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

Jia-Fei Cheng, Yue-Ji Ning, Wei Zhang, Zong-Hai Lu, Lin Lin

Jia-Fei Cheng, Yue-Ji Ning, Wei Zhang, Zong-Hai Lu, Lin Lin, Department of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Author contributions: Cheng JF and Lin L designed the research; Cheng JF, Ning YJ, Zhang W and Lu ZH performed the research; Cheng JF and Ning YJ analyzed the data; Cheng JF and Lin L wrote the paper.

Correspondence to: Lin Lin, Professor, Department of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China. lin9100@yahoo.com.cn

Received: October 20, 2009 Revised: December 22, 2009 Accepted: December 29, 2009 Published online: March 14, 2010

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.

© 2010 Baishideng. All rights reserved.

Key words: ATG16L1; Inflammatory bowel diseases; Crohn’s disease; Ulcerative colitis; Meta-analysis

Peer reviewer: Dr. Marco Scarpa, PhD, Department of Surgical & Gastroenterological Sciences (Gastroenterology section), University of Padova, via Giustiniani 2, Padova, 35128, Italy

Cheng JF, Ning YJ, Zhang W, Lu ZH, Lin L. T300A polymorphism of ATG16L1 and susceptibility to inflammatory bowel diseases: A meta-analysis. World J Gastroenterol 2010; 16(10): 1258-1266 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i10/1258.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i10.1258

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 countries1). 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[3]. 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, 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 $\chi^2$-based $I^2$ 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 $\chi^2$ 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[31-46] 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[3,10,13] evaluated more than one study. Two of these studies contained relevant data and were combined[3,10], while the third was excluded[13]. 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], Wicce et al[40], Parks et al[41] | Other SNPs rather than rs2241880 SNP of ATG16L1 were analyzed |
| Beckley et al[39], Barrett et al[42], Libioulle et al[43], Raelson et al[46], Kugathasan et al[47] | Data could not be extracted |

Table 2 Studies included in the meta-analysis

| No. | First author | Year | Population | Number of participants used in analysis |
|-----|--------------|------|------------|----------------------------------------|
|     |              |      | CD         | UC                                      | Controls                     |
| 1   | Hampe et al[5] | 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] | 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] | 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] | 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 |          |      | 14095 | 7331 | 15849, 13852 for CD, UC respectively |

1Data were extracted from the combination of panel A, panel B, panel C, and UC cohort; 2Only GWA study and replication cohort 2 study were included; 3Data from the cohort 1 study were not included in the analysis based on the use of data for controls from 251 families with IBD; 4Data 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.

Figure 1 A flowchart illustrating the selection of published studies included in this meta-analysis.

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

25 articles included in this meta-analysis
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\(^{[5,25,29]} \) 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 \( et \) \( al \)\(^{[25]} \) study was eliminated. As shown in Table 3 and Figure 3, a significant association between 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 \( et \) \( al \)\(^{[24]} \) were excluded, similar results were obtained: OR = 1.05, 95% CI: 1.01-1.10, \( P < 0.00001 \).

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

The pooled analysis for child-onset cases of CD identified a significant association between the T300A polymorphism of ATG16L1 and child-onset CD (OR = 1.35, 95% CI: 1.16-1.57, \( P = 0.0001 \)) (Table 3 and Figure 4). When the data of Zhi \( et \) \( al \)\(^{[24]} \) 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

**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)    | <0.0001      |
| UC vs control        | 14             | F                       | 1.06 (1.01-1.10)    | 0.02         |
| Child-onset vs control | 7             | R                       | 1.35 (1.16-1.57)    | 0.0001       |
| Child-onset vs control | 2             | F                       | 0.98 (0.81-1.19)    | 0.84         |

**Figure 2** Meta-analysis of the association between the rs2241880 polymorphism of the ATG16L1 gene and CD for the G allele vs 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. 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. In addition, enteric flora has been found to be more commonly associated with IBD patients than with control groups. IBD animal model studies also have demonstrated that gut inflammation does not develop in a germ-free environment. 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.

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. 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. 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. have generated hypomorphic ATG16L1 (ATG16L1<sup>−/−</sup>) 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. 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. 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<sup>T300A</sup> protein is unstable under conditions of high microbial load. These observations suggest the effect of this mutation has a direct impact on autophagic effectiveness.
summary, the T300A mutation of ATG16L1 impairs specific autophagic, innate resistance mechanisms to gut commensals, which facilitate inflammation after infection.\cite{39}

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.\cite{19}

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.\cite{60,61} Autophagy and apoptosis share many common triggers and cross-inhibitory interactions\cite{62}, 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.

