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ATG16L1 rs2241880/T300A increases susceptibility to perianal Crohn's disease: An updated meta-analysis on inflammatory bowel disease risk and clinical outcomes

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Abstract

Background: *ATG16L1* plays a fundamental role in the degradative intracellular pathway known as autophagy, being a mediator of inflammation and microbial homeostasis. The variant rs2241880 can diminish these capabilities, potentially contributing to inflammatory bowel disease (IBD) pathogenesis.

Objectives: To perform an updated meta-analysis on the association between *ATG16L1* rs2241880 and IBD susceptibility by exploring the impact of age, ethnicity, and geography. Moreover, to investigate the association between rs2241880 and clinical features.

Methods: Literature searches up until September 2022 across 7 electronic public databases were performed for all case-control studies on *ATG16L1* rs2241880 and IBD. Pooled odds ratios (OR_P) and 95% CI were calculated under the random effects model.

Results: Our analyses included a total of 30,606 IBD patients, comprising 21,270 Crohn's disease (CD) and 9336 ulcerative colitis (UC) patients, and 33,329 controls. *ATG16L1* rs2241880 was significantly associated with CD susceptibility, where the A allele was protective (OR_P: 0.74, 95% CI: 0.72–0.77, *p*-value: <0.001), while the G allele was a risk factor (OR_P: 1.23, 95% CI: 1.09–1.39, *p*-value: 0.001), depending on the minor allele frequencies observed in this multi-ancestry study sample. rs2241880 was predominantly relevant in Caucasians from North America and Europe, and in Latin American populations. Importantly, CD patients harbouring the G allele were significantly more predisposed to perianal disease (OR_P: 1.21, 95% CI: 1.07–1.38, *p*-value: 0.003).

Conclusions: *ATG16L1* rs2241880 (G allele) is a consistent risk factor for IBD in Caucasian cohorts and influences clinical outcomes. As its role in non-Caucasian populations remains ambiguous, further studies in under-reported populations are necessary.

KEYWORDS
ATG16L1, autophagy, biomarker, Crohn disease, IBD, inflammatory bowel disease, perianal, risk factor, rs2241880, ulcerative colitis

INTRODUCTION

Inflammatory bowel disease (IBD) describes the collection of chronic and relapsing inflammatory disorders afflicting the gastrointestinal (GI) tract, whereby there is an absence of a discernible aetiological agent or clear underlying cellular process contributing to inflammation.[1] The two predominant clinical manifestations of IBD are Crohn's disease (CD), and ulcerative colitis (UC). Typical symptoms suffered by patients include diarrhoea, abdominal cramps, rectal bleeding, weight loss and fatigue. The occurrence of extraintestinal manifestations (EIM) is not uncommon, potentially affecting the musculoskeletal, dermatological, ocular, oral, metabolic, and renal systems.[2]

In 2017, it was estimated that there were more than 6.8 million people globally living with IBD, and the age-standardised prevalence rate sat at 79.5 per 100,000.[3] Traditionally, IBD has been labelled as a Western disease, as the highest incidence and prevalence rates have been consistently reported in North America and Europe.[3,4] However, an epidemiological shift over the past decades has seen the emergence of IBD as a global disease with rapidly increasing incidence rates in newly industrialised or 'westernised' regions such as South America, Asia, and Africa.[3,4] The impact of ethnicity on the risk of IBD remains somewhat elusive due to the paucity of data in non-Caucasian populations, although current evidence suggests that non-Hispanic Caucasians are the most at risk of developing IBD in their lifetime.[5]

The aetiology of IBD remains largely enigmatic due to its complexity and the lack of an identifiable cause-and-effect relationship. As a multifactorial disorder, IBD is hypothesised to stand firmly at the crossroads between host genetic susceptibility, an aberrant immune response, the gut microbiota (both commensal and pathogenic), and environmental interactions (e.g., diet and smoking).

The pursuit to identify those host genetic factors that are implicated in IBD susceptibility remains heavily focussed on immunogenetics, including microbial recognition. Over the years, GWAS studies have identified some 200 IBD susceptibility loci.[6,7] An early candidate in IBD GWAS studies was rs2241880 (A > G, also known as T300A), situated in the autophagy-related protein 16-like 1 (*ATG16L1)* locus.[8,9] ATG16L1 plays a fundamental role in the degradative intracellular pathway known as autophagy. Autophagy is critical for the maintenance of overall cellular homeostasis, especially during periods of nutrient starvation, as it is involved in the recycling of old or damaged organelles and proteins.[10] Autophagy can also be directed towards the eradication of pathogens, rendering it an important component of the innate immune response, intimately linked to inflammation through its influence on inflammatory cells (i.e., macrophages, lymphocytes, and neutrophils) and modulation of pro-inflammatory cytokine production.[11] *ATG16L1* rs2241880 is a missense substitution at position 300 on the polypeptide (Threonine to Alanine), altering protein polarity[8] and susceptibility to caspase-3 degradation, which is suspected to contribute to abnormal autophagic functions.[12–14] Consequently, autophagy, one of the principal mechanisms by which the host can maintain intestinal homeostasis, both immunologically and microbially, is defective and potentially contributes to IBD pathogenesis.[15]

Previous meta-analyses[16,17] have been published on the matter; however, these have generally only focussed on an association with CD susceptibility. What we present here, thus, is the most comprehensive meta-analysis to date on the association of *ATG16L1* rs2241880 and IBD, including both CD and UC, determining any relevant demographic susceptibility patterns based on age (paediatric vs. adult), ethnicity (Caucasian, East Asian, South Asian, Middle Eastern, Latin American), and geographical origin (Northern Europe, Eastern Europe, Southern Europe, Western Europe, North America, South America, Oceania, Middle East, East Asia, South Asia). Importantly, we attempted to associate rs2241880 with relevant clinical features and outcomes including disease location, disease behaviour,

Key summary

Summarise the established knowledge on this subject

- *ATG16L1* rs2241880/T300A is a predisposition locus for Crohn's disease; however, the susceptibility pattern is not universal, especially in non-Caucasian populations.
- The locus has been linked to predisposition for the ileal subphenotype of Crohn's disease, but not any other biomarker, clinical phenotype, or outcome of inflammatory bowel disease.

What are the significant and/or new findings of this study?

- *ATG16L1* rs2241880 has relevance as a biomarker for Crohn's disease in Caucasians, particularly from North America and Europe, as well as in populations of Latin American ancestry.
- Clinically, rs2241880 influences predisposition to Crohn's disease associated perianal disease.
- As *ATG16L1* rs2241880 can influence predisposition to perianal disease, this study provides a better understanding of the pathogenesis, with the potential to identify at-risk patients and initiate disease surveillance resulting in more effective treatment.

presence of perianal disease and presence of extraintestinal manifestations (EIM).

METHODS

Literature search strategy and screening

This study was conducted using the Preferred Reporting Items for Systematic Review and Meta-Analysis protocols (PRISMA)[18] with a systematic literature search conducted independently by two authors (IS & NCR). Any discrepancies in study selection or data collection were discussed between the authors to reach a consensus. A systematic search of publicly available scientific literature databases (Pubmed, Science Direct, Scopus, Web of Science, Cochrane, Lilacs and Scielo) was conducted up until 30 September 2022. The search terms utilised are presented in Supplementary Table S1. Retrievals were also identified through hand-searching and manual review of reference lists from selected articles. Articles were initially screened based on title, keywords and abstract to identify records of relevance.

Study selection criteria

Inclusion criteria

Full text articles were selected for eligibility based on the following inclusion criteria: (1) full original peer-reviewed article available (no abstracts or conference articles) in either English, Spanish or Portuguese, (2) a clear case–control study design with a diagnosis of IBD (CD and UC) denoted as 'cases', and (3) population-based or hospital-based controls denoted as healthy or otherwise (non-IBD), (4) paediatric (early-onset) or adult populations, (5) study evaluates the association of polymorphism with any IBD outcome or phenotype, and (6) accessibility to raw data (genotypic and allelic frequencies).

Exclusion criteria

Studies were excluded (1) should their methodology include familial (related) data, (2) presence of duplicated data, and (3) the control group deviated from Hardy-Weinberg Equilibrium (HWE).

Study quality assessment and data extraction

To further assess eligibility and quality of the full-text selected articles, the Newcastle-Ottawa Scale (NOS) Score[19] was implemented using the standard 9-point system. Studies were judged based on subject selection, comparability of subject groups (cases and controls), and the ascertainment of exposure (genotyping). The final selected studies were extracted for author, year, journal, geographical location, ethnicity, age category (adult or paediatric (early-onset) population), total genotype numbers, and *ATG16L1* rs2241880 genotypic (if applicable) and allelic frequencies.

For those articles which also performed genotype-phenotype correlations, genotypic/allelic data were extracted and stratified by one or more of the following variables: Montreal/Vienna classification (disease location and disease behaviour), presence of EIM, or perianal disease. Corresponding authors were contacted if studies indicated such analyses were conducted but data was not accessible/shown.

Statistical analysis

The pooled odds ratio (OR_P) was calculated using the generic inverse variance method where the OR from each individual study was weighted by the inverse of their variance. The random-effects model was employed to calculate ORp and 95% confidence intervals (CI) to account for the assumed variation in the true effects due to study-level heterogeneity, which is summarised by estimating the mean and variance of a distribution of true effects.[20] A meta-regression was also performed to assess the impact of multiple independent variables on the effect size, allowing us to identify potential sources of heterogeneity or confounding variables influencing the association between *ATG16L1* rs2241880 and CD. The variables included in the regression model were ethnicity (Caucasian, East Asian, South Asian, Middle Eastern, Latin American), age of onset (paediatric vs. adult) and study NOS score (≤ 5 vs. ≥ 6). Stratified analyses were further conducted based on the age of onset, ethnicity, geographical origin (Northern Europe, Eastern Europe, Southern Europe, Western Europe, North America, South America, Oceania, Middle East, East Asia, South Asia) and study NOS score. To test for heterogeneity, the Cochran's-Q test was applied, where a p-value <0.1 was suggestive of heterogeneity. Since some of the stratified analyses comprised only a small number of studies, which ultimately undermined the power of the Cochran's-Q test, the Higgins test (I^2) was also employed in parallel for all analyses. The Higgins test defines the percentage of total variation across all studies due to heterogeneity rather than chance, such that I^2 lies between 0% and 100%, where 0% indicates the absence of heterogeneity and increasing values indicate greater heterogeneity.[21] The following categories for I^2 values were assigned: low heterogeneity: <25%, moderate heterogeneity: 25%–75% and high heterogeneity: >75%. To further identify influential studies and examine statistical robustness, leave-one-out sensitivity analysis was performed. To assess for potential publication bias, funnel plots were generated, and Egger's regression asymmetry tests were conducted. All statistical tests were carried out as two-tailed, with a p-value of <0.05 deemed statistically significant. All statistical analyses were conducted using the Comprehensive Meta-Analysis (CMA) Software package V. 4.0 (Biostat, Englewood, New Jersey). Genotype-phenotype correlation analysis was further controlled via the application of the FDR method (Benjamini-Hochberg) at level α (0.05) using an available online tool (https://tools.carbocation.com/FDR).

