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Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations

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Ulcerative colitis and Crohn's disease are the two main forms of inflammatory bowel disease (IBD). Here we report the first trans-ancestry association study of IBD, with genome-wide or Immunochip genotype data from an extended cohort of 86,640 European individuals and Immunochip data from 9,846 individuals of East Asian, Indian or Iranian descent. We implicate 38 loci in IBD risk for the first time. For the majority of the IBD risk loci, the direction and magnitude of effect are consistent in European and non-European cohorts. Nevertheless, we observe genetic heterogeneity between divergent populations at several established risk loci driven by differences in allele frequency (*NOD2*) or effect size (*TNFSF15* and *ATG16L1*) or a combination of these factors (*IL23R* and *IRGM*). Our results provide biological insights into the pathogenesis of IBD and demonstrate the usefulness of trans-ancestry association studies for mapping loci associated with complex diseases and understanding genetic architecture across diverse populations.

IBD is composed of chronic, relapsing intestinal inflammatory diseases affecting more than 2.5 million people in Europe, with increasing prevalence in Asia and developing countries^{1,2}. IBD is thought to arise from inappropriate activation of the intestinal mucosal immune system in response to commensal bacteria in a genetically susceptible host.

Thus far, 163 genetic loci have been associated with IBD via large-scale genome-wide association studies (GWAS) in cohorts of European descent. Smaller GWAS performed in populations from Japan, India and Korea have reported six new genome-wide significant associations

outside of the human leukocyte antigen (HLA) region. Three of these loci (13q12, *FCGR2A* and *SLC26A3*) subsequently achieved genome-wide significant evidence of association in European cohorts. The remaining three loci demonstrated a consistent direction of effect and nominally significant evidence of association ($P < 1 \times 10^{-4}$) in previous European GWAS analyses^{3–6}. A number of loci initially associated with IBD in European cohorts have now also been shown to underlie risk in non-Europeans, including *JAK2*, *IL23R* and *NKX2-3*. The evidence of shared IBD risk loci across diverse populations

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suggests that combining genotype data from cohorts of different ancestry will enable the detection of additional IBD-associated loci. Such trans-ancestry association studies have successfully identified susceptibility loci for other complex diseases, including type 2 diabetes and rheumatoid arthritis^{7,8}.

In this study, we aggregate genome-wide or ImmunoChip genotype data from 96,486 individuals. In comparison to our previously published GWAS meta-analysis, this study includes an additional 11,535 individuals of European ancestry and 9,846 individuals of non-European ancestry. Using these data, we aim to identify new IBD risk loci and compare the genetic architecture of IBD susceptibility across ancestrally divergent populations.

RESULTS

Study design

After quality control and 1000 Genomes Project imputation (Phase I–August 2012), we used 5,956 Crohn's disease cases, 6,968 ulcerative colitis cases and 21,770 population controls of European descent to perform GWAS of Crohn's disease, ulcerative colitis and IBD (Crohn's disease and ulcerative colitis together) (Online Methods). Replication was undertaken using an additional 16,619 Crohn's disease cases, 13,449 ulcerative colitis cases and 31,766 population controls genotyped on the ImmunoChip. The replication cohort included 2,025 Crohn's disease cases, 2,770 ulcerative colitis cases and 5,051 population controls of non-European ancestry (Table 1 and Supplementary Figs. 1 and 2), so principal-component analysis was used to assign individuals to 1 of 4 ancestral groups (European, Iranian, Indian or East Asian) (Supplementary Fig. 3). Case-control association tests were performed within each ancestry group using a linear mixed model (MMM)⁹ (Online Methods). A fixed-effects meta-analysis was undertaken to combine the summary statistics from our European-only GWAS meta-analysis with those from the European replication cohort. We next performed a Bayesian trans-ancestry meta-analysis, as implemented in MANTRA, to enable heterogeneity in effect sizes to be correlated with the genetic distance between populations, as estimated by the mean fixation index (F_{ST}) across all SNPs¹⁰ (Online Methods). For the trans-ancestry meta-analysis, the 6,392 cases and 7,262 population controls of European ancestry that were present in both the GWAS and replication cohorts were excluded from the ImmunoChip replication study (Supplementary Fig. 2). To maximize power for our solely ImmunoChip-based comparisons across ancestral groups, the mixed-model association analysis was repeated after reinstating these individuals in the ImmunoChip cohort.

Trans-ancestry meta-analysis identifies 38 new IBD loci

In total, 38 new disease-associated loci were identified at genome-wide significance in either the association analysis of individual ancestry groups ($P < 5 \times 10^{-8}$) or the trans-ancestry meta-analysis that included all ancestries ($\log_{10}(\text{Bayes factor}) > 6$) for ulcerative colitis,

Crohn's disease or IBD (Table 2, Supplementary Figs. 4–7 and Supplementary Tables 1 and 2). To reduce false positive associations, we required all loci only implicated in disease risk via the trans-ancestry meta-analysis (with $\log_{10}(\text{Bayes factor}) > 6$ but $P > 5 \times 10^{-8}$ in each individual ancestry cohort) to show no significant evidence of heterogeneity across all four ancestry groups ($I^2 > 85.7\%$) (Online Methods and Supplementary Table 3).

Twenty-five of the 38 newly associated loci overlapped with loci previously reported for other traits, including immune-mediated diseases, whereas 13 had not previously been associated with any disease or trait (Online Methods and Supplementary Table 4). A likelihood-modeling approach showed that 27 of the 38 newly identified loci were associated with both Crohn's disease and ulcerative colitis (designated here as IBD-associated loci), with 7 of these loci demonstrating evidence of heterogeneity of effect between the 2 diseases. Of the remaining 11 loci, 7 were classified as specific to Crohn's disease and 4 were classified as specific to ulcerative colitis (Table 2 and Supplementary Table 1).

