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

Lynn Cotterill MSc[1], Debbie Payne BSc[2], Scott Levison MRCP[3], John McLaughlin PhD FRCP[3], Emma Wesley MRCP[4], Mark Feeney FRCP[4], Hilary Durbin RGN[4], Simon Lal PhD MRCP[5], Alistair Makin FRCP[6], Simon Campbell MRCP[6], Stephen A Roberts PhD[7], Catherine O'Neill PhD[3], Cathryn Edwards DPhil FRCP[4], William G Newman PhD FRCP[1]

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BACKGROUND/OBJECTIVE: Variants in the interleukin-23 receptor (*IL23R*) and the autophagy-related 16-like 1 (*ATG16L1*) genes have been associated with an increased risk of Crohn's disease (CD). Both genes were identified through genome-wide association scans and subsequent studies have validated these associations. To assess the effect size of these variants, an independent case-control association study and meta-analysis were performed.

METHODS: British Caucasian subjects with inflammatory bowel disease (n=500) and 877 ethnically matched controls were genotyped for the disease-associated variants in *IL23R* and *ATG16L1*. In addition, meta-analyses of 12,991 patients and 14,598 controls, and 11,909 patients and 15,798 controls, were conducted on independently published data for the associations between *IL23R* and *ATG16L1* variants and CD, respectively.

RESULTS: In the present cohort, both susceptibility variants showed highly significant associations, including *IL23R* (rs11209026, P=0.0006; OR 0.37; 95% CI 0.21 to 0.67) and *ATG16L1* (rs2241880, P=0.0017; OR 1.36; 95% CI 1.12 to 1.66). The meta-analysis based on the random effects model showed similar combined effects for rs11209026 (n=26, OR 0.41; 95% CI 0.37 to 0.46) and rs2241880 (n=25, OR 1.33; 95% CI 1.28 to 1.39). There was no statistically significant gene-gene interaction between caspase recruitment domain (*CARD15*) variants and the *IL23R* or *ATG16L1* polymorphisms (P=0.44 and P=0.24, respectively).

CONCLUSION: The present cohort and meta-analysis provides strong evidence that, in addition to *CARD15*, polymorphisms in both *IL23R* and *ATG16L1* alter susceptibility to CD and that these effects are consistent across all populations of European ancestry; however, only *ATG16L1* is relevant to inflammatory bowel disease in the Asian population.

Key Words: ATG16L1; *Crohn's disease*; IL23R; *Inflammatory bowel disease; Meta-analysis*

La réplication et la méta-analyse de 13 000 cas définissent le risque des variantes du gène du récepteur de l'interleukine 23 et du gène 1 de type 16 lié à l'autophagie dans la maladie de Crohn

HISTORIQUE ET OBJECTIF : Des variantes du gène du récepteur de l'interleukine 23 (*IL23R*) et du gène 1 de type 16 lié à l'autophagie (ATG16L1) s'associent à une augmentation du risque de maladie de Crohn (MC). Ces deux gènes ont été repérés par balayages d'association sur tout le génome, et des études subséquentes ont validé ces associations. Pour évaluer l'ampleur de l'effet de ces variantes, les chercheurs ont procédé à une étude d'association cas-témoins et à une méta-analyse.

MÉTHODOLOGIE : Des sujets britanniques blancs atteints d'une maladie inflammatoire de l'intestin (MII) (n=500) et 877 sujets témoins appariés selon l'ethnie ont subi un génotypage pour déceler les variantes associées à la maladie dans les gènes *IL23R* et *ATG16L1*. De plus, les chercheurs ont effectué des méta-analyses de 12 991 patients et 14 598 sujets-témoins ainsi que de 11 909 patients et 15 798 sujets-témoins à partir de données indépendantes pour déceler les associations entre les variantes des gènes *IL23R* et *ATG16L1* et la MC, respectivement.

