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Abstract

Background & Aims—RAC1 is a GTPase that has an evolutionarily conserved role in coordinating immune defenses, from plants to mammals. Chronic inflammatory bowel diseases (IBD) are associated with dysregulation of immune defenses. We studied the role of RAC1 in IBD using human genetic and functional studies and animal models of colitis.

Methods—We used a candidate gene approach to HapMap-Tag single nucleotide polymorphisms (SNPs) in a discovery cohort; findings were confirmed in 2 additional cohorts. *RAC1* mRNA expression was examined from peripheral blood cells of patients. Colitis was induced in mice with conditional disruption of *Rac1* in phagocytes by administration of dextran sulphate sodium (DSS).

Results—We observed a genetic association between *RAC1* with ulcerative colitis (UC) in a discovery cohort, 2 independent replication cohorts, and in combined analysis for the SNPs rs10951982 (Pcombined UC = 3.3×10^{-8}, odds ratio [OR]=1.43 [1.26–1.63]) and rs4720672 (Pcombined UC=4.7×10^{-6}, OR=1.36 [1.19–1.58]). Patients with IBD who had the rs10951982 risk allele had increased expression of *RAC1*, compared to those without this allele. Conditional disruption of *Rac1* in macrophage and neutrophils of mice protected them against DSS-induced colitis.

Conclusion—Studies of human tissue samples and knockout mice demonstrated a role for the GTPase RAC1 in the development of UC; increased expression of *RAC1* was associated with susceptibility to colitis.

Keywords

innate immunity; Crohn's disease; CD; Rac-1 knockout

Introduction

IBD is a chronic relapsing and remitting disease that affects 1 in 250 individuals of European ancestry. Although the exact etiology of IBD is unknown, it is speculated that IBD occurs in genetically susceptible individuals as a result of dysregulated immune response to gut flora after exposure to an as yet unidentified environmental stimulus[1]. Genetic association studies have identified innate immunity as a critical component in the development of IBD[2–8]. However, these studies have identified only 23% of the susceptibility determinants for CD[9] and 16% for UC[10].

RAC1 belongs to the Ras superfamily of GTP-binding proteins that act as "molecular switches" and influence a number of key cellular functions critical for innate immunity[11] including NOD2 and TLR2 regulation[12], leukocyte chemotaxis[13], barrier defence[14], and bacterial killing[15]. RAC1 function is also disrupted by multiple bacterial cytotoxins[16].

Furthermore, RAC1 is a target of the commonly used IBD treatment azathioprine, the effects of which are thought to be mediated through T cell apoptosis[17] and altered phagocyte chemotaxis to sites of intestinal inflammation[18]. The importance of RAC1 in innate immunity as seen through its conservation from plants[19] to mammals led to our detailed exploration of the *RAC1* gene involvement in IBD.

Materials and Methods

SNP Analysis and Genotyping

International HapMap project[20] (http://www.hapmap.org) Caucasian (CEU) Phase II data were used to select tag SNPs (MAF > 1%) that span the *RAC1* gene and flanking regions through the "Tagger" software program[21]. Twelve tag SNPs covering *RAC1* region (Chromosome 7, 6386318 to 6411760) were captured with $r^2 > 0.8$ (1 SNP was removed during QC because the MAF was < 1%; See Supplemental Methods). Genotype analysis of samples was done using the Illumina® Goldengate Custom Chip genotyping system (Toronto discovery) and Taqman (North America Replication and Scotland Validation) at The Centre for Applied Genomics, Hospital for Sick Children, Toronto and the University of Edinburgh.

Subjects (for Quality Control, and Population Stratification please see Supplemental Methods)

All subjects in this study were of European descent by self-reporting of ethnic heritage. All probands had a confirmed diagnosis of IBD and fulfilled standard diagnostic criteria. Phenotypic characterization was based on the Montreal classification[22]. Perianal disease included only those CD patients with perianal abscess and/or fistulae. Study subject phenotypic information and DNA samples were obtained with institutional review ethics board approval for IBD genetic studies at the Hospital for Sick Children and Mount Sinai Hospital in Toronto. Replication cohorts had REB approval for genetic and phenotypic studies at the individual institutions. Written informed consent was obtained from all participants.