RESULTS

Literature search and study characteristics

Our systematic search of seven publicly available scientific literature databases revealed collective 5891 records across all search terms used (Figure 1). Hand-searching yielded an additional 9 articles, totalling 5900 records. After duplicate removal, 520 records remained. Initial screening of title, keywords and abstracts, and application of our inclusion criteria, led to 83 studies for qualitative analysis using the NOS Scoring system. We ascertained a total of 61 studies for data extraction and inclusion in the quantitative meta-analysis, consisting of 30,606 IBD cases (21,270 CD and 9336 UC), and 33,329 controls. Reasoning for the exclusion of articles at the data extraction stage is highlighted in Supplementary Table S2. The final selected studies and their general characteristics as well as allele and/or genotype frequencies are presented in Tables 1 and 2 for CD and UC, respectively.

Study quality

By employing the NOS Scoring system, we determined that the overall quality of studies included in the quantitative meta-analysis was high with the majority (47/61; 77%) attaining ≥6 stars. In special circumstances where a single study reported on multiple independent cohorts, a NOS score was applied for each independent cohort, and the final NOS score for the article was determined by averaging the score of those cohorts.

Association of *ATG16L1* rs221880 and IBD

To preface, each population was evaluated to determine the minor allele, G or A, using their respective control cohort, and grouped accordingly for the following analyses. This was done to account for the genetic variations between both ethnic and geographical populations, as evidenced by public databases (International HapMap Project), which report that the frequency of the A allele is 0.458 in European (Caucasian) samples, 0.830 in Japanese (East Asian) samples, and 0.611 in Han Chinese (East Asian) samples.[9]

Our unique approach in segregating the study populations based on the minor allele frequency (MAF) in their respective controls provides consideration of the genetic effects on population differentiation across diverse ethnic groups. It is well known that human allele frequencies of single nucleotide polymorphisms (SNPs) diverge based on geography and ethnicity,[81–83] which is influenced by several factors including natural selection, whereby the selective pressures of

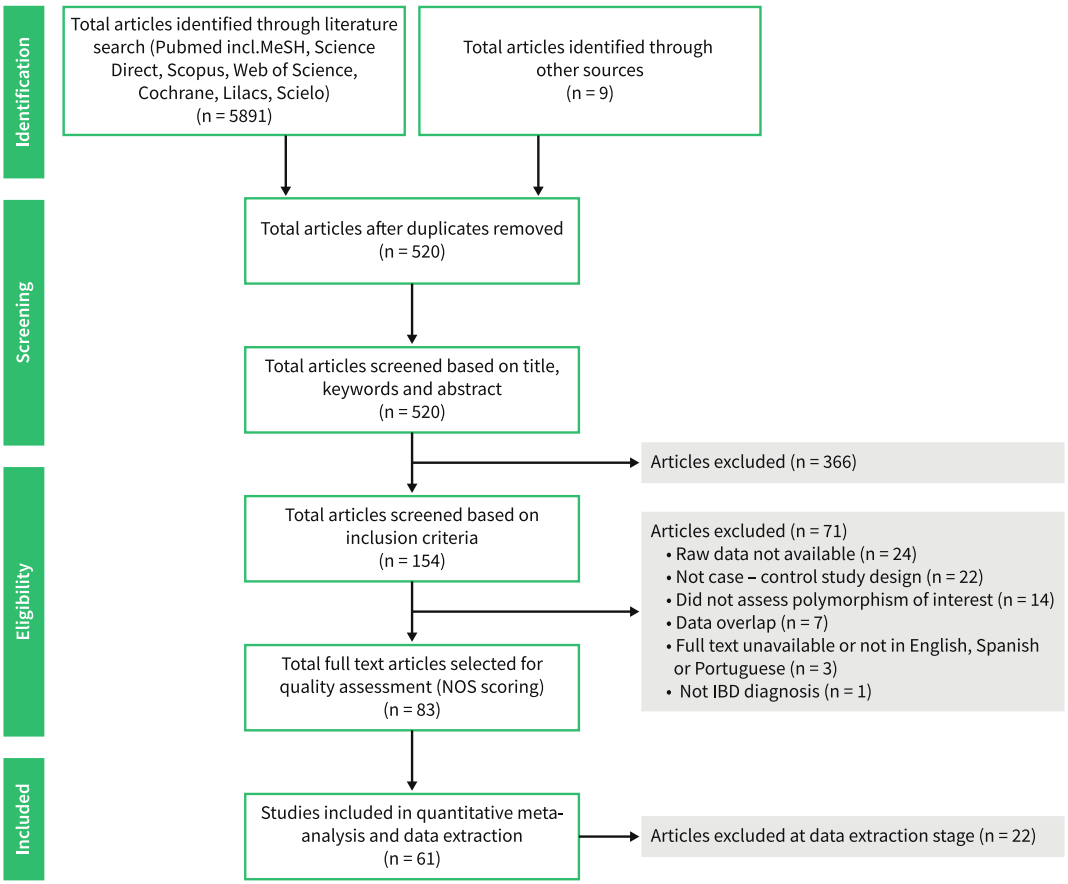

FIGURE 1 PRISMA workflow diagram of the literature search strategy.

TABLE 1 Summary of included studies in quantitative meta-analysis examining the association between *ATG16L1* rs2241880 and Crohn's disease in adult and paediatric-onset populations.

Author	Study design	Ethnicity	Sum (cases/controls)	Genotypic frequencies (cases/controls)			Allelic frequencies (cases/controls)		HWE	NOS Score
				AA	AG	GG	A	G	χ2	
Adult-onset										
Aida *et al.*[22]	Case-control	African	118/161	21/44	54/78	43/39	96/166	140/156	0.146	7
Baptista *et al.*[23]	Case-control	Caucasian	180/189	40/57	94/90	46/42	174/204	186/174	0.327	7
Baradaran Ghavami *et al.*[24]	Case-control	Middle Eastern	26/100	5/14	12/56	9/30	22/84	30/116	0.14	7
Buning *et al.* (Cohort 1)[25]	Case-control	Caucasian	310/285	63/74	149/143	98/68	275/291	345/279	0.004	7
Buning *et al.* (Cohort 2)[25]	Case-control	Caucasian	147/207	23/49	86/109	38/49	162/207	132/207	0.584	7
Buning *et al.* (Cohort 3)[25]	Case-control	Caucasian	157/215	19/47	78/102	60/66	116/196	198/234	0.41	7
Cotterill *et al.*[26]	Case-control	Caucasian	273/834	–/–	–/–	–/–	229/828	317/840	-	6
Csongei *et al.*[27]	Case-control	Caucasian	315/314	56/72	151/163	108/79	263/307	367/321	0.47	7
Dalton *et al.*[28]	Case-control	Caucasian	83/55	12/14	49/33	22/8	73/61	93/49	2.53	5
Deuring *et al.*[29]	Case-control	Caucasian	78/12	17/3	38/3	23/6	72/9	84/15	2.61	5
Dusatkova *et al.*[30]	Case-control	Caucasian	333/499	68/128	158/239	107/132	294/495	372/503	0.881	8
Eglington *et al.*[31]	Case-control	Caucasian	507/600	–/–	–/–	–/–	425/596	589/604	-	9
Fabio *et al.*[32]	Case-control	Caucasian	279/190	51/43	134/97	94/50	236/183	322/197	0.096	7
Fowler *et al.* (Study 1)[33]	Case-control	Caucasian	669/1244	111/304	315/601	243/339	537/1209	801/1279	1.35	8
Gaj *et al.*[34]	Case-control	Caucasian	59/140	11/38	25/70	23/32	47/146	71/134	0.0004	6
Gazouli *et al.*[35]	Case-control	Caucasian	364/539	46/104	177/274	141/161	269/482	459/596	0.429	7
Glas *et al.*[36]	Case-control	Caucasian	768/1615	–/–	–/–	–/–	630/1557	906/1673	-	8
Gutierrez *et al.*[37]	Case-control	Caucasian	179/27	73/22	84/5	22/0	230/49	128/5	0.281	6
Hampe *et al.* (Study 1)[8]	GWAS	Caucasian	1233/1400	–/–	–/–	–/–	986/1316	1480/1484	-	6
Hampe *et al.* (Study 2)[8]	GWAS	Caucasian	509/656	–/–	–/–	–/–	417/630	601/682	-	3
Hirano *et al.*[38]	Case-control	East Asian	1311/6585	–/–	–/–	–/–	1993/10,141	629/3029	-	5
Hong *et al.*[39]	Case-control	East Asian	1000/2000	–/–	–/–	–/–	1278/2788	722/1212	-	6
Jung *et al.* (Exploratory)[40]	Case-control	Caucasian	798/960	–/–	–/–	–/–	958/1056	638/864	-	6
Kee *et al.* (Cohort 1)[41]	Case-control	East Asian	27/86	12/41	14/39	1/6	38/121	16/51	0.65	7
Kee *et al.* (Cohort 2)41	Case-control	South Asian	38/90	5/23	22/46	11/21	32/92	44/88	0.046	7
Kee *et al.* (Cohort 3)[41]	Case-control	South East Asian	20/74	4/34	11/34	5/6	19/102	21/46	0.389	7

(Continues)

TABLE 1 (Continued)

