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Genetic Susceptibility to Inflammation and Colonic Transit in Lower Functional Gastrointestinal Disorders: Preliminary Analysis

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Abstract

Background—Irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) coexist in some patients. We observed an association between Crohn's disease (CD) candidate gene *TNFSF15* and IBS symptom phenotype. Three genes (*TLR9*, *IL6* and *CDH1*) have been associated with post-infectious (PI) IBS. Our **aim** was to preliminarily assess association between 30 susceptibility loci for CD, three genes associated with PI-IBS, and *PARM1*, with colonic transit in lower functional gastrointestinal disorders (FGID).

Methods—A 665 person cohort was assembled in prior studies. TaqMan assay was used for all single nucleotide polymorphisms (SNPs) associated with the loci of interest. Data were analyzed for univariate associations with symptoms phenotype and colonic transit (nominal p values, uncorrected) using dominant and co-dominant genetic models.

Results—Carriers of the rs5743836 risk allele had increased odds for IBS-D (vs control, p=0.02, uncorrected). Among the CD risk loci, rs7927894 (p=0.007), rs7746082 (p=0.011), rs2872507 (p=0.014), together with rs5743836 (p=0.010) were univariately associated with colonic transit at 24 or 48 h. Specific tests for genetic interactions between loci revealed potential association of genes that influence neural, barrier or mast cell function with colonic transit.

Conclusion—Genetic variations that may influence local mucosal immune functions are univariately associated with altered colonic transit in lower FGID.

Keywords

CDH1; c11orf30; ORMDL3; PRDM1; TLR9

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Authors' contributions: M. Camilleri: study conceptualization, writing protocol and paper; P. Carlson: genotyping; A.R. Zinsmeister: database management and statistical analysis; S. McKinzie: patient recruitment and classification through questionnaires and medical record review; I. Busciglio: rectal sensation studies; D. Burton: scintigraphic transit; M. Zucchelli: statistical/association analysis; M. D'Amato: expertise on genotyping

INTRODUCTION

Intestinal dysmotility and sensation, abnormal gut-brain interactions, diet, stressful life experiences, prior gastrointestinal infections, and mucosal immune activation have been proposed as pathogenetic factors in irritable bowel syndrome [IBS (1)]. Changes in mucosal functions include enterochromaffin cell hyperplasia, low-grade mucosal inflammation, increased intestinal mucosal permeability (2–5), and a dysregulated immune response to intestinal bacteria (6,7). Some patients with inflammatory bowel disease (IBD) experience symptoms suggestive of IBS. For example, among over 1500 patients with IBD, Zaman et al (8) identified 30 with ileal Crohn's disease and 25 with ulcerative colitis involving only the rectosigmoid, and stable symptoms, no changes in medications or use of chronic steroids for 3 months, and no prior bowel surgery.

Rome II IBS criteria were met by 67% of patients with Crohn's disease and 60% of ulcerative colitis, with similar symptoms in the two diseases. Thus, symptoms of IBS are common in IBD patients whose inflammation appears to be stable or in remission. However, the magnitude and the nature of the inflammatory infiltrate in IBS is different from that observed in Crohn's disease (CD) and ulcerative colitis (UC). Studies of candidate genes addressing diverse mechanisms (detailed in reference 9) have not convincingly proven a genetic component in IBS; in contrast, several risk loci have been identified in inflammatory bowel disease (IBD), in particular, CD (10,11). Some of these genes ultimately impact on Th17 lymphocyte function (12).

In a prior study, in sigmoid and rectal mucosa we showed that two mucin-related genes, that is, prostate androgen-regulated mucin-like protein 1 or *PARM 1* [which at the time of our publication (13) was documented as the uncharacterized gene, DKFZP564O0823] and *MUC20* are expressed more abundantly in IBS patients than in healthy controls (13).

Recently, four other genes have been associated with post-infectious IBS (14). One of these, *CDH1* (rs 1728785) is also associated with ulcerative colitis (15). In a study (16) that combined 1992 individuals (861 IBS patients and 1131 controls) from Sweden and USA, we detected strong association with the SNP rs4263839 in the tumor necrosis factor (ligand) superfamily member 15 gene *TNFSF15*. The strongest effect of *TNFSF15* was in IBS-C, in whom the A allele was associated with nearly 2-fold reduced risk of disease in the combined cohort from the two countries.

Among biomarkers associated with functional constipation, functional diarrhea and IBS as a group of lower FGIDs, the most robust is colonic transit. For example, accelerated colonic transit was observed in ~50% of patients with IBS-D, and ~25% of patients with IBS-C and patients presenting with diarrhea or constipation (2,17,18). In addition, the primary intermediate phenotype colonic transit geometric center [GC] at 24 hours has been shown to be significantly associated with looser stool consistency, and increased stool frequency. For example, a 1 unit difference in colonic GC at 24 h is associated with ~0.6 difference in consistency on the Bristol stool form scale and ~0.45 stool/day (19).

Our current study extends the previous genotype-symptom phenotype association study. Our hypothesis is that the candidate genes associated with Crohn's disease or post-infectious IBS are significantly associated with the intermediate phenotype colonic transit in patients with lower functional gastrointestinal disorders (FGID). Our aims were: first, to extend the symptom phenotype-genotype association study to include recently described association between post-infectious IBS and *TLR9*, *IL-6*, and *CDH1* (2 SNPs) and the mucin-related *PARM1* gene; second, to conduct a preliminary association study between the 34 distinct candidate loci and the quantitative motor and sensory traits of stomach, intestinal and

colonic functions measured in 665 participants (healthy and FGID), with colonic transit at 24 hours as the primary endpoint.