RÉSULTATS : Dans la présente cohorte, les deux variantes de susceptibilité ont révélé des associations hautement significatives, incluant les gènes *IL23R* (rs11209026, P=0,0006; RRR 0,37; 95 % IC 0,21 à 0,67) et *ATG16L1* (rs2241880, P=0,0017; RRR 1,36; 95 % IC 1,12 à 1,66). La méta-analyse fondée sur le modèle d'essais aléatoires a révélé des effets combinés similaires pour le rs11209026 (n=26, RRR 0,41; 95 % IC 0,37 à 0,46) et le rs2241880 (n=25, RRR 1,33; 95 % IC 1,28 à 1,39). Les chercheurs n'ont constaté aucune interaction statistiquement significative d'un gène à l'autre entre les variantes du domaine de recrutement de la caspase (*CARD15*) et les polymorphismes des gènes *IL23R* ou *ATG16L1* (P=0,44 et P=0,24, respectivement).

CONCLUSION : La présente cohorte et la présente analyse fournissent des données probantes solides selon lesquelles en plus du *CARD15*, les polymorphismes des gènes *IL23R* et *ATG16L1* modifient la susceptibilité à la MC et que ces effets sont constants entre toutes les populations d'origine européenne. Cependant, seul le gène *ATG16L1* est pertinent pour la maladie inflammatoire de l'intestin au sein de la population asiatique.

Inflammatory bowel disease (IBD) is a chronic gastrointestinal disease that includes the subtypes Crohn's disease (CD) and ulcerative colitis (UC). Both disorders have complex etiologies involving an inadequately defined relationship among microbial insult, genetic predisposition and altered intestinal barrier permeability (1). Genetic studies have explored the molecular pathogenesis of CD and UC using several strategies. Initial genome-wide linkage approaches led, in

[1]*Department of Medical Genetics, Manchester Academic Health; Science Centre, St Mary's Hospital and University of Manchester;* [2]*Centre for Integrated Genomic Medical Research (CIGMR), University of Manchester;* [3]*Department of Gastrointestinal Sciences, University of Manchester, Manchester;* [4]*Gastroenterology Unit, Torbay Hospital, Torbay;* [5]*Gastroenterology Unit, University Hospital Aintree, Liverpool;* [6]*Department of Gastroenterology, Manchester Royal Infirmary;* [7]*Health Research Methodology Group, University of Manchester, Manchester, United Kingdom*

Correspondence: Dr William G Newman, Department of Medical Genetics, St Mary's Hospital, Manchester Academic Health Science Centre, University of Manchester, Manchester M13 0JH, United Kingdom. Telephone 44-161-276-6264, fax 44-161-276-6145, e-mail william.newman@manchester.ac.uk

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2001, to two different groups identifying the caspase recruitment domain (*CARD15*) family (nucleotide-binding oligomerization domain containing 2) of protein variants that were associated with an increased risk for CD (2,3). The association between the three common *CARD15* variants has been replicated in a large meta-analysis (4) of many different populations, with a threefold increased risk of CD conferred by carriage of one or more minor alleles. Subsequent positional cloning studies (5) based on the original linkage scans led to the identification of additional susceptibility genes including discs large homologue 5 (*DLG5*) and a locus on chromosome 5q31 (6) predominantly associated with CD. Several candidate gene association studies have defined relationships between other gene variants of both UC and CD. However, replication studies for the majority of these variants have yielded conflicting results. IBD susceptibility gene identification has been revolutionized by the recent publication of genome-wide association (GWA) scans. These studies have been able to analyze hundreds of thousands of polymorphisms in thousands of IBD patients and compare the allelic frequencies with those in healthy, ethnically matched controls. The advantages of these studies were that no a priori assumptions were required regarding the function or expression pattern of the genes, and that the entire genome could be interrogated at high density in a single screen. Associations between variants of genes including interleukin (IL)-23 receptor (*IL23R*) and autophagy-related 16-like 1 (*ATG16L1*), not previously associated with IBD, were strongly significant in the initial studies of CD. In a study of 500 patients with ileal CD, Duerr et al (7) identified an arginine to glutamine missense variant (*p.R381Q*, rs11209026) in the subunit of the *IL23R* gene. The minor allele of this variant was strongly protective for CD. Importantly, a number of other variants within the gene were also independently associated with CD, suggesting that the Arg381→Gln change did not account for all of the association at this locus and that other variants are relevant to CD risk. Subsequent studies have validated the protective role of this variant in different CD populations (8-23). In addition, some groups have also demonstrated a weaker association with UC (11,17,18,21-23). *IL23R* is an excellent candidate for a role in CD pathogenesis because it forms a subunit of the IL-12 receptor, and studies of monoclonal antibodies directed against IL-12R (24) showed promising therapeutic results in a cohort of patients with CD.