Author	Study design	Ethnicity	Sum (cases/controls)	Genotypic frequencies (cases/controls)			Allelic frequencies (cases/controls)		HWE	NOS Score
				AA	AG	GG	A	G	χ2	
Khan *et al.*[42]	Case-control	South Asian	69/41	17/15	18/19	34/7	52/49	86/33	0.055	5
Kiely *et al.*[43]	Case-control	Unknown	6/10	1/2	1/6	4/2	4/10	10/10	0.4	5
Lakatos *et al.*[44]	Case-control	Caucasian	266/149	49/33	125/83	92/33	223/149	309/149	1.939	8
Lappalainen *et al.*[45]	Case-control	Caucasian	240/190	–/–	–/–	–/–	248/201	232/179	-	5
Latiano *et al.*[46]	Case-control	Caucasian	491/749	72/159	254/376	165/214	398/694	584/804	0.067	6
Lauriola *et al.*[47]	Case-control	Caucasian	18/20	3/3	9/11	6/6	15/17	21/23	0.314	6
Marquez *et al.*[48]	Case-control	Caucasian	344/745	63/177	156/347	125/221	282/701	406/789	3.16	7
Mentzer *et al.*[49]	Case-control	Unknown	90/1023	–/–	–/–	–/–	93/1050	87/996	-	6
Nakagome *et al.*[50]	Case-control	East Asian	129/163	68/89	51/63	10/11	187/241	71/85	0.001	6
Okazaki *et al.*[51]	Case-control	Caucasian	208/314	28/76	103/150	77/88	159/302	257/326	0.586	7
Palomino-Morale *et al.*[52]	Case-control	Caucasian	544/666	75/167	253/316	216/183	403/650	685/682	1.699	7
Perricone *et al.*[53]	Case-control	Caucasian	163/160	33/30	73/76	57/54	139/136	187/184	0.127	8
Peter *et al.*[54]	Case-control	Middle Eastern	369/503	–/–	–/–	–/–	273/412	465/594	-	4
Prescott *et al.*[55]	Case-control	Caucasian	727/579	142/144	338/282	247/153	622/570	832/588	0.382	6
Pugazhendhi *et al.*[56]	Case-control	South Asian	211/361	48/96	135/180	47/85	231/372	229/350	0.0002	6
Quiroz-Cruz *et al.*[57]	Case-control	Latin American	15/200	11/124	2/65	2/11	24/313	6/87	0.409	6
Rioux *et al.*[9]	GWAS	Caucasian	946/977	–/–	–/–	–/–	689/885	1203/1069	-	5
Rioux *et al.* (Replication)[9]	Case-control	Unknown	625/207	–/–	–/–	–/–	466/198	784/216	-	5
Scharl *et al.*[58]	Case-control	Caucasian	12/8	2/7	6/1	4/0	10/15	14/1	0.036	6
Scolaro *et al.*[59]	Case-control	Latin American	106/238	28/84	53/106	25/48	109/274	103/202	1.86	5
Sventoraityte *et al.*[60]	Case-control	Caucasian	55/186	11/53	28/89	16/44	50/195	60/177	0.309	7
Teimoori-Toolabi *et al.*[61]	Case-control	Middle Eastern	132/86	21/11	54/48	57/27	96/70	168/102	2.1	8
Tsianos *et al.*[62]	Case-control	Caucasian	108/223	11/43	55/113	42/67	77/199	139/247	0.143	7
Van Limbergen *et al.*[63]	Case-control	Caucasian	360/345	60/71	163/176	137/98	283/318	437/372	0.243	6
Wang *et al.*[64]	Case-control	African American	349/352	141/179	164/140	44/33	446/498	252/206	0.543	6
Waterman *et al.*[65]	Case-control	Caucasian	1144/1057	–/–	–/–	–/–	1381/1082	907/1032	-	6
Weersma *et al.*[66]	Case-control	Caucasian	286/871	40/163	125/428	121/280	205/754	367/988	0.0006	6
Wei *et al.*[67]	Case-control	East Asian	39/100	17/27	18/47	4/26	52/101	26/99	0.359	5

TABLE 1 (Continued)

Author	Study design	Ethnicity	Sum (cases/controls)	Genotypic frequencies (cases/controls)			Allelic frequencies (cases/controls)		HWE	NOS Score
				AA	AG	GG	A	G	χ2	
Yamazaki *et al.*[68]	Case-control	East Asian	481/437	274/238	184/167	23/32	732/643	230/231	0.131	6
Yang *et al.*[69]	Case-control	East Asian	377/372	178/186	156/146	43/40	512/518	242/226	1.935	8
Zhang *et al.* (2019)[70]	Case-control	East Asian	490/260	–/–	–/–	–/–	613/337	367/183	-	7
Zhang *et al.* (2012)[71]	Case-control	Caucasian	34/23	1/5	19/8	14/10	21/18	47/28	1.675	4
TOTAL			**19,215/30,692**							
Paediatric-onset										
Amre *et al.*[72]	Case-control	Caucasian	286/290	47/91	137/135	102/64	231/317	341/263	1.07	4
Baldassano *et al.*[73]	GWAS	Caucasian	142/281	19/67	65/136	58/78	103/270	181/292	0.262	4
Chinnadurai *et al.*[74]	Case-control	Unknown	6/6	0/3	4/2	2/1	4/8	8/4	0.375	6
Gazouli *et al.*[35]	Case-control	Caucasian	110/539	17/104	45/274	48/161	79/482	141/596	0.429	7
Jakobsen *et al.*[75]	Case-control	Caucasian	244/543	–/–	–/–	–/–	195/520	293/566	-	6
Lacher *et al.*[76]	Case-control	Caucasian	152/253	19/69	73/128	60/56	111/266	193/240	0.053	6
Latiano *et al.*[46]	Case-control	Caucasian	176/749	33/159	81/376	62/214	147/694	205/804	0.067	6
Na *et al.*[77]	Case-control	East Asian	65/72	–/–	–/–	–/–	76/93	54/51	-	7
Peterson *et al.*[78]	Case-control	Caucasian	555/486	–/–	–/–	–/–	455/467	655/505	-	6
Pranculiene *et al.*[79]	Case-control	Caucasian	31/157	10/40	14/78	7/39	34/158	28/156	0.006	8
Pugazhendhi *et al.*[56]	Case-control	South Asian	19/361	5/96	9/180	5/85	19/372	19/350	0.0002	6
Van Limbergen *et al.*[63]	Case-control	Caucasian	269/345	58/71	131/176	80/98	247/318	291/372	0.243	6
Total			**2055/4082**							

Abbreviations: HWE, Hardy Weinberg Equilibrium; NOS, Newcastle-Ottawa Scale.

TABLE 2 Summary of included studies in quantitative meta-analysis examining the association between *ATG16L1* rs2241880 and ulcerative colitis in adult and paediatric-onset populations.

Author	Study design	Ethnicity	Sum (cases/controls)	Genotypic frequencies (cases/controls)			Allelic frequencies (cases/controls)		HWE	NOS score
				AA	AG	GG	A	G	χ2	
Adult-onset										
Baradaran Ghavami *et al.*[24]	Case-control	Middle Eastern	75/100	10/14	35/56	30/30	55/84	95/116	2.23	7
Buning *et al.* (Cohort 1)[25]	Case-control	Caucasian	179/285	43/74	88/143	48/68	174/291	184/279	0.004	7
Buning *et al.* (Cohort 2)[25]	Case-control	Caucasian	117/207	26/49	60/109	31/49	122/207	112/207	0.584	7
Cotterill *et al.*[26]	Case-control	Caucasian	188/834	–/–	–/–	–/–	184/828	192/840	-	6
Dalton *et al.*[28]	Case-control	Caucasian	64/55	18/14	27/33	19/8	63/61	65/49	2.53	5
Fowler *et al.* (Study 1)[33]	Case-control	Caucasian	543/1244	131/304	303/601	109/339	565/1209	521/1279	1.35	8
Glas *et al.*[36]	Case-control	Caucasian	507/1615	–/–	–/–	–/–	455/1557	559/1673	-	8
Hampe *et al.* (Study 1)[8]	GWAS	Caucasian	788/1400	–/–	–/–	–/–	725/970	851/1094	-	6
Kiely *et al.*[43]	Case-control	Unknown	2/10	0/2	2/6	0/2	2/10	2/10	0.4	5
Lakatos *et al.*[44]	Case-control	Caucasian	149/149	32/33	72/83	45/33	136/149	162/149	1.939	8
Lappalainen *et al.*[45]	Case-control	Caucasian	459/190	–/–	–/–	–/–	495/201	423/179	-	5
Latiano *et al.*[46]	Case-control	Caucasian	506/749							
Marquez *et al.*[48]	Case-control	Caucasian	368/745	87/177	185/347	96/221	359/701	377/789	3.16	7
Nakagome *et al.*[50]	Case-control	East Asian	82/163	50/89	26/63	6/11	126/241	38/85	0.001	6
Okazaki *et al.*[51]	Case-control	Caucasian	113/314	27/76	58/150	28/88	112/302	114/326	0.586	7
Palomino-Morale *et al.*[52]	Case-control	Caucasian	414/666	95/167	194/316	125/183	384/650	444/682	1.699	7
Prescott *et al.*[55]	Case-control	Caucasian	877/579	–/–	–/–	–/–	793/570	961/588	0.382	6
Pugazhendhi *et al.*[56]	Case-control	Caucasian	235/361	47/96	125/180	63/85	226/372	256/350	0.0002	6
Quiroz-Cruz *et al.*[57]	Case-control	Latin American	78/200	63/124	9/65	6/11	135/313	21/87	0.409	6
Rioux *et al.* (Replication)[9]	Case-control	Caucasian	353/207	–/–	–/–	–/–	293/198	413/216	-	5
Roberts *et al.*[80]	Case-control	Caucasian	466/549	118/134	223/285	125/130	459/553	473/545	0.806	6
Sventoraityte *et al.*[60]	Case-control	Caucasian	119/186	25/53	61/89	33/44	111/195	127/177	0.309	7
Tsianos *et al.*[62]	Case-control	Caucasian	97/223	14/43	52/113	31/67	80/199	114/247	0.143	7
Van Limbergen *et al.*[63]	Case-control	Caucasian	495/345	–/–	–/–	–/–	465/318	525/372	0.243	6
Waterman *et al.*[a,65]	Case-control	Caucasian	1230/1057	–/–	–/–	–/–	1298/1082	1162/1032	-	6

TABLE 2 (Continued)

Author	Study design	Ethnicity	Sum (cases/controls)	Genotypic frequencies (cases/controls)			Allelic frequencies (cases/controls)		HWE	NOS score
				AA	AG	GG	A	G	χ2	
Weersma *et al.*[66]	Case-control	Caucasian	187/871	27/163	91/428	69/280	145/754	229/988	0.0006	6
Zhang *et al.* (2012)[71]	Case-control	Caucasian	27/23	5/5	11/8	11/10	21/18	33/28	1.675	4
Total			**8718/13,327**							
Paediatric-onset										
Jakobsen *et al.*[75]	Case-control	Caucasian	318/543	–/–	–/–	–/–	298/520	338/566	-	6
Latiano *et al.*[46]	Case-control	Caucasian	162/749	36/159	71/376	55/214	143/694	181/804	0.067	6
Pranculiene *et al.*[79]	Case-control	Caucasian	45/157	10/40	24/78	11/39	44/158	46/156	0.006	8
Pugazhendhi *et al.*[56]	Case-control	South Asian	6/361	2/96	3/180	1/85	7/372	5/350	0.0002	6
Van Limbergen *et al.*[63]	Case-control	Caucasian	87/345	–/–	–/–	–/–	90/318	84/372	0.243	6
Total			**618/2155**							

Abbreviations: HWE, Hardy Weinberg Equilibrium; NOS, Newcastle-Ottawa Scale.