The discovery cohort included patients recruited from the Hospital for Sick Children and Mt Sinai Hospital in Toronto with local and NIDDK control individuals. The first replication cohort consisted of 1836 Caucasian individuals from North American including 443 CD and 477 UC patients, and 916 controls (NIDDK patients recruited from Chicago and Pittsburgh with North American control individuals obtained from the Centre for Applied Genomics). The second cohort consisted of 2449 individuals exclusively recruited from Scotland including 691 CD and 615 UC patients, and 1143 controls. All patients and controls individuals were non-related Caucasian individuals. Only two *RAC1* SNPs were genotyped in the replication cohorts and both had call rates greater than 98% in both cohorts. Part of these cohorts have been used in previous GWAS including the all the NIDDK patients in the North American replication[5,23] and 374 individual from Scotland in the Pediatric IBD GWAS[24]. None of the replication cohort control individuals were genotyped in previous IBD GWAS.

Preliminary Analysis

HAPLOVIEW[25] was used to obtain LD patterns, obtaining descriptive statistics and summaries of the SNPs. PLINK version 1.06[26] was used to test for Hardy-Weinberg equilibrium (HWE) for each marker based on Pearson's chi-square test. Descriptive statistics of demographic variables were generated using SAS version 9.2 (SAS institute, Cary, NC). The Wilcoxon Rank Sum Test and Chi-square test were used to identify differences in demographic variables between subgroups.

Association Analysis

The analysis was applied in three stages. In stage one, association analyses were applied to detect the associations with the 11 *RAC1* SNPs (1 SNP was removed during QC because the MAF was < 1%; See Supplemental Methods) and IBD, CD, and UC vs. healthy controls (HC). Logistic regression models were applied for the additive genetic model, and Pearson chi-square tests were applied for dominant and recessive genetic models. Although we used an additive genetic model for primary analysis[27] we also explored dominant and recessive genetic models for sensitive analysis. Throughout the report the p-values are the dominant genetic model p-value. Odds ratios (OR) and 95% confidence intervals (CI) were estimated for the disease compared to the control group. The association, adjusting for selected principal component vectors from the EIGENSTRAT analysis, was tested using conditional logistic regression (SAS v9.2, Cary, NC).

In stage two, the 2 *RAC1* SNPs identified from the discovery cohort were genotyped in a replication cohort (North America) and independent validation cohort (Scotland). Independent analysis was applied on the replication cohort and validation cohort. Combined effect estimates from all three IBD cohorts were estimated using a logistic regression model. All P-values are two-sided.

In stage 3, the confirmed *RAC1* SNPs were further explored. Haplotype analysis was applied on this region on multiple marker blocks. Imputation of an additional 73 ungenotyped SNPs in the *RAC1* region was performed using released Phase II/III CEU HapMap data (BEAGLE). The imputed SNPs were analyzed on the IBD phenotypes using an additive genetic model.

Subgroup Analysis

In addition to comparing IBD, CD, and UC to HC, we applied subgroup analysis to evaluate the genetic effect on the disease risk of the IBD sub-population according to the Montreal Classification system. The sub-population comparisons were applied for each of the genetic markers on ileal only (L1), colonic only (L2), ileo-colonic (L3), ileal any (L1/L3), colon only (L2 plus UC), colon any (L2/L3 plus UC), perianal, and early onset IBD patients (diagnosis age < 19) vs. HC. Different genetic models were used to test single marker associations between each of the subgroups. The analyses were applied for the discovery cohort, North American replication cohort, Scottish replication cohort, and the pooled samples separately.

