Special Issue: Biomarkers for pathophysiology

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2017, Vol. 45(6) 1636–1650

DOI: 10.1177/0300060516662404
imr.sagepub.com
SAGE

Association between *ATG16L1* gene polymorphism and the risk of Crohn's disease

Bei-Bei Zhang[1], Yu Liang[2], Bo Yang[1] and Ying-Jun Tan[1]

Abstract

Objective: To perform a meta-analysis to evaluate studies investigating the association between *ATG16L1* gene polymorphism and Crohn's disease.

Methods: PubMed, Embase and Web of Science databases were searched for all studies focusing on the association of *ATG16L1* and Crohn's disease. Combined odds ratios with 95% confidence intervals were calculated for four genetic models (allelic model: G allele versus A allele; additive model: GG versus AA; dominant model: GA + GG versus AA; recessive model: GG versus GA + AA) using either a random effects or fixed effects model.

Results: A total of 47 case–control studies involving 18 638 cases and 30 181 controls were included in the final meta-analysis. There was a significant association between *ATG16L1* and Crohn's disease for all four genetic models. Significant associations were also shown in subgroup analyses when stratified by study design (population- or hospital-based).

Conclusion: In this meta-analysis, the *ATG16L1* genotype was significantly associated with the risk of developing Crohn's disease.

Keywords

ATG16L1, autophagy, Crohn's disease, meta-analysis

Date received: 9 April 2016; accepted: 12 July 2016

Introduction

Crohn's disease is a type of inflammatory bowel disease associated with chronic relapsing inflammation of the digestive tract anywhere from the mouth to the anus.[1] Although its aetiopathogenesis remains unclear, it is well established that Crohn's disease is a complex disorder resulting from

[1]Department of Medical Affairs, General Hospital of PLA Chengdu Military Area Command, Chengdu, China
[2]Department of Thoracic Surgery, General Hospital of PLA Chengdu Military Area Command, Chengdu, China

Corresponding author:
Bei-Bei Zhang, Department of Medical Affairs, General Hospital of PLA Chengdu Military Area Command, Chengdu 610083, China.
Email: zbb1983918@163.com

the interactions of genetic, environmental and microbial factors. Among these, genetic factors may be responsible for a major component of disease susceptibility.[2]

The role of autophagy processes in the development of inflammatory bowel disease is attracting increasing attention.[3] It is possible that genes involved in the autophagy pathway may contribute to the pathogenesis of Crohn's disease. The autophagy-related 16-like 1 (*ATG16L1*) gene encodes an important protein involved in the formation of autophagosomes during autophagy.[4] Genome-wide association studies have shown an association between *ATG16L1* polymorphism involving an amino acid change at position 300 and increased susceptibility to Crohn's disease.[5,6] This substitution of threonine with alanine is the result of a single nucleotide polymorphism in which adenine (A) is replaced with guanine (G). This association has been examined in numerous studies, but the results have been inconsistent. The present meta-analysis was designed to evaluate the association between *ATG16L1* and Crohn's disease using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) criteria.[7]

Materials and methods

Literature search

Two investigators (B.B.Z and B.Y.) systematically searched the databases PubMed (up to June 2016), Embase (1966 to June 2016) and Web of Science (2003 to June 2016), and also references from articles, reviews and abstracts presented at meetings of related scientific societies. The following search terms were used: (''*ATG16L1*'') AND (''Crohn's disease'' OR ''inflammatory bowel diseases'') AND (''polymorphism'' OR ''mutation'' OR ''variant'' OR ''genotype''). Studies were limited to those published in English.

Inclusion criteria and quality assessment

The same two investigators independently screened each of the titles, abstracts and full texts to determine whether the studies met the following criteria: (i) evaluation of the association of Crohn's disease and *ATG16L1* polymorphism; (ii) case–control design; (iii) sufficient data for the estimation of odds ratios (ORs) and 95% confidence intervals (CIs). In addition, a quality assessment was performed on all included studies using the Newcastle–Ottawa Scale (NOS) as described elsewhere.[8]

Data extraction

The following data were collected from each study included in the meta-analysis: first author's name, publication date, country, total numbers of cases and controls, and frequency of *ATG16L1* genotypes in cases and controls.

Statistical analyses

Strength of agreement between the investigators regarding study selection was evaluated using the Kappa statistic. The combined ORs and 95% CIs were calculated for the allelic model (G allele versus A allele), the additive model (GG versus AA), the dominant model (GA + GG versus AA) and the recessive model (GG versus GA + AA) using either the random effects model[9] or the fixed effects model.[10] Galbraith plots were created to graphically assess the source of any heterogeneity. Publication bias was analyzed using Begg's funnel plots and Egger's test, with a P-value < 0.05 being considered representative of statistically significant publication bias.[11] Conformity with the Hardy–Weinberg equilibrium amongst the controls was determined using the χ^2-square test and was considered to be in agreement when the P-value is ≥ 0.05. All statistical analyses