[a]Cases included those with UC and IBD-Unclassified.

environmental conditions can modulate the allelic balance across populations. In addition, except in instances of Mendelian diseases, the role of the minor allele in complex diseases has a natural tendency to be inherently attributed to be the risk allele.[84] Furthermore, association tests were reported more likely to be statistically significant if the minor allele in the population was considered the risk allele instead of the major allele.[84] Notwithstanding, the major allele may of course be recognised as the risk allele in certain populations, as was the case in the current meta-analysis, which identified that almost half of the included study populations harbours the *ATG16L1* rs2241880 risk allele (G) as the major allele in their respective controls. By conducting the analyses independently for each allele and population, based on their respective MAF, we can provide a neutral evaluation of the role of rs2241880 in IBD. In this respect, analysis for CD and UC susceptibility will be presented for each allele independently.

Effect of *ATG16L1* rs2241880 A allele on CD susceptibility

Populations with the rs2241880 minor allele denoted as A included 33 independent case-control studies across 30 articles (Figure 2a). Overall, OR_P was determined to be a highly significant 0.74 (0.72–0.77; *p*-value: <0.001) under the random effects model, indicating that the A allele is favourable against CD development (Figure 2a). There was no evidence of heterogeneity in this analysis (Figure 2a).

A meta-regression for the A allele in CD included 27 studies in the model since 6 studies were excluded from this analysis due to missing data for one or more independent variables. The regression model showed that these variables (ethnicity, age of onset, and study NOS score) did not influence the overall association between the A allele and CD susceptibility (*p*-value: 0.623, Supplementary Table S3). However, as the magnitude of the effect size can still differ based on some of these variables, we also conducted stratified analyses.

Limiting included studies in these analyses to those of the highest quality (n = 26, study NOS score ≥6) did not influence the significance nor effect size of the A allele on CD susceptibility (OR: 0.75, 95% CI: 0.72–0.78, *p*-value <0.001; data not shown).

The onset of IBD can occur at any point within a lifetime, where paediatric-onset IBD (before the age of 16) is often treated as a separate entity from that of adult-onset IBD due to differences in clinical presentation and the natural course of disease.[85,86] On this account, we evaluated the effect of rs2241880 in the context of age onset, broadly, adult-onset versus paediatric-onset. The A allele reported a highly significant OR_P of 0.74 (0.71–0.77; *p*-value: <0.001) and 0.77 (0.68–0.87; *p*-value: <0.001) for CD adult-onset and paediatric onset, respectively (Table 3). Heterogeneity was detected only in the paediatric-onset subgroup (I^2: 40.14%) (Table 3).

Our approach to evaluating the impact of ethnicity on rs2241880 and IBD susceptibility involved categorising each population crudely into Caucasian, East Asian, South Asian, Middle Eastern and Latin American categories. When stratified by ethnicity, only populations of Caucasian and Middle Eastern ancestry appeared

(a)

Study name	OR	Lower limit	Upper limit	*p*-value
Baldassano et al.	0.615	0.459	0.825	0.001
Baradaran Ghavami et al.	1.013	0.546	1.878	0.968
Buning et al. (Cohort 2)	0.815	0.604	1.100	0.181
Buning et al. (Cohort 3)	0.699	0.519	0.942	0.019
Cotterill et al.	0.733	0.603	0.891	0.002
Csongei et al.	0.749	0.600	0.936	0.011
Deuring et al.	1.429	0.590	3.459	0.429
Dusatkova et al.	0.803	0.660	0.978	0.029
Eglinton et al.	0.731	0.618	0.865	0.000
Fabio et al.	0.789	0.607	1.025	0.076
Fowler et al. (Study 1)	0.709	0.620	0.811	0.000
Gazouli et al.	0.717	0.600	0.857	0.000
Glas et al.	0.747	0.661	0.845	0.000
Hampe et al. (Study 1)	0.751	0.673	0.838	0.000
Hampe et al. (Study 2)	0.751	0.637	0.886	0.001
Jakobsen et al.	0.724	0.583	0.900	0.004
Lakatos et al.	0.722	0.543	0.960	0.025
Latiano et al.	0.800	0.689	0.929	0.003
Lauriola et al.	0.966	0.388	2.406	0.941
Marquez et al.	0.782	0.651	0.939	0.008
Okazaki et al.	0.668	0.519	0.859	0.002
Palomino-Morales et al.	0.617	0.524	0.727	0.000
Perricone et al.	1.006	0.736	1.374	0.972
Peter et al.	0.846	0.696	1.029	0.094
Peterson et al.	0.751	0.631	0.894	0.001
Prescott et al.	0.771	0.660	0.901	0.001
Rioux et al.	0.692	0.608	0.787	0.000
Rioux et al. (Rep)	0.648	0.518	0.812	0.000
Teimoori-Toolabi et al.	0.833	0.561	1.235	0.363
Tsianos et al.	0.688	0.492	0.961	0.029
Van Limbergen et al.	0.852	0.706	1.027	0.092
Weersma et al.	0.732	0.602	0.890	0.002
Zhang et al. (2012)	0.695	0.317	1.523	0.363
Random	0.743	0.718	0.769	0.000

OR and 95% CI

0.1 0.2 0.5 1 2 5 10

Heterogeneity: *Q*: 24.78, *p*- Value: 0.815, I^2: 0.00%

(b)

Study name	OR	Lower limit	Upper limit	*p*-value
Aida et al.	1.552	1.105	2.179	0.011
Amre et al.	1.779	1.408	2.248	0.000
Baptista et al.	1.253	0.939	1.674	0.126
Buning et al. (Cohort 1)	1.309	1.042	1.644	0.021
Chinnadurai et al.	4.000	0.733	21.838	0.109
Dalton et al.	1.586	0.976	2.576	0.062
Gaj et al.	1.646	1.063	2.548	0.025
Gutierrez et al.	5.454	2.119	14.035	0.000
Hirano et al.	1.057	0.958	1.166	0.272
Hong et al.	1.300	1.160	1.456	0.000
Jung et al. (Exploratory)	0.814	0.711	0.931	0.003
Kee et al. (Cohort 1)	0.999	0.511	1.951	0.998
Kee et al. (Cohort 2)	1.438	0.837	2.469	0.189
Kee et al. (Cohort 3)	2.451	1.203	4.993	0.014
Khan et al.	2.456	1.403	4.298	0.002
Kiely et al.	3.000	0.622	14.469	0.171
Lacher et al.	1.927	1.440	2.579	0.000
Lappalainen et al.	1.050	0.802	1.375	0.720
Mentzer et al.	0.986	0.727	1.338	0.929
Na et al.	1.296	0.795	2.111	0.298
Nakagome et al.	1.077	0.745	1.556	0.695
Pranculiene et al.	0.834	0.483	1.441	0.516
Pugazhendhi et al.	1.054	0.834	1.331	0.661
Quiroz-Cruz et al.	0.899	0.356	2.270	0.822
Scharl et al.	21.000	2.372	185.930	0.006
Scolaro et al.	1.282	0.926	1.774	0.134
Sventoraityte et al.	1.322	0.863	2.026	0.200
Wang et al.	1.366	1.092	1.709	0.006
Waterman et al.	0.689	0.611	0.776	0.000
Wei et al.	0.510	0.295	0.881	0.016
Yamazaki et al.	0.875	0.708	1.080	0.213
Yang et al.	1.083	0.871	1.348	0.473
Zhang et al. (2019)	1.103	0.883	1.376	0.388
Random	1.229	1.090	1.385	0.001

OR and 95% CI

0.1 0.2 0.5 1 2 5 10

Heterogeneity: *Q*: 184.63, *p*- Value: 0.000, I^2: 82.67%

FIGURE 2 Forest plots of the meta-analysis assessing the association between *ATG16L1* rs2241880 CD susceptibility. (a) Analysis in respect to the A allele. (b) Analysis in respect to the G allele. Pooled odds ratios (OR_P) with 95% confidence intervals (CI) were calculated under the random effects model (coloured diamond). Squares denote the contributing weight of each study to analyses. *Q*, Cochran's Q test, I^2; Higgins test.

TABLE 3 Pooled effect size and heterogeneity for the meta-analyses assessing the association between the *ATG16L1* rs2241880 A allele and IBD susceptibility.

				Heterogeneity		
Stratified analysis	**OR_P***	**95% CI**	***p*-value**	***Q*–value**	***p*-value**	**I^2**
Crohn's disease						
Age of disease onset*						
Adult - onset	0.740	0.713–0.767	<0.001	19.952	0.866	0.000
Paediatric - onset	0.770	0.680–0.871	<0.001	8.352	0.138	40.135
Ethnicity**						
Caucasian	0.749	0.722–0.778	<0.001	20.730	0.836	0.000
Middle Eastern	0.881	0.632–1.229	0.456	0.274	0.601	0.000
Geographical origin**						
Eastern Europe	0.774	0.687–0.873	<0.001	0.561	0.905	0.000
Middle East	0.881	0.632–1.229	0.456	0.274	0.601	0.000
North America	0.672	0.610–0.739	<0.001	0.647	0.958	0.000
Northern Europe	0.767	0.708–0.832	<0.001	1.747	0.782	0.000
Oceania	0.718	0.646–0.797	<0.001	0.077	0.781	0.000
Southern Europe	0.751	0.680–0.829	<0.001	10.687	0.153	34.497
Western Europe	0.747	0.695–0.804	<0.001	2.305	0.680	0.000
Ulcerative colitis						
Overall						
	0.945	0.898–0.995	0.031	19.74	0.288	13.89
Age of disease onset*						
Adult–onset	0.943	0.893–0.995	0.032	19.000	0.269	15.790
Paediatric - onset	0.995	0.851–1.163	0.949	2.431	0.297	17.719
Ethnicity**						
Caucasian	0.956	0.909–1.005	0.078	16.377	0.357	8.411
Geographical origin**						
Eastern Europe	0.878	0.700–1.102	0.262	0.149	0.700	0.000
North America	0.893	0.711–1.122	0.331	2.591	0.274	22.806
Northern Europe	0.945	0.855–1.045	0.273	3.544	0.315	15.361
Southern Europe	0.957	0.874–1.049	0.351	2.317	0.509	0.000
Western Europe	0.906	0.829–0.990	0.029	1.575	0.455	0.000

Note: The random effects model using two-tailed *p*-value was applied to ascertain pooled analysis results. *For age of disease onset analysis, those studies which included both types of subpopulations but failed to provided data discriminating between them were excluded from this stratified analysis. **Studies where the ethnicity or the geographical origin of the population was not stated or could not be comfortably deduced were excluded from this stratified analysis.