Animal Experimentation

Rac1-KO mice were generated, bred and genotyped (mixed Sv129 black 6 and Balb/c background) as previously described[28]. Mice used in these experiments were between 8 and 12 wks old, and all control mice were littermates of the *Rac1*-KO mice. *Rac1*-KO mice exhibited no obvious phenotype. Mice were housed in a pathogen free environment and fed standard diet with free access to water. All experiments were approved by the Animal Care Ethics panel at the University of Toronto.

DSS Model

Dextran sodium sulphate (DSS) experiments were performed as previously described[29]. For the *Rac1*-KO experiments 12 KO and 18 WT mice were treated with 5% DSS in their drinking water for 7 days. Mice were monitored daily for weight loss, stool consistency, rectal bleeding and general appearance. For further DSS, Cytokine, and MPO methods please see Supplemental Methods.

Results

Genetic Studies

Candidate Gene Approach—We first examined the role of *RAC1* in IBD using a candidate gene approach. After strict quality control (QC; 1 SNP was removed during QC because the MAF was < 1%; See Supplemental Methods) measures, 11 *RAC1* tag SNPs (Supplemental Figure 1) were successfully genotyped in the discovery cohort consisting of 2049 subjects (656 CD, 544 UC, and 849 controls; Supplementary Table 1A). The rs10951982 *RAC1* SNP was significantly associated with IBD after Bonferroni correction threshold for 11 SNPs examined for IBD, CD and UC ($\alpha = 1.5 \times 10^{-3}$; Table 1 shows the association for discovery SNPs based on the minor allele). rs10951982 is not located in a known regulatory element or in strong LD ($r^2 > 0.25$) with any HapMap SNP located in other genes on Chromosome 7.

Genetic Replication—To confirm this association, we examined rs10951982 and rs4720672 in two independent cohorts. The replication cohort was comprised of 1836 Caucasian subjects including 443 CD and 477 UC patients, and 916 controls recruited from North America and a second validation cohort from Scotland comprised of 2449 Caucasian subjects including 691 CD and 615 UC patients, and 1143 controls (Supplementary Table 1B and 1C). The signal for IBD and UC was replicated in both cohorts with similar ORs and minor allelic frequency (Table 2). Further exploration of genetic models showed that the signal was strongest in the dominant model (reported here and Supplemental Table 3). Combined analysis (1790 CD and 1636 UC patients, and 2908 controls) showed that both SNPs were significantly associated with IBD, CD, and UC ($P_{combined\ IBD} = 4.2 \times 10^{-7}$, OR = 1.29 (1.17–1.44), $P_{combined\ CD} = 3.7 \times 10^{-3}$, OR = 1.19 (1.06–1.34), and $P_{combined\ UC} = 3.3 \times 10^{-8}$, OR = 1.43 (1.26–1.63) for rs10951982; and $P_{combined\ IBD} = 7.7 \times 10^{-7}$, OR = 1.34 (1.17–1.47), $P_{combined\ CD} = 3.7 \times 10^{-4}$, OR = 1.26 (1.11–1.44), and $P_{combined\ UC} = 4.7 \times 10^{-6}$, OR= 1.36 (1.19–1.58) for rs4720672).

As rs10951982 and rs4720672 are in LD ($r^2 = 0.64$, controls), we applied haplotype analysis[30]. The haplotype of this two-SNP block is significantly associated with IBD, CD, and UC ($P_{IBD} = 9.1 \times 10^{-3}$, $P_{CD} = 5.0 \times 10^{-2}$, and $P_{UC} = 3.1 \times 10^{-2}$, haplotype omnibus test). Further haplotype analysis on different *RAC1* SNP blocks did not show significant associations (data not shown).