In a GWA study of approximately 20,000 coding variants in 498 German CD patients and 1032 controls, Hampe et al (25) identified a threonine to alanine missense variant (*p.T300A*, rs2241880) in the *ATG16L1* gene that was associated with increased risk of CD. This finding was replicated in independent CD patient cohorts from Germany and the United Kingdom (26), and in subsequent studies (8,11,17,18,27-33). *ATG16L1* is involved in the autophagosome pathway that engulfs intracellular bacteria (25), a disruption of which could alter the microbial processing and inflammation characteristic of CD. In contrast to *IL23R*, only one causal risk variant accounts for all of the signal at this locus and only one study (33) reports a strong association with UC susceptibility.

We undertook a replication study to determine the relevance of *ATG16L1* and *IL23R* variants in cohorts of British Caucasian CD patients. Furthermore, although the majority of replication studies have been positively associated with the variants in both *ATG16L1* and *IL23R*, some reports have generated conflicting results (9,11,12,21,26,28,34,35). Because these studies have been undertaken in populations with different ethnic backgrounds, phenotypes and in individuals with different ages of disease onset, we conducted a comprehensive meta-analysis to determine a more accurate approximation of the risk estimates of these two susceptibility variants.

METHODS

Subjects

A total of 500 individuals with IBD (295 with CD and 205 with UC) were recruited. All individuals met standard diagnostic criteria for IBD and were classified according to the Montreal criteria (36). Individuals were recruited at several hospitals in the United Kingdom. All study participants were of Caucasian British ancestry.

Control samples (n=877) from ethnically matched individuals with no history or family history of inflammatory disease were obtained from a bank of anonymized, healthy, unrelated individuals through the Regional Molecular Genetics Laboratory of St Mary's Hospital. The present study was approved by the Central Manchester NHS and University of Manchester Ethics Committees (Manchester, United Kingdom).

Genotyping and analysis

DNA was extracted from 5 mL to 10 mL of blood lymphocytes in EDTA by the automated AutoPure system (Gentra Systems, USA). The CD susceptibility variants in *IL23R* (rs11209026, *c.1142G→A*, *p.R381Q*) and *ATG16L1* (rs2241880, *c.1338A→G*, *p.T300A*) were selected for genotyping. Genotyping of CD-associated *CARD15* *c.2104C→T* (*p.R702W*, rs2066844), *c.2722G→C* (*p.G908R*, rs2066845), and *c.3020insC* (*p.L1007fs*, rs2066847) variants was also performed. AssayDesigner (Sequenom, USA) was used to design the assays and genotyping was performed using the MassArray iPLEX platform (Sequenom, USA). BCSNPmax (Biocomputing Platforms Ltd, Finland) was used to determine allelic associations. Associations with $P \leq 0.05$ were considered to be statistically significant. Genotypes were also tested for consistency with Hardy-Weinberg equilibrium (HWE) ($P > 0.05$) by a contingency test analysis. Power calculations determined that the study had greater than 96% and greater than 79% power to replicate the previous associations between CD and *IL23R* and *ATG16L1*, respectively (7,25).

Eligibility criteria for meta-analysis

A systematic literature search was conducted in Medline and EMBASE using the following key words: "Crohn's disease", "inflammatory bowel disease", "IL23R", "rs11209026", "ATG16L1" and "rs2241880". Studies corresponding with these key words were filtered by further examination of the titles and abstracts. All relevant publications were read completely.

Studies in each meta-analysis were required to fulfill the following criteria:

1. Case-control association studies;
2. Must report rs11209026 *(IL23R)* and/or rs2241880 *(ATG16L1)* variants;
3. Published in English language;
4. Each study was independent and presented original data;
5. Patients and controls were unrelated;
6. Published (in print or online) before March 10, 2009;
7. Published in a peer-reviewed journal or in press;
8. Included allelic frequencies and/or ORs, 95% CIs and the size of the patient/control samples; and
9. Must be consistent with the HWE in control populations.

