BRIEF ARTICLE

T300A polymorphism of *ATG16L1* and susceptibility to inflammatory bowel diseases: A meta-analysis

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Abstract

AIM: To evaluate the association of the autophagy-related 16-like 1 (*ATG16L1*) T300A polymorphism (rs2241880) with predisposition to inflammatory bowel diseases (IBD) by means of meta-analysis.

METHODS: Publications addressing the relationship between rs2241880/T300A polymorphism of *ATG16L1* and Crohn's disease (CD) and ulcerative colitis (UC) were selected from the MEDLINE and EMBASE databases. To make direct comparisons between the data collected in these studies, the individual authors were contacted when necessary to generate a standardized set of data from these studies. From these data, odds ratio (OR) with 95% confidence interval (CI) were calculated.

RESULTS: Twenty-five studies of CD were analyzed, 14 of which involved cases of UC. The variant G allele of *ATG16L1* was positively associated with CD (OR = 1.32, 95% CI: 1.26-1.39, $P < 0.00001$) and UC (OR = 1.06, 95% CI: 1.01-1.10, $P = 0.02$). For child-onset IBD, a higher G allele frequency was found for cases of CD (OR = 1.35, 95% CI: 1.16-1.57, $P = 0.0001$) than for cases of UC (OR = 0.98, 95% CI: 0.81-1.19, $P = 0.84$) relative to controls.

CONCLUSION: The *ATG16L1* T300A polymorphism contributes to susceptibility to CD and UC in adults, but different in children, which implicates a role for autophagy in the pathogenesis of IBD.

INTRODUCTION

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the gastrointestinal tract that have an increasing incidence and prevalence in developing countries[1]. CD can affect any part of the alimentary tract, although it most commonly involves the terminal ileum, and is characterized by transmural discontinuous lesions. UC is a continuous disease that commences in the rectum and extends for a varying distance proximally in the colon, and its continuous inflammation is limited to mucosal and submucosal layers. Currently, the etiology and pathogenesis of IBD are not completely understood. IBDs are complex diseases with a number of contributing factors such as genetic predisposition, environmental factors, intestinal

microbial flora, and aberrant immune responses[2].

Advances in genetic research, especially the genome-wide association (GWA) studies, have identified a number of IBD susceptibility loci. One of these loci, the autophagy-related 16-like 1 (*ATG16L1*) gene, has been shown to have a role in IBD. The *ATG16L1* gene is located on chromosome 2, at 2q37.1, and encodes a protein that is involved in the formation of autophagosomes during autophagy. Autophagy is a non-selective degradation system that has roles in starvation adaptation, intracellular protein and organelle clearance, development, anti-aging processes, elimination of microorganisms, cell death, tumor suppression, and antigen presentation[3]. Autophagy has also been implicated in the innate and adaptive immune responses[4].

Hampe *et al*[5] first identified *ATG16L1* as a susceptibility gene for CD in a GWA study of 19 779 non-synonymous single nucleotide polymorphisms (SNPs) present in 735 individuals with CD and 368 controls. Further analysis showed that the marker, rs2241880, was an SNP that encodes a threonine to alanine substitution (T300A) at amino acid position 300, which was correlated with the incidence of CD in two German and one British studies of CD. The rs2241880 SNP directly correlated with the majority of risk associated with this locus. In contrast, no correlation between rs2241880 and UC was detected. Many other studies[6-29] have assessed the association of rs2241880 with predisposition to CD and UC, however, the results of these studies are inconsistent. In addition, a number of studies included child-onset IBD cases, the results of which were also confusing.

A meta-analysis was recently published that focused on the relationship between the T300A polymorphism and the incidence of CD, but not on the relationship between the T300A polymorphism and risk of UC, or the role of this polymorphism in child-onset IBD[30]. With the publication of additional studies related to the T300A polymorphism, sufficient data were available to perform a comprehensive meta-analysis of these studies in order to identify consistencies in the data to determine disease susceptibility, as well as to identify areas that need to be further addressed.

MATERIALS AND METHODS

Study selection
As of June 1, 2009, published studies in MEDLINE and EMBASE containing the following key words were included in this study: "inflammatory bowel disease", "IBD", "Crohn's disease", "CD", "Ulcerative colitis", "UC", "autophagy related 16 like 1", and "ATG16L1". The references of the eligible publications were searched manually to identify additional relevant studies. Relevant publications were also identified using the "Related Articles" option in PubMed. Studies reported by the same authors, yet published in different journals, were checked for possible overlapping participant groups. No language restrictions were made. When pertinent data were not included, or data that were presented were unclear, the authors were directly contacted.

Inclusion criteria
To be eligible for inclusion in this meta-analysis, the following criteria were established: (1) the study must include a case-control study that addressed IBD (i.e. CD or UC) patients and unrelated controls; (2) the study must have evaluated the ATG16L1 T300A polymorphism and the risk of CD or UC; and (3) the study must have included sufficient data for extraction.