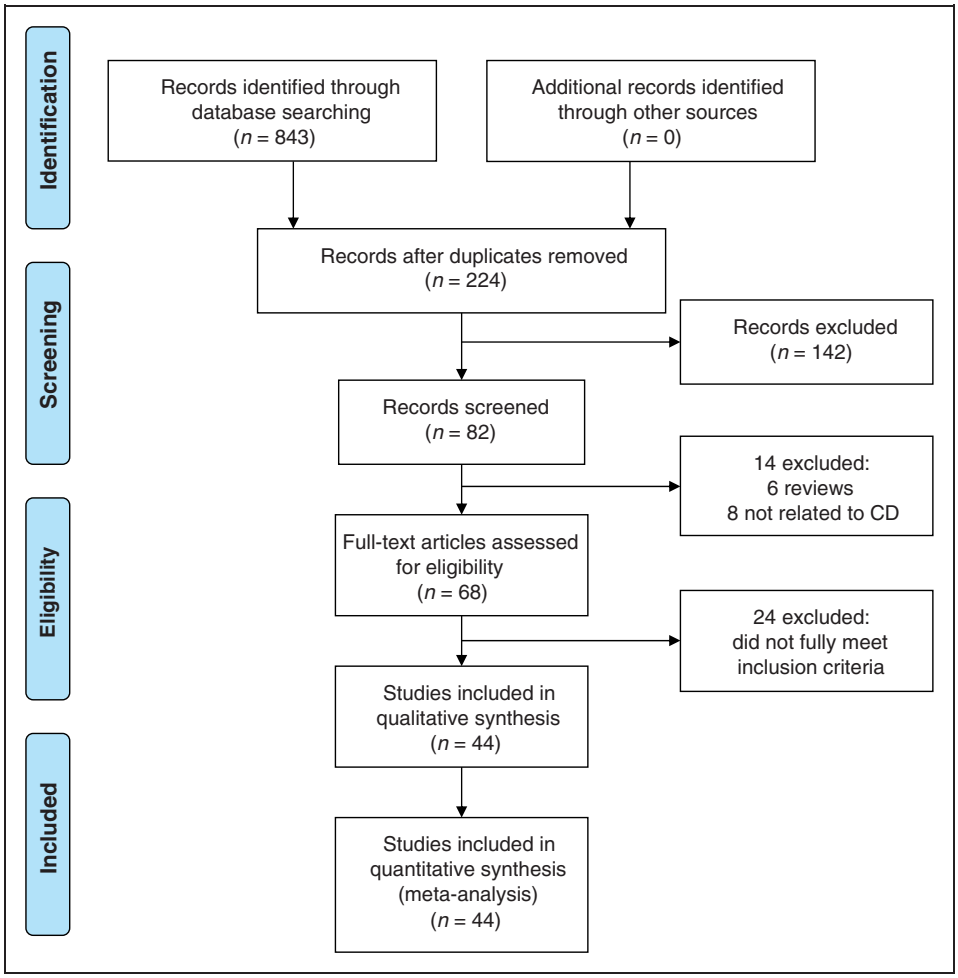

Figure 1. Flow diagram of the study selection process. CD, Crohn's disease.

were performed using Stata statistical software version 11.0 (StataCorp, College Station, TX, USA).

Results

Study characteristics

A total of 843 potentially relevant articles were initially identified. After exclusion of duplicate studies and application of the inclusion criteria, a total of 44 articles[12–55] were included in the qualitative synthesis (Figure 1). Büning et al.[13] contained three separate case–control studies and Fowler et al.[19] contained two separate case–control studies; therefore, a total of 47 case–control studies involving 18 638 cases and 30 181 controls were included in the final meta-analysis. The main characteristics of these studies are given in Table 1.

Quantitative synthesis

When all the studies were pooled in the meta-analysis, a significant association was seen between *ATG16L1* and Crohn's disease

Table 1. Main characteristics of studies included in the meta-analysis.

Reference	Source of subjects	Genotype and allele distribution (case/control)				HWE		NOS score
		GG	GA	AA	G	Conforms	Statistical significance	
Baldassano et al., 2007[12]	Population-based	58/78	65/136	19/67	181/292	Yes	NS	6
Büning et al., 2007:[13] study 1	Population-based	98/68	149/143	63/74	345/279	Yes	NS	6
Büning et al., 2007:[13] study 2	Population-based	38/49	86/109	23/49	162/207	Yes	NS	6
Büning et al., 2007:[13] study 3	Population-based	60/66	78/102	19/47	198/234	Yes	NS	6
Cummings et al., 2007[14]	Hospital-based	209/196	282/330	81/157	700/722	Yes	NS	6
Prescott et al., 2007[15]	Population-based	435/321	565/626	236/288	1435/1268	Yes	NS	6
Roberts et al., 2007[16]	Population-based	166/130	243/285	87/134	575/545	Yes	NS	7
Yamazaki et al., 2007[17]	Population-based	23/32	184/167	274/238	230/231	Yes	NS	6
Baptista et al., 2008[18]	Population-based	46/42	94/90	40/57	186/174	Yes	NS	8
Fowler et al., 2008:[19] study 1	Population-based	243/339	315/601	111/304	801/1279	Yes	NS	6
Fowler et al., 2008:[19] study 2	Population-based	59/110	73/189	22/121	191/409	No	$P = 0.04$	6
Gaj et al., 2008[20]	Population-based	24/32	25/70	11/37	73/134	Yes	NS	8
Glas et al., 2008[21]	Population-based	—	—	—	906/1673	N/A	N/A	8
Hancock et al., 2008[22]	Population-based	216/321	288/569	82/266	720/1211	Yes	NS	7
Lakatos et al., 2008[23]	Population-based	92/33	125/83	49/33	309/149	Yes	NS	7
Lappalainen et al., 2008[24]	Population-based	—	—	—	232/179	N/A	N/A	6
Latiano et al., 2008[25]	Population-based	227/214	335/376	105/159	789/804	Yes	NS	7
Okazaki et al., 2008[26]	Population-based	77/88	103/150	28/76	257/326	Yes	NS	8
Perricone et al. 2008[27]	Population-based	33/30	73/76	57/54	139/136	Yes	NS	7
Peterson et al., 2008[28]	Population-based	—	—	—	655/505	N/A	N/A	6
Van Limbergen et al., 2008[29]	Population-based	217/98	294/176	118/71	728/372	Yes	NS	6
Weersma et al., 2008[30]	Population-based	121/280	125/428	40/163	367/988	Yes	NS	7
Amre et al., 2009[31]	Population-based	102/64	137/135	47/91	341/263	Yes	NS	8
Dema et al., 2009[32]	Population-based	178/246	314/407	115/206	670/899	Yes	NS	7
Dusatkova et al., 2009[33]	Population-based	107/132	158/239	68/128	372/503	Yes	NS	7

(continued)

Table 1. Continued.

Reference	Source of subjects	Genotype and allele distribution (case/control)				HWE		NOS score
		GG	GA	AA	G	Conforms	Statistical significance	
Lacher et al., 2009[34]	Population-based	60/56	73/128	19/69	193/240	Yes	NS	7
Márquez et al., 2009[35]	Population-based	125/221	156/347	63/177	406/789	Yes	NS	7
Newman et al., 2009[36]	Population-based	159/253	204/415	72/227	522/921	No	$P = 0.03$	9
Palomino-Morales et al., 2009[37]	Hospital-based	216/183	253/316	75/167	685/682	Yes	NS	7
Cotterill et al., 2010[38]	Population-based	—	—	—	317/840	N/A	N/A	7
Csöngei et al., 2010[39]	Population-based	108/79	151/163	56/72	367/321	Yes	NS	7
Gazouli et al., 2010[40]	Population-based	189/161	222/274	63/104	600/596	Yes	NS	6
Sventoraityte et al., 2010[41]	Population-based	16/44	28/89	11/53	60/177	Yes	NS	8
Fabio et al., 2011[42]	Population-based	94/50	134/97	51/43	322/197	Yes	NS	6
Frank et al., 2011[43]	Hospital-based	25/17	22/19	14/23	72/53	No	$P = 0.007$	5
Lauriola et al., 2011[44]	Population-based	6/6	9/11	3/3	21/23	Yes	NS	6
Jung et al., 2012[45]	Population-based	—	—	—	638/864	N/A	N/A	6
Wang et al., 2012[46]	Population-based	44/33	164/140	141/179	252/206	Yes	NS	6
Hirano et al., 2013[47]	Population-based	—	—	—	1993/10141	N/A	N/A	6
Dalton et al., 2014[48]	Population-based	22/8	49/33	12/14	93/49	Yes	NS	6
Jakobsen et al., 2014[49]	Population-based	—	—	—	293/566	N/A	N/A	7
Scolaro et al., 2014[50]	Population-based	25/48	53/106	28/84	103/202	Yes	NS	8
Serbati et al., 2014[51]	Population-based	10/9	43/76	16/30	63/94	No	$P < 0.001$	6
Zhang et al., 2014[52]	Population-based	77/62	134/166	209/272	288/290	No	$P < 0.001$	7
Na et al., 2015[53]	Population-based	—	—	—	54/51	N/A	N/A	7
Salem et al., 2015[54]	Hospital-based	108/29	78/13	50/15	294/71	No	$P < 0.001$	6
Yang et al., 2015[55]	Population-based	226/211	838/1033	745/1192	1290/1455	Yes	NS	7

