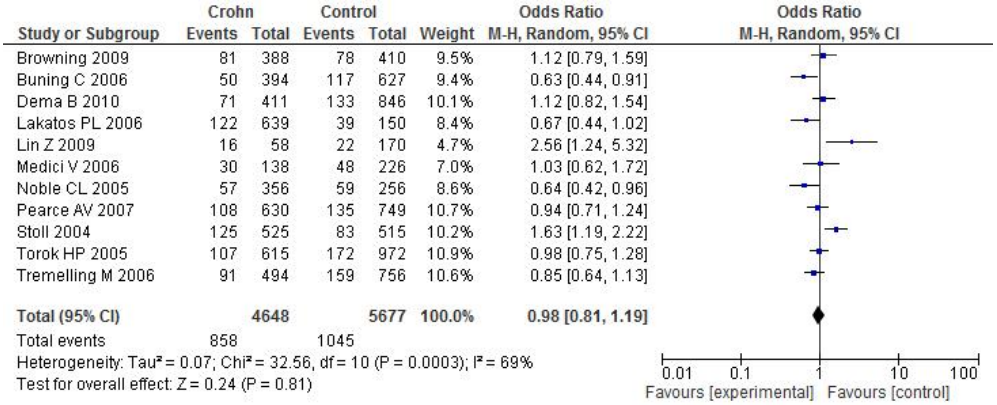

Figure 2. The meta-analysis of DLG5 113G/A gene polymorphism genotypes in Crohn's disease: there is no association between AG genotype and Crohn's disease. Events show the number of positive cases for the mentioned genotypes under the total number of cases in IBS or healthy control groups. Weight shows how much each study contributes to the pooled estimated odds ratio. M-H, Mantel-Haenszel; CI, confidence interval

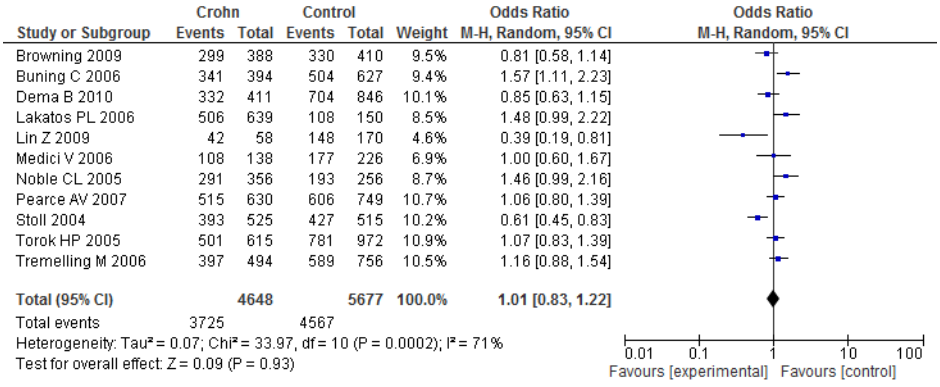

Figure 3. The meta-analysis of DLG5 113G/A gene polymorphism genotypes in Crohn's disease: there is no association between GG genotype and Crohn's disease. Events show the number of positive cases for the mentioned genotypes under the total number of cases in IBS or healthy control groups. Weight shows how much each study contributes to the pooled estimated odds ratio. M-H, Mantel-Haenszel; CI, confidence interval

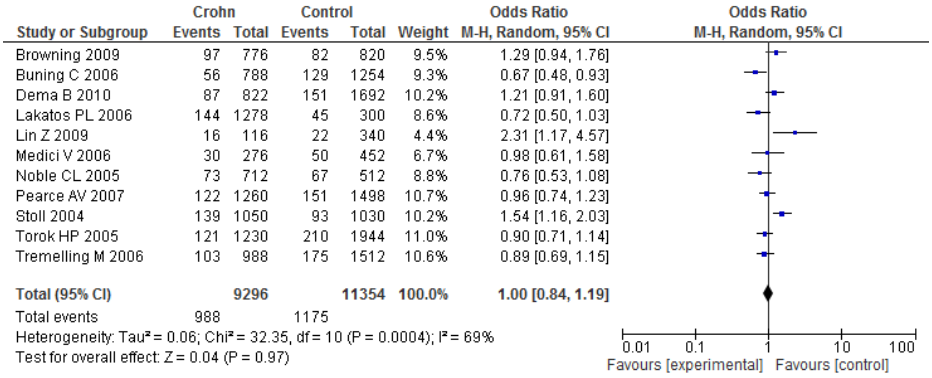

Figure 4. The meta-analysis of DLG5 113G/A gene polymorphism alleles in Crohn's disease: there is no association between A allele and Crohn's disease. Events show the number of positive cases for the mentioned genotypes under the total number of cases in IBS or healthy control groups. Weight shows how much each study contributes to the pooled estimated odds ratio. M-H, Mantel-Haenszel; CI, confidence interval