### REFERENCES
1. Zheng JJ, Zhu XS, Huangfu Z, Gao ZX, Guo ZR, Wang Z, Cheng JF et al. ATG16L1 and inflammatory bowel diseases
2. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007; 369: 1627-1640
3. Mizushima N. The pleiotropic role of autophagy: from protein metabolism to bactericide. *Cell Death Differ* 2005; 12 Suppl 2: 1535-1541
4. Deretic V. Autophagy in innate and adaptive immunity. *Trends Immunol* 2005; 26: 523-528
5. Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Günther S, Prescott NJ, Onnie CM, Häselr S, Sipos B, Fölsch UR, Lengauer T, Plätzker M, Mathew CG, Krawczak M, Schreiber S. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in Crohn's Disease. *Nat Genet* 2007; 39: 207-211
6. Baldassano RN, Bradfield JP, Monos DS, Kim CE, Glessner JT, Casalunovalo T, Frackelton EC, O’Toole FG, Canakaris S, Shaffer JL, Smith RM, Eckert AW, Robinson LJ, Onyiah CC, Abrams DJ, Chivauci RM, Skraban R, Devoto M, Grant SF, Hakonarson H. Association of the T300A non-synonymous variant of the ATG16L1 gene with susceptibility to paediatric Crohn’s disease. *Gut* 2007; 56: 1171-1173
7. Bünning C, Durnus T, Molnar T, de Jong DJ, Drenth JPH, Fiedler T, Gentz E, Todorov T, Haas V, Buhner S, Sturm A, Baumgart DC., Nagy F, Lonovics J, Landt O, Kage A, Bünning H, Nickel R, Büttner J, Lochs H, Schmidt HH-J, Witt H. A study in three European IBD cohorts confirms that the ATG16L1 c.898A>G (pThr300Ala) variant is a susceptibility factor for Crohn’s disease. *J Crohn’s Colitis* 2007; 1: 70-76
8. Cummings JR, Cooney R, Pathan S, Anderson CA, Barrett JC, Beckly J, Geremia A, Hancock L, Guo C, Ahmad T, Cardon LR, Jewell DP. Confirmation of the role of ATG16L1 as a Crohn’s disease susceptibility gene. *Inflamm Bowel Dis* 2007; 13: 941-946
9. Prescott NJ, Fisher SA, Franke A, Hampe J, Onnie CM, Sears D, Bagnall R, Mirza MM, Sanderson J, Forbes A, Mansfield JC, Lewis CM, Schreiber S, Mathew CG. A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn’s disease and is independent of CARD15 and IBD5. *Gastroenterology* 2007; 132: 1665-1671
10. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huettt A, Green T, Kabbah P, Baradma MM, Datta LW, Shugart YY, Griffisht AM, Targan SR, Ippoliti AF, Bernard EF, Mei L, NicolaL DL, Requeiro M, Schumm LP, Steinhardt AH, Rotter JI, Duerph RH, Cho JH, Daly MJ, Brant SR, Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; 39: 596-604
11. Roberts RL, Gearry RB, Hollis-Moffatt JE, Miller AL, Reid J, Abkevich V, Timms KM, Gutin A, Lanchbury JS, Merriman TR, Barclay ML, Kennedy MA. IL23R 8381Q and ATG16L1 T300A are strongly associated with Crohn’s disease in a study of New Zealand Caucasians with inflammatory bowel disease. *Am J Hum Genet* 2007; 80: 2754-2761
12. Yamazaki K, Onouchi Y, Takazoe M, Kubo M, Nakamura Y, Hata A. Association analysis of genetic variants in IL23R, ATG16L1 and 5p13.1 loci with Crohn’s disease in mainland China: a systematic analysis of 50 years of research. *Chin J Dig Dis* 2005; 6: 175-181
13. Amre DK, Mack DR, Morgan K, Krupoves A, Costea I, Lambrette P, Grimard G, Dong J, Feguery H, Bucioni V, Deslandres C, Levy E, Seidman EG. Autophagy gene ATG16L1 but not IRGM is associated with Crohn’s disease in Canadian children. *Inflamm Bowel Dis* 2009; 15: 501-507
14. Baptista ML, Amarante H, Pichetich G, Sdepanian VL, Peterson N, Babasukumar U, Lima HC, Kugathasan S. CARD15 and IL23R influence Crohn’s disease susceptibility but not disease phenotype in a Brazilian population. *Inflamm Bowel Dis* 2008; 14: 674-679
15. Haddow NK, Whitehead DC, Florin TH, Montgomery
GW, Cavanaugh JA, Radford-Smith GL. ATG16L1 T300A shows strong association with disease subgroups in a large Australian IBD population: further support for significant disease heterogeneity. *Am J Gastroenterol* 2008; 103: 2519-2526.