Genotype-Phenotype Analysis—As the initial analysis indicated that the genetic signal was strongest in IBD and UC, the role of these SNPs was examined by disease location using the Montreal Classification[22] (Table 3). We analyzed "any" colonic IBD (combined UC, colonic only CD (L2), and ileo-colonic CD (L3)) and found significant association with both SNPs in the combined analysis ($P_{combined} = 7.8 \times 10^{-8}$, OR= 1.33 (1.20–1.49) for rs10951982; and $P_{combined} = 1.6 \times 10^{-7}$, OR= 1.35 (1.20–1.51) for rs4720672). These SNP remained significant after Bonferroni correction (Bonferroni correction threshold: for 11 SNP examined in 8 IBD sub-phenotypes; $\alpha = 5.6 \times 10^{-4}$). Further significant association was found with colonic only IBD (combined UC and colonic only CD (L2)) and early onset IBD (diagnosis < 19 years of age).

Imputation Analysis—We next imputed 73 SNPs in the *RAC1* region not initially genotyped using released Phase II/III CEU HapMap data to identify any additional significant SNPs in our discovery cohort. Imputation analysis revealed multiple HapMap SNPs with nominal significant levels of association (Figure 1A for IBD and Supplemental Figure 2 for UC and CD). Three SNPs located in intron 3 of *RAC1* in strong LD ($r^2 > 0.80$) with rs10951982 showed the strongest association with IBD and UC (for IBD: rs836472, P

$= 1.2 \times 10^{-3}$, OR = 1.33; rs1880118, P = 9.8×10^{-4}, OR = 1.33; and rs12536544, P = 1.2×10^{-3}, OR = 1.33). Together these data implicate this region of *RAC1* as harboring the causal variant; however, the imputed SNPs are based on *in silico* analysis and further sequencing in this region is required to determine the causal variant(s).

***RAC1* Expression**—Our study indicates that *RAC1* rs10951982 risk allele increases the susceptibility to the development of colonic IBD. A previous study demonstrated that SNPs in the *RAC1* gene region are in an expression quantitative trait loci (eQTLs) in lymphoblastoid cell lines (Figure 2A)[31]. The area of maximal LOD score (3.3 – 4.3) on chromosome 7 occurs in the *RAC1* gene region with several SNPs (rs37279790, rs836472, rs1880118, rs12536544, and rs10951983) in strong linkage disequilibrium to rs10951982 (r^2 of 0.82 to 1.0). The additive effect for the major allele corresponded to a *cis*-acting regulatory effect with an increase in standard deviation units for *RAC1*[31] (Figure 2A and Supplemental Table 4). Furthermore, mRNA from freshly isolated peripheral blood cells (PBCs) in 16 IBD patients with colonic disease showed increased *RAC1* expression based on genotype (Figure 2B). Carriage of the risk `G' allele resulted in increased *RAC1* expression (comparison of normalized expression for GG (7 patients) / AG (5 patients) to AA (4 patients) using non-parametric comparison and Wilcoxon exact test P = 0.008; and AA vs GG Wilcoxon exact test P = 0.012). These results indicate that carriage of the risk allele of rs10951982 results in increased *RAC1* expression. There were no differences in *RAC1* splicing based on genotype in these patients (data not shown). Further expression analysis is required to determine the effect of these SNPs on *RAC1* expression, splicing, and mRNA stability in both healthy individuals and IBD patients.