HWE, Hardy–Weinberg equilibrium; N/A, not available; NOS, Newcastle–Ottawa scale.
NS, not statistically significant ($P \geq 0.05$).

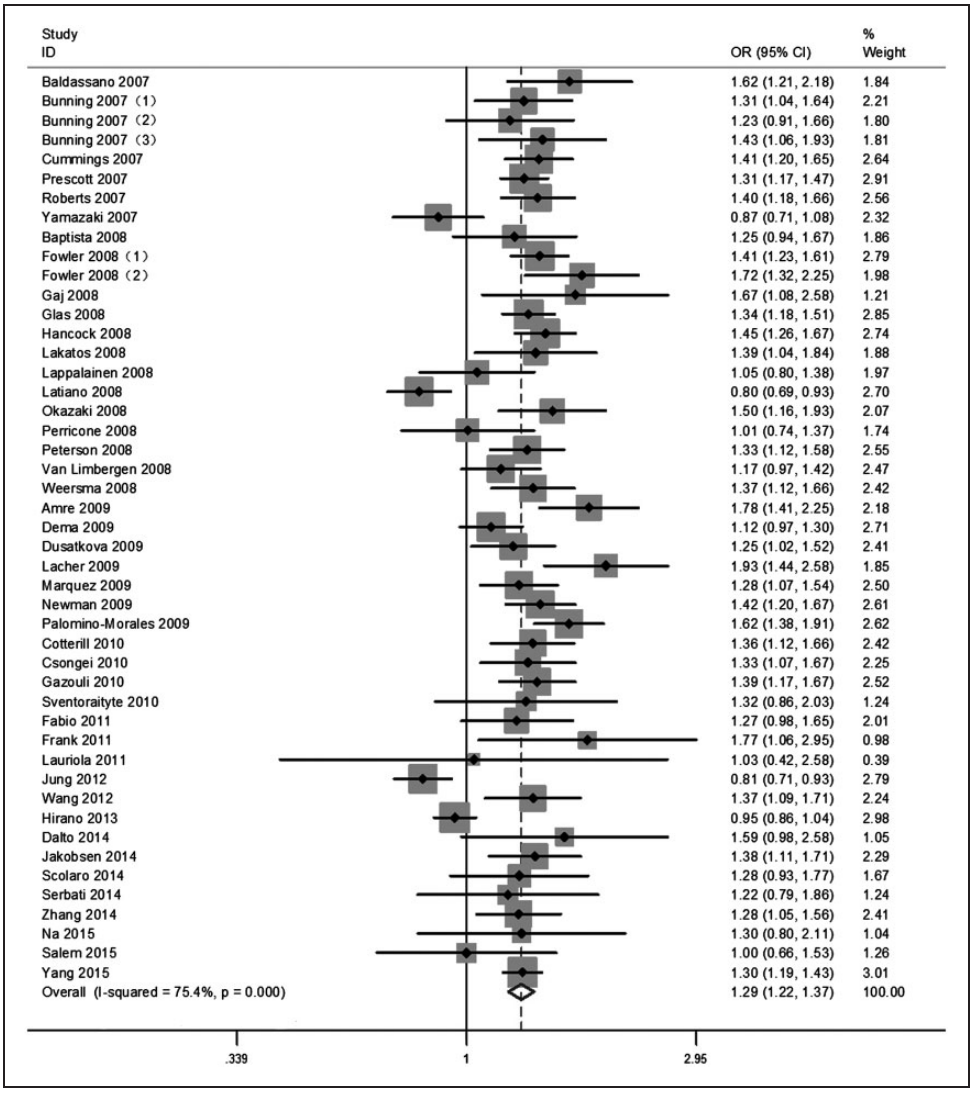

Figure 2. Forest plot of the association between *ATG16L1* and Crohn's disease using the allelic model (G allele versus A allele). The pooled odds ratio (OR) and 95% confidence intervals (CI) are indicated by the diamond. Percentage weights were calculated using a random effects model.

in all four genetic models (allelic model: OR = 1.29, 95% CI = 1.22, 1.37, Figure 2; additive model: OR = 1.80, 95% CI = 1.68, 1.92, Figure 3; dominant model: OR = 1.47, 95% CI = 1.39, 1.55, Figure 4; recessive model: OR = 1.46, 95% CI = 1.39, 1.54, Figure 5). When stratified by study design (population- or hospital-based), a significant association between *ATG16L1* and Crohn's disease was still seen in all four genetic models (Table 2).

Sensitivity analyses

Sensitivity analyses were conducted to determine whether modification of the inclusion