Exclusion criteria
Studies were excluded from consideration if: (1) the study was based on family data; (2) the study did not have the outcomes of comparison reported or it was not possible to determine them; or (3) the study contained a smaller sample size and overlapped others meanwhile.

Data extraction
Using a standardized form, data from published studies were extracted independently by two investigators to populate the needed information. Data collected included first author, year of publication, inclusion and exclusion criteria, study population characteristics, and sample size. Any discrepancies between the two sets of data collected were resolved.

Statistical analysis
Odds ratio (OR) and 95% confidence interval (CI) were calculated for each study using Review Manager version 4.2 software. Between-study heterogeneity was estimated using the χ^2-based Q statistic. Heterogeneity was considered statistically significant when $P < 0.05$. I^2 was also tested. If heterogeneity existed, data were analyzed using a random effects model. In the absence of heterogeneity, a fixed effects model was used.

A χ^2 test was performed to examine Hardy-Weinberg equilibrium when genotype data were available. $P < 0.05$ was considered statistically significant. If Hardy-Weinberg disequilibrium existed, or it was impossible to evaluate this equilibrium, sensitivity analysis was performed.

RESULTS

Patients and controls
Of the 41 papers identified in a literature search of MEDLINE and EMBASE databases relevant to IBD, CD and UC, 25[5-29] were included in this meta-analysis, while 16 were excluded[31-46] (Figure 1). The reasons for exclusion are listed in Table 1. Three of the 41 papers[5,10,15] evaluated more than one study. Two of these studies contained relevant data and were combined[5,10], while the third was excluded[15]. Each of the 25 studies (Table 2) included cases of CD, with various subsets of papers considering different ethnic populations. For example, there were three studies of Asian popula-

Table 1 Reasons for study exclusion

Studies	Reasons
Glas *et al*[31], Török *et al*[32]	Data overlapped those of another article[17]
Cooney *et al*[33]	Data overlapped those of another article[8]
Weersma *et al*[34], Weersma *et al*[35]	Data overlapped those of another article[26]
Franke *et al*[36], Franke *et al*[37]	Data overlapped those of another article[5]
Fisher *et al*[38], Wtccc *et al*[40], Parkes *et al*[43], Yamazaki *et al*[45]	Other SNPs rather than rs2241880 SNP of *ATG16L1* were analyzed
Beckly *et al*[39], Barrett *et al*[41], Libioulle *et al*[42], Raelson *et al*[44], Kugathasan *et al*[46]	Data could not be extracted

Table 2 Studies included in the meta-analysis

No.	First author	Year	Population	CD	UC	Controls
1	Hampe *et al*[5]1	2007	Germany, UK	2122	1227	2056, 1032 for CD, UC respectively
2	Baldassano *et al*[6]	2007	USA	142	NA	281
3	Büning *et al*[7]	2007	Germany, Hungary, Netherlands	614	296	707
4	Cummings *et al*[8]	2007	UK	645	676	1190
5	Prescott *et al*[9]	2007	UK	727	877	579
6	Rioux *et al*[10]2	2007	North-America	1571	353	1184, 207 for CD, UC respectively
7	Roberts *et al*[11]	2007	New Zealand	496	466	549
8	Yamazaki *et al*[12]	2007	Japan	481	NA	437
9	Amre *et al*[13]	2009	Canada	286	NA	290
10	Baptista *et al*[14]	2008	Brazil	180	NA	189
11	Fowler *et al*[15]3	2008	Australia	154	NA	420
12	Gaj *et al*[16]	2008	Poland	59	NA	140
13	Glas *et al*[17]	2008	Germany	768	507	1615
14	Lakatos *et al*[18]	2008	Hungary	266	149	149
15	Lappalainen *et al*[19]	2008	Finland	240	459	190
16	Latiano *et al*[20]	2008	Italy	667	668	749
17	Okazaki *et al*[21]	2008	Canada	208	113	314
18	Perricone *et al*[22]	2008	Italy	163	NA	160
19	Peterson *et al*[23]	2008	USA	555	NA	486
20	Van Limbergen *et al*[24]4	2008	Scotland	629	580	345
21	Zhi *et al*[25]	2008	China	40	40	50
22	Weersma *et al*[26]	2009	Holland	1684	1120	1350
23	Hancock *et al*[27]	2008	UK	586	NA	1156
24	Yang *et al*[28]	2009	Korea	377	NA	372
25	Newman *et al*[29]	2009	Canada	435	NE	895
Total				14 095	7531	15 849, 13 852 for CD, UC respectively

[1]Data were extracted from the combination of panel A, panel B, panel C, and UC cohort; [2]Only GWA study and replication cohort 2 study were included; [3]Data from the cohort 1 study were not included in the analysis based on the use of data for controls from 251 families with IBD; [4]Data of the UC group were obtained by communication with the authors of this publication. CD: Crohn's disease; UC: Ulcerative colitis; NA: Not applicable (no such group); NE: Non-extractable or unavailable data.