Abbreviations: CI, confidence intervals; I^2, Higgins test; OR_P, pooled odds ratio; *Q*, Cochran's Q test; Std, standard.

to harbour the A allele as the minor allele (Table 3). Stratification by ethnicity revealed relevance for the A allele in CD susceptibility only in Caucasian cohorts, with a highly significant OR_P of 0.75 (0.72–0.78, *p*-value: <0.001) (Table 3). No heterogeneity was found (I^2: 0.00%) in both subgroups (Table 3).

It is clear in genetic association studies, including this one, that the impact of susceptibility variants not only vary across ethnicities but also across geographical regions of the same ethnicity.[9] To this end, we also investigated the impact of geographical origin on rs2241880 and IBD risk to determine any susceptibility patterns. Populations were stratified according to the following geographical regions: Northern Europe, Eastern Europe, Southern Europe, Western Europe, North America, South America, Oceania, Middle East, East Asia, South Asia. This categorisation was conducted in line with the United Nations geoscheme for regions and individual countries.[87]

Given our findings in the ethnicity-stratified analysis, unsurprisingly, we found a pattern where those populations with the A allele as the minor allele were concentrated in regions of the globe with a majority Caucasian ethnicity (Europe and North America). The A allele was a highly significant protective factor across all of Europe (Table 3). Furthermore, the A allele held the most protective value in North America with an OR_P of 0.67 (0.61–0.74, *p*-value: <0.001). No significant evidence of heterogeneity was reported across all subgroups, except Southern Europe, which showed the presence of moderate heterogeneity (I^2: 34.50%). In summary, the A allele appears to have significant relevance in Caucasian ethnic groups, from both the North American and European regions, which confers protection against CD development.

Effect of *ATG16L1* rs2241880G allele on CD susceptibility

Populations with the rs2241880 minor allele denoted as G included 33 independent case-control studies across 31 articles (Figure 2b). For CD, the G allele showed a significant OR_P of 1.23 (1.09–1.36, *p*-value: 0.001) (Figure 2b). There was a high level of heterogeneity noted in this analysis (I^2: 82.67% (Figure 2b).

A meta-regression for the G allele in CD included 29 studies in the model since 4 studies were excluded due to missing data for one or more of the independent variables included (age of onset and study NOS score). Ethnicity was not included in the model due to colinearity. The regression model showed that neither of these independent variables influenced the overall association between the G allele and CD susceptibility (*p*-value: 0.36; Supplementary Table S3). However, given that the magnitude of the effect size can still differ based on some of these variables, we also conducted stratified analyses.

Stratification based on high study quality ($n = 25$, study NOS score ≥ 6) did not undermine the association between the G allele and CD, still reaching a highly significant OR of 1.22 (95% CI: 1.06–1.41, *p*-value: 0.007; data not shown).

Following stratification by age, the G allele was shown to be a highly significant risk factor in both adult-onset and paediatric-onset CDs. The OR_P for adult-onset was reported to be 1.19 (1.06–1.34, *p*-value: 0.003), while the OR_P for paediatric-onset was reported to be 1.47 (1.12–1.95, *p*-value: 0.006) (Table 4). Moderate levels of heterogeneity were reported for both adult-onset and paediatric-onset analyses (I^2: 72.67% and I^2: 55.11%, respectively, Table 4).

When stratified by ethnicity, the G allele remained significant in the Caucasian and Latin American subgroups with OR_P of 1.36 (1.03–1.79, *p*-value: 0.029) and 1.24 (1.01–1.54, *p*-value: 0.04), respectively (Table 4). Moderate to high levels of heterogeneity were reported in the Caucasian, East Asian and South Asian subgroup analyses (I^2: 91.17%, I^2:62.95% and I^2:74.55%, respectively, Table 4).

When stratified by geographical origin, we emulated the ethnicity findings that in South American populations, the G allele is a significant risk factor for CD (OR: 1.27, 95% CI: 1.02–1.57, *p*-value: 0.032). The remaining geographical regions, including Caucasian-based regions, reported non-significant findings for the G allele in CD susceptibility. There was high heterogeneity reported across several subgroups (Table 4).

Effect of *ATG16L1* rs2241880 on UC susceptibility

Our analyses on the impact of rs2241880 on UC susceptibility revealed largely negligible effects in both allelic analysis (Tables 3 and 4; Supplementary materials) and across all analysis types. Thus, no further analysis (clinic manifestations) was conducted on the UC populations.

Effect of *ATG16L1* rs2241880 on clinical manifestation and outcomes of CD

The clinical manifestation of IBD is highly heterogeneous, and the potential for accurate molecular diagnosis and prognosis has always remained a lucrative clinical application. Here, we attempted to evaluate the applicability of *ATG16L1* rs2241880 as a biomarker of different clinical features and outcomes of CD. This included disease location (ileum involvement vs. no ileum involvement), disease behaviour (only inflammatory (i.e., non-stricturing and non-penetrating) versus. stricturing or penetrating), presence of perianal disease, or EIM. As confirmed in the meta-analysis, the G allele is the risk allele and as such, all our genotype-phenotype analyses were conducted with the A allele as reference.

A significant clinical outcome of CD is perianal disease which can be characterised by inflammation and injury at or near the anus, including fistulae, abscesses, and skin tags.[88] Stratification based on the presence or absence of perianal disease included 860 and 1720 patients, respectively, across seven independent case-control studies. Here, we identified a significant association with increased susceptibility to perianal disease in CD patients carrying the G risk allele (OR_P: 1.21, 95% CI: 1.07–1.38, adjusted *p*-value: 0.003, Table 5.) with an adjusted *p*-value of 0.015 after FDR correction (Benjamini-Hochberg). The remaining comparisons failed to yield any significant role for the G allele with other clinical manifestations (Table 5).

Sensitivity analysis and publication bias

Both the *ATG16L1* rs2241880 A and G allele remained a statistically significant risk factor for CD irrespective of what cohort was removed from the analysis each time. Statistical significance for the impact of an allele in UC susceptibility is lost when six out of the 18 studies are removed in the random effects model. This is most likely a by-product of the weak association observed. The G allele is not significantly associated with UC irrespective of what study was removed at a time. The significance of the G allele in CD associated perianal disease associated is achieved irrespective of which paper was removed for sensitivity analysis, inferring robustness. There was

TABLE 4 Pooled effect size and heterogeneity for the meta-analyses assessing the association between the *ATG16L1* rs2241880 G allele and IBD susceptibility.

				Heterogeneity		
Stratified analysis	**OR_P**	**95% CI**	***p*–value**	***Q*–value**	***p*–value**	**I^2**
Crohn's disease						
Age of disease onset*						
Adult - onset	1.191	1.062–1.336	0.003	87.825	0.000	72.673
Paediatric - onset	1.474	1.115–1.948	0.006	11.137	0.049	55.105
Ethnicity**						
Caucasian	1.357	1.031–1.785	0.029	124.552	0.000	91.168
East Asian	1.058	0.932–1.200	0.383	21.595	0.006	62.954
Latin American	1.244	1.008–1.535	0.042	0.507	0.776	0.000
South Asian	1.477	0.894–2.438	0.128	7.860	0.020	74.554
Geographical origin**						
East Asia	1.058	0.932–1.200	0.383	21.595	0.006	62.954
North America	1.129	0.646–1.972	0.669	65.110	0.000	95.392
Northern Europe	1.100	0.922–1.311	0.289	4.484	0.344	10.786
South America	1.266	1.020–1.571	0.032	0.010	0.919	0.000
South Asia	1.477	0.894–2.438	0.128	7.860	0.020	74.554
Western Europe	1.449	0.852–2.465	0.171	41.056	0.000	92.693
Ulcerative colitis						
Overall						
	1.054	0.947–1.173	0.339	10.338	0.324	12.943
Age of disease onset*						
Adult–onset	1.052	0.933–1.187	0.407	10.545	0.229	24.137
Paediatric - onset	1.010	0.654–1.560	0.963	0.273	0.601	0.000
Ethnicity**						
Caucasian	1.070	0.958–1.194	0.231	2.374	0.795	0.000
Geographical origin**						
Northern Europe	1.079	0.912–1.277	0.376	2.247	0.523	0.000

Note: The random effects model using two-tailed *p*-value was applied to ascertain pooled analysis results. *For age of disease onset analysis, those studies which included both types of subpopulations but failed to provided data discriminating between them were excluded from this stratified analysis. Authors of these studies were contacted in an attempt to stratify the data accordingly; however, this was largely unsuccessful. **Studies where the ethnicity or the geographical origin of the population was not stated or could not be comfortably deduced were excluded from this stratified analysis.

Abbreviations: CI, confidence intervals; I^2, Higgins test; OR_P, pooled odds ratio; *Q*, Cochran's Q test; Std, standard.

no evidence of publication bias in these meta-analyses, except in the G allele analysis for CD susceptibility (*p*-value: 0.008, Supplementary Table S4, Figures S1-S4).

DISCUSSION

As one of the first identified susceptibility loci from GWAS studies, *ATG16L1* rs2241880 has since provided conflicting evidence over its inclusion and applicability in the molecular profile of IBD susceptibility. To the best of the authors' knowledge, the last meta-analysis conducted on this topic was in 2017,[17] and since then several more studies have been reported including those in under-studied or minority populations, contributing to the growing literature on the impact of rs2241880 in the pathogenesis of IBD. Thus, we sought to provide an updated, comprehensive meta-analysis of the available literature on *ATG16L1* rs2241880 on IBD susceptibility. Moreover, we aimed to identify any trends in clinical presentation of CD as well as attributable susceptibility demographics (age, ethnicity, or geography).

TABLE 5 Meta-analysis and heterogeneity of *ATG16L1* rs2241880 G allele and CD clinical manifestations and outcomes.

				Heterogeneity		
Stratified analysis	**OR_P**	**95% CI**	***p*–value**	***Q*–value**	***p*–value**	**I^2**
Disease location*						
Ileum non-involved	Ref	-	-	-	-	-
Ileum-involved	1.154	0.934–1.426	0.185	53.970	0.000	77.765
Disease behaviour**						
B1 –inflammatory only (non-stricturing/penetrating)	Ref	-	-	-	-	-
B2–stricturing	1.086	0.967–1.219	0.164	4.278	0.831	0.000
B3–penetrating	1.110	0.979–1.258	0.104	8.955	0.346	10.665
Perianal disease						
Absent	Ref	-	-	-	-	-
Present	1.21	1.069–1.375	0.003	4.952	0.550	0.000
EIM						
Absent	Ref	-	-	-	-	-
Present	0.797	0.575–1.105	0.173	0.265	0.607	0.000

Note: The random effects model using two-tailed *p*-value was applied to ascertain pooled analysis results. *Disease location was classified as ileum-involved (either ileum only or ileocolonic) or ileum non-involved (colon only and/or upper GI). **Disease behaviour was classified according to both the Montreal and Vienna classification systems which were the most frequently utilised classification systems where; B1 indicated non-stricturing/penetrating OR inflammatory only, B2 indicated stricturing only and B3 indicated penetrating only.