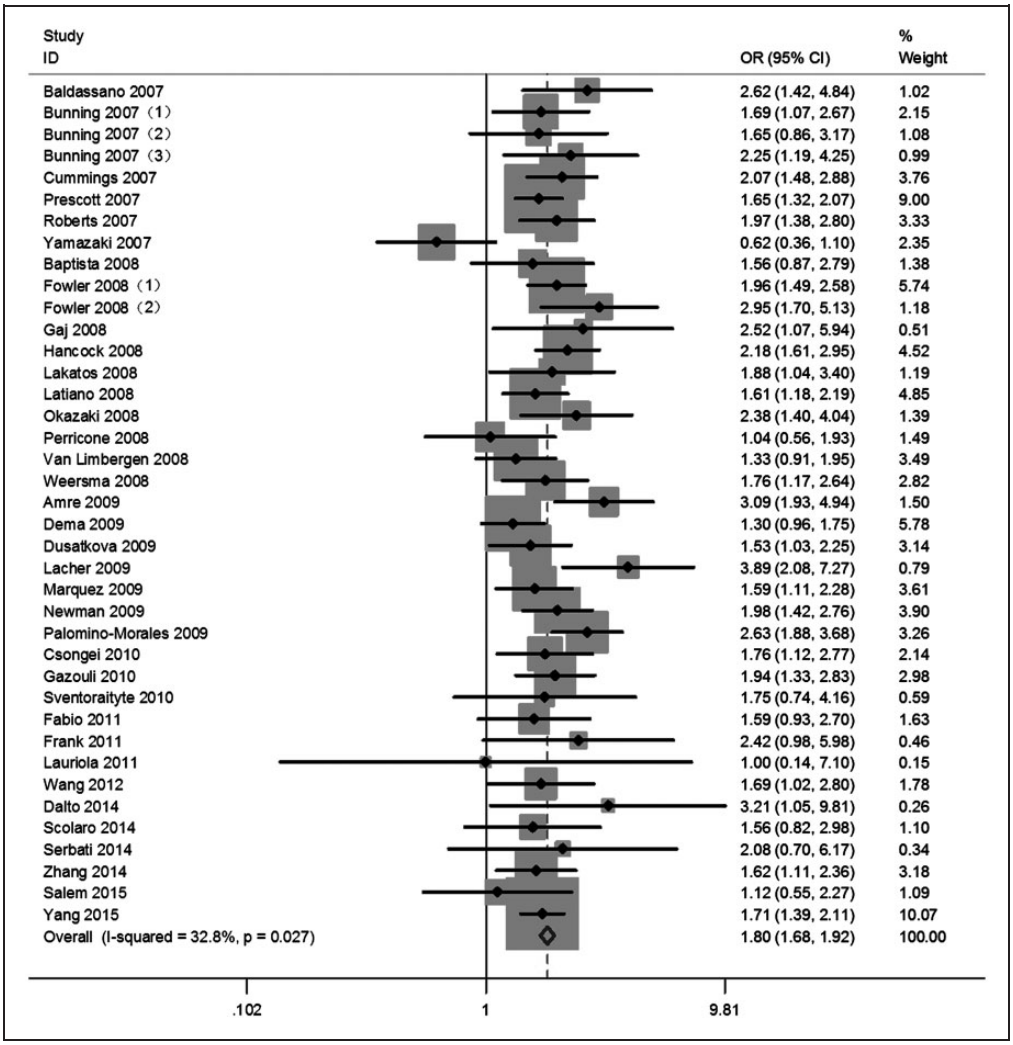

Figure 3. Forest plot of the association between *ATG16L1* and Crohn's disease using the additive model (GG versus AA). The pooled odds ratio (OR) and 95% confidence intervals (CI) are indicated by the diamond. Percentage weights were calculated using a fixed effects model.

criteria of the meta-analysis affected the final results. When the included studies were limited to those conforming to the Hardy–Weinberg equilibrium ($P \geq 0.05$), the pooled ORs of these 33 studies were not materially different from those of the full meta-analysis (Table 2). Likewise, when the included studies were limited to those with a high NOS score (≥ 7), the pooled ORs of these 22 studies were not materially different from those of the full meta-analysis (Table 2).

Analysis of heterogeneity

Significant heterogeneity existed in the allelic model ($I^2 = 75.4\%$). A Galbraith plot was created to graphically assess the source of heterogeneity (Figure 6). The studies by

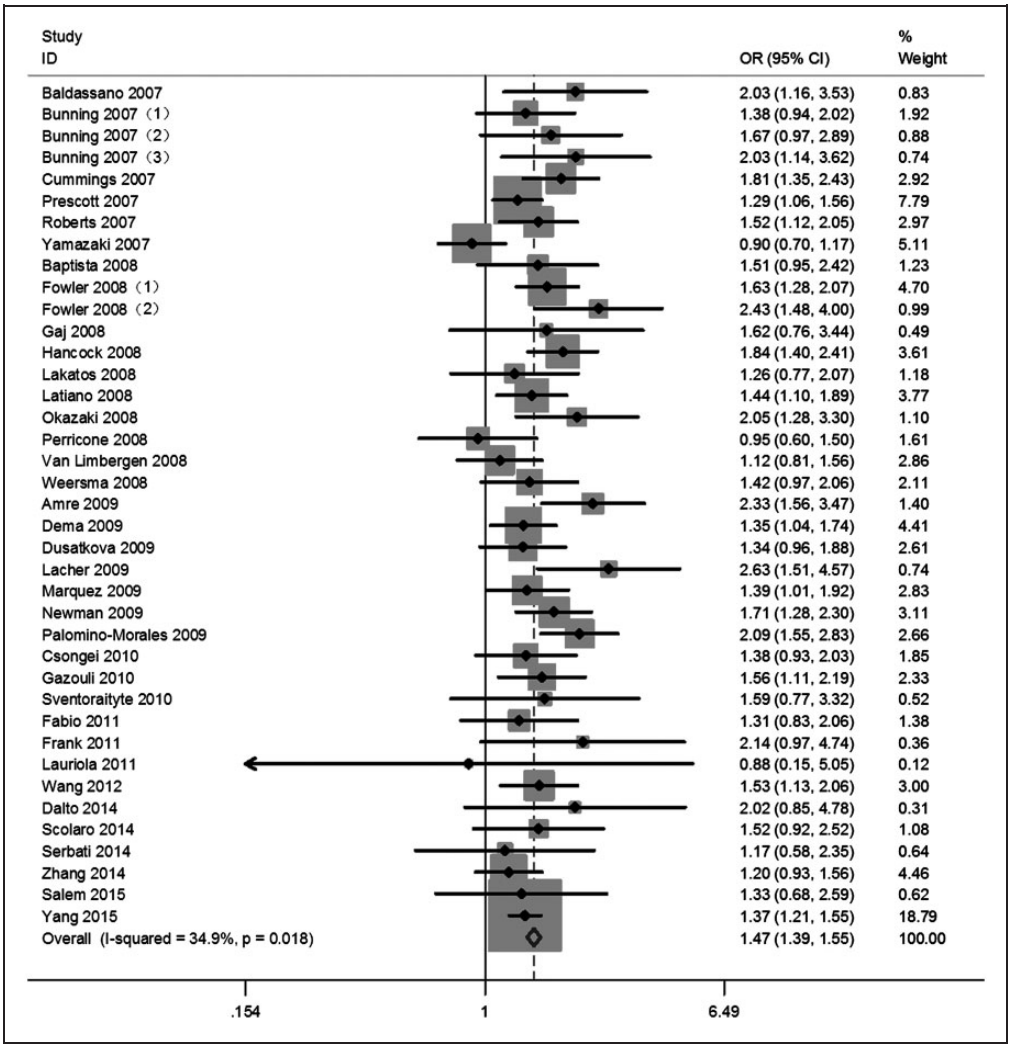

Figure 4. Forest plot of the association between *ATG16L1* and Crohn's disease using the dominant model (GG + GA versus AA). The pooled odds ratio (OR) and 95% confidence intervals (CI) are indicated by the diamond. Percentage weights were calculated using a fixed effects model.