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│ 41 papers from MEDLINE, EMBASE and manual reference list search │
└──────────────────────────────────────────────────────────────┘
                    │ ──────────────► ┌──────────────────────────────────────┐
                    │                  │ 4 excluded: Examined other SNPs of ATG16L1 │
                    ▼                  └──────────────────────────────────────┘
┌──────────────────────────────────────────────────────────────┐
│ 37 explored the relationship between ATG16L1 T300A polymorphism and IBD │
└──────────────────────────────────────────────────────────────┘
                    │ ──────────────► ┌──────────────────────────────────────┐
                    │                  │ 5 excluded: Data could not be extracted │
                    ▼                  └──────────────────────────────────────┘
          ┌────────────────────┐
          │ 32 available articles │
          └────────────────────┘
                    │ ──────────────► ┌──────────────────────────────────────┐
                    │                  │ 7 excluded: Overlapped other articles │
                    ▼                  └──────────────────────────────────────┘
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          │ 25 articles included in this meta-analysis │
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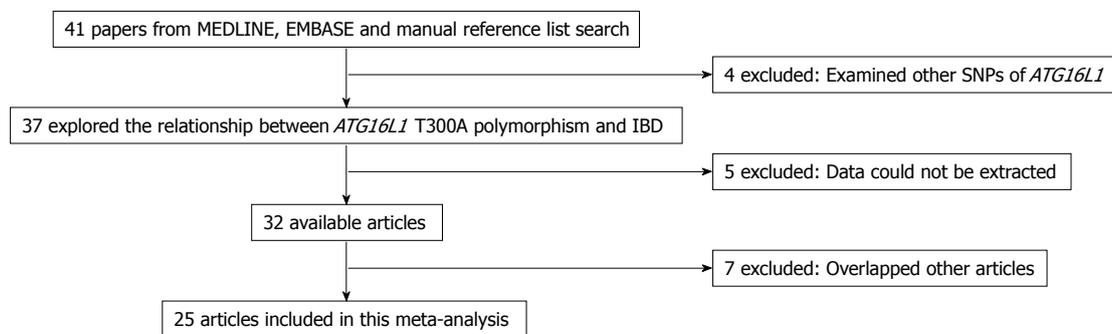
Figure 1 A flowchart illustrating the selection of published studies included in this meta-analysis.