As a result of our updated sample quality control procedure, 17 of the 194 independent SNPs reported at genome-wide significance in our previous European-only GWAS meta-analysis⁶ failed to reach this significance threshold in the present study. Sixteen of these loci still demonstrated strong suggestive evidence of association in the current European cohort ($5 \times 10^{-8} < P < 8.7 \times 10^{-6}$, representing a false discovery rate (FDR) of ~ 0.001) (Supplementary Table 1). SNP rs2226628 on chromosome 11 failed to achieve even suggestive evidence of association in our current European association analysis ($P = 0.0024$). Our previous European-only meta-analysis incorporated a number of principal components as covariates in a logistic regression test of association, and, interestingly, if we adopted the approach taken by Jostins *et al.*⁶, we observed a more significant P value of 7.38×10^{-6} for this SNP. This observation, together with the divergent allele frequencies at this SNP across European populations (1000 Genomes Project release 14: GBR (British in England and Scotland), 0.20; CEU (Utah residents of Western European ancestry), 0.28; IBS (Spanish Iberian), 0.39; FIN (Finnish), 0.47) suggests that the previously reported signal of association might have been driven, at least in part, by population stratification (which is now better accounted for in the linear mixed-model analysis)⁶. In summary, we now consider 231 independent SNPs within 200 loci to be associated with IBD risk (Supplementary Table 2).

Forty-one of the 163 IBD-associated SNPs originally identified in our previous European-only GWAS meta-analysis replicated in at least one non-European cohort if we considered a one-tailed Bonferroni-corrected significance threshold of $P < 6.1 \times 10^{-4}$ (0.05/163) (Supplementary Table 1). Nine of the 14 non-HLA loci (10 for Crohn's disease and 4 for ulcerative colitis) that had been identified at genome-wide levels of significance in previous non-European GWAS cohorts from Japan, India and Korea^{3,4,11–13} were associated with either Crohn's disease or ulcerative colitis in the East Asian, Indian and/or Iranian cohorts with $P < 1.0 \times 10^{-5}$ (Supplementary Table 5). Four of the five remaining SNPs (or reliable proxy SNPs) were not present on the ImmunoChip. The previously reported association at rs2108225 (*SLC26A3*) on chromosome 7 showed an association signal at $P = 2.64 \times 10^{-3}$ in the current East Asian cohort but was strongly associated with IBD in the European cohort ($P = 1.04 \times 10^{-18}$).

We next performed a series of analyses to prioritize genes within the newly associated loci for causality. *Cis*-eQTL (expression quantitative trait locus) analysis from two data sets of peripheral blood samples from a total of 1,240 individuals showed that 12 of the 38 newly associated SNPs had *cis*-eQTL effects (FDR < 0.05) (Online Methods and Supplementary Table 6). Two SNPs showed *trans*-eQTL effects.

Table 1 Cohort sample sizes for GWAS and ImmunoChip trans-ancestry meta-analysis

Population	Crohn's disease		Ulcerative colitis		IBD	
	Cases	Controls	Cases	Controls	Cases	Controls
European GWAS	5,956	14,927	6,968	20,464	12,882	21,770
European ImmunoChip	14,594	26,715	10,679	26,715	25,273	26,715
Non-European ImmunoChip	2,025	5,051	2,770	5,051	4,795	5,051
Total	22,575	46,693	20,417	52,230	42,950	53,536