Yamazaki et al.,[17] Fowler et al.[19] (study 1), Latiano et al.,[25] Amre et al.,[31] Lacher et al.,[34] Palomino-Morales et al.,[37] Jung et al.[45] and Hirano et al.[47] were identified as contributors to the heterogeneity. When these eight studies were excluded, the I^2 was 0.0% and the OR (95% CI) was 1.33 (1.28, 1.37).

Publication bias

The shapes of the Begg's funnel plots did not reveal any evidence of obvious asymmetry (Figure 7). No statistical evidence of publication bias was found using Egger's regression test ($P = 0.09$ for the allelic model; $P = 0.62$ for the additive model; $P = 0.08$

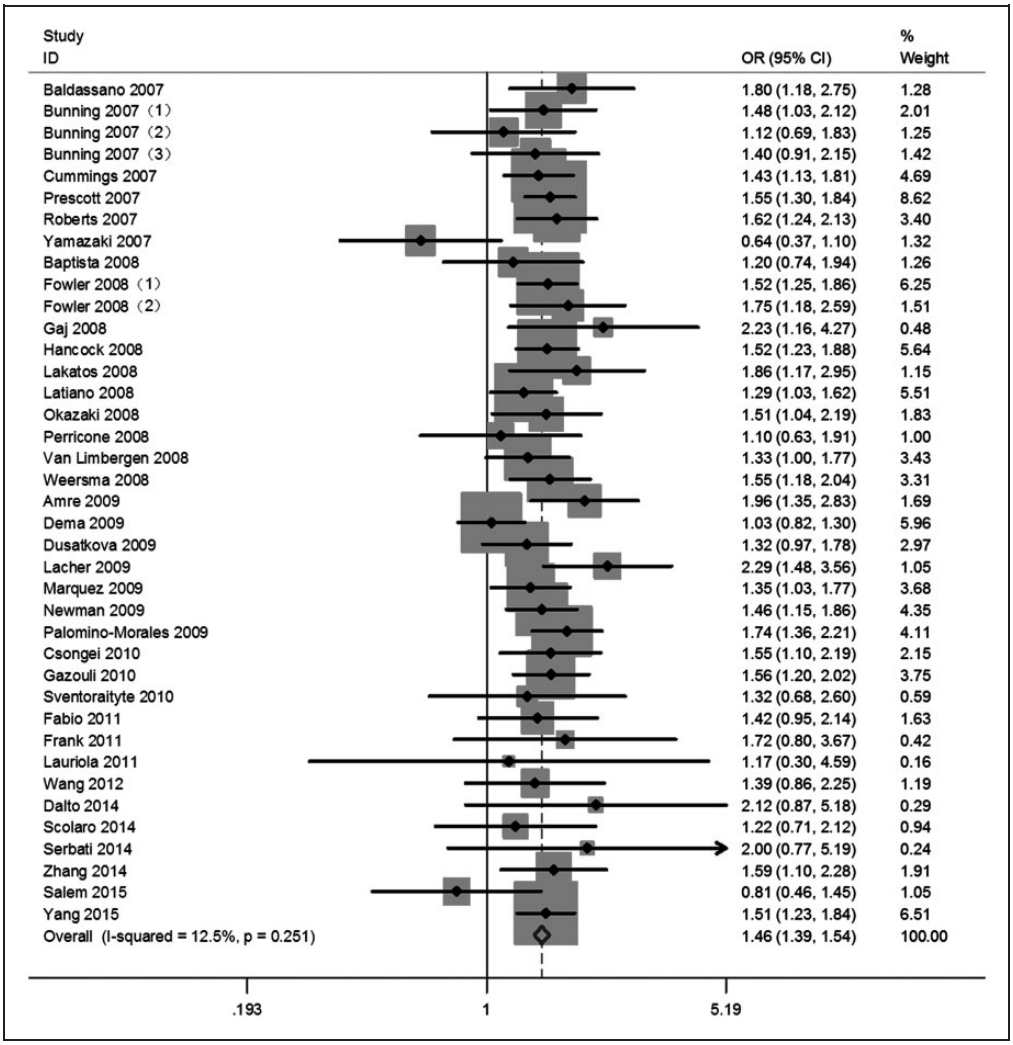

Figure 5. Forest plot of the association between *ATG16L1* and Crohn's disease using the recessive model (GG versus GA + AA). The pooled odds ratio (OR) and 95% confidence intervals (CI) are indicated by the diamond. Percentage weights were calculated using a fixed effects model.

for the dominant model; and $P = 0.83$ for the recessive model).

Discussion

Since Hampe et al.[5] reported in 2007 that *ATG16L1* gene polymorphism was associated with Crohn's disease, many studies have evaluated the relationship between *ATG16L1* and the risk of Crohn's disease.[56] However, the results are inconsistent. As the strength of results from a single case–control study is weak due to small sample sizes, the combination of many studies in a meta-analysis has the benefit of overcoming this limitation by increasing the sample size and

Table 2. Results of meta-analysis and subgroup analysis for the association between *ATG16L1* and Crohn's disease according to the allelic, additive, dominant and recessive models.

Group analysed	Allelic model (G vs A)		Additive model (GG vs AA)		Dominant model (GG + GA vs AA)		Recessive model (GG vs GA + AA)	
	OR (95% CI)	Analysis model	OR (95% CI)	Analysis model	OR (95% CI)	Analysis model	OR (95% CI)	Analysis model
All	1.29 (1.22, 1.37)	Random effects	1.80 (1.68, 1.92)	Fixed effects	1.47 (1.39, 1.55)	Fixed effects	1.46 (1.39, 1.54)	Fixed effects
Population-based	1.28 (1.20, 1.37)	Random effects	1.76 (1.64, 1.89)	Fixed effects	1.44 (1.36, 1.52)	Fixed effects	1.46 (1.38, 1.54)	Fixed effects
Hospital-based	1.46 (1.31, 1.62)	Fixed effects	2.18 (1.76, 2.70)	Fixed effects	1.86 (1.54, 2.26)	Fixed effects	1.51 (1.29, 1.76)	Fixed effects
NOS score ≥ 7	1.33 (1.24, 1.43)	Random effects	1.83 (1.68, 1.99)	Fixed effects	1.49 (1.39, 1.59)	Fixed effects	1.47 (1.37, 1.57)	Fixed effects
Conform to HWE	1.32 (1.24, 1.40)	Random effects	1.79 (1.67, 1.92)	Fixed effects	1.46 (1.38, 1.54)	Fixed effects	1.46 (1.38, 1.54)	Fixed effects

OR, odds ratio; CI, confidence intervals; NOS, Newcastle–Ottawa Scale; HWE, Hardy–Weinberg equilibrium.

generating more robust results. Meta-analysis has been widely used in genetic association studies.[57,58] The present meta-analysis was performed to assess whether the combined evidence supports an association between *ATG16L1* and Crohn's disease.