Animal Studies: *Rac1* Conditional Knockout Mice Studies—As *Rac1* knockouts are embryonic lethal in mice[32], we next examined the neutrophil and macrophage conditional *Rac1* knockout (*Rac1*-KO) mice[28]. These mice had no obvious colonic phenotype with normal bowel histology although they did show increased MPO activity (Figure 3B). As shown in Figure 3A, the *Rac1*-KO mice were protected from developing DSS-induced colitis. *Rac1*-KO mice show decreased weight loss after 6 days (P < 0.01; Wilcoxon rank sum test). WT mice began bleeding after day 4 with 10/18 mice bleeding on day 7. In comparison, only 1/12 *Rac1*-KO was bleeding on day 7 (P = 0.0051; log rank test = 7.3, hazard ratio 4.6, 95% CI 1.5–14). WT mice had significantly worse DAI scores starting on day 4 (P < 0.05 on day 4, and P < 0.001 on days 5, 6, and 7 Wilcoxon rank sum test). Comparison of the distal colon on day seven of DSS treatment showed reduced histological scores with fewer ulcers in the distal colonic in the *Rac1*-KO group (P=0.008 analyzed as a dichotomous variable with a cutoff of 0 – 2 or > 3 ulcers using Fisher's exact test; Supplemental Figure 3). Neutrophil migration into the colon was reduced in the *Rac1*-KO mice compared to WT as assessed by MPO activity (Figure 3C. P < 0.01; Wilcoxon rank sum test). Cytokine profiles for colonic lystates from *Rac1*-KO mice treated with DSS showed significantly reduced level of the proinflammatory cytokine IL-1β and the neutrophil chemokine KC compared to WT mice (Figure 3C; P < 0.05; Mann Whitney test Two Tailed). Furthermore, there was impaired up-regulation of proinflammatory cytokines including IL-1β, IL-12 and TNFα in response to DSS induced colitis as compared to WT (P < 0.05, Mann Whitney test Two Tailed; Supplemental Figure 4). Splenocytes isolated from DSS-treated *Rac1*-KO mice and stimulated with lipopolysaccharide (LPS) also showed a similar decrease in production of inflammatory cytokines (Supplemental Figure 5) further demonstrating the reduced inflammation in these KO mice.

Discussion

Recent IBD GWAS have identified innate immunity as a critical component in the pathogenesis of IBD[2–8]. The identification of *RAC1's* association with IBD further

strengthens this link with innate immunity. The number of IBD susceptibility genes has increased dramatically due to meta-analysis of recent GWAS and the signal for *RAC1* has not been reported to date. However, in the 1st International IBD Genetics Consortium (IIBDGC) meta-analysis for UC, SNPs in strong LD ($r^2 > 0.80$; and identified in our imputation analysis Figure 1A) to our reported SNP (rs10951982) showed strong association to UC (rs12536544, $P_{meta} = 8.80 \times 10^{-5}$; rs836472, $P_{meta} = 2.60 \times 10^{-5}$; rs1880118, $P_{meta} = 6.3 \times 10^{-4}$; and rs10951983, UC $P_{meta} = 1.2 \times 10^{-4}$)[10]. Although these SNPs have not yet been reported due to the stringent criteria for replication in that UC meta-analysis[10], these meta-analysis results strongly implicate *RAC1* as a novel UC susceptibility gene. Furthermore, the *RAC1* SNP rs12536544 genotyped in the meta-analysis was at the maximum eQTL for *RAC1* as shown in Figure 2A ($P = 10^{-7}$) indicating that the risk allele for these SNPs increased *RAC1* expression. Interestingly, this novel genetic association is also found with the colonic IBD (UC and colonic CD) phenotype, a phenotype that has yet to be explored in IBD GWAS.

We observed that reduction or loss of Rac1 expression through conditional neutrophil/ macrophage KO resulted in protection from the development of DSS induced colitis. We chose the DSS model as it has recently been shown to be a reasonable model of UC[33]. This protection from DSS induced colitis was accompanied by a reduction in MPO, an indirect marker of neutrophil colonic infiltration, and a reduction in the colonic proinflammatory cytokine IL-1β and the neutrophil chemokine KC. We have previously shown that loss of Rac1 in these mice results in profound defects in inflammatory recruitment and migration to chemotactic stimuli[28]. The down-regulation of the neutrophil chemokine KC would also result in decreased neutrophil migration and reduced inflammation into the intestine[34]. Therefore, it is possible that the protection from DSS induced colitis in these mice is due to delayed neutrophil infiltration as observed when neutrophil recruitment is impaired through CXCR2 blocking antibodies[35]. This is also consistent with other animal models of inflammation where loss of Rac1 function leads to decreased neutrophil recruitment leading to attenuation of inflammation[28, 36, 37].