TABLE 1
Allelic frequencies and association analysis of interleukin-23 receptor (*IL-23R*) and autophagy-related 16-like 1 (*ATG16L1*) gene variants in British Caucasian inflammatory bowel disease patients and controls

Variant, single nucleotide polymorphism	Gene	Group	n	Allele frequency, n (%)		P (allele)	OR (95%CI)
				A	G		
rs11209026,	*IL23R*						
c.1142G→A p.R381Q		Crohn's disease	236	13 (2.8)	459 (97.2)	0.0006	0.37 (0.21–0.67)
		Ulcerative colitis	160	16 (5.0)	304 (95.0)	0.18	0.69 (0.40–1.18)
		Controls	813	115 (7.10)	1511 (92.9)	N/A	N/A
rs2241880,	*ATG16L1*			A	G		
c.1338A→G p.T300A		Crohn's disease	273	229 (41.9)	317 (58.1)	0.0017	1.36 (1.12–1.66)
		Ulcerative colitis	188	184 (48.9)	192 (51.1)	0.81	1.03 (0.82–1.29)
		Controls	834	828 (49.6)	840 (50.4)	N/A	N/A

A Adenine; G Guanine; N/A Not available

Data extraction

The data from each study were extracted according to the following variables: year of publication, authors, genotyping method, total patients, total controls, ethnicity, P value, OR and 95% CI. Allelic frequencies were used to calculate P values, OR and 95% CIs if these data were not available. Any other differences were noted – for example, any deviations from HWE in pediatric/adult populations were recorded. Data were extracted by one researcher and supervised by at least one senior researcher.

Statistical analysis

The statistical program STATA v9.0 (StataCorp, USA) was used to perform the meta-analysis using the function *metan*. A random effects model used by DerSimonian and Laird (37) was adopted to create a forest plot analysis. The fixed effects model by Mantel and Haenszel (38) was also used for a direct comparison. Each of these analyses gave a combined OR value with 95% CIs. The more conservative random effects model assumes between-study heterogeneity and may, therefore, yield wider CIs than the fixed effects model. Sampling and genotyping error can affect both random and fixed effect models.

The Higgins' I^2 value was used to determine percentage variation across studies in which low, medium and high heterogeneity was 25%, 50% and 75%, respectively (39), and showed heterogeneity rather than variation by chance. The Egger linear regression funnel plot or test for asymmetry (40), and Begg-Mazumdar rank correlation (41) plots (metabias) were used to assess study publication bias.

A sensitivity analysis was performed to determine if any individual studies significantly affected the heterogeneity by sequentially removing each study for each analysis.

Potential interactions between *CARD15* carrier status and the susceptibility genes were investigated in a logistic regression model with an additive genetic model for the susceptibility genes and likelihood ratio tests for the specific interactions. These analyses were implemented using the R environment for statistical computing (42).

RESULTS

Validation study

The *IL23R* and *ATG16L1* susceptibility variants were genotyped in 500 IBD (295 CD and 205 UC) patients and 877 controls, with an 89% and 95% genotyping success rate, respectively. Neither variant deviated from HWE (P>0.05) in patients or controls.

Table 1 shows the allelic frequencies and association analysis of *IL23R* (*p.R381Q*, rs11209026) and *ATG16L1* (*p.T300A*, rs2241880) in IBD patients and controls. The minor protective *c.1142G→A* allele of the *IL23R* variant occurred significantly less in CD patients (P=0.0006; OR 0.37; 95% CI 0.21 to 0.67) than in controls. The minor allele also occurred less frequently in UC patients than in controls; however, statistical significance was not reached (P=0.18). The minor allele of the *ATG16L1* variant *c.1338A→G* also showed a significant association in CD patients compared with controls (P=0.0017; OR 1.36; 95% CI 1.12 to 1.66), but not with UC (P=0.81).

The three common CD-associated *CARD15* variants were also genotyped in the IBD cohort. *CARD15* genotyping was incomplete for all samples genotyped for *IL23R* and *ATG16L1* variants due to genotype assay failure and the nonavailability of samples. A composite analysis determined that individuals carrying one or more *CARD15* susceptibility variants had a greater than 2.5-fold increased risk of CD (P=0.0001; OR 2.69; 95% CI 1.59 to 4.54).