Table 2 Newly associated IBD risk loci

Chr.	SNP	Position (bp)	Reference allele ^a	Best phenotype ^b	LR phenotype ^c	log ₁₀ (Bayes factor) ^d	Het. (ρ^2) ^e	European OR	European <i>P</i>	Candidate gene(s)
1	rs1748195	63,049,593	G	CD	CD	6.08	0	1.07 (1.04–1.10)	7.13×10^{-8}	<i>USP1</i>
1	rs34856868	92,554,283	A	IBD	IBD_U	6.16	0	0.82 (0.77–0.88)	9.80×10^{-9}	<i>BTBD8</i>
1	rs11583043	101,466,054	A	UC	IBD_U	8.34	66.5	1.08 (1.05–1.11)	6.05×10^{-8}	<i>SLC30A4</i> , <i>EDG1</i>
1	rs6025	169,519,049	A	IBD	IBD_U	6.43	0	0.84 (0.79–0.89)	2.51×10^{-8}	<i>SELP</i> , <i>SELE</i> , <i>SELL</i>
1	rs10798069	186,875,459	A	CD	IBD_S	7.24	0	0.93 (0.91–0.95)	4.25×10^{-9}	<i>PTGS2</i> , <i>PLA2G4A</i>
1	rs7555082	198,598,663	A	CD	IBD_U	7.97	0	1.13 (1.09–1.17)	1.47×10^{-10}	<i>PTPRC</i>
2	rs11681525	145,492,382	C	CD	CD	8.8	59.3	0.86 (0.82–0.90)	4.08×10^{-11}	–
2	rs4664304	160,794,008	A	IBD	IBD_U	6.34	0	1.06 (1.04–1.08)	2.61×10^{-8}	<i>MARCH7</i> , <i>LY75</i> , <i>PLA2R1</i>
2	rs3116494	204,592,021	G	UC	IBD_S	7.03	0	1.08 (1.05–1.11)	1.30×10^{-7}	<i>ICOS</i> , <i>CD28</i> , <i>CTLA4</i>
2	rs111781203	228,660,112	G	IBD	IBD_U	10.04	0	0.94 (0.92–0.96)	2.16×10^{-10}	<i>CCL20</i>
2	rs35320439	242,737,341	G	CD	IBD_S	7.71	0	1.09 (1.06–1.12)	9.89×10^{-10}	<i>PDCD1</i> , <i>ATG4B</i>
3	rs113010081	46,457,412	G	UC	IBD_U	7.45	0	1.14 (1.09–1.19)	9.02×10^{-10}	<i>FLJ78302</i> , <i>LTF</i> , <i>CCR1</i> , <i>CCR2</i> , <i>CCR3</i> , <i>CCR5</i>
3	rs616597	101,569,726	A	UC	UC	6.68	54.7	0.93 (0.90–0.96)	9.34×10^{-6}	<i>NFKBIZ</i>
3	rs724016	141,105,570	G	CD	CD	7.41	70.9	1.06 (1.04–1.09)	3.36×10^{-6}	–
4	rs2073505	3,444,503	A	IBD	IBD_U	6.87	0	1.10 (1.06–1.14)	1.46×10^{-7}	<i>HGFAC</i>
4	rs4692386	26,132,361	A	IBD	IBD_U	6.47	0	0.94 (0.92–0.96)	1.21×10^{-8}	–
4	rs6856616	38,325,036	G	IBD	IBD_U	9.78	61.6	1.10 (1.06–1.14)	9.72×10^{-7}	–
4	rs2189234	106,075,498	A	UC	UC	8.85	0	1.08 (1.05–1.11)	1.95×10^{-10}	–
5	rs395157	38,867,732	A	IBD	IBD_U	19.5	0	1.10 (1.08–1.12)	2.22×10^{-20}	<i>OSMR</i> , <i>FYB</i> , <i>LIFR</i>
5	rs4703855	71,693,899	A	IBD	IBD_U	6.83	70.3	0.93 (0.91–0.95)	7.16×10^{-11}	–
5	rs564349	172,324,978	G	IBD	IBD_U	8.12	37.5	1.06 (1.04–1.08)	1.54×10^{-7}	<i>C5orf4</i> , <i>DUSP1</i>
6	rs7773324	382,559	G	CD	IBD_U	7.67	0	0.92 (0.90–0.94)	1.06×10^{-9}	<i>IRF4</i> , <i>DUSP22</i>
6	rs13204048	3,420,406	G	CD	IBD_S	7.23	53.5	0.93 (0.91–0.95)	2.89×10^{-8}	–
6	rs7758080	149,577,079	G	CD	IBD_S	7.88	0	1.08 (1.05–1.11)	7.27×10^{-9}	<i>MAP3K7IP2</i>
7	rs1077773	17,442,679	G	UC	UC	5.86	76.7	0.93 (0.91–0.95)	5.96×10^{-9}	<i>AHR</i>
7	rs2538470	148,220,448	A	IBD	IBD_U	10.93	54.6	1.07 (1.05–1.09)	3.00×10^{-11}	<i>CNTNAP2</i>
8	rs17057051	27,227,554	G	IBD	IBD_U	6.74	15.9	0.94 (0.92–0.96)	5.50×10^{-8}	<i>PTK2B</i> , <i>TRIM35</i> , <i>EPHX2</i>
8	rs7011507	49,129,242	A	UC	IBD_U	7.49	39.3	0.90 (0.87–0.93)	6.40×10^{-8}	–
10	rs3740415	104,232,716	G	IBD	IBD_U	6.26	0	0.95 (0.93–0.97)	1.03×10^{-7}	<i>NFKB2</i> , <i>TRIM8</i> , <i>TMEM180</i>
12	rs7954567	6,491,125	A	CD	CD	8.25	0	1.09 (1.06–1.12)	1.30×10^{-9}	<i>CD27</i> , <i>TNFRSF1A</i> , <i>LTBR</i>
12	rs653178	112,007,756	G	IBD	IBD_U	6.57	49.7	1.06 (1.04–1.08)	1.11×10^{-8}	<i>SH2B3</i> , <i>ALDH2</i> , <i>ATXN2</i>
12	rs11064881	120,146,925	A	IBD	IBD_U	7.02	31.7	1.10 (1.06–1.14)	5.95×10^{-8}	<i>PRKAB1</i>
13	rs9525625	43,018,030	A	CD	CD	8.55	37.3	1.08 (1.05–1.11)	1.41×10^{-9}	<i>AKAP1</i> , <i>TFSF11</i>
17	rs3853824	54,880,993	A	CD	IBD_S	8.46	50.4	0.92 (0.90–0.94)	1.17×10^{-10}	–
17	rs17736589	76,737,118	G	UC	UC	6.53	53.4	1.09 (1.06–1.12)	4.34×10^{-8}	–
18	rs9319943	56,879,827	G	CD	CD	6.33	33.4	1.08 (1.05–1.11)	9.05×10^{-7}	–
18	rs7236492	77,220,616	A	CD	IBD_S	6.6	0	0.91 (0.88–0.94)	9.09×10^{-9}	<i>NFATC1</i> , <i>TST</i>
22	rs727563	41,867,377	G	CD	CD	7.1	76	1.10 (1.07–1.13)	1.88×10^{-10}	<i>TEF</i> , <i>NHP2L1</i> , <i>PMM1</i> , <i>L3MBTL2</i> , <i>CHADL</i>

Loci for IBD, ulcerative colitis or Crohn's disease were identified through a trans-ancestry analysis of genome-wide and Immunochip genotype data from a cohort of 86,682 European individuals and 9,846 individuals of non-European descent. Loci achieving genome-wide significance ($P < 5 \times 10^{-8}$) in one of the individual cohorts of European, East Asian, Indian or Iranian descent or $\log_{10}$ (Bayes factor) > 6 in the combined trans-ancestry association analysis were considered to be significantly associated loci. Loci having $\log_{10}$ (Bayes factor) > 6 but $P > 5 \times 10^{-8}$ in each individual ancestral cohort were required to show no significant evidence of heterogeneity across all four ancestry groups ($\rho^2 > 85.7\%$). Association *P* values and odds ratios for the non-European cohorts are given in **Supplementary Table 1**. Candidate genes were identified by at least one of the gene prioritization methods we performed (eQTL, GRAIL, DAPPLE and coding SNP annotation (cSNP); see the main text and Online Methods). Genes in bold were prioritized by >2 gene prioritization strategies. UC, ulcerative colitis; CD, Crohn's disease; IBD, inflammatory bowel disease; chr., chromosome; OR, odds ratio.

^aThe minor allele in the European cohort was chosen to be the reference allele. ^bPhenotype with the largest MANTRA Bayes factor. ^cThe preferred phenotype (ulcerative colitis, Crohn's disease or IBD) from our likelihood-modeling approach classifying loci according to their relative strength of association. LR, likelihood ratio. IBD_S and IBD_U refer to the IBD saturated and IBD unsaturated models, respectively (see the main text and Online Methods). ^dMANTRA $\log_{10}$ (Bayes factor). ^eHeterogeneity ρ^2 percentage.