Abbreviations: CI, confidence intervals; I^2, Higgins test; OR_P, pooled odds ratio; *Q*, Cochran's Q test; Std, standard.

Our meta-analysis involved a total of 30,606 IBD patients, comprising 21,270 CD patients and 9336 UC patients, and 33,329 controls, across 68 populations from 61 different articles: the largest meta-analysis on this subject to date. We present a confirmation of the highly significant association of *ATG16L1* rs2241880 with CD susceptibility, and to a lesser extent with UC susceptibility. The A allele was determined to be protective against CD with an OR_P of 0.74 (0.72–0.77), and complementarily, the G allele was determined to be a risk factor with an OR_P of 1.23 (1.09–1.39). With regard to UC susceptibility, we report only an association with the A allele, which holds a very mild protective value with an OR_P of 0.95 (0.90–1.00).

While early-onset and adult-onset IBD may carry differing underlying pathogenesis, we report here that rs2241880 contributes significantly to CD development, regardless of age of onset. Compared to adult-onset, in early-onset IBD genetic predisposition is suspected to carry greater influence in aetiology due to the more limited exposure to environmental risk factors.[89,90] This is reflected in our finding that the G allele held a higher OR for paediatric-onset CD compared with adult-onset CD (OR_P: 1.47 vs. 1.18).

The influence of ethnicity on IBD phenotype and outcomes has been demonstrated across Caucasians, Blacks, Hispanics, and Asians.[5,91] Whether this is attributed to true differences in genetics, or rather environmental and lifestyle factors coupled with socio-economic disparities, is yet to be clearly established. As described previously, the genetic implication of ethnic diversity plays a major role in establishing the validity of such genetic markers in complex diseases such as IBD. The current literature describes a lack of a universal susceptibility pattern for *ATG16L1* rs2241880 in IBD pathogenesis. With the incidence rates of IBD varying greatly between different ethnic groups, it is conceivable to credit this, at least in part, to the differences in allele frequencies of disease-associated SNPs. The current meta-analysis demonstrates almost exclusive relevance in Caucasian cohorts, and more specifically in high-risk 'Westernised' regions (North America and Europe). Interestingly, Caucasian populations displayed great variability in the assignment of the minor allele, while Asian populations largely remained undivided (i.e., the minor allele was almost always designated as G). This would suggest that despite being a risk allele, the rs2241880 G allele may evolutionarily serve a homeostatic purpose. Furthermore, the lower G allele frequency in East Asian populations[9] coincides with a lower rate of IBD incidence rates.[92] We also present evidence, for the first time, of the relevance of *ATG16L1* rs2241880 (G) allele in CD development in patients from Latin American populations, which failed to be achieved by single studies.

Importantly, a novel significant association between rs2241880 and perianal disease was identified in the current meta-analysis. The manifestation of perianal disease in the clinical course of CD is commonly reported in 25%–80% of patients.[93] The literature is increasingly supporting an underlying genetic predisposition to the development of perianal fistulae.[88] Our findings indicate that rs2241880 is a part of this susceptibility profile, as CD patients harbouring the G allele were found to be at increased risk of developing perianal disease (Table 5; OR_P: 1.21, 95% CI: 1.07–1.38).

ATG16L1 is not the first member of the autophagic pathway to be associated with perianal disease, as variants in another key autophagy protein, IRGM (rs4958847 and rs1000113), were associated with perianal fistulas in an Italian population.[94] Due to the role of autophagy in mediating inflammation, hinderance of the function of these proteins could lead to aberrant inflammation, conducive to perianal disease.

On the contrary to the above findings for CD, our conclusions for the significance of rs2241880 on UC susceptibility are more unassuming. A very modest protective relationship was established for the A allele, which was restricted to adult-onset UC (Table 3). Notably, we observed a significant protective role for the A allele in UC susceptibility for Caucasians from Western Europe (Table 3). With larger sample sizes available from these regions, it does set a precedent for the numbers required to fully illustrate the more limited role of rs2241880 in UC susceptibility. Differences in the pathophysiology between CD and UC are suspected to dictate the relevance of rs2241880 in their susceptibility. While the influence of genetic predisposition is viewed to lesser magnitude in UC pathogenesis, disease-specific risk loci do exist for UC such as *ECM1*,[95] yet the impact of ethnicity is again pronounced.[96]

Most case-control studies underscore a modest contribution of *ATG16L1* rs2241880 to IBD susceptibility which is undermined by the lack of statistical power. The current meta-analysis is able to overcome this obstacle to provide a more comprehensive examination of the role of rs2241880 in IBD susceptibility. We are acutely aware of potential population stratification in these analyses due to the ethnic bias of Caucasian populations prevalent in the majority of these studies, which is more than likely driving some significance. We attempted to circumvent these issues by stratifying not only by ethnicity but also by geographical origin. Further, a drawback of meta-analyses is the often-high level of heterogeneity reported, which is a natural and routine phenomenon in meta-analyses involving population studies due to their clinical and methodological differences.[97] The majority of the included studies had a NOS score between 6 and 8. The main study limitation noted within individual studies was a lack of specificity on the definition and selection of controls. Further, study design varied greatly, with almost 90% of studies not age- and gender-matching their controls during recruitment, which undermines their comparability. Beyond any clinical or methodological differences between studies, publication bias could also be a contributing factor, as was noted for the analyses for the G allele in CD susceptibility (p-value: 0.008), which also carried high levels of heterogeneity (I^2: 82.67%, p-value: 0.000). By carefully implementing a NOS scoring system, applying the random effects model, a dual test of heterogeneity (Cochran's Q-statistic and I^2-statistic), and performing a meta-regression, we endeavoured to minimise subjectivity and the effects of heterogeneity but cannot entirely exclude residual biases. In addition, we note other limitations in our approach of this meta-analysis; (1) not all studies clearly denoted what minor allele they were reporting on, and (2) some of the stratified analyses were constrained due to limited statistical power. More epidemiological data on IBD incidence in understudied populations and ethnicities (African, Latin American) are needed to supplement the findings here, as the current data is still too heavily saturated in Western regions (North America and Europe). We also cannot ignore environmental influence and its role in the increasing rates of IBD in these new regions, which has been deemed too fast in the past 60 years to be purely explained by changes in the genetic make-up of these populations.[92,98]

To summarise, we provide increasing evidence for the use of *ATG16L1* rs2241880 as a clinical biomarker in IBD susceptibility and clinical outcomes, especially for patients of Caucasian ethnicities residing in North America and Europe, and those of Latin American ancestry. The G allele remains a significant risk factor for CD susceptibility, whereas the A allele illustrates a protective role in both CD and UC development. Due to the large number of studies and patients included, we provide novel insights into the role of rs2241880 on the clinical features of CD, that earlier meta-analyses failed to achieve[52] which includes a significant role for the G allele in the predisposition to perianal disease.

AUTHORS' CONTRIBUTIONS

IS and NCR were involved in conception and design of the study, including the development of research questions and inclusion/exclusion criteria, acquisition of data, analysis, and interpretation of the data, and drafting of the article. IH, RT, KSC, SYW, WSL and SR were involved in conception of the study and in critical revisions of the manuscript. All authors approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and in the accompanying online supplementary material.

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REFERENCES

1. Podolsky DK. Inflammatory bowel disease. N Engl J Med. 1991; 325(13):928–37. https://doi.org/10.1056/nejm199109263251306
2. Levine JS, Burakoff R. Extraintestinal manifestations of inflammatory bowel disease. Gastroenterol Hepatol. 2011;7(4):235–41.
3. Collaborators GBDIBD, Sepanlou SG, Ikuta K, Vahedi H, Bisignano C, Safiri S, et al. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol. 2020;5(1):17–30. https://doi.org/10.1016/s2468-1253(19)30333-4
4. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2018;390(10114):2769–78. https://doi.org/10.1016/s0140-6736(17)32448-0
5. Nguyen GC, Chong CA, Chong RY. National estimates of the burden of inflammatory bowel disease among racial and ethnic groups in the United States. J Crohns Colitis. 2014;8(4):288–95. https://doi.org/10.1016/j.crohns.2013.09.001
6. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012;491(7422):119–24. https://doi.org/10.1038/nature11582
7. Liu JZ, van Sommeren S, Huang H, Ng SC, Alberts R, Takahashi A, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet. 2015;47(9):979–86. https://doi.org/10.1038/ng.3359
8. Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet. 2007;39(2):207–11. https://doi.org/10.1038/ng1954
9. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet. 2007;39(5):596–604. https://doi.org/10.1038/ng2032
10. Mizushima N. Autophagy: process and function. Genes Dev. 2007;21(22):2861–73. https://doi.org/10.1101/gad.1599207
11. Qian M, Fang X, Wang X. Autophagy and inflammation. Clin Transl Med. 2017;6(1):24. https://doi.org/10.1186/s40169-017-0154-5
12. 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(10):e3391. https://doi.org/10.1371/journal.pone.0003391
13. Lassen KG, Kuballa P, Conway KL, Patel KK, Becker CE, Peloquin JM, et al. Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense. Proc Natl Acad Sci U S A. 2014;111(21):7741–6. https://doi.org/10.1073/pnas.1407001111
14. Murthy A, Li Y, Peng I, Reichelt M, Katakam AK, Noubade R, et al. A Crohn's disease variant in Atg16l1 enhances its degradation by caspase 3. Nature. 2014;506(7489):456–62. https://doi.org/10.1038/nature13044
15. Larabi A, Barnich N, Nguyen HTT. New insights into the interplay between autophagy, gut microbiota and inflammatory responses in IBD. Autophagy. 2020;16(1):38–51. https://doi.org/10.1080/15548627.2019.1635384
16. Grigoras CA, Ziakas PD, Jayamani E, Mylonakis E. ATG16L1 and IL23R variants and genetic susceptibility to crohn's disease: mode of inheritance based on meta-analysis of genetic association studies. Inflamm Bowel Dis. 2015;21(4):768–76. https://doi.org/10.1097/mib.0000000000000305
17. Zhang B.-B, Liang Y, Yang B, Tan Y.-J. Association between ATG16L1 gene polymorphism and the risk of Crohn's disease. J Int Med Res. 2017;45(6):1636–50. https://doi.org/10.1177/0300060516662404
18. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097. https://doi.org/10.1371/journal.pmed.1000097
19. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Oxford; 2000. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 10 August 2022.
20. Chang BH, Hoaglin DC. Meta-analysis of odds ratios: current good practices. Med Care. 2017;55(4):328–35. https://doi.org/10.1097/mlr.0000000000000696
21. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539–58. https://doi.org/10.1002/sim.1186
22. Aida I, Meddour Y, Kadiri H, Smara M, Bousseloub A, Kecili L, et al. T300A variant of AT16L1 gene in a cohort of Algerian Crohn disease patients. Curr Res Transl Med. 2018;66(1):9–14. https://doi.org/10.1016/j.retram.2018.01.002
23. Baptista ML, Amarante H, Picheth G, Sdepanian VL, Peterson N, Babasukumar U, et al. CARD15 and IL23R influences Crohn's disease susceptibility but not disease phenotype in a Brazilian population. Inflamm Bowel Dis. 2008;14(5):674–9. https://doi.org/10.1002/ibd.20372
24. Baradaran Ghavami S, Kabiri F, Nourian M, Balaii H, Shahrokh S, Chaleshi V, et al. Association between variants of the autophagy related gene ATG16L1 in inflammatory bowel diseases and clinical statues. Gastroenterol Hepatol Bed Bench. 2019;12((Suppl 1)):S94s100.
25. Büning C, Durmus T, Molnar T, de Jong DJ, Drenth JP, Fiedler 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(2):70–6. https://doi.org/10.1016/j.crohns.2007.08.001
26. Cotterill L, Payne D, Levinson S, McLaughlin J, Wesley E, Feeney M, 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(5):297–302. https://doi.org/10.1155/2010/480458
27. Csöngei V, Járomi L, Sáfrány E, Sipeky C, Magyari L, Polgár N, et al. Interaction of the major inflammatory bowel disease susceptibility alleles in Crohn's disease patients. World J Gastroenterol. 2010;16(2):176–83. https://doi.org/10.3748/wjg.v16.i2.176
28. Dalton JP, Desmond A, Shanahan F, Hill C. 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(3):195–205. https://doi.org/10.1007/s00430-014-0332-7
29. Deuring JJ, Fuhler GM, Konstantinov SR, Peppelenbosch MP, Kuipers EJ, de Haar C, et al. Genomic ATG16L1 risk allele-restricted Paneth cell ER stress in quiescent Crohn's disease. Gut. 2014;63(7):1081–91. https://doi.org/10.1136/gutjnl-2012-303527
30. Dusatkova P, Hradsky O, Lenicek M, Bronsky J, Nevoral J, Kotalova R, et al. Association of IL23R p.381Gln and ATG16L1 p.197Ala with Crohn disease in the Czech population. J Pediatr Gastroenterol Nutr. 2009;49(4):405–10. https://doi.org/10.1097/mpg.0b013e31819344ee
31. Eglinton TW, Roberts R, Pearson J, Barclay M, Merriman TR, Frizelle FA, et al. Clinical and genetic risk factors for perianal Crohn's disease in a population-based cohort. Am J Gastroenterol. 2012;107(4):589–96. https://doi.org/10.1038/ajg.2011.437