The present meta-analysis examined *ATG16L1* gene polymorphism and its relationship with the risk of Crohn's disease based on data from 47 case–control studies involving 18 638 cases and 30 181 controls. Most of these studies reported that *ATG16L1* was associated with the risk of Crohn's disease, but not all. The results of the meta-analyses demonstrated that overall there was evidence of a significant association between *ATG16L1* gene polymorphism and Crohn's disease. This significant association remained in all four genetic models when subgroup analyses were performed based on study design (population-based or hospital-based).

When considering the potential mechanisms linking *ATG16L1* polymorphism with an increased risk of Crohn's disease, it has been shown that *ATG16L1* polymorphism impairs the autophagy processing of pathogenic bacteria and the function of intestinal Paneth cells.[59,60] In addition, it has been shown that *ATG16L1* polymorphism is associated with increased susceptibility to *Helicobacter pylori* infection.[61] In patients with Crohn's disease, it has been reported that homozygosity of the *ATG16L1* risk allele (GG) was associated with a reduced ability to clear pathosymbionts.[62] Paneth cells in *ATG16L1*-deficient mice have been shown to be dysfunctional and to demonstrate increased expression of pro-inflammatory cytokines.[63,64]

When interpreting the results of this meta-analysis, a number of limitations should be acknowledged. First, it is well known that both environmental factors and individual genetic predisposition contribute to the development of Crohn's disease. Due to the lack of original data, however,

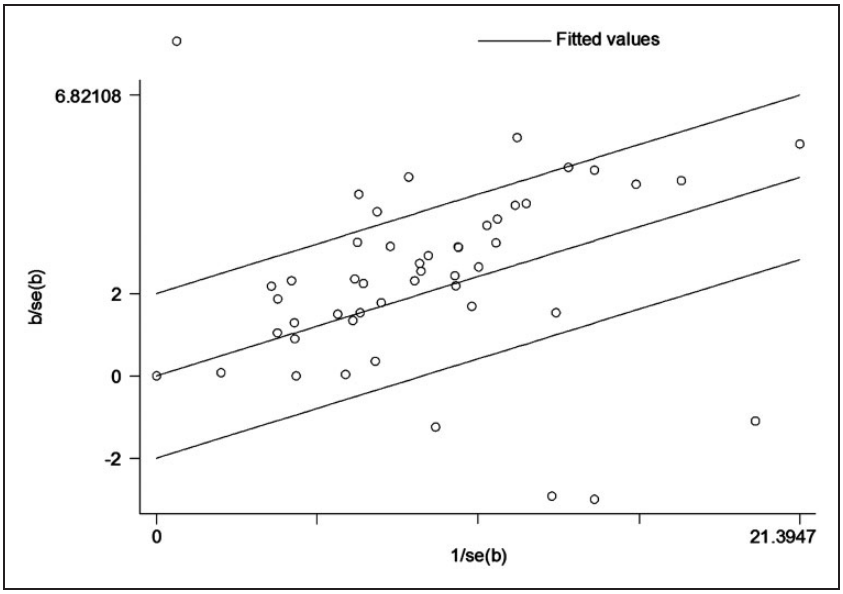

Figure 6. Galbraith plot of the allelic model. The outliers were the studies by Yamazaki et al.,[17] Fowler et al.[19] (study 1), Latiano et al.,[25] Amre et al.,[31] Lacher et al.,[34] Palomino-Morales et al.,[37] Jung et al.[45] and Hirano et al.[47] b, effect estimate; se, standard error.

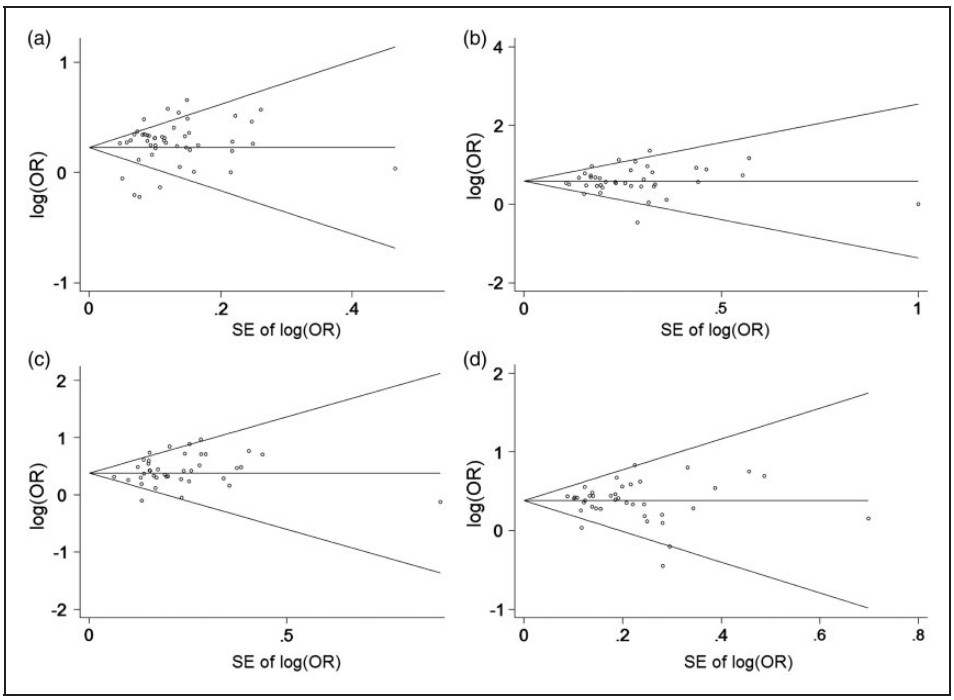

Figure 7. Begg's funnel plots with pseudo 95% confidence limits of all studies in the meta-analysis using the four model types: (a) allelic model (G allele versus A allele); (b) additive model (GG versus AA); (c) dominant model (GG + GA versus AA); (d) recessive model (GG versus GA + AA). SE, standard error; OR, odds ratio.

potential interactions between these two types of influence has not been evaluated. Secondly, *ATG16L1* seems to exert a close functional correlation with other genes in regulating autophagy. For example, the interaction of *ATG16L1* and *NOD2* has been implicated in the pathogenesis of Crohn's disease.[63] Potential gene–gene interactions require further evaluation. Thirdly, the *ATG16L1* genotype has been reported to be associated with disease phenotype,[65] which has clinical significance. Further combined analyses are needed to clarify the association between the *ATG16L1* genotype and Crohn's disease phenotype.