tions[12,25,28], two of Oceanic populations[11,15], seven studies based in the United States[6,10,13,14,21,23,29], and 13 studies of European populations[5,7-9,16-20,22,24,26,27]. Of the 25 studies, 14 included both CD and UC cases and
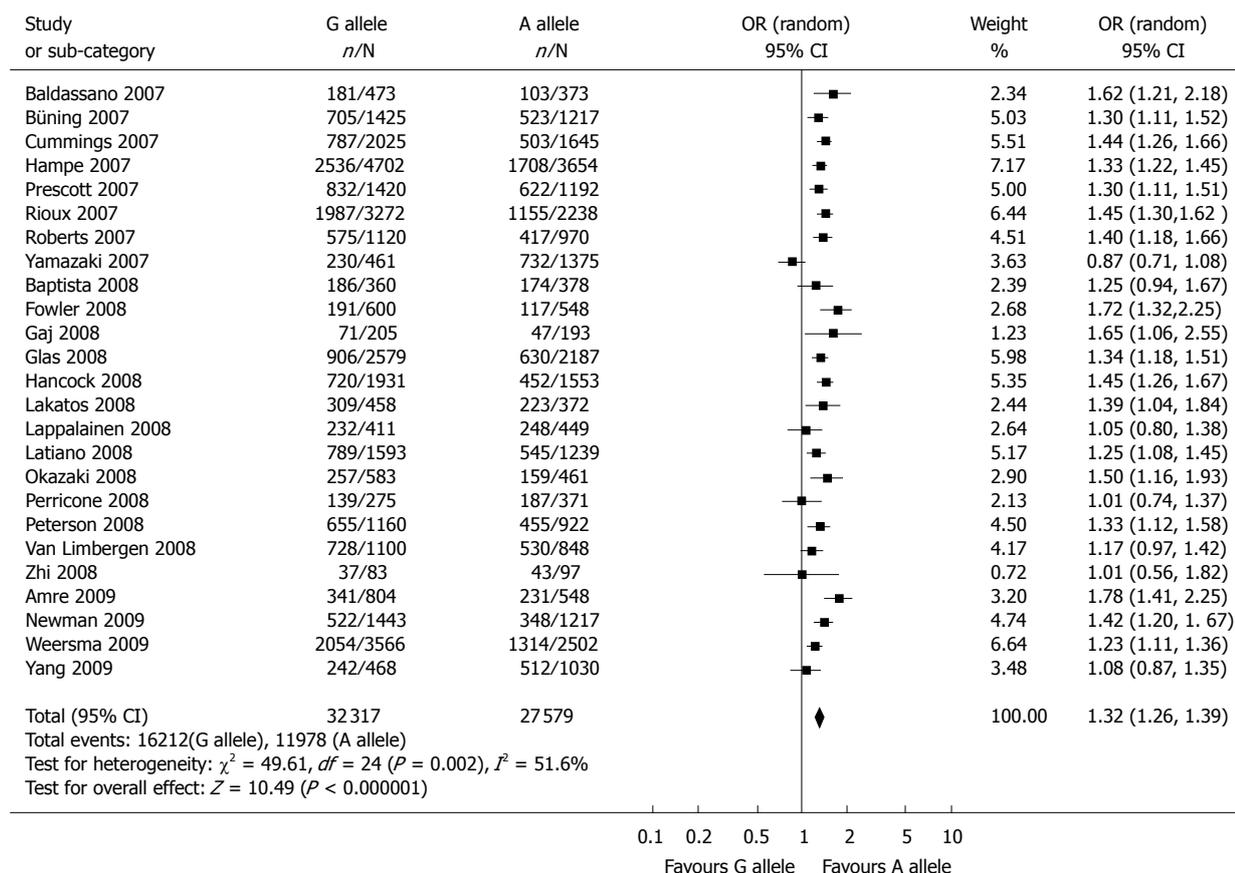
Study or sub-category	G allele n/N	A allele n/N	OR (random) 95% CI	Weight %	OR (random) 95% CI
Baldassano 2007	181/473	103/373		2.34	1.62 (1.21, 2.18)
Büning 2007	705/1425	523/1217		5.03	1.30 (1.11, 1.52)
Cummings 2007	787/2025	503/1645		5.51	1.44 (1.26, 1.66)
Hampe 2007	2536/4702	1708/3654		7.17	1.33 (1.22, 1.45)
Prescott 2007	832/1420	622/1192		5.00	1.30 (1.11, 1.51)
Rioux 2007	1987/3272	1155/2238		6.44	1.45 (1.30,1.62)
Roberts 2007	575/1120	417/970		4.51	1.40 (1.18, 1.66)
Yamazaki 2007	230/461	732/1375		3.63	0.87 (0.71, 1.08)
Baptista 2008	186/360	174/378		2.39	1.25 (0.94, 1.67)
Fowler 2008	191/600	117/548		2.68	1.72 (1.32,2.25)
Gaj 2008	71/205	47/193		1.23	1.65 (1.06, 2.55)
Glas 2008	906/2579	630/2187		5.98	1.34 (1.18, 1.51)
Hancock 2008	720/1931	452/1553		5.35	1.45 (1.26, 1.67)
Lakatos 2008	309/458	223/372		2.44	1.39 (1.04, 1.84)
Lappalainen 2008	232/411	248/449		2.64	1.05 (0.80, 1.38)
Latiano 2008	789/1593	545/1239		5.17	1.25 (1.08, 1.45)
Okazaki 2008	257/583	159/461		2.90	1.50 (1.16, 1.93)
Perricone 2008	139/275	187/371		2.13	1.01 (0.74, 1.37)
Peterson 2008	655/1160	455/922		4.50	1.33 (1.12, 1.58)
Van Limbergen 2008	728/1100	530/848		4.17	1.17 (0.97, 1.42)
Zhi 2008	37/83	43/97		0.72	1.01 (0.56, 1.82)
Amre 2009	341/804	231/548		3.20	1.78 (1.41, 2.25)
Newman 2009	522/1443	348/1217		4.74	1.42 (1.20, 1. 67)
Weersma 2009	2054/3566	1314/2502		6.64	1.23 (1.11, 1.36)
Yang 2009	242/468	512/1030		3.48	1.08 (0.87, 1.35)
Total (95% CI)	32 317	27 579		100.00	1.32 (1.26, 1.39)

Total events: 16212(G allele), 11978 (A allele)
Test for heterogeneity: χ^2 = 49.61, df = 24 (P = 0.002), I^2 = 51.6%
Test for overall effect: Z = 10.49 (P < 0.000001)

0.1 0.2 0.5 1 2 5 10
Favours G allele   Favours A allele

Figure 2 Meta-analysis of the association between the rs2241880 polymorphism of the *ATG16L1* gene and CD for the G allele *vs* the A allele.

were based on data from an Asian population[25], an Oceanic population[11], two studies from the United States[10,21], and 10 European populations[5,7-9,17-20,24,26]. Seven of the included studies explored the relationship between the incidence of the T300A polymorphism of *ATG16L1* and child-onset CD[6,11,13,17,20,23,24], while only two reported cases of child-onset UC[20,24]. For six of the total studies, genotype data were not available even after authors were contacted for further inquiry. Therefore, a Hardy-Weinberg equilibrium test was performed on the remaining 19 studies, and three of them showed disequilibrium[15,25,29] (P = 0.04, P = 0.00, and P = 0.03, respectively). Among the six untested studies due to unavailable data, five[5,8,10,17,26] reported Hardy-Weinberg equilibrium data in the original paper.