32. Fabio RR, Concetta RM, Giuseppe C, Sara R, Ambrogio O, Aurelio M, et al. ATG16L1 contribution to Crohn's disease risk in Sicily. Inflamm Bowel Dis. 2011;17(7):1635–6. https://doi.org/10.1002/ibd.21558
33. Fowler EV, Doecke J, Simms LA, Zhao ZZ, Webb PM, Hayward NK, 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(10): 2519–26. https://doi.org/10.1111/j.1572-0241.2008.02023.x
34. 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(1):81. https://doi.org/10.1186/1471-2350-9-81
35. Gazouli M, Pachoula I, Panayotou I, Mantzaris G, Chrousos G, Anagnou NP, et al. NOD2/CARD15, ATG16L1 and IL23R gene polymorphisms and childhood-onset of Crohn's disease. World J Gastroenterol. 2010;16(14):1753–8. https://doi.org/10.3748/wjg.v16.i14.1753
36. Glas J, Konrad A, Schmechel S, Dambacher J, Seiderer J, Schroff F, 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(3): 682–91. https://doi.org/10.1111/j.1572-0241.2007.01694.x
37. Gutiérrez A, Scharl M, Sempere L, Holler E, Zapater P, Almenta I, et al. Genetic susceptibility to increased bacterial translocation influences the response to biological therapy in patients with Crohn's disease. Gut. 2014;63(2):272–80. https://doi.org/10.1136/gutjnl-2012-303557
38. Hirano A, Yamazaki K, Umeno J, Ashikawa K, Aoki M, Matsumoto T, et al. Association study of 71 European Crohn's disease susceptibility loci in a Japanese population. Inflamm Bowel Dis. 2013;19(3): 526–33. https://doi.org/10.1097/mib.0b013e31828075e7
39. Hong SN, Park C, Park SJ, Lee CK, Ye BD, Kim YS, et al. Deep resequencing of 131 Crohn's disease associated genes in pooled DNA confirmed three reported variants and identified eight novel variants. Gut. 2016;65(5):788–96. https://doi.org/10.1136/gutjnl-2014-308617
40. Jung C, Colombel JF, Lemann M, Beaugerie L, Allez M, Cosnes J, et al. Genotype/phenotype analyses for 53 Crohn's disease associated genetic polymorphisms. PLoS One. 2012;7(12):e52223. https://doi.org/10.1371/journal.pone.0052223
41. Kee BP, Ng JG, Ng CC, Hilmi I, Goh KL, Chua KH. Genetic polymorphisms of ATG16L1 and IRGM genes in Malaysian patients with Crohn's disease. J Dig Dis. 2020;21(1):29–37. https://doi.org/10.1111/1751-2980.12829
42. Khan IA, Nayak B, Markandey M, Bajaj A, Verma M, Kumar S, et al. Differential prevalence of pathobionts and host gene polymorphisms in chronic inflammatory intestinal diseases: crohn's disease and intestinal tuberculosis. PLoS One. 2021;16(8):e0256098. https://doi.org/10.1371/journal.pone.0256098
43. Kiely CJ, Pavli P, O'Brien CL. The microbiome of translocated bacterial populations in patients with and without inflammatory bowel disease. Intern Med J. 2018;48(11):1346–54. https://doi.org/10.1111/imj.13998
44. Lakatos PL, Szamosi T, Szilvasi A, Molnar E, Lakatos L, Kovacs A, et al. ATG16L1 and IL23 receptor (IL23R) genes are associated with disease susceptibility in Hungarian CD patients. Dig Liver Dis. 2008;40(11):867–73. https://doi.org/10.1016/j.dld.2008.03.022
45. Lappalainen M, Halme L, Turunen U, Saavalainen P, Einarsdottir E, Färkkilä M, 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(8): 1118–24. https://doi.org/10.1002/ibd.20431
46. Latiano A, Palmieri O, Valvano MR, D'Incà R, Cucchiara S, Riegler G, 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(29):4643–51. https://doi.org/10.3748/wjg.14.4643
47. Lauriola M, Ugolini G, Rivetti S, Nanì S, Rosati G, Zanotti S, et al. IL23R, NOD2/CARD15, ATG16L1 and PHOX2B polymorphisms in a group of patients with Crohn's disease and correlation with subphenotypes. Int J Mol Med. 2011;27(3):469–77.
48. Márquez A, Núñez C, Martínez A, Mendoza JL, Taxonera C, Fernández-Arquero M, 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(11): 1697–704. https://doi.org/10.1002/ibd.21001
49. Mentzer A, Nayee S, Omar Y, Hullah E, Taylor K, Goel R, et al. Genetic association analysis reveals differences in the contribution of NOD2 variants to the clinical phenotypes of orofacial granulomatosis. Inflamm Bowel Dis. 2016;22(7):1552–8. https://doi.org/10.1097/mib.0000000000000844
50. Nakagome S, Takeyama Y, Mano S, Sakisaka S, Matsui T, Kawamura S, et al. Population-specific susceptibility to Crohn's disease and ulcerative Colitis; Dominant and recessive relative risks in the Japanese population. Ann Hum Genet. 2010;74(2):126–36. https://doi.org/10.1111/j.1469-1809.2010.00567.x
51. Okazaki T, Wang MH, Rawsthorne P, Sargent M, Datta LW, Shugart YY, 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(11): 1528–41. https://doi.org/10.1002/ibd.20512
52. Palomino-Morales RJ, Oliver J, Gómez-García M, López-Nevot MA, Rodrigo L, Nieto A, et al. Association of ATG16L1 and IRGM genes polymorphisms with inflammatory bowel disease: a meta-analysis approach. Genes Immun. 2009;10(4):356–64. https://doi.org/10.1038/gene.2009.25
53. Perricone C, Borgiani P, Romano S, Ciccacci C, Fusco G, Novelli G, et al. ATG16L1 Ala197Thr is not associated with susceptibility to Crohn's disease or with phenotype in an Italian population. Gastroenterology. 2008;134(1):368–70. https://doi.org/10.1053/j.gastro.2007.11.017
54. Peter I, Mitchell AA, Ozelius L, Erazo M, Hu J, Doheny D, et al. Evaluation of 22 genetic variants with Crohn's disease risk in the Ashkenazi Jewish population: a case-control study. BMC Med Genet. 2011;12(1):63. https://doi.org/10.1186/1471-2350-12-63
55. Prescott NJ, Fisher SA, Franke A, Hampe J, Onnie CM, Soars D, et al. A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5. Gastroenterology. 2007;132(5):1665–71. https://doi.org/10.1053/j.gastro.2007.03.034
56. Pugazhendhi S, Baskaran K, Santhanam S, Ramakrishna BS. Association of ATG16L1 gene haplotype with inflammatory bowel disease in Indians. PLoS One. 2017;12(5):e0178291. https://doi.org/10.1371/journal.pone.0178291
57. Quiroz-Cruz S, Posada-Reyes B, Alatorre-García T, Del Real-Calzada CM, García-Samper X, Escobar-Gutiérrez A, et al. Genetic polymorphisms present in IL10, IL23R, NOD2, and ATG16L1 associated with susceptibility to inflammatory bowel disease in Mexican population. Eur J Gastroenterol Hepatol. 2020;32(1):10–6. https://doi.org/10.1097/meg.0000000000001540
58. Scharl M, Wojtal KA, Becker HM, Fischbeck A, Frei P, Arikkat J, et al. Protein tyrosine phosphatase nonreceptor type 2 regulates autophagosome formation in human intestinal cells. Inflamm Bowel Dis. 2012;18(7):1287–302. https://doi.org/10.1002/ibd.21891
59. Scolaro BL, dos Santos E, Ferreira LE, França PHC, Kleinubing H, Kotze PG, et al. T300A genetic polymorphism: a susceptibility factor for Crohn's disease? Arq Gastroenterol. 2014;51(2):97–101. https://doi.org/10.1590/s0004-28032014000200005
60. Sventoraityte J, Zvirbliene A, Franke A, Kwiatkowski R, Kiudelis G, Kupcinskas L, et al. NOD2, IL23R and ATG16L1 polymorphisms in