SNP rs653178 in a locus harboring *SH2B3* and *ATXN2* is associated with multiple other immune-mediated diseases, including celiac disease and rheumatoid arthritis. It had *trans*-eQTL effects on 14 genes, including genes within IBD-associated loci (*TAGAP* and *STAT1*). rs616597 had a *cis*-eQTL effect on *NFKBIZ* and had *trans*-eQTL effects on *FLXB13* (**Supplementary Table 6**) (ref. 14). Both SNPs reside in known DNase I hypersensitivity and histone modification sites in multiple cell lines (**Supplementary Table 7**). In contrast to the high number of SNPs tagging eQTLs, only 3 of the 38 SNPs were in high linkage disequilibrium (LD; $r^2 > 0.8$) with known missense coding variants (**Supplementary Table 8**).

To enable a meaningful comparison with our previously published results, we recreated the GRAIL connectivity network using all loci that now achieved genome-wide significant evidence of association (**Supplementary Fig. 8**). Twelve genes in the previous GRAIL network were removed in this new network. We found that these genes had significantly larger GRAIL *P* values (Wilcoxon *P* value = 6×10^{-4}) and fewer interaction partners (11.2 versus 16.0) than genes remaining in the network. Sixty-two genes were connected into the GRAIL network for the first time, only 36 of which were located within the newly associated loci (including *NFKBIZ*, *CD28* and *OSMR*). Thus, 26 genes from previously established IBD loci are brought into the network

Box 1 Select candidate genes in the newly associated IBD susceptibility loci

PTGS2 encodes COX-2, an enzyme that converts arachidonic acid into prostaglandins and that is the pharmacological target of non-steroidal anti-inflammatory drugs (NSAIDs). Prostaglandins were once thought to be exclusively proinflammatory (hence the anti-inflammatory moniker of NSAIDs), although there is now increasing evidence that some may have important anti-inflammatory roles through inhibiting T cell activation and promoting regulatory T cell development²⁵. Consistent with this new evidence, NSAIDs are generally avoided in IBD, as they are known to precipitate disease flares.

LY75 encodes DEC-205 (also known as CD205), a cell surface receptor that is highly expressed on dendritic cells and is involved in the endocytosis of extracellular antigens and their presentation on major histocompatibility complex (MHC) class I molecules²⁶. This receptor has been shown to have an important role in T cell function and homeostasis²⁷.

CD28 encodes a key co-stimulatory molecule that has an important role in T cell activation. The corresponding locus contains other genes that are also involved in T cell co-stimulation, including *ICOS* and *CTLA4*. Stimulation of T cells in the absence of co-stimulatory signal typically leads to anergy—one of the three main processes that can bring about tolerance, an important means of preventing aberrant immunological responses to intestinal antigens.

CCL20 encodes a chemokine that is produced by the intestinal epithelium²⁸ and that binds and activates CCR6. This interaction is important in regulating the migration of T cells (especially regulatory T cells) and dendritic cells to the gut, with increased production of CCL20 being detectable during inflammation²⁹. Consistent with this evidence, IBD in mouse models is modulated by the absence of CCR6 (ref. 30). The *CCR6* locus is itself associated with IBD.

NFKBIZ encodes NF- κ B inhibitor ζ (NFKBIZ), an inducible regulator of NF- κ B. This protein has been shown to have several functions, including roles in natural killer cell activation³¹ and monocyte recruitment³². Recently, however, NFKBIZ has also been shown to be a critical regulator of T_H17 cell development through its interaction with ROR nuclear receptors³³. Accordingly, this association further underlines the importance of T_H17 cells in IBD pathogenesis.

OSMR encodes the oncostatin M receptor, a cytokine receptor component that heterodimerizes with other proteins to form both the oncostatin M receptor and the IL-31 receptor. Oncostatin M is present at elevated levels in biopsies from patients with active IBD and is thought to promote intestinal epithelial cell proliferation and wound healing, thereby augmenting the barrier function of the intestinal epithelium in intestinal inflammation¹⁶.

AHR encodes the aryl hydrocarbon receptor, a ligand-activated transcription factor that can bind to a range of aromatic hydrocarbons, including several compounds derived from dietary components. This receptor is highly expressed on T_H17 cells, and its ligation leads to their expansion and enhanced production of cytokines, including IL-22 (ref. 34). Moreover, deficiency of this receptor (or its ligands) also disrupts intraepithelial lymphocyte homeostasis, leading to failure to control intestinal microbial load and composition and aberrant immune activation resulting in epithelial damage³⁵. Accordingly, this association further highlights the importance of the interaction between genes and the environment in IBD pathogenesis.

PTK2B encodes protein tyrosine kinase β 2 (also known as Pyk2), an important intracellular kinase for diverse signaling pathways, including mitogen-activated protein kinase (MAPK) and JNK signaling. Its functions include roles in monocyte migration and neutrophil degranulation.

NFATC1 encodes nuclear factor of activated T cells, cytoplasmic 1, an NFAT transcription factor that is specifically expressed upon activation of T and B cells following ligation of their respective receptors. This expression supports lymphocyte proliferation and inhibits activation-induced cell death, leading to enhanced immune responses³⁶. NFAT transcription factors are the main molecular targets of calcineurin inhibitors, such as cyclosporine, which are used in the treatment of IBD.

for the first time, 12 of which are the only GRAIL gene reported for the corresponding locus, including *TAGAP* and *IKZF1*. Genes within the 16 previously associated loci that failed to reach genome-wide significance in our current study had similar average connectivities as other genes in the network (17.8 versus 16.4, respectively; Wilcoxon P value = 0.94), thus further supporting their likely involvement in IBD risk. Thirty-seven of 56 DAPPLE candidate genes were identified as candidates in the GRAIL analysis (**Supplementary Table 9**).

Biological implications of newly associated IBD loci

Previous GWAS analyses have highlighted components in several key pathways underlying IBD susceptibility, many involved in innate immunity, T cell signaling and epithelial barrier function. Accepting the need for fine mapping to pinpoint causal variants within the newly identified loci, the current study expands the range of pathways implicated.