Association between ATG16L1 T300A polymorphism and IBD

Meta-analysis of the 25 studies that fulfilled the inclusion criteria identified a significant association between the G allele of *ATG16L1* and susceptibility to CD (OR = 1.32, 95% CI: 1.26-1.39, P < 0.00001) (Table 3 and Figure 2). Sensitivity analysis was performed with the omission of three studies[15,25,29] and Hardy-Weinberg disequilibrium showed similar results (data not shown). No notable change in the results of the statistic analyses was obtained when the Peterson *et al*[23] study was eliminated. As shown in Table 3 and Figure 3, a significant association between

Table 3 Summary of the association of the rs2241880 polymorphism and IBD determined in the meta-analysis

Comparisons	No. of studies	Effects model selection	OR (95% CI)	*P*-value
CD *vs* control	25	R	1.32 (1.26-1.39)	< 0.00001
UC *vs* control	14	F	1.06 (1.01-1.10)	0.02
Child-onset CD *vs* control	7	R	1.35 (1.16-1.57)	0.0001
Child-onset UC *vs* control	2	F	0.98 (0.81-1.19)	0.84

R: Random effects model; F: Fixed effects model.

the G allele of *ATG16L1* and the risk of UC was also detected (OR = 1.06, 95% CI: 1.01-1.10, P = 0.02). When the data of Zhi *et al*[25] were excluded, similar results were obtained: OR = 1.05, 95% CI: 1.01-1.10, P = 0.02.

Association between ATG16L1 T300A polymorphism and child-onset CD and UC

The pooled analysis for child-onset cases of CD identified the T300A polymorphism of *ATG16L1* as a factor for predisposition to child-onset CD in a total of seven studies[6,11,13,17,20,23,24] (OR = 1.35, 95% CI: 1.16-1.57, P = 0.0001) (Table 3 and Figure 4). When the study of Peterson *et al*[23] was excluded, similar data were acquired: OR = 1.35, 95% CI: 1.11-1.64, P = 0.003. Two studies[20,24] discussed the association of the T300A polymorphism of *ATG16L1* and
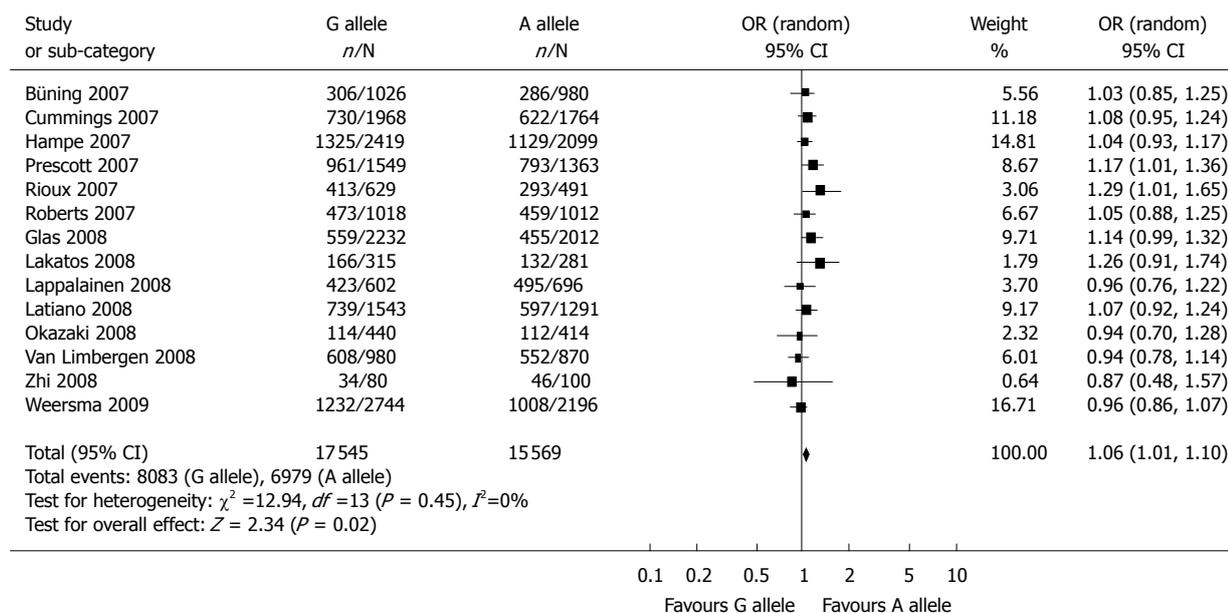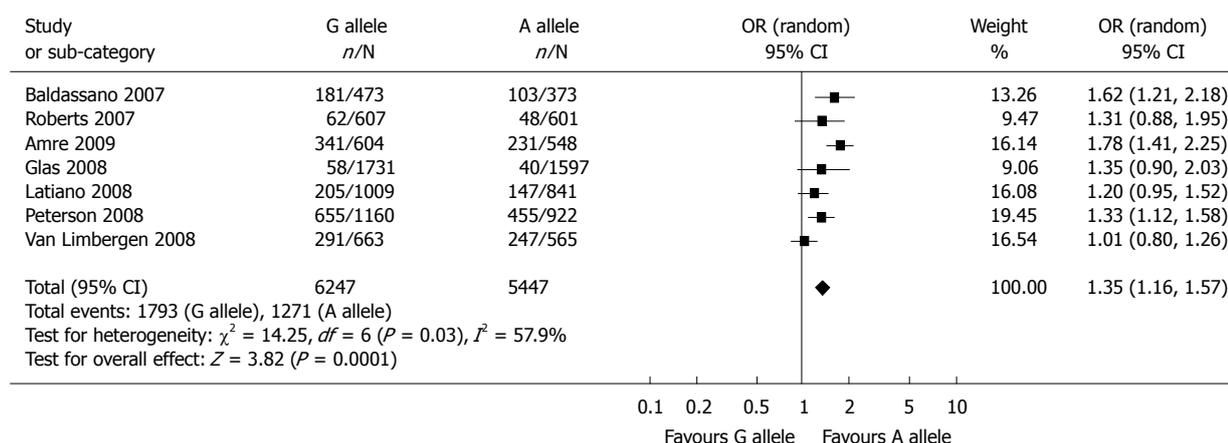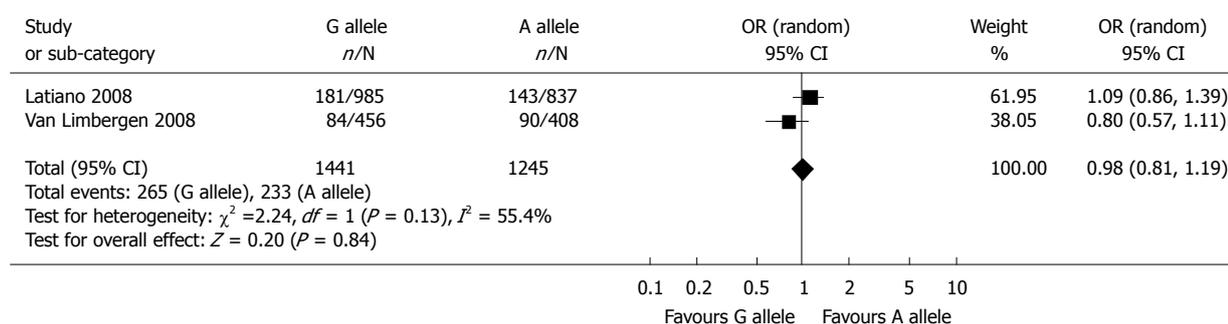
Study or sub-category	G allele n/N	A allele n/N	OR (random) 95% CI	Weight %	OR (random) 95% CI
Büning 2007	306/1026	286/980		5.56	1.03 (0.85, 1.25)
Cummings 2007	730/1968	622/1764		11.18	1.08 (0.95, 1.24)
Hampe 2007	1325/2419	1129/2099		14.81	1.04 (0.93, 1.17)
Prescott 2007	961/1549	793/1363		8.67	1.17 (1.01, 1.36)
Rioux 2007	413/629	293/491		3.06	1.29 (1.01, 1.65)
Roberts 2007	473/1018	459/1012		6.67	1.05 (0.88, 1.25)
Glas 2008	559/2232	455/2012		9.71	1.14 (0.99, 1.32)
Lakatos 2008	166/315	132/281		1.79	1.26 (0.91, 1.74)
Lappalainen 2008	423/602	495/696		3.70	0.96 (0.76, 1.22)
Latiano 2008	739/1543	597/1291		9.17	1.07 (0.92, 1.24)
Okazaki 2008	114/440	112/414		2.32	0.94 (0.70, 1.28)
Van Limbergen 2008	608/980	552/870		6.01	0.94 (0.78, 1.14)
Zhi 2008	34/80	46/100		0.64	0.87 (0.48, 1.57)
Weersma 2009	1232/2744	1008/2196		16.71	0.96 (0.86, 1.07)
Total (95% CI)	17 545	15 569		100.00	1.06 (1.01, 1.10)