Although the causal variant has not been established in this study, expression datasets[22] and our patient's expression studies demonstrates that the *RAC1* risk allele identified here increases *RAC1* expression. These human expression experiments are complemented by our animal model experiments that show that decreased Rac1 activity or loss *Rac1* expression leads to protection from the development of DSS induced colitis. The cytokine profile observed from colonic lysates from DSS treated animals with conditional macrophage/ neutrophil *Rac1*-KO demonstrated defective proinflammatory production. Overall, these results suggest that carriage of the *RAC1* risk allele results in higher expression of *RAC1* leading to increased neutrophil recruitment into the colon and subsequent increased proinflammatory cytokine expression in colonic. Along with RAC1 dependent T-cell apoptosis[17], one of the proposed mechanisms for the medication commonly used to maintain IBD patients in remission, azathioprine, is through inhibition of neutrophil trafficking into the colon[18]. Therefore it is intriguing to speculate that patients carrying the *RAC1* risk allele have higher levels of *RAC1* expression with increased neutrophil migration into the colon and that these patients may benefit from treatment with azathioprine.

RAC1 is expressed in most cell types and has divergent and critical role in numerous cellular pathways. The functional studies presented here focus on its role in the neutrophil-macrophage cell lineage. It is also plausible that the association of *RAC1* with IBD is mediated through an alternate pathway not examined here. The divergent functions of RAC1 in innate immunity, barrier defense, and T cell apoptosis makes further study of this protein important to our understanding of IBD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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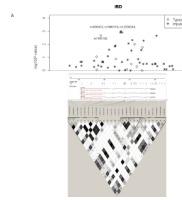
Figure 1. *RAC1* Imputation Analysis
Regional blots plot of the negative decadic logarithm of the P-values of SNPs in the *RAC1* region including both genotyped (diamond) and imputation (*) SNPs, based on the 2049 individuals from the discovery cohort. In IBD the strongest signal comes from 3 SNPs (rs836472, rs1880118, rs12536544) located at 6401429 to 6402426 of Chromosome 7 that are in strong LD ($r^2 > 0.80$) with rs10951982. *RAC1* gene is depicted below LD plot with genotyed SNP in bold and r^2 SNP map.
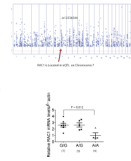
Figure 2. *RAC1* Expression Based on Genotype

(A) Plot of LOD score (y-axis) and SNP position of each chromosome (x-axis). The eQTL maximal LOD score on chromosome 7 is the *RAC1* SNP (rs12536544) that is in strong disequilibrium to rs10951982 (r^2 of 0.82 to 1.0).

(B) *RAC1* expression from mRNA isolated from PBCs from 16 IBD patients with colonic disease based on rs10951982 genotype. `G' allele is the risk allele. Expression was normalized to β-actin expression. Wilcoxon exact test.
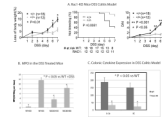
Figure 3. Impact of impairing Rac1 function in DSS induced colitis in mice
(A) *Rac1*-KO. Left Panel graphs changes in weight with DSS treatment. Middle Panel shows changes in rectal bleeding. # at risk indicates number of mice not bleeding at each time point. Right Panel shows Disease Activity Index. (Error bars represent SEM).
(B) Colonic MPO Activity. Colonic neutrophil infiltration was measured by MPO activity. MPO activity was assessed in the distal colon of WT mice (WT + water), *Rac1*-KO mice (Rac1-KO + water), and *Rac1*-KO mice treated with 5% DSS (*Rac1*-KO + DSS). (Wilcoxon rank sum test; Error bars represent SEM).
(C) Colonic Cytokine Assay. Cytokine assays performed using MESO scale discovery mouse TH1/TH2 9-plex assay kit. (Mann Whitney test Two Tailed; Error bars represent SEM).