Gaj P, Habior A, Mikula M, Ostrowski J. Lack of evidence for association of primary sclerosing cholangitis and primary biliary cirrhosis with risk alleles for Crohn’s disease in Polish patients. *BMC Med Genet* 2008; 9: 81

Glas J, Konrad A, Schmechel S, Dambacher J, Seiderer J, Schorr F, Wetzeck M, Roese D, Török HP, Tonenchl L, Pfennig S, Haller D, Griga T, Klein W, Epplen JT, Folwaczny C, Lohse P, Göke B, Ochsenkühn T, Mussack T, Folwaczny M, Müller-Myskob B, Brand S. The ATG16L1 gene variants rs2241879 and rs2241880 (T300A) are strongly associated with susceptibility to Crohn’s disease in the German population. *Am J Gastroenterol* 2008; 103: 682-691

Lakatos PL, Szamoty T, Szilvéi Á, Molnár E, Lakatos K, Kovacs A, Molnar T, Altorjai J, Papp M, Tulassay Z, Mihéller P, Papp J, Tordai A, Andrikovics H. ATG16L1 and IL23 receptor (IL23R) genes are associated with disease susceptibility in Hungarian CD patients. *Dig Liver Dis* 2008; 40: 867-873

Lappalainen M, Halme L, Turunen U, Saavalainen P. Association of IL23R, TNFRSF1A, and HLA-DRB1*0103 allele variants with inflammatory bowel disease phenotypes in the Finnish population. *Inflamm Bowel Dis* 2008; 14: 1118-1124

Latiano A, Palmieri O, Valvano MR, D’Inca R, Cucchiara S, Riegler G, Staiano AM, Ardizzone S, Accomando S, de Angelais GL, Corritore G, Bossa F, Annese V. Replication of interleukin 23 receptor and autophagy-related 16-like 1 association in adult- and pediatric-onset inflammatory bowel disease in Italy. *World J Gastroenterol* 2008; 14: 4643-4651

Okazaki T, Wang MH, Rawsthorne P, Sargent M, Datta LW, Sh Lugthart Y, Bernstein CN, Brant SR. Contributions of IBD5, IL23R, ATG16L1, and NOD2 to Crohn’s disease risk in a population-based case-control study: evidence of gene-gene interactions. *Inflamm Bowel Dis* 2008; 14: 1528-1541

Perricone C, Borgiani P, Romano S, Cicciacci C, Fusco G, Novelli G, Biancone L, Calabrese E, Pallone F. ATG16L1 Ala197Thr is not associated with susceptibility to Crohn’s disease or with phenotype in an Italian population. *Gastroenterology* 2008; 134: 368-370

Peterson N, Guthery S, Dersohn L, Lee J, Saeed S, Prahalad S, Blain V, EIhert R, Tomer G, Grand R, Rudolph C, Kugathasan S. Genetic variants in the autophagy pathway contribute to paediatric Crohn’s disease. *Gut* 2008; 57: 1336-1337; author reply 1337

Van Limbergen J, Russel RK, Nîmmo ER, Drummond HE, Smith L, Anderson NH, Davies G, Gillett PM, McGrogan P, Weaver LT, Bisset WM, Mahdi G, Arndt I, Wilson DC, Satsangi J. Autophagy gene ATG16L1 influences susceptibility and disease location but not childhood-onset in Crohn’s disease in Northern Europe. *Inflamm Bowel Dis* 2008; 14: 338-346

Zhi J, Zhi FC, Chen ZY, Yao GP, Guan J, Lin Y, Zhang YC. Correlation of the autophagosome gene ATG16L1 polymorphism and inflammatory bowel disease [published online ahead of print] *Zhonghua Yi Xue Za Zhi* 2008; 58: 388-395

Weersma RK, Stokkers PC, van Bodegraven AA, van Hogezand RA, Verspaget HW, de Jong DJ, van der Woude LW, Shugart YY, Bernstein CN, Brant SR. Contributions of ATG16L1, IL23R, NOD2 to Crohn’s disease risk in a population-based case-control study: evidence of gene-gene interactions. *Am J Gastroenterol* 2009; 104: 665-672