Total events: 8083 (G allele), 6979 (A allele)
Test for heterogeneity: χ^2 =12.94, *df* =13 (*P* = 0.45), I^2=0%
Test for overall effect: *Z* = 2.34 (*P* = 0.02)

0.1 0.2 0.5 1 2 5 10
Favours G allele   Favours A allele

Figure 3 Meta-analysis of the association between the rs2241880 polymorphism of the *ATG16L1* gene and UC for the G allele *vs* the A allele.

Study or sub-category	G allele n/N	A allele n/N	OR (random) 95% CI	Weight %	OR (random) 95% CI
Baldassano 2007	181/473	103/373		13.26	1.62 (1.21, 2.18)
Roberts 2007	62/607	48/601		9.47	1.31 (0.88, 1.95)
Amre 2009	341/604	231/548		16.14	1.78 (1.41, 2.25)
Glas 2008	58/1731	40/1597		9.06	1.35 (0.90, 2.03)
Latiano 2008	205/1009	147/841		16.08	1.20 (0.95, 1.52)
Peterson 2008	655/1160	455/922		19.45	1.33 (1.12, 1.58)
Van Limbergen 2008	291/663	247/565		16.54	1.01 (0.80, 1.26)
Total (95% CI)	6247	5447		100.00	1.35 (1.16, 1.57)

Total events: 1793 (G allele), 1271 (A allele)
Test for heterogeneity: χ^2 = 14.25, *df* = 6 (*P* = 0.03), I^2 = 57.9%
Test for overall effect: *Z* = 3.82 (*P* = 0.0001)

0.1 0.2 0.5 1 2 5 10
Favours G allele   Favours A allele

Figure 4 Meta-analysis of the association between the rs2241880 polymorphism of the *ATG16L1* gene and child-onset CD for the G allele *vs* the A allele.