Table 1

Genetic Association in Discovery Cohort.

rs#	Position	MAF Controls	P_{IBD}	OR (95% CI)	P_{CD}	OR (95% CI)	P_{UC}	OR (95% CI)
rs836488	6,386,318	0.0366032	1.1×10^{-1}	1.33 (0.93–1.87)	5.4×10^{-1}	1.13 (0.75–1.71)	4.0×10^{-2}	1.51 (1.02–1.74)
rs10499343	6,386,519	0.122743	1.1×10^{-1}	0.98 (0.81–1.19)	2.5×10^{-1}	0.86 (0.67–1.11)	1.5×10^{-1}	1.10 (0.92–1.11)
rs10951982	6,389,081	0.217911	1.3×10^{-3}	0.78 (0.67–0.91)	4.0×10^{-3}	0.73 (0.59–0.96)	1.0×10^{-2}	0.75 (0.63–0.96)
rs10238136	6,389,871	0.034163	2.2×10^{-1}	0.81 (0.57–1.14)	3.5×10^{-1}	0.82 (0.54–1.23)	3.6×10^{-1}	0.81 (0.52–1.23)
rs702484	6,398,458	0.275012	1.1×10^{-1}	0.89 (0.77–1.02)	8.1×10^{-1}	0.83 (0.67–1.02)	2.7×10^{-1}	0.88 (0.74–1.02)
rs836547	6,405,443	0.0961445	8.7×10^{-1}	1.01 (0.82–1.25)	8.7×10^{-2}	0.96 (0.73–1.25)	7.8×10^{-1}	1.04 (0.80–1.25)
rs2303364	6,408,213	0.0471883	4.6×10^{-1}	1.11 (0.82–1.51)	76×10^{-1}	0.97 (0.67–1.39)	4.6×10^{-1}	1.30 (0.89–1.39)
rs4720672	6,410,364	0.155198	1.1×10^{-2}	0.76 (0.63–0.94)	2.5×10^{-2}	0.77 (0.61–0.96)	4.0×10^{-2}	0.78 (0.65–0.96)
rs836554	6,411,760	0.229136	5.1×10^{-1}	0.95 (0.82–1.10)	1.3×10^{-1}	0.85 (0.69–1.05)	4.5×10^{-1}	1.08 (0.88–1.05)
rs3813517	6,415,057	0.0258663	9.9×10^{-2}	1.00 (0.67–1.48)	8.9×10^{-1}	0.96 (0.64–1.55)	7.1×10^{-1}	1.09 (0.66–1.55)
rs836559	6,415,288	0.435334	1.8×10^{-2}	0.85 (0.75–0.97)	1.5×10^{-3}	0.70 (0.56–0.87)	1.0×10^{-1}	0.82 (0.79–0.87)

MAF – minor allelic frequency. P-vale and OR based on Dominant genetic model and MAF.

Bonferroni correction threshold: for 11 SNP examined in 3 IBD (CD, UC and IBD); $\alpha = 1.5 \times 10^{-3}$

Table 2

Discovery, replication, and pooled analysis showing association between *RAC1* SNPs and IBD, CD, and UC.

