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.


MÉTHODES : 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 décoder 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 décoder les associations entre les variantes des gènes IL23 et ATG16L1 et la MC, respectivement.

RÉSULTATS : Dans la présente cohorte, les deux variantes de susceptibility ont révélé des associations hautement significatives, incluant les gènes IL23R (rs11209026, P=0,0006; OR 0,37; 95 % IC 0,31 à 0,47) et ATG16L1 (rs2241880, P=0,0017; OR 1,36; 95 % IC 1,12 à 1,66). La méta-analyse basée sur le random effects model a montré des effets combinés similaires pour le rs11209026 (n=26, OR 0,41; 95 % IC 0,37 à 0,46) et le rs2241880 (n=25, OR 1,33; 95 % IC 1,28 à 1,39). Il n’y avait pas de variantes gène-gène en interaction entre les variantes du domaine de recrutement de la caspase (CARD15) et les gènes IL23R et ATG16L1 polymorphismes (P=0,44 et P=0,24, respectivement).

CONCLUSION : La présente cohorte et la méta-analyse fournissent des données probantes solides selon lesquelles 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.
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 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 (8,11,17,18,21-33). 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.3200insC (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). BCSNmax (Biocomputing Platforms Ltd, Finland) was used to design allelic associations. Associations with P<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

<table>
<thead>
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<th>Variant, single nucleotide polymorphism</th>
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<td>rs2241880, ATG16L1</td>
<td>Controls</td>
<td>813</td>
<td>115</td>
<td>698</td>
<td>N/A</td>
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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 95% CIs. The more conservative random effects model used by DerSimonian and Laird (37) was used to perform the meta-analysis using the function metan.

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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 1.12 to 1.66), but not with UC (P=0.81).

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, rs2241880) and ATG16L1 (rs2241880, rs11209026, 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).

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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.
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), Baklassano 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 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 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.1142G→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 allele 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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