Török HP, Glas J, Endres J, Tonenchl L, Teshome MY, Wetzeck M, Klein W, Lohse P, Ochsenkühn T, Folwaczny M, Müller-Myskob B, Brand S. Epistasis between Toll-like receptor-9 polymorphisms and variants in NOD2 and IL23R modulates susceptibility to Crohn’s disease. *Am J Gastroenterol* 2009; 104: 1723-1733

Weersma RK, Stokkers PC, Cleynen I, Wolf K, Henschke L, Schreiber S, Dijkstra G, Franke A, Nolte IM, Rutgeerts P, Wijmenga C, Vermeire S. Confirmation of multiple Crohn’s disease susceptibility loci in a large Dutch-Belgian cohort. *Am J Gastroenterol* 2009; 104: 630-638

Franke A, Hampe J, Rosenstiel P, Becker C, Wagner F, Häser R, Little RD, Huse K, Ruethe A, Balschun T, Wittig M, Elsharawi A, Mayr G, Albrecht M, Prescott NJ, Onnie CM, Fournier H, Keith T, Radoluf U, Platzer M, Mathew CG, Stoll M, Krawczak M, Nürnberg P, Schreiber S. Systematic association mapping identifies NELLI as a novel IBD disease gene. *PLoS One* 2007; 2: e691

Franke A, Balschun T, Karlson TH, Hedderich J, May S, Lu T, Schultd D, Nikolaus S, Rosenstiel P, Krawczak M, Schreiber S. Replication of signals from recent studies of Crohn’s disease identifies previously unknown disease loci for ulcerative colitis. *Nat Genet* 2008; 40: 713-715

Fisher SA, Tremain T, Anderson CA, Gwilliam R, Bumpstead S, Prescott NJ, Nimmo ER, Massey D, Berzuci C, Johnson C, Barrett JC, Cummings FR, Drummond H, Lees CW, Onnie CM, Hanson CE, Blazczak K, Inouye M, Ewels P, Ravindrarajah R, Keniry A, Hunt S, Carter M, Watkins N, Ouwehand W, Lewis CM, Cardon L, Lobo A, Forbes A, Anderson J, Jewell DP, Mansfield JC, Deloukas P, Mathew CG, Fournier H, Keith T, Radoluf U, Platzer M, Mathew CG, Stoll M, Krawczak M, Nürnberg P, Mathew CG, Parkes M, Satsangi J. Genetic determinants of ulcerative colitis include the ECMI locus and five loci implicated in Crohn's disease. *Nat Genet* 2008; 40: 711-712

Beckly JB, Hancock L, Geremia A, Cummings JR, Morris A, Rooney S, Balschun T, Krawczak M, Schreiber S. Replication of signals from recent studies of Crohn’s disease identifies previously unknown disease loci for ulcerative colitis. *Nat Genet* 2008; 40: 500-507

Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; 447: 661-678

Cheng JF et al. ATG16L1 and inflammatory bowel diseases

31

29

28

27

26

25

24

23

22

21

20

16

15

14

13

12

11

10

9
Barrett JC, Hansoul S, Nicolaë DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MO, Rotter JI, Schummm LP, Steinhart AH, Targan SR, Xavier RJ, Libioiu C, Sandor C, Lathrop M, Belaiche J, Dewit G, Got I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghot J, Bumpstead SW, Gilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn’s disease. Nature Genet 2008; 40: 955-962

Libioiu C, Louis E, Hansoul S, Sandor C, Farnir F, Franchimont D, Vermeire S, Dewit G, de Vos M, Dixon A, Demarche B, Gut I, Heath S, Foglio M, Liang L, Laukens D, Mni M, Zelenika D, Van Gossum A, Rutgeerts P, Belaiche J, Lathrop M, Georges M. Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 13q12.3 and contains expression of PTGER4. PLoS Genet 2007; 3: e58

Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, Roberts RG, Nimmo ER, Cummings FR, Soars D, Drummond H, Lees CW, Khawaja SA, Bagnall R, Burke DA, Todhunter CE, Ahmad T, Onnie CM, McArindle W, Strachan D, Bethel G, Bryan C, Lewis CM, Deloukas P, Forbes A, Sanderson J, Jewell DP, Satsangi J, Mansfield JC, Cardon L, Mathew CG. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn’s disease susceptibility. Nat Genet 2007; 39: 830-832