The associations of *ATG16L1* and *IL23R* with CD were stratified by the presence or absence of the *CARD15* susceptibility variants, based on an additive genetic model. There were no significant gene-gene interactions between the *CARD15* variants and either *IL23R* (P=0.44) or *ATG16L1* (P=0.24).

Meta-analysis study inclusion

All case-control association studies from a comprehensive literature search that met the inclusion criteria were used in the analysis. For the meta-analyses, a total of 26 and 25 studies were included for both *IL23R* (rs11209026 *p.R381Q)* and *ATG16L1* (rs2241880 *p.T300A*) polymorphisms, respectively. Studies were not included due to noncase-control study design (eg, transmission disequilibrium test [43]); if the genotypic frequencies deviated from or did not report HWE (44,45); consideration of other single-nucleotide polymorphisms in *IL23R* and *ATG16L1* other than rs11209026 and rs2241880 (46); and for any possible duplication of samples between studies (17,44); for example, samples from the Wellcome Trust Case Control Consortium were used in more than one publication (23,45). A study by Yamazaki et al (35) was removed from the *IL23R* meta-analysis because the *c.1142G→A* minor allele was not present in the Japanese cohort. One published paper was excluded because it was only available in Chinese but did not report a significant association with *ATG16L1* (46).

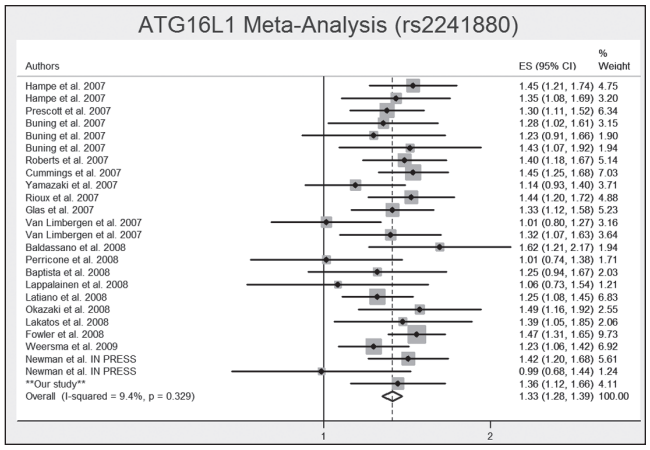

Figure 1) *Meta-analysis of the random effects model of the association between the autophagy-related 16-like 1 (*ATG16L1*) variant and Crohn's disease in 25 individual studies. ES Epidemiological study*

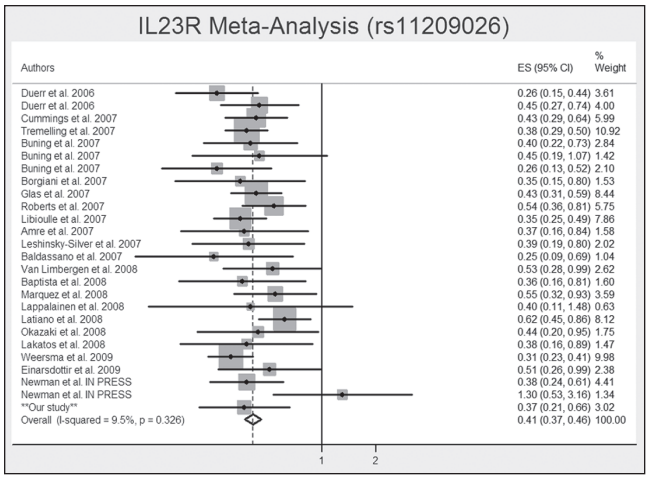

Figure 2) *Meta-analysis of the random effects model of the association between the interleukin-23 receptor (*IL23R*) variant and Crohn's disease in 26 individual studies. ES Epidemiological study*

The Lappalainen et al (9) study was included because consistency with HWE was measured, although the result was not reported and the value could not be calculated with the available data. Some studies included only pediatric populations, such as those by Van Limbergen et al (13,28), Baldassano et al (27,47) and Amre et al (15). The results for the *ATG16L1* variant in the study by Okazaki et al (48) were recalculated because the presented ORs deviated from calculations made in the current study using their allelic data. Taylor et al (49) performed a case-control study of *IL23R* variants but only performed haplotype analysis and did not provide allelic data; therefore, that study was excluded.