The process of autophagy, which is an intracellular process during which cytoplasmic content is engulfed by double-membrane autophagosomes and delivered to the vacuole or lysosome for degradation and recycling, has been implicated in Crohn's disease pathogenesis since the identification of *ATG16L1* and *IRGM* as Crohn's disease susceptibility genes¹⁵. The newly identified Crohn's disease gene *ATG4B* encodes a cysteine protease with a central role in this process, reinforcing the importance of autophagy in Crohn's disease pathogenesis. Likewise, the importance of epithelial barrier function in IBD pathogenesis (previously highlighted by associations with *LAMB1* and *HNF4A*¹⁶) is underscored by the new association at *OSMR*, which modulates a barrier-protective host response in intestinal inflammation.

Many of the newly identified candidate genes, including *LY75*, *CD28*, *CCL20*, *NFKBIZ*, *AHR* and *NFATC1*, modulate specific aspects

of the T cell response. Thus, beyond the involvement of type 17 helper T (T_H17) cells (previously identified through associations with, for example, *IL23R*), our results now implicate all three components of T cell activation (TCR ligation, co-stimulation and interleukin (IL)-2 signaling). Notably, these processes are critical for the development of immunological memory and are common to both CD4⁺ and CD8⁺ T cells.

The functions of leading new positional candidate genes are discussed in **Box 1**.

Comparing non-European IBD with European IBD

Recent large-scale trans-ancestry genetic studies of complex diseases have shown that the majority of risk-associated loci are shared across divergent populations^{8,17,18}. The true extent of sharing is difficult to characterize because the sizes of non-European cohorts are often much smaller than their European counterparts, limiting power to detect associated loci. Despite our study including a large cohort of 9,846 non-European samples and being the largest non-European study of IBD thus far, this sample size is still small in comparison with the European cohort of 86,640 individuals. As such, we expect that the majority of known risk loci will not be associated in the non-European populations at genome-wide significance. Nevertheless, we observed a striking positive correlation in the direction of effect when comparing the 231 independently associated SNPs in the European and East Asian cohorts ($P < 1.0 \times 10^{-22}$ for Crohn's disease and $P < 1.0 \times 10^{-31}$ for ulcerative colitis) (**Fig. 1**). Furthermore, of 3,900 suggestively associated SNPs ($5 \times 10^{-5} \leq P < 5 \times 10^{-8}$) from the European-only IBD association analysis, 2,566 had the same direction of effect in the East Asian analysis ($P = 5.92 \times 10^{-88}$). Consistent with

Figure 1 Comparison of odds ratios for Crohn's disease and ulcerative colitis risk variants in Europeans and East Asians. (a,b) For each SNP, odds ratios (on a log scale) were estimated within each population for Crohn's disease (a) and ulcerative colitis (b). The color of each point denotes the association *P* value for that phenotype in East Asians. The red line denotes the best-fitting least-squares regression line, weighted by the inverse of the variance of the log(ORs) in East Asians. Significance and goodness-of-fit are shown in red.

the concordant direction of effect at associated SNPs, there was high genetic correlation (r_G) between the European and East Asian cohorts when considering the additive effects of all SNPs genotyped on the Immunochip¹⁹ (Crohn's disease $r_G = 0.76$ and ulcerative colitis $r_G = 0.79$) (Supplementary Table 10). Given that rare SNPs (minor allele frequency (MAF) < 1%) are more likely to be specific to a particular population, these high r_G values also support the notion that the majority of causal variants are common (MAF > 5%). Although the Indian and Iranian cohort sizes were small in comparison to the East Asian cohort, we observed similar trends for homogeneity of odds ratios at associated loci (Supplementary Figs. 9 and 10) and high genetic correlations with the European cohort (Supplementary Table 10). Together with the strong effect size correlations at known risk loci, these results indicate that the majority of IBD risk loci are shared across ancestral populations. Therefore, ancestry-matched groups of IBD cases and controls can be combined from divergent populations to amass the large sample sizes needed to detect further disease-associated loci.

Not all IBD risk loci are shared across populations, as evidence by r_G being significantly less than 1 ($P < 8.2 \times 10^{-4}$) for all pairwise population comparisons. In most cases, apparent differences in genetic risk were explained by different allele frequencies across populations. For instance, consistent with previous genetic studies of Crohn's disease in East Asians², the three coding variants in *NOD2* (encoding nucleotide-binding oligomerization domain-containing protein 2) that had a large effect on IBD risk in Europeans (odds ratio (OR) = 2.13–3.03) had a risk allele frequency (RAF) of 0 in East Asians. Beyond these three coding variants, there is also evidence of at least four additional low-frequency independent *NOD2* variants on the Immunochip that are associated with Crohn's disease in Europeans (H.H., unpublished data). In the East Asian cohort, two of these variants had a RAF of 0, whereas we were not powered to detect association at the other two variants because we observed fewer than four copies of the risk allele (MAF < 0.0004). Furthermore, no SNP within *NOD2* achieved even suggestive evidence of association in the East Asian cohort (all $P > 7.18 \times 10^{-4}$). Larger sample sizes and more

complete ascertainment of variants (particularly in non-European cohorts) will be required to better assess the genetic architecture of *NOD2* across divergent populations. Similarly, at the *IL23R* gene (encoding IL-23 receptor), previous studies have shown that there is substantial genetic heterogeneity between European and East Asian individuals in IBD risk². In line with these observations, the *IL23R* SNP with the largest effect on risk of Crohn's disease and ulcerative colitis in Europeans (rs80174646) had a RAF of 1 in East Asians, and secondary *IL23R* variants observed in Europeans were also not significantly associated with disease in the East Asian population (rs6588248, $P = 0.65$; rs7517847, $P = 0.04$). These two secondary variants are common in East Asians (rs6588248, MAF = 0.39; rs7517847, MAF = 0.42), and, assuming the effect sizes observed in Europeans, we had 100% power to detect association with rs7517847 at $P < 5 \times 10^{-8}$ but only 84% power to detect association with rs6588248 at $P < 0.05$. Therefore, we cannot rule out the possibility that rs6588248 is involved in Crohn's disease susceptibility in East Asians. Both variants showed significant heterogeneity of effect between the European and East Asian Crohn's disease cohorts ($P < 2.44 \times 10^{-4}$). However, *IL23R* clearly has a role in IBD in the East Asian population, as evidenced by the association at rs76418789 with both Crohn's disease and ulcerative colitis in East Asians (IBD $P = 1.83 \times 10^{-13}$). The same variant was previously implicated in a GWAS of Crohn's disease in Koreans (Supplementary Table 5) (ref. 4). This variant, which has a much lower allele frequency in Europeans (MAF = 0.004) than East Asians (MAF = 0.07), demonstrated suggestive evidence of association with IBD in Europeans ($P = 3.99 \times 10^{-6}$; OR = 0.66) and became genome-wide significant ($P = 2.31 \times 10^{-10}$; OR = 0.53) after conditioning on the three known European risk variants (rs11209026, rs6588248 and rs7517847).