Lithuanian patients with inflammatory bowel disease. World J Gastroenterol. 2010;16(3):359–64. https://doi.org/10.3748/wjg.v16.i3.359
61. Teimoori-Toolabi L, Samadpoor S, Mehrtash A, Ghadir M, Vahedi H. Among autophagy genes, ATG16L1 but not IRGM is associated with Crohn's disease in Iranians. Gene. 2018;675:176–84. https://doi.org/10.1016/j.gene.2018.06.074
62. Tsianos VE, Kostoulas C, Gazouli M, Frillingos S, Georgiou I, Christodoulou DK, et al. ATG16L1 T300A polymorphism is associated with Crohn's disease in a Northwest Greek cohort, but ECM1 T130M and G290S polymorphisms are not associated with ulcerative colitis. Ann Gastroenterol. 2020;33(1):38–44.
63. Van Limbergen J, Russell RK, Nimmo ER, Drummond HE, Smith L, Anderson NH, 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(3):338–46. https://doi.org/10.1002/ibd.20340
64. Wang MH, Okazaki T, Kugathasan S, Cho JH, Isaacs KL, Lewis JD, et al. Contribution of higher risk genes and European admixture to Crohn's disease in African Americans. Inflamm Bowel Dis. 2012;18(12):2277–87. https://doi.org/10.1002/ibd.22931
65. Waterman M, Xu W, Stempak JM, Milgrom R, Bernstein CN, Griffiths AM, et al. Distinct and overlapping genetic loci in crohn's disease and ulcerative colitis: correlations with pathogenesis. Inflamm Bowel Dis. 2011;17(9):1936–42. https://doi.org/10.1002/ibd.21579
66. Weersma RK, Zhernakova A, Nolte IM, Lefebvre C, Rioux JD, Mulder F, et al. ATG16L1 and IL23R are associated with inflammatory bowel diseases but not with celiac disease in The Netherlands. Am J Gastroenterol. 2008;103(3):621–7. https://doi.org/10.1111/j.1572-0241.2007.01660.x
67. Wei SC, Ni YH, Yang HI, Su YN, Chang MC, Chang YT, et al. A hospital-based study of clinical and genetic features of Crohn's disease. J Formos Med Assoc. 2011;110(9):600–6. https://doi.org/10.1016/j.jfma.2011.07.009
68. 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 Japanese patients. J Hum Genet. 2007;52(7):575–83. https://doi.org/10.1007/s10038-007-0156-z
69. Yang SK, Park M, Lim J, Park SH, Ye BD, Lee I, et al. Contribution of IL23R but not ATG16L1 to Crohn's disease susceptibility in Koreans. Inflamm Bowel Dis. 2009;15(9):1385–90. https://doi.org/10.1002/ibd.20921
70. Zhang M, Wang XY, Jiang XD, Yang X, Wen C, Zhi M, et al. Polymorphisms of the tnf gene and three susceptibility loci are associated with crohn's disease and perianal fistula crohn's disease: a study among the han population from South China. Med Sci Monit. 2019;25:9637–50. https://doi.org/10.12659/msm.917244
71. Zhang T, DeSimone RA, Jiao X, Rohlf FJ, Zhu W, Gong QQ, et al. Host genes related to Paneth cells and xenobiotic metabolism are associated with shifts in human ileum-associated microbial composition. Plos One Article. 2012;7(6):e30044. https://doi.org/10.1371/journal.pone.0030044
72. Amre DK, Mack DR, Morgan K, Krupoves A, Costea I, Lambrette P, et al. Autophagy gene ATG16L1 but not IRGM is associated with Crohn's disease in Canadian children. Inflamm Bowel Dis. 2009;15(4):501–7. https://doi.org/10.1002/ibd.20785
73. Baldassano RN, Bradfield JP, Monos DS, Kim CE, Glessner JT, Casalunovo T, et al. Association of the T300A non-synonymous variant of the ATG16L1 gene with susceptibility to paediatric Crohn's disease. Gut. 2007;56(8):1171–3. https://doi.org/10.1136/gut.2007.122747
74. Chinnadurai R, Copland IB, Ng S, Garcia M, Prasad M, Arafat D, et al. Mesenchymal stromal cells derived from crohn's patients deploy indoleamine 2,3-dioxygenase-mediated immune suppression, independent of autophagy. Mol Ther. 2015;23(7):1248–61. https://doi.org/10.1038/mt.2015.67
75. Jakobsen C, Cleynen I, Andersen PS, Vermeire S, Munkholm P, Paerregaard A, et al. Genetic susceptibility and genotype-phenotype association in 588 Danish children with inflammatory bowel disease. J Crohns Colitis. 2014;8(7):678–85. https://doi.org/10.1016/j.crohns.2013.12.010
76. Lacher M, Schroepf S, Ballauff A, Lohse P, von Schweinitz D, Kappler R, et al. Autophagy 16-like 1 rs2241880 G allele is associated with Crohn's disease in German children. Acta Paediatr. 2009;98(11):1835–40. https://doi.org/10.1111/j.1651-2227.2009.01438.x
77. Na SY, Park SS, Seo JK. Genetic polymorphisms in autophagy-associated genes in Korean children with early-onset crohn disease. J Pediatr Gastroenterol Nutr. 2015;61(3):285–91. https://doi.org/10.1097/mpg.0000000000000796
78. Peterson N, Guthery S, Denson L, Lee J, Saeed S, Prahalad S, et al. Genetic variants in the autophagy pathway contribute to paediatric Crohn's disease. Gut. 2008;57(9):1336–7. https://doi.org/10.1136/gut.2008.152207
79. Pranculienė G, Steponaitienė R, Skiecevičienė J, Kučinskienė R, Kiudelis G, Adamonis K, et al. Associations between NOD2, IRGM and ORMDL3 polymorphisms and pediatric-onset inflammatory bowel disease in the Lithuanian population. Med Kaunas. 2016;52(6):325–30. https://doi.org/10.1016/j.medici.2016.11.006
80. Roberts RL, Gearry RB, Hollis-Moffatt JE, Miller AL, Reid J, Abkevich V, 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(12):2754–61. https://doi.org/10.1111/j.1572-0241.2007.01525.x
81. Lan Q, Shen M, Garcia-Rossi D, Chanock S, Zheng T, Berndt SI, et al. Genotype frequency and F ST analysis of polymorphisms in immunoregulatory genes in Chinese and Caucasian populations. Immunogenetics. 2007;59(11):839–52. https://doi.org/10.1007/s00251-007-0253-3
82. Mattei J, Parnell LD, Lai CQ, Garcia-Bailo B, Adiconis X, Shen J, et al. Disparities in allele frequencies and population differentiation for 101 disease-associated single nucleotide polymorphisms between Puerto Ricans and non-Hispanic whites. BMC Genet. 2009;10(1):45. https://doi.org/10.1186/1471-2156-10-45
83. Ioannidis JP, Ntzani EE, Trikalinos TA. Racial' differences in genetic effects for complex diseases. Nat Genet. 2004;36(12):1312–8. https://doi.org/10.1038/ng1474
84. Kido T, Sikora-Wohlfeld W, Kawashima M, Kikuchi S, Kamatani N, Patwardhan A, et al. Are minor alleles more likely to be risk alleles? BMC Med Genomics. 2018;11(1):3. https://doi.org/10.1186/s12920-018-0322-5
85. Kelsen J, Baldassano RN. Inflammatory bowel disease: the difference between children and adults. Inflamm Bowel Dis. 2008;14(Suppl l_2):S9–11. https://doi.org/10.1002/ibd.20560
86. Duricova D, Burisch J, Jess T, Gower-Rousseau C, Lakatos PL, Epi-Com E. Age-related differences in presentation and course of inflammatory bowel disease: an update on the population-based literature. J Crohns Colitis. 2014;8(11):1351–61. https://doi.org/10.1016/j.crohns.2014.05.006
87. United Nations Statistics Division. Standard country or area codes for statistical use (m49). 2019. https://unstats.un.org/unsd/methodology/m49/. Accessed 7 December 2022.
88. de Zoeten EF, Pasternak BA, Mattei P, Kramer RE, Kader HA. Diagnosis and treatment of perianal Crohn disease: NASPGHAN clinical report and consensus statement. J Pediatr Gastroenterol Nutr. 2013;57(3):401–12. https://doi.org/10.1097/mpg.0b013e3182a025ee
89. Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med. 2009;361(21):2033–45. https://doi.org/10.1056/nejmoa0907206

90. Denson LA. The role of the innate and adaptive immune system in pediatric inflammatory bowel disease. Inflamm Bowel Dis. 2013;19(9):2011–20. https://doi.org/10.1097/mib.0b013e318281f590
91. Shi HY, Levy AN, Trivedi HD, Chan FKL, Ng SC, Ananthakrishnan AN. Ethnicity influences phenotype and outcomes in inflammatory bowel disease: a systematic review and meta-analysis of population-based studies. Clin Gastroenterol Hepatol. 2018;16(2):190–7. https://doi.org/10.1016/j.cgh.2017.05.047
92. Ng SC. Emerging trends of inflammatory bowel disease in Asia. Gastroenterol Hepatol. 2016;12(3):193–6.
93. Vermeire S, Van Assche G, Rutgeerts P. Perianal Crohn's disease: classification and clinical evaluation. Dig Liver Dis. 2007;39(10):959–62. https://doi.org/10.1016/j.dld.2007.07.153
94. Latiano A, Palmieri O, Cucchiara S, Castro M, D'Incà R, Guariso G, et al. Polymorphism of the IRGM gene might predispose to fistulizing behavior in Crohn's disease. Am J Gastroenterol. 2009;104(1):110–6. https://doi.org/10.1038/ajg.2008.3
95. Fisher SA, Tremelling M, Anderson CA, Gwilliam R, Bumpstead S, Prescott NJ, et al. Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. Nat Genet. 2008;40(6):710–2. https://doi.org/10.1038/ng.145
96. Shi J, Zhou L, Zhernakova A, Qian J, Zhu F, Sun G, et al. Haplotype-based analysis of ulcerative colitis risk loci identifies both IL2 and IL21 as susceptibility genes in Han Chinese. Inflamm Bowel Dis. 2011;17(12):2472–9. https://doi.org/10.1002/ibd.21652
97. Melsen WG, Bootsma MC, Rovers MM, Bonten MJ. The effects of clinical and statistical heterogeneity on the predictive values of results from meta-analyses. Clin Microbiol Infect. 2014;20(2):123–9. https://doi.org/10.1111/1469-0691.12494
98. Benchimol EI, Fortinsky KJ, Gozdyra P, Van den Heuvel M, Van Limbergen J, Griffiths AM. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. Inflamm Bowel Dis. 2011;17(1):423–39. https://doi.org/10.1002/ibd.21349

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