SNP	Position	Cohort	Number of Individuals (Risk Allele Frequency)	Number of Patients (Risk Allele Frequency)	P-value	OR (95% CI)
rs10951982	6389081			**IBD**		
		Toronto	849 (0.78)	1200 (0.80)	1.3×10^{-3}	1.34 (1.12–1.62)
		North America	916 (0.76)	918 (0.79)	4.0×10^{-3}	1.31 (1.08–1.62)
		Scotland	1106 (0.74)	1288 (0.75)	2.0×10^{-2}	1.21 (1.03–1.44)
		Combined	2871 (0.75)	3346 (0.79)	$\mathbf{4.2 \times 10^{-7}}$	**1.29 (1.17–1.44)**
				CD		
		Toronto		656 (0.80)	4.0×10^{-3}	1.36 (1.09–1.69)
		North America		441 (0.77)	1.9×10^{-1}	1.16 (1.08–1.47)
		Scotland		650 (0.75)	4.7×10^{-1}	1.07 (1.11–1.34)
		Combined		1747 (0.77)	$\mathbf{3.7 \times 10^{-3}}$	**1.19 (1.06–1.34)**
				UC		
		Toronto		544 (0.81)	1.4×10^{-2}	1.34 (1.09–1.63)
		North America		477 (0.80)	8.1×10^{-4}	1.49 (1.17–1.89)
		Scotland		578 (0.78)	4.0×10^{-2}	1.43 (1.75–0.87)
		Combined		1599 (0.80)	$\mathbf{3.3 \times 10^{-8}}$	**1.43 (1.26–1.63)**
rs4720672	6410364			**IBD**		
		Toronto	849 (0.83)	1200 (0.86)	1.1×10^{-2}	1.28 (1.06–1.56)
		North America	916 (0.82)	918 (0.85)	5.5×10^{-3}	1.33 (1.08–1.62)
		Scotland	1106 (0.80)	1288 (0.83)	5.0×10^{-3}	1.28 (1.08–1.53)
		Combined	2871 (0.82)	3346 (0.85)	$\mathbf{7.7 \times 10^{-7}}$	**1.34 (1.17–1.47)**
				CD		
		Toronto		656 (0.86)	2.6×10^{-2}	1.29 (1.03–1.62)
		North America		441 (0.84)	5.0×10^{-2}	1.28 (1.00–1.63)
		Scotland		650 (0.82)	9.0×10^{-2}	1.14 (1.031.44)
		Combined		1747 (0.84)	$\mathbf{3.7 \times 10^{-4}}$	**1.26 (1.11–1.44)**
				UC		

SNP	Position	Cohort	Number of Individuals (Risk Allele Frequency)	Number of Patients (Risk Allele Frequency)	P-value	OR (95% CI)
		Toronto		544 (0.86)	4.9×10^{-2}	1.26 (1.03–1.62)
		North America		477 (0.86)	1.0×10^{-2}	1.36 (1.07–1.75)
		Scotland		578 (0.85)	3.0×10^{-3}	1.41 (1.12–1.75)
		Combined		1599 (0.85)	$\mathbf{4.7 \times 10^{-6}}$	**1.36 (1.19–1.58)**

Table 3

Genotype phenotype analysis *RAC1* SNPs in the combined analysis.

Phenotype (Montreal Classification)	# Patients	SNP	P	OR (95% CI)	SNP	P	OR (95% CI)
Ileal "only" CD (L1)	476	rs10951982	1.0×10^{-1}	1.19 (1.03–1.47)	rs4720672	1.8×10^{-1}	1.16 (1.07–1.44)
Ileal "any" (L1/L3)	1260		3.6×10^{-3}	1.23 (1.07–1.40)		1.9×10^{-3}	1.26 (1.08–1.47)
Colonic "only" CD (L2)	513		1.0×10^{-1}	1.17 (1.03–1.44)		1.0×10^{-2}	1.31, (1.06–1.61)
Ileo-colonic CD (L3)	784		6.1×10^{-3}	1.36 (1.06–1.49)		$1.5 \times^{-4}$	1.33 (1.12–1.61)
Colon "only" IBD (UC/L2 CD)	2112		1.5×10^{-7}	1.36 (1.21–1.53)		1.3×10^{-6}	1.35 (1.55–1.53)
Colon "any" IBD (UC/L2/L3 CD)	2896		7.8×10^{-8}	1.33 (1.20–1.49)		1.6×10^{-7}	1.35 (1.20–1.51)
Early onset IBD	1132		2.9×10^{-6}	1.38 (1.20–1.61)		2.0×10^{-5}	1.33 (1.19–1.61)
CD with perianal disease (p)	446		6.0×10^{-3}	1.35 (1.08–1.66)		1.6×10^{-3}	1.44 (1.14–1.85)

P-value based on combined analysis. Early onset IBD (diagnosis > 19 years of age). Bonferroni correction threshold: for 11 SNP examined in 8 IBD sub-phenotypes; $\alpha = 5.6 \times 10^{-4}$