We were well powered to detect genetic heterogeneity between our East Asian and European cohorts at several alleles of large effect in Europeans (Fig. 2 and Supplementary Fig. 10). For example, at *ATG16L1*, the reported Crohn's disease risk variant in Europeans (rs12994997) had a RAF of 0.53 and an OR of 1.27. The variant showed no evidence of association in East Asians ($P = 0.21$), a finding driven at

Figure 2 Comparison of variance explained per risk variant for Crohn's disease and ulcerative colitis between East Asians and Europeans. **(a,b)** Each box represents an independently associated SNP for Crohn's disease **(a)** and ulcerative colitis **(b)**. The size of each box is proportional to the amount of variance in disease liability explained by that variant. Only SNPs with association $P < 0.01$ are included in the East Asian panel. The color of each box denotes whether any difference in variance explained is due to differences in allele frequency ($F_{ST} > 0.1$ /monomorphic in East Asians), significant heterogeneity of odds ratios ($P < 2.5 \times 10^{-4}$) or both.

least in part by a significant difference in allele frequency (RAF = 0.24 in East Asians; $F_{ST} = 0.15$). However, assuming the effect size at this SNP in the East Asian cohort was equal to that seen in the European cohort, we would still have more than 80% power to detect suggestive evidence of association ($P < 5 \times 10^{-5}$). In addition to differences in allele frequency, we also observed evidence of heterogeneity of odds at this SNP (East Asian OR = 1.06; $P = 8.45 \times 10^{-4}$). The previously reported lead SNP at the *IRGM* locus in Europeans also showed only nominally significant evidence of association with Crohn's disease in East Asians (rs11741861: European $P = 5.89 \times 10^{-44}$, East Asian $P = 2.62 \times 10^{-3}$) as well as evidence of heterogeneity of effect (European OR = 1.33 versus East Asian OR = 1.13; heterogeneity $P = 1.20 \times 10^{-3}$). However, not all loci demonstrating significant heterogeneity of odds had lower effect sizes in the non-European cohort: two of the three independent signals at *TNFSF15-TNFSF8* had much larger effects on IBD risk in East Asians (rs4246905: European OR = 1.15 and East Asian OR = 1.75; rs13300483: European OR = 1.14 and East Asian OR = 1.70), despite similar allele frequencies in the two populations.

The third European risk variant was not significantly associated in East Asians (rs11554257: $P = 0.21$), although this might reflect a lack of power (76% power to detect this variant at $P < 0.05$ when assuming an identical odds ratio).

Although the incidence of IBD is rising in developing countries, comparable data on the clinical phenotype of disease in European and non-European populations are limited. We collected subphenotype data on 4,686 patients with IBD from East Asia, India and Iran and compared these data with available clinical phenotypes for 35,128 Europeans. Given that the current cohort is the largest available for clinical comparisons of IBD in Europeans and non-Europeans, we performed basic comparative statistical analyses. Overall, our data showed some demographic differences between the European and non-European populations, with male predominance in Crohn's disease (67% of non-European patients with Crohn's disease were male in comparison to 45% of European patients; $P = 7.09 \times 10^{-78}$). Furthermore, we observed more stricturing behavior ($P = 2.02 \times 10^{-33}$) and perianal disease ($P = 5.36 \times 10^{-33}$) and less inflammatory Crohn's

disease ($P = 4.28 \times 10^{-32}$) in the non-European population. In ulcerative colitis, there was a lower rate of extensive colitis reported in the non-European population ($P = 1.52 \times 10^{-34}$), which was also reflected in a lower rate of colectomy ($P = 1.23 \times 10^{-69}$) (Supplementary Table 11). Although these data have been collected retrospectively, the current findings are in line with previously reported prospectively collected clinical findings in incident cases of IBD in non-Europeans².

DISCUSSION

We identified 38 additional IBD susceptibility loci by adding an extra 11,535 individuals of European descent and 9,846 individuals of non-European descent to our previously reported European-only cohort of 75,105 samples. Given that trans-ancestry association studies principally identify risk loci shared across populations, we would expect to identify a similar number of associated loci had all the individuals in this study been of the same ancestry. Our analyses suggest that significant differences in effect size are minimal at all but a handful of associated loci, further indicating that trans-ancestry association studies represent a powerful means of identifying new loci in complex diseases such as IBD. Furthermore, the nearly complete sharing of genetic risk among individuals of diverse ancestry has important consequences for association studies and disease risk prediction in non-European populations. First, a significant association in one population makes the locus in question a very strong candidate for involvement in IBD risk worldwide. Second, our data suggest that odds ratios estimated from a very large association study are likely to better represent the effect size of the associated variants in a second, ancestrally diverse population than those estimated from a substantially smaller study in the second population itself (because of the larger sampling variance in the second study). Finally, because rare alleles are more likely than common variants to be specific to a particular population, the substantial number of IBD risk loci shared across ancestral populations implies that the underlying causal variants at these loci are common. This adds further weight to the growing number of arguments against the 'synthetic association' model explaining a large proportion of GWAS loci^{20–22}.

Although the majority of risk-associated loci are shared across populations, we were able to detect a handful of loci demonstrating heterogeneity of effect between populations. Major European risk variants in *NOD2* and *IL23R* are not present in individuals of East Asian ancestry. The relatively small sample size of the non-European cohorts and the fact that Immunochip SNP selection was only based on resequencing data from individuals of European ancestry hinder our ability to identify association with sites that are monomorphic in Europeans but polymorphic in non-Europeans. Targeted resequencing efforts in large numbers of non-European IBD cases and controls, similar to those undertaken in European cohorts, may identify such associations and thus provide further insight into the genetic architecture of IBD^{23,24}. The much smaller number of individuals in the non-European cohorts also reduces power to detect heterogeneity of effect versus the European cohort, and we therefore may be overestimating the degree of sharing between the various ancestry groups.