Raelson JV, Litttle RD, Ruether A, Fournier H, Paquin B, Van Eerdegwen P, Bradley WE, Croteau P, Nguyen-Huu Q, Segal J, Debrus S, Allard R, Rosenstiel P, Franke A, Jacobs CA, Fisher SA, Roberts RG, Nimmo ER, Cummings FR, Soars D, Drummond H, Lees CW, Khawaja SA, Bagnall R, Burke DA, Todhunter CE, Ahmad T, Onnie CM, McArindle W, Strachan D, Bethel G, Bryan C, Lewis CM, Deloukas P, Forbes A, Sanderson J, Jewell DP, Satsangi J, Mansfield JC, Cardon L, Mathew CG. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn’s disease susceptibility. Nat Genet 2007; 39: 830-832

Kugathasan S, Baldassano RN, Bradfiepd JP, Sleiman PM, Imielsinski M, Gauthery SL, Cucchiara S, Kim CE, Frackelton EC, Annaiah K, Glessner JT, Santa E, Willson T, Eckert AW, Bonkowski E, Shaner JL, Smith RM, Otieno FG, Peterson N, Abrams DJ, Chiavacci RM, Grundmeier R, Tamura Y, Hata A. Positive association of genetic variants in the upstream region of NKX2-3 with Crohn’s disease in Japanese patients. Proc Natl Acad Sci USA 2007; 104: 14747-14752

Yamazaki K, Takahashi A, Takazoe J, Kubo M, Onouchi Y, Fujino A, Kamatani N, Nakamura Y, Hata A. Positive association of genetic variants in the upstream region of NKX2-3 with Crohn’s disease in Japanese patients. Proc Natl Acad Sci USA 2007; 104: 14747-14752

Kugathasan S, Fiocchi C. Progress in basic inflammatory bowel disease research. Semin Paliativ Surg 2007; 16: 146-153

Barnich N, Carvalho FA, Glasser AL, Darcha C, Jantscheff P, Allez M, Peeters H, Bommelaer G, Desreumaux P, Colombel JF, Darfeuille-Michaud A. CEACAM6 acts as a receptor for adherent-invasive E. coli, supporting ileal mucosa colonization in Crohn disease. J Clin Invest 2007; 117: 1566-1574

Darfeuille-Michaud A, Neut C, Barnich N, Lederman E, Di Martino P, Desreumaux P, Gambiez L, Joly B, Cortot A, Colombel JF. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn’s disease. Gastroenterology 1998; 115: 1405-1413

Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. Annu Rev Immunol 2002; 20: 495-549

Mizushima N. Autophagy: process and function. Genes Dev 2007; 21: 2861-2873

Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. Nat Rev Immunol 2007; 7: 767-777

Saitoh T, Fujita N, Jiang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M, Tanaka K, Kawai T, Tsujimura T, Takeuchi O, Yoshihiro T, Akira S. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature 2008; 456: 264-268

Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Ke W, Carrero JA, Hunt S, Stone CD, Brun MT, Xavier RJ, Sleckman BP, Li E, Mizushima N, Stappenbeck TS, Virgin HW 4th. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature 2008; 456: 259-263

Xavier RJ, Huett A, Rioux JD. Autophagy as an important process in gut homeostasis and Crohn’s disease pathogenesis. Gut 2008; 57: 717-720

Kuballa P, Huett A, Rioux JD, Daly MJ, Xavier RJ. Impaired autophagy of an intracellular pathogen induced by a Crohn’s disease associated ATG16L1 variant. PLoS One 2008; 3: e3391

Munz C. Enhancing immunity through autophagy. Annu Rev Immunol 2009; 27: 423-449

Zeissig S, Bojarski C, Burgel N, Mankertz J, Zeitz M, Fromm M, Schulzke JD. Downregulation of epithelial apoptosis and barrier repair in active Crohn’s disease by tumour necrosis factor alpha antibody treatment. Gut 2004; 53: 1295-1302

Boirivant M, Marin M, Di Felice G, Pronio AM, Montesani C, Tersigni R, Strober W, Lamina propria T cells in Crohn’s disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. Gastroenterology 1999; 116: 557-565

Yukawa M, Izuoka M, Horie Y, Yoneyama K, Shirasaki T, Iitou H, Komatsu M, Fukushima T, Watanabe S. Systemic and local evidence of increased Fas-mediated apoptosis in ulcerative colitis. Int J Colorectal Dis 2002; 17: 70-76

Karamanolis DG, Kyrlagkitsis I, Konstantinou K, Papa-theodoridis GV, Karamanolis DG, Di Martino P, Desreumaux P, Gambiez L, Joly B, Cortot A, Colombel JF. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn’s disease. Gastroenterology 1998; 115: 1405-1413

Mauri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol 2007; 8: 741-752

S-Editor Tian L, L-Editor Kerr C, E-Editor Ma WH