Risk estimates

The meta-analysis was based on the random effects model. The meta-analysis for *IL23R* (*p.R381Q,* rs11209026) derived data from 26 studies including a total of 12,991 patients and 14,598 controls, and confirmed the protective effect of the minor allele (n=26, OR 0.41; 95% CI 0.37 to 0.46). Data from 25 studies were included in the *ATG16L1* meta-analysis, which had combined totals of 11,909 patients and 15,798 controls. The *ATG16L1* variant (*p.T300A,* rs2241880*)* increased the risk of CD (n=25, OR 1.33; 95% CI 1.28 to 1.39). The combined ORs for both variants were similar to those presented in the present replication study (Figures 1 and 2). The combined estimates for the fixed effects model (data not shown) showed only slight divergence from these values, suggesting a low level of between-study heterogeneity; however, interstudy variation may still exist. Both the *IL23R* and *ATG16L1* meta-analyses had low heterogeneity, with I^2 values of 9.5% and 9.4%, respectively (an I^2 value of less than 25% suggests low heterogeneity [50]). A sensitivity analysis showed that no single study from either analysis significantly affected the meta-analysis. Populations with different ethnicities and age of onset (pediatric studies) were included in the meta-analyses, but these did not significantly affect the combined estimates in either the random or fixed model. Funnel plots, including Egger linear regression and Begg-Mazumdar rank correlation plots, were created to assess for publication bias. No publication bias was observed using these tests for either variant (data not shown).

DISCUSSION

A number of genetic variants have been associated with increased susceptibility to IBD (51). The most robust association previously defined has been between variants in *CARD15* and CD, in which carriage of one or more common *CARD15* susceptibility variants increased the risk of CD by threefold (4). The recent GWA studies have identified two additional genes – *IL23R* (7) and *ATG16L1* (25) – that have been widely validated as being associated with increased risk of CD. Our study adds an additional independent cohort of IBD patients in whom positive associations between CD and both *IL23R* and *ATG16L1* variants have been confirmed. Consistent with the majority of other studies (7,25), we did not find an association between *ATG16L1* variants and UC. However, the lack of replication of an association between *IL23R* with UC in our study probably reflects a lack of statistical power to detect this more modest effect.

In our study, there was no interaction between *CARD15* variants and *IL23R* or *ATG16L1* that was associated with an increased risk of CD. This is consistent with the majority of published studies, although some groups (25,28,33) have suggested that the risks for CD are confined to individuals dependent upon whether they harbour *CARD15* variants. Clearly, larger CD cohorts will be required to develop robust risk estimate algorithms that combine clinical and genetic susceptibility factors, which will be important to translate these data into meaningful clinical risk estimations (52).

Genotype-phenotype studies in CD have confirmed that *CARD15* variants are associated with ileal disease, stronger family history and stenotic disease (4). In contrast, apart from a study indicating an increased risk of CD for smokers who carry two *ATG16L1* variants and an increased risk of ileal disease (33), the majority of genotype-phenotype correlation studies have been negative for both *IL23R* and *ATG16L1* (23,31). Therefore, no meta-analysis of associations with subphenotypes was conducted.

A meta-analysis is a powerful technique to combine multiple studies to potentially establish the true effect of an association between a genetic variant and disease while correcting for potential biases (52). A comprehensive meta-analysis of *CARD15* variants has established a robust association with an increased risk of CD (4). In the meta-analysis presented here for *IL23R,* with data from nearly 13,000 patients and 14,500 controls, the combined OR of 0.41 supports a significant protective effect of the *c.1142*G→A minor allele for CD. The majority of

studies were consistent with respect to overall risk, except for some small studies (16,20) and one of a Jewish CD population (11) that found no association but had a small sample size and wide CIs; therefore, appropriately, in both analytical models, this study had a small study weight. Data from approximately 12,000 patients and nearly 16,000 controls were included in the *ATG16L1* meta-analysis confirming that the *ATG16L1 c.1338A→G* minor allele is a susceptibility factor for CD (OR 1.34). The *ATG16L1* variant was not associated with CD in studies of Italian (34), Brazilian (12) and Jewish-Canadian CD (11) populations, but these had overlapping risk estimates and small sample sizes, and each study lacked adequate power to definitively detect a positive association.