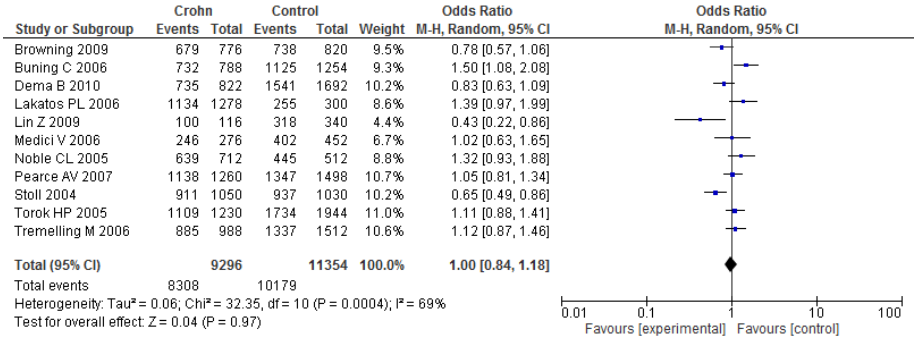

Figure 5. The meta-analysis of DLG5 113G/A gene polymorphism alleles in Crohn's disease: there is no association between G allele and Crohn's disease. Events show the number of positive cases for the mentioned genotypes under the total number of cases in IBS or healthy control groups. Weight shows how much each study contributes to the pooled estimated odds ratio. M-H, Mantel-Haenszel; CI, confidence interval

Discussion

Studies of the association between DLG5 113G/A polymorphism and the risk of CD have extrapolated inconsistent and inconclusive results. Variable population allele frequencies, variable effect sizes in different populations, gene-gene or gene-environment interactions, allelic heterogeneity, phenotypic heterogeneity and gender-specific effects may describe the discrepancy observed in the findings of studies on this gene polymorphism.

Therefore, we performed this meta-analysis to understand the relationship between DLG5 113G/A polymorphism and susceptibility to CD. A total of 11 publications describing thirteen case-control studies in Caucasians were included in this meta-analysis. We did not include research that incorporated Asian ethnicity considering this polymorphism was absent in such studies.

Although this meta-analysis did not reveal an association between this gene polymorphism and the susceptibility to CD; the role of DLG5 protein in the pathogenesis of IBD including CD cannot be ruled out on account that this gene is expressed in many human tissues such as the colon and small bowel (25). DLG5 is one of the members of the cell polarity complexes, SCRIB complex (Scribble,LGL, and DLG) that is responsible for maintenance of the basolateral membrane integrity. These complexes have a vital role in maintaining and the establishment of epithelial cell polarity and integrity (26). Moreover, DLG5 gene is crucial for many physiological processes which include: maintenance of cell polarity, cell proliferation, invasion, migration and cell division (27-30)

Likewise, J.K. Yamamoto-Furusho *et al*., showed increased colonic mucosa DLG5 mRNA expression in patients with active UC as compared to the healthy control group (P<0.0001). Moreover, there was upregulation seen in the UC remission group when compared to the healthy control group (P<0.0001) (31).

Sporadic studies have shown the association of DLG5 gene polymorphism in alternative loci rather than

113G/A and CD, suggesting some potential roles for DLG5 in CD. Table 2 shows some of the studies investigating the role of other DLG5 gene polymorphisms rather than 113G/A (rs1248696) (20,22,23,28).

Friedrichs *et al*., found that 30Q allele was a risk factor for CD in men, but not women, and that the 30Q has a lower population allele frequency in men than in women (32). Based on two other studies, no significant difference between allele frequencies in male and female was detected.

In conclusion, no association between DLG5 113G/A polymorphism and CD was detected in this study. However, the findings of this meta-analysis should be interpreted with caution, as a result of the small number of studies and subjects who were included in this meta-analysis. Such meta-analysis studies could also be done on other gene SNPs, where single studies showed signification association (35-37).

Future works should include investigations that examine other populations such as Asians, Latinos, etc., and the association of this polymorphism based on disease phenotype and gender.

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