In conclusion, the present meta-analysis of robust data and unbiased results demonstrated an association between *ATG16L1* genotype and the development of Crohn's disease. These findings will be helpful in understanding the aetiology of Crohn's disease and indicate that the *ATG16L1* gene might have potential as a therapeutic or diagnostic target.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002; 347: 417–429.
2. McGovern DP, Kugathasan S and Cho JH. Genetics of inflammatory bowel diseases. *Gastroenterology* 2015; 149: 1163–1176.e2.
3. Mizushima N, Levine B, Cuervo AM, et al. Autophagy fights disease through cellular self-digestion. *Nature* 2008; 451: 1069–1075.
4. Fujioka Y, Noda NN, Nakatogawa H, et al. Dimeric coiled-coil structure of *Saccharomyces cerevisiae* Atg16 and its functional significance in autophagy. *J Biol Chem* 2010; 285: 1508–1515.
5. Hampe J, Franke A, Rosenstiel P, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007; 39: 207–211.
6. Rioux JD, Xavier RJ, Taylor KD, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; 39: 596–604.
7. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; 6: e1000097.
8. Zhang BB, Li Y, Feng JQ, et al. No association between IL-1RN VNTR and the risk of duodenal ulcer: a meta-analysis. *Hum Immunol* 2013; 74: 1170–1178.
9. DerSimonian R and Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7: 177–188.
10. Mantel N and Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959; 22: 719–748.
11. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315: 629–634.
12. Baldassano RN, Bradfield JP, Monos DS, et al. Association of the T300A nonsynonymous variant of the ATG16L1 gene with susceptibility to paediatric Crohn's disease. *Gut* 2007; 56: 1171–1173.
13. Büning C, Durmus T, Molnar T, et al. A study in three European IBD cohorts confirms that the ATG16L1 c.898A>G (p.Thr300Ala) variant is a susceptibility factor for Crohn's disease. *J Crohns Colitis* 2007; 1: 70–76.
14. Cummings JR, Cooney R, Pathan S, et al. Confirmation of the role of ATG16L1 as a Crohn's disease susceptibility gene. *Inflamm Bowel Dis* 2007; 13: 941–946.
15. Prescott NJ, Fisher SA, Franke A, et al. A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is

independent of CARD15 and IBD5. *Gastroenterology* 2007; 132: 1665–1671.
16. Roberts RL, Gearry RB, Hollis-Moffatt JE, et al. IL23R R381Q and ATG16L1 T300A are strongly associated with Crohn's disease in a study of New Zealand Caucasians with inflammatory bowel disease. *Am J Gastroenterol* 2007; 102: 2754–2761.
17. Yamazaki K, Onouchi Y, Takazoe M, et al. Association analysis of genetic variants in IL23R, ATG16L1 and 5p13.1 loci with Crohn's disease in Japanese patients. *J Hum Genet* 2007; 52: 575–583.
18. Baptista ML, Amarante H, Picheth G, et al. CARD15 and IL23R influences Crohn's disease susceptibility but not disease phenotype in a Brazilian population. *Inflamm Bowel Dis* 2008; 14: 674–679.
19. Fowler EV, Doecke J, Simms LA, et al. ATG16L1 T300A shows strong associations with disease subgroups in a large Australian IBD population: further support for significant disease heterogeneity. *Am J Gastroenterol* 2008; 103: 2519–2526.
20. Gaj P, Habior A, Mikula M, et al. 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.
21. Glas J, Konrad A, Schmechel S, et al. 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.
22. Hancock L, Beckly J, Geremia A, et al. Clinical and molecular characteristics of isolated colonic Crohn's disease. *Inflamm Bowel Dis* 2008; 14: 1667–1677.
23. Lakatos PL, Szamosi T, Szilvasi A, et al. ATG16L1 and IL23 receptor (IL23R) genes are associated with disease susceptibility in Hungarian CD patients. *Dig Liver Dis* 2008; 40: 867–873.
24. Lappalainen M, Halme L, Turunen U, et al. 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.
25. Latiano A, Palmieri O, Valvano MR, et al. 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.
26. Okazaki T, Wang MH, Rawsthorne P, et al. 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.
27. Perricone C, Borgiani P, Romano S, et al. ATG16L1 Ala197Thr is not associated with susceptibility to Crohn's disease or with phenotype in an Italian population. *Gastroenterology* 2008; 134: 368–370.
28. Peterson N, Guthery S, Denson L, et al. Genetic variants in the autophagy pathway contribute to paediatric Crohn's disease. *Gut* 2008; 57: 1336–1337; author reply 1337.
29. Van Limbergen J, Russell RK, Nimmo ER, et al. 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.
30. Weersma RK, Zhernakova A, Nolte IM, et al. ATG16L1 and IL23R are associated with inflammatory bowel diseases but not with celiac disease in the Netherlands. *Am J Gastroenterol* 2008; 103: 621–627.
31. Amre DK, Mack DR, Morgan K, et al. Autophagy gene ATG16L1 but not IRGM is associated with Crohn's disease in Canadian children. *Inflamm Bowel Dis* 2009; 15: 501–507.
32. Dema B, Fernández-Arquero M, Maluenda C, et al. Lack of association of NKX2-3, IRGM, and ATG16L1 inflammatory bowel disease susceptibility variants with celiac disease. *Hum Immunol* 2009; 70: 946–949.
33. Dusatkova P, Hradsky O, Lenicek M, et al. Association of IL23R p.381Gln and ATG16L1 p.197Ala with Crohn disease in the Czech population. *J Pediatr Gastroenterol Nutr* 2009; 49: 405–410.
34. Lacher M, Schroepf S, Ballauff A, et al. Autophagy 16-like 1 rs2241880 G allele is associated with Crohn's disease in German children. *Acta Paediatr* 2009; 98: 1835–1840.