In addition to allele frequencies differing between ancestral populations, patterns of LD can also vary greatly; such differences further complicate comparisons of genetic architecture for complex disease across diverse populations. For example, we observed significant heterogeneity of odds at the *TNFSF15-TNFSF8* and *ATG16L1* loci, potentially suggesting that gene-environment interactions increase the variance explained by these associations in either European (*ATG16L1*) or non-European (*TNFSF15-TNFSF8*) populations. Although this hypothesis is attractive, the heterogeneity in effect

sizes could also be underpinned by differential tagging of untyped causal variants at these loci in one or both populations. Although the Immunochip provides dense coverage of 186 previously associated loci, SNP selection was based on low-coverage sequence data from a pilot release of the 1000 Genomes Project. Approximately 240,000 SNPs were selected for inclusion, with an assay design success rate of approximately 80%. Therefore, it is possible that causal variants could remain untyped, even within the dense fine-mapping regions of the Immunochip, and the chances of this occurring are greater still in populations of non-European ancestry. Until the causal variants that underlie these associated loci have been identified (or all SNPs within these loci are included in the association tests), we cannot rule out the possibility that differential tagging of untyped causal variants is driving the observed heterogeneity in effects.

In summary, we have performed the first trans-ancestry association study of IBD and identified 38 risk loci, increasing the number of known IBD risk loci to 200. Together, these loci explain 13.1% and 8.2% of the variance in disease liability for Crohn's disease and ulcerative colitis, respectively. The majority of these loci are shared across diverse ancestry groups, with only a handful demonstrating population-specific effects driven by heterogeneity in RAF (for example, *NOD2*) or effect size (for example, *TNFSF15-TNFSF8*). Concordance in direction of effect is significantly enriched among SNPs demonstrating only suggestive evidence of association, indicating that larger trans-ancestry association studies may represent a powerful means of identifying more risk loci for IBD. By leveraging imputation based on tens of thousands of reference haplotypes or directly sequencing large numbers of cases and controls, these studies will more thoroughly survey causal variants and thus have increased ability to model the genetic architecture of IBD across diverse ancestral populations.

URLs. National Human Genome Research Institute (NHGRI) GWAS Catalog, <http://www.genome.gov/admin/gwascatalog.txt>; functionGVS, <http://snp.gs.washington.edu/SeattleSeqAnnotation141/>; Variant Explorer, <http://molgenis70.target.rug.nl/index.htm>.

METHODS

Methods and any associated references are available in the [online version of the paper](#).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Study design: J.Z.L., S.v.S., H.H., A.P.M., J.C.B., B.Z.A., M.P., T.B.K., M.J.D., A.F., C.A.A. and R.K.W. Collection of samples and clinical information: S.C.N., J.C.L.,

S.A., J.H.C., N.E.D., Y.F., A.H., R.C.J., G.J., W.H.K., H.P., W.G.N., V.M., T.R.O., H.V., A.S., J.J.Y.S., R.M., K.Y., S.-K.Y., M.K., T.B.K., A.F. and R.K.W. Quality control and genotype calling: J.Z.L., S.v.S., B.Z.A., H.H., L.J., T.S. and C.A.A. Statistical analyses: J.Z.L., S.v.S., H.H., A.T., L.J., R.A., S.R., H.-J.W., L.F., C.A.A. and R.K.W. Writing of the manuscript: J.Z.L., S.v.S., H.H., J.C.L., J.C., B.Z.A., M.P., C.A.A. and R.K.W.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Ethical approval. The recruitment of study subjects was approved by the ethics committees or institutional review boards of all individual participating centers or countries. Written informed consent was obtained from all study participants.

GWAS cohort, quality control and analysis. *Cohorts and quality control.* The GWAS cohorts and quality control are described in detail in Jostins *et al.*⁶. Briefly, seven Crohn's disease and eight ulcerative colitis collections with genome-wide SNP data were combined. Samples were genotyped on a combination of the Affymetrix GeneChip Human Mapping 500K, Affymetrix Genome-Wide Human SNP Array 6.0, Illumina HumanHap300 BeadChip and Illumina HumanHap550 BeadChip arrays. After SNP and sample quality control, the Crohn's disease data consisted of 5,956 cases and 14,927 controls, the ulcerative colitis data consisted of 6,968 cases and 20,464 controls, and the data for Crohn's disease and ulcerative colitis combined (IBD) consisted of 12,882 cases and 21,770 controls. The number of SNPs per collection varied between 290,000 and 780,000.

Imputation. Genotype imputation was performed using the prephasing/imputation stepwise approach implemented in IMPUTE2/SHAPEIT (chunk size of 3 Mb and default parameters)^{37,38}. The imputation reference set consisted of 2,186 phased haplotypes from the full 1000 Genomes Project data set (August 2012, 30,069,288 variants, release v3.macGT1).

Association analysis. Genome-wide association analyses was carried out for Crohn's disease, ulcerative colitis and IBD (the Crohn's disease and ulcerative colitis cases combined). After applying filters requiring MAF > 1% and imputation INFO score > 0.6 to all imputed variants, around 9 million variants were found to be suitable for association analysis. Association tests were carried out in PLINK, using the post-imputation genotype dosage data and using 10, 7 and 15 principal components for Crohn's disease, ulcerative colitis and IBD, respectively, as covariates, chosen from the first 20 principal components. The Crohn's disease, ulcerative colitis and IBD scans had genomic inflation (λ_{GC}) values of 1.129, 1.114 and 1.160, respectively. Accounting for inflation due to sample size and polygenic effects, these Crohn's disease, ulcerative colitis and IBD λ_T values are equivalent to $\lambda_{GC,1,000}$ (the inflation factor from a sample size of 1,000 cases and 1,000 controls)³⁹ values of 1.015, 1.011 and 1.010, respectively.