Study or sub-category	G allele n/N	A allele n/N	OR (random) 95% CI	Weight %	OR (random) 95% CI
Latiano 2008	181/985	143/837		61.95	1.09 (0.86, 1.39)
Van Limbergen 2008	84/456	90/408		38.05	0.80 (0.57, 1.11)
Total (95% CI)	1441	1245		100.00	0.98 (0.81, 1.19)

Total events: 265 (G allele), 233 (A allele)
Test for heterogeneity: χ^2 =2.24, *df* = 1 (*P* = 0.13), I^2 = 55.4%
Test for overall effect: *Z* = 0.20 (*P* = 0.84)

0.1 0.2 0.5 1 2 5 10
Favours G allele   Favours A allele

Figure 5 Meta-analysis of the association between the rs2241880 polymorphism of *ATG16L1* gene and child-onset UC for the G allele *vs* the A allele.

child-onset UC, yet the results of the combined analysis indicated there was no correlation (Table 3 and Figure 5).

DISCUSSION

Over the past few years, knowledge of the genetics of IBD has advanced tremendously since the first IBD GWA study was published in 2006[47]. Hence, several novel susceptibility loci have been identified; one of them being the *ATG16L1* gene. Hampe *et al*[5] first identified the association of the rs2241880 SNP in *ATG16L1* with cases of CD, but not with UC. Several subsequent studies

have tried to verify these results, however, the results of these latter studies were inconsistent, especially regarding the relationship between *ATG16L1* and UC, further perplexing our understanding of the role of *ATG16L1* in IBD. Thus, we conducted a meta-analysis of the currently published reports of CD and UC to attempt to clarify the correlation between *ATG16L1* and IBD. In order to obtain as much relevant data as possible, we retained some studies that presented a Hardy-Weinberg disequilibrium, however, there were no notable change in the results of our analyses whether these studies were included or omitted.

Our meta-analysis confirmed a positive association between the rs2241880 polymorphism of *ATG16L1* and susceptibility to CD. Meanwhile, a modest but significant association of the rs2241880 polymorphism with predisposition to UC was also observed. Taken together, these outcomes demonstrate that the *ATG16L1* variant containing the T300A substitution confers risk for both CD and UC.

Many aspects of child-onset IBD differ from adult-onset IBD, especially in regard to the type, location, and behavior of the disease, as well as sex preponderance and genetically attributable risk[48]. Considering the relatively short time of exposure to environmental factors (e.g. smoking) in children compared to adults with IBD, genetic background is hypothesized to be a more important factor in early-onset IBD. Therefore, we further analyzed the association between *ATG16L1* and child-onset IBD. Our results indicated that *ATG16L1* was associated with the risk of child-onset CD, but not with child-onset UC. Regarding the negative finding in early-onset UC, small sample sizes must be recognized as a contributing factor. Only two studies were included in the final analysis, therefore, this may have precluded the detection of a significant association. Additional studies are needed to determine whether an association exists in this cohort or not.

There were some limitations in the present meta-analysis. For example, because of publication limitations, some relevant studies could not be included in our analysis. Secondly, heterogeneity existed among studies of CD and child-onset CD, which had the potential to influence the results of our meta-analysis. The lack of information provided by some published studies, especially with regard to genotype data, was another limitation. Although we contacted authors of publications that did not provide a comprehensive set of data, genotype data remained unavailable for six of the total number of studies. Finally, there were only two studies that examined the relationship between *ATG16L1* and child-onset UC. As a result, the small sample size available was not ideal for detecting small genetic effects.