A recent meta-analysis of three CD GWA (3230 patients and 4829 controls) and replication studies (3664 independent patients) confirmed the associations between *IL23R* and *ATG16L1* and CD, in addition to identifying a number of other susceptibility genes (53). Importantly, their study analyzed different intronic variants of *IL23R*, rs11465804, *ATG16L1* and rs3828309, compared with our analysis of coding variants. The interpretation of their replication of *IL23R* in the context of our study is also complicated by the fact that they considered the risk associated with the major allele (54). However, their reported combined OR of 1.28 in case-control and 1.3 in family-based replication between *ATG16L1* rs3828309 and CD, is very similar to the combined OR of 1.33 generated in the present study. This is not surprising considering the strong linkage disequilibrium across *ATG16L1*, and reflects a tight homogeneity across studies investigating *ATG16L1* and CD. We acknowledge that a number of the replicated cases considered in the Barrett et al (53) study were also included in our analysis. However, this analysis also incorporated studies of non-European ancestry to broadly establish the relevance of these variants.

The presence of the *ATG16L1*, but not the *IL23R*, variant in the Japanese population (35) indicates that the *p.T300A* variant in *ATG16L1* is likely to have appeared more than 50,000 years ago, before the divergence of European and Asian populations. Although Yamazaki et al (35) did not demonstrate a positive association between *ATG16L1* and CD, possibly due to the lower minor allelic frequency in this population (31%), the study had less than 80% power to detect an association. Therefore, because variants in *CARD15* or *IL23R* have not been found in Asian populations (35), *ATG16L1* may have wider relevance to CD pathogenesis on a worldwide scale. This requires further investigation in larger Asian CD populations.

Van Limbergen et al (28) demonstrated that *ATG16L1* was not associated with CD in a pediatric Scottish population (P=0.95), but this contrasted with the study by Baldassano et al (27), which reported a highly significant association (P=0.0007) in a CD pediatric cohort from Pennsylvania (USA). In addition, a recent Italian study (54) confirmed the association between both *ATG16L1* and *IL23R* variants and CD in a large pediatric population. Therefore, the differential effects of these variants in pediatric and adult CD cohorts cannot be confirmed across different studies. Both meta-analyses included studies representing both pediatric and adult populations. However, random and fixed model analyses showed only low heterogeneity, suggesting that these differences did not significantly affect the combined estimates. Furthermore, it was suggested (55) that meta-analyses with fewer than 20 studies may not be efficient at detecting true heterogeneity; however, both meta-analyses exceeded this number.

Our validation of the association between *IL23R* and *ATG16L1* variants with CD represents very similar risk estimates to the pooled combined estimates from the meta-analysis. This indicates that our study population is representative of a wider Caucasian CD population. We confirm, both through our own replication study and a comprehensive meta-analysis of published case-control association studies, the association between variants in both *IL23R* and *ATG16L1* and CD.

CONCLUSION

Variants in three genes, *CARD15*, *IL23R* and *ATG16L1* are important in the pathogenesis of CD. Further studies are needed to determine the overall phenotypic effects of these genes and their relationships with the other genes recently associated with CD (53). Meta-analysis will be an increasingly important tool to establish the relevance of risk factors of modest effect in IBD and other common complex disorders.

CONFLICTS OF INTEREST: The authors have no conflicts of interest to declare.

SPECIFIC AUTHOR CONTRIBUTIONS: Lynn Cotterill and William Newman wrote the manuscript. Lynn Cotterill, Catherine O'Neill and William Newman designed the study. Lynn Cotterill and Debbie Payne performed the genotyping. Lynn Cotterill, Stephen Roberts and William Newman conducted the statistical analysis. Simon Lal, Alistair Makin, Simon Campbell, Scott Levison, Emma Wesley, Mark Feeney and Cathryn Edwards recruited and phenotyped the study participants. Hilary Durbin and John McLaughlin recruited study participants. Cathryn Edwards, Catherine O'Neill and William Newman supervised the study and provided research funding. All authors critically reviewed the manuscript and provided comments.

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