35. Márquez A, Núñez C, Martínez A, et al. Role of ATG16L1 Thr300Ala polymorphism in inflammatory bowel disease: a study in the Spanish population and a meta-analysis. *Inflamm Bowel Dis* 2009; 15: 1697–1704.
36. Newman WG, Zhang Q, Liu X, et al. Genetic variants in IL-23R and ATG16L1 independently predispose to increased susceptibility to Crohn's disease in a Canadian population. *J Clin Gastroenterol* 2009; 43: 444–447.
37. Palomino-Morales RJ, Oliver J, Gómez-Garcia M, et al. Association of ATG16L1 and IRGM genes polymorphisms with inflammatory bowel disease: a meta-analysis approach. *Genes Immun* 2009; 10: 356–364.
38. Cotterill L, Payne D, Levinson S, et al. Replication and meta-analysis of 13,000 cases defines the risk for interleukin-23 receptor and autophagy-related 16-like 1 variants in Crohn's disease. *Can J Gastroenterol* 2010; 24: 297–302.
39. Csöngei V, Járomi L, Sáfrány E, et al. Interaction of the major inflammatory bowel disease susceptibility alleles in Crohn's disease patients. *World J Gastroenterol* 2010; 16: 176–183.
40. Gazouli M, Pachoula I, Panayotou I, et al. NOD2/CARD15, ATG16L1 and IL23R gene polymorphisms and childhood-onset of Crohn's disease. *World J Gastroenterol* 2010; 16: 1753–1758.
41. Sventoraityte J, Zvirbliene A, Franke A, et al. NOD2, IL23R and ATG16L1 polymorphisms in Lithuanian patients with inflammatory bowel disease. *World J Gastroenterol* 2010; 16: 359–364.
42. Fabio RR, Concetta RM, Giuseppe C, et al. ATG16L1 contribution to Crohn's disease risk in Sicily. *Inflamm Bowel Dis* 2011; 17: 1635–1636.
43. Frank DN, Robertson CE, Hamm CM, et al. Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. *Inflamm Bowel Dis* 2011; 17: 179–184.
44. Lauriola M, Ugolini G, Rivetti S, et al. IL23R, NOD2/CARD15, ATG16L1 and PHOX2B polymorphisms in a group of patients with Crohn's disease and correlation with sub-phenotypes. *Int J Mol Med* 2011; 27: 469–477.
45. Jung C, Colombel JF, Lemann M, et al. Genotype/phenotype analyses for 53 Crohn's disease associated genetic polymorphisms. *PloS One* 2012; 7: e52223.
46. Wang MH, Okazaki T, Kugathasan S, et al. Contribution of higher risk genes and European admixture to Crohn's disease in African Americans. *Inflamm Bowel Dis* 2012; 18: 2277–2287.
47. Hirano A, Yamazaki K, Umeno J, et al. Association study of 71 European Crohn's disease susceptibility loci in a Japanese population. *Inflamm Bowel Dis* 2013; 19: 526–533.
48. Dalton JP, Desmond A, Shanahan F, et al. Detection of *Mycobacterium avium* subspecies *paratuberculosis* in patients with Crohn's disease is unrelated to the presence of single nucleotide polymorphisms rs2241880 (ATG16L1) and rs10045431 (IL12B). *Med Microbiol Immunol* 2014; 203: 195–205.
49. Jakobsen C, Cleynen I, Andersen PS, et al. Genetic susceptibility and genotype-phenotype association in 588 Danish children with inflammatory bowel disease. *J Crohns Colitis* 2014; 8: 678–685.
50. Scolaro BL, dos Santos E, Ferreira LE, et al. T300A genetic polymorphism: a susceptibility factor for Crohn's disease? *Arq Gastroenterol* 2014; 51: 97–101.
51. Serbati N, Senhaji N, Diakite B, et al. IL23R and ATG16L1 variants in Moroccan patients with inflammatory bowel disease. *BMC Res Notes* 2014; 7: 570.
52. Zhang J, Chen J, Gu J, et al. Association of IL23R and ATG16L1 with susceptibility of Crohn's disease in Chinese population. *Scand J Gastroenterol* 2014; 49: 1201–1206.
53. Na SY, Park SS and Seo JK. Genetic polymorphisms in autophagy-associated genes in Korean children with early-onset Crohn disease. *J Pediatr Gastroenterol Nutr* 2015; 61: 285–291.
54. Salem M, Nielsen OH, Nys K, et al. Impact of T300A variant of ATG16L1 on antibacterial response, risk of culture positive infections, and clinical course of Crohn's

disease. *Clin Transl Gastroenterol* 2015; 6: e122.
55. Yang SK, Ye BD and Song K. ATG16L1 contributes to Crohn's disease susceptibility in Koreans: overmuch concern for ethnic difference? *Gut* 2015; 64: 687–688.
56. Salem M, Ammitzboell M, Nys K, et al. ATG16L1: a multifunctional susceptibility factor in Crohn disease. *Autophagy* 2015; 11: 585–594.
57. Xiao H, Gao H, Zheng T, et al. Plate fixation versus intramedullary fixation for midshaft clavicle fractures: meta-analysis of complications and functional outcomes. *J Int Med Res* 2016; 44: 201–215.
58. Zhang S, Kong YL, Li YL, et al. Interleukin-10 gene -1082 G/A polymorphism in cervical cancer and cervical intraepithelial neoplasia: meta-analysis. *J Int Med Res* 2014; 42: 1193–1201.
59. Strisciuglio C, Duijvestein M, Verhaar AP, et al. Impaired autophagy leads to abnormal dendritic cell-epithelial cell interactions. *J Crohns Colitis* 2013; 7: 534–541.
60. Strisciuglio C, Miele E, Wildenberg ME, et al. T300A variant of autophagy ATG16L1 gene is associated with decreased antigen sampling and processing by dendritic cells in pediatric Crohn's disease. *Inflamm Bowel Dis* 2013; 19: 2339–2348.
61. Raju D, Hussey S and Jones NL. Crohn disease ATG16L1 polymorphism increases susceptibility to infection with *Helicobacter pylori* in humans. *Autophagy* 2012; 8: 1387–1388.
62. Sadaghian Sadabad M, Regeling A, de Goffau MC, et al. The ATG16L1-T300A allele impairs clearance of pathosymbionts in the inflamed ileal mucosa of Crohn's disease patients. Gut 2015; 64: 1546–1552.
63. Homer CR, Richmond AL, Rebert NA, et al. ATG16L1 and NOD2 interact in an autophagy-dependent antibacterial pathway implicated in Crohn's disease pathogenesis. *Gastroenterology* 2010; 139: 1630–1641.e2.
64. Cadwell K, Liu JY, Brown SL, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 2008; 456: 259–263.
65. Strisciuglio C, Auricchio R, Martinelli M, et al. Autophagy genes variants and paediatric Crohn's disease phenotype: a single-centre experience. *Dig Liver Dis* 2014; 46: 512–517.