ImmunoChip cohort, quality control and analysis. *Description of the ImmunoChip.* The ImmunoChip is an Illumina Infinium microarray comprising 196,524 SNPs and small indel markers selected on the basis of results from GWASs of 12 different immune-mediated diseases. The ImmunoChip enables replication of all nominally associated SNPs ($P < 0.001$) from the index GWAS scans and fine mapping of 186 loci associated at genome-wide significance with at least 1 of the 12 index immune-mediated diseases. Within fine-mapping regions, SNPs from the 1000 Genomes Project pilot Phase 1 (European cohorts), plus selected autoimmune disease resequencing efforts, were selected for inclusion (with a design success rate of around 80%). The chip also contains around 3,000 SNPs added as part of the Wellcome Trust Case Control Consortium 2 (WTCCC2) project replication phase. These SNPs are useful for quality control purposes because they have not previously been associated with immune-mediated diseases ('null' SNPs).

Cohorts of European ancestry. Recruitment of patients and matched controls genotyped with the ImmunoChip was performed in 15 countries in Europe, North America and Oceania (Table 1). Diagnosis of IBD was based on accepted radiological, endoscopic and histopathological evaluation. All included cases fulfilled clinical criteria for IBD. Genotyping was performed across 36 batches and included a total of 19,802 Crohn's disease cases, 14,864 ulcerative colitis cases and 34,872 population controls. The ImmunoChip cohort included 3,424 Crohn's disease cases, 3,189 ulcerative colitis cases and 7,379 population controls present in the GWAS cohort. The overlapping ImmunoChip samples were excluded from the trans-ancestry association analysis but included in the modeling of European versus non-European IBD because this was based solely on ImmunoChip data.

Cohorts of East Asian, Indian and Iranian ancestry. East Asian patients with IBD and controls were recruited from the following countries: Japan (Institute of Medical Science, University of Tokyo, RIKEN Yokohama Institute and Japan

Biobank), Korea (Yonsei University College of Medicine and Asan Medical Centre, Seoul) and Hong Kong (Chinese University of Hong Kong). Indian IBD cases and controls were recruited from Dayanand Medical College and Hospital, Ludhiana, and the University of Delhi South Campus. Iranian cases and controls were recruited from the Tehran University of Medical Sciences. Samples recruited as part of a European cohort but that clustered with a non-European cohort in principal-component analysis were reassigned to the non-European cohort. In total, 6,598 East Asian, 3,088 Indian and 1,393 Iranian individuals were genotyped on the ImmunoChip (Table 1, Supplementary Figs. 1 and 2, and Supplementary Table 12).

Phenotype data. Detailed phenotype data (including sex, ancestry, age of disease onset, smoking status, family history, extraintestinal manifestations and surgery) were available for 47,799 European IBD cases and 3,986 non-European IBD cases (Supplementary Table 11). Disease location and behavior were assessed with the Montreal classification. Clinical demographics and disease phenotype were compared in the European and non-European cohorts using χ^2 analysis (SPSS 20).

Genotyping and calling. The ImmunoChip samples were genotyped in 36 batches. Normalized intensities for all samples were centrally called using the optiCall clustering program⁴⁰ with Hardy-Weinberg equilibrium blanking disabled and the no-call cutoff set to 0.7. Before calling all data, we first established the optimal composition of sample sets. Calling per genotyping batch turned out to give the most reliable genotype clustering (in comparison to calling individual ancestral populations separately within each genotyping batch, calling all individuals per ancestry group together or calling all available data together).

Quality control. Quality control was performed separately in each population (East Asian, Iranian, Indian and European) using PLINK⁴¹. Individuals were assigned to populations on the basis of principal-component analysis. This analysis was performed using EIGENSTRAT⁴² on a set of 15,552 ImmunoChip SNPs that had pairwise $r^2 < 0.2$ and MAF > 0.05 and were present in 1000 Genomes Project Phase 2 data. The first two principal components were estimated for the 1000 Genomes Project individuals and projected onto all ImmunoChip cases and controls. As expected, a clear separation between the different populations was seen (Supplementary Fig. 3). Samples were assigned to the population with which they clustered, and those that did not cluster with any of the reported populations were removed.

Marker quality control. SNPs were removed if they (i) were not on autosomes; (ii) had a call rate lower than 98% across all genotyping batches in the population and/or lower than 90% in one of the genotyping batches; (iii) were not present in 1000 Genomes Project Phase 1 data; (iv) failed Hardy-Weinberg equilibrium ($FDR < 1 \times 10^{-5}$ across all samples or within each genotyping batch); (v) had heterogeneous allele frequencies between the different genotyping batches within one population ($FDR < 1 \times 10^{-5}$; in genotyping batches with more than 100 samples); (vi) had different missing genotype rates for cases and controls ($P < 1 \times 10^{-5}$); and (vii) were monomorphic in the population. After marker quality control, 125,141 SNPs remained in the East Asian data set, 145,857 SNPs remained in the Indian data set, 152,232 SNPs remained in the Iranian data set and 144,245 SNPs remained in the European data set.

Sample quality control. Samples with a low call rate (<98%) and samples with an outlying heterozygosity rate ($FDR < 0.01$) were removed. Identity by descent was calculated using an LD-pruned set of SNPs with MAF > 0.05. Sample pairs with identity by descent of >0.8 were considered duplicates, and pairs with identity by descent of >0.4 and <0.8 were considered related. For all duplicate and related pairs, the sample with the lowest genotype call rate was removed. After sample quality control, 6,543 (2,824 cases, 3,719 controls) East Asian samples, 2,413 (1,423 cases, 990 controls) Indian samples, 890 (548 cases, 342 controls) Iranian samples and 65,642 (31,664 cases, 33,977 controls) European samples remained.

Per-population association analysis. Case-control association tests for Crohn's disease, ulcerative colitis and IBD were performed in each ancestry group (European, East Asian, Indian and Iranian) using a linear mixed model as implemented in MMM⁹. A covariance genetic relatedness matrix, R , was included as a random-effects component in the model to account for population stratification. To avoid biases in the estimation of R due to the design of the ImmunoChip, SNPs were first pruned for LD (pairwise $r^2 < 0.2$). Of the remaining SNPs, we then removed those that lay in the HLA region or had