Although the precise impact of the *ATG16L1* variant on the pathogenesis of IBD remains unknown, accumulating evidence suggests that microbes play an important role in the initiation and etiopathogenesis of IBD. IBD lesions have been shown to develop preferentially in regions with the highest concentrations of bacteria[49]. In addition, enteric flora has been found to be more commonly associated with IBD patients than with control groups[50,51]. IBD animal model studies also have demonstrated that gut inflammation does not develop in a germ-free environment[52]. Autophagy is a fundamental intracellular degradation system that protects cells against various bacterial pathogens and the cytotoxic effect of bacterial toxins. Autophagy also has been identified to play a role in both innate and adaptive immune responses[53,54]. The specific role of *ATG16L1* in these processes remains unclear. ATG16L1 is expressed not only in intestinal epithelial cells, but also in lymphocytes and macrophages. It interacts with two other autophagy proteins, ATG5 and ATG12, to form a complex essential for the process of autophagy. Several studies have characterized the influence of ATG16L1 deficiency. Rioux *et al*[10] have shown that knockdown of *ATG16L1* mRNA in HeLa cells with siRNA reduced targeting of *Salmonella typhimurium* to autophagic vacuoles, which implicates a role for ATG16L1 in the clearance of intracellular bacteria *via* autophagy. Saitoh *et al*[55] have reported that ATG16L1 deficiency disrupts the recruitment of the ATG12-ATG5 conjugate to the isolation membrane, which results in loss of microtubule-associated protein 1 light chain 3 binding to phosphatidylethanolamine. Consequently, autophagosome formation and degradation of proteins with a long half-life are severely impaired in ATG16L1-deficient cells. ATG16L1-deficient macrophages also have been shown to produce large amounts of the inflammatory cytokines, IL-1 and IL-18, and mice that lack ATG16L1 expression in hematopoietic cells are highly predisposed to dextran sulfate sodium-induced acute colitis. Cadwell *et al*[56] have generated hypomorphic *ATG16L1* (ATG16L1^{HM}) mice that express ATG16L1 at 30% of its normal level. In this model, notable abnormalities have been observed in Paneth cells. These ileal epithelial cells are hypothesized to play a role in the control of intestinal microbiota by secreting granules that contained antimicrobial peptides and lysozymes. Aberrant Paneth cells exhibit deficiencies in their granule exocytosis pathway, which results in a decreased capacity to eliminate microbes. The mammalian ATG16L1 protein consists of three distinct domains: the N-terminal region mediates interactions with other autophagy proteins; a coiled-coil domain provides the capacity for ATG16L1 oligomerization and ATG5-ATG12 association; and there is a region of 7 WD repeats[57]. The T300A mutation is located within the WD repeats, which are associated with protein interactions. Therefore, the T300A mutation is hypothesized to affect protein interactions necessary for the formation of autophagosomes, which results in dysfunction of the autophagy pathway and a subsequent decrease in pathogen clearance[9]. It has been shown that the ATG16L1 coding variant is defective in mediating efficient antibacterial autophagy in cultured HeLa and Caco2 cells, and the ATG16L1*300A protein is unstable under conditions of high microbial load[58]. These observations suggest the effect of this mutation has a direct impact on autophagic effectiveness. In

summary, the T300A mutation of *ATG16L1* impairs specific autophagic, innate resistance mechanisms to gut commensals, which facilitate inflammation after invasion[59].

In addition to decreased ability to remove intestinal microbes directly, there may be other mechanisms that underlie the association between the *ATG16L1* variant and increased risk for IBD. For example, since autophagy contributes to immune tolerance against self tissues, it seems likely that decreased autophagy may lead to a failure of immune tolerance by autoantigen presentation on major histocompatibility complex class II molecules, which causes immune inflammation[59]. Another possible mechanism involves autophagy and apoptosis. Accumulating studies have detected an acceleration in the rate of epithelial cell apoptosis and inhibition of inflammatory cell apoptosis in CD and UC[60-63]. Autophagy and apoptosis share many common triggers and cross-inhibitory interactions[64], thus, it is hypothesized that defective autophagy might alter the process of intestinal cell apoptosis, ultimately contributing to IBD pathogenesis.

In conclusion, our meta-analysis of published cases of CD and UC suggests that the ATG16L1 T300A polymorphism is associated with susceptibility to CD and UC. However, the effect of this polymorphism differs between child-onset CD and UC. These findings implicate the role of autophagy and intestinal microbes in the pathogenesis of IBD, and demonstrate the need for further studies.

COMMENTS

Background
Inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are polygenic disorders. An A to G transition of the autophagy-related 16-like 1 gene (*ATG16L1*) has been implicated as a risk factor for IBD, but individual studies have been inconclusive or controversial.

Research frontiers
Much attention has been paid to the potential role of autophagy in the pathogenesis of IBD, since Hampe *et al* identified *ATG16L1* as a CD susceptibility gene in 2007. Some of them have been trying to confirm the definite relationship between *ATG16L1* polymorphism and IBD, and meanwhile, others attempting to uncover underlying mechanisms.

Innovations and breakthroughs
The current study demonstrated that *ATG16L1* is a susceptibility gene for CD and UC in adults, but different in children.

Applications
It is seen from this study that the ATG16L1 T300A polymorphism contributes to susceptibility to CD and UC in adults, which provides an insight into the role of autophagy in the pathogenesis of IBD. Studies for further exploring the mechanism by which altered ability of autophagy predisposes to IBD are needed.

Terminology
Autophagy is a cytoplasmic homeostasis process that cleanses the interior of all eukaryotic cells. It has an important role in cell and tissue homeostasis, and has been implicated in a range of human diseases.

Peer review
This study is an interesting meta-analysis about T300A polymorphism of ATG16L1 and susceptibility to inflammatory bowel diseases. The authors carefully reviewed the available literture and oncluded that ATG16L1 T300A polymorphism contributes to susceptibility to both CD and UC in adults, but not in children, implicating a role for autophagy in the pathogenesis of IBD.

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