We were also interested in preliminarily exploring genetic interactions at selected loci, given the observation that susceptibility genes generally contribute only a small proportion of the variance in any disease [e.g., diabetes mellitus type I (20) or multiple sclerosis (21) or Crohn's disease (14)], for example, relative to the HLA type. The biological actions of the proteins encoded by candidate genes at different loci may interact biologically to alter an underlying mechanism, phenotype or pathophysiology such as, for instance, nerve or barrier functions or mast cell control. Therefore, we also tested the genetic interactions at selected loci with the quantitative trait of colonic transit.

MATERIALS AND METHODS

Overall Design

We studied symptom phenotype in 665 participants using validated questionnaires of gastrointestinal symptoms (22) and intermediate phenotype colonic transit functions by a validated scintigraphic method that has been used extensively in health and disease states. Colonic transit of solid residue was measured in 172 participants, that is 142 patients and 30 healthy volunteers.

Participants

The participants who were considered in the association between symptom phenotypes and genotypes have been extensively described in prior publications (23–28). There were 433 patients with Rome II positive FGIDs [19 chronic abdominal pain (14 F, 5 M, mean age 47 y); 175 diarrhea-predominant IBS (IBS-D) or functional diarrhea (154 F, 21 M, mean age 45 y); 155 constipation-predominant IBS (IBS-C) or functional constipation (148 F, 7 M, mean age 45 y); 84 IBS-mixed or alternating (IBS-Alt) (77 F and 7 M, mean age 41 y); and 233 HV (165 F, and 68 M, mean age 37 y)]. All were recruited to studies of symptom phenotype and genotype from 2000–2007 (18–23). All resided within 150 miles of Rochester, Minnesota. Participants had been recruited for the original studies by means of letters or public advertisements and had signed informed consent for the respective studies; all patients fulfilled Rome II criteria (29). The study was approved by the Mayo Clinic Institutional Review Board; all participants had provided written permission for research studies based on their medical records and DNA samples. An experienced coordinator (SM) used the validated bowel symptom questionnaire, electronic medical record, and information from direct physician interview and examination (MC) to characterize the subtype of FGID.

Measurement of Colonic Transit

Colonic transit was measured by scintigraphy using a validated method that involves ileocolonic delivery of ^{111}In bound to activated charcoal in a methacrylate-coated, delayed release capsule. The performance characteristics have been published elsewhere (19,30).

Genotyping

Selection of SNPs—The SNPs selected were based on published CD meta-analysis as previously done for the study of IBS symptom-phenotype and on additional genetic risk factors that have been proposed to be associated with post-infectious IBS (14); these are listed in Table I. Single nucleotide polymorphisms (SNPs) tested for *PARM1* are non-synonymous, and may result in missense mutations with potentially functional changes in protein amino acid sequence. We kept SNPs close to *IL23* and *LRRK2* in the analysis despite the low MAF (<10%) in view of the evidence of association with IBD (15).

Based on the biological effects attributed to potential candidate genes at risk loci, we evaluated the associations for specific sets of 2-gene combinations to examine their joint associations with colonic transit. Thus:

- a. *PRDMI* and *TLR9* are both involved in barrier function, innate immunity and development of immunocytes such as lymphocytes and plasma cells (31,32).
- b. *PRDMI* and *ORMDL3* are both involved in development of neural elements: *PRDMI* with development of the enteric nervous system and afferent nerves (33) and *ORMDL3* with the synthesis of sphingolipid, a critical component of nerve structures.
- c. *ORMDL3* and *c11orf30* are both associated with atopy, allergy and mast cell function (34).

Method—Genomic DNA was isolated from blood using standard methods. Genotyping was carried out with TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions using 10–20ng of genomic DNA for each sample. Primers and probes were Assay-by Design (Applied Biosystems). Following PCR amplification, end reactions are read on the 7300 Real-Time PCR System using Sequence Detection Software (Applied Biosystems). Hardy-Weinberg Equilibrium (HWE) calculations were performed to verify that each marker was within allelic equilibrium in controls.

Statistical Analyses

The pre-specified quantitative trait of primary interest was colonic transit (geometric center [GC]) at 24 hours. The overall univariate associations of SNPs with colonic transit were assessed by coding the genotypes based on the dominant genetic model and using the two-sample Wilcoxon rank sum test. In addition, linear regression analyses using the general (additive) genetic model were also examined. This corresponds to using the number of minor allele copies (0,1,2) as the predictor variable in the regression models for colonic transit values (e.g. GC at 24 hours). Since 34 genes were assessed, a correction for multiple comparisons would require a Bonferroni adjusted alpha level ($\alpha = 0.05/34$ or 0.0015) to assess statistical significance over all individual genes tested for their association with the quantitative trait of primary interest. The results are presented as uncorrected p values for univariate associations of individual genes.

Associations of 2-gene combinations were examined using a nonparametric approach [Kruskal-Wallis test for overall associations among the 4 genetic subgroups (that is, homozygous major versus combined heterozygous plus homozygous minor allele) per pair, based on the dominant genetic model and the Wilcoxon rank sum test for 6 possible pairwise comparisons]. For the later pairwise tests, statistical significance was only noted when the corresponding p value was <0.0083 (that is an alpha level of 0.05/6).

RESULTS

Genotyping

Thirty-four SNPs (Table I) were genotyped in 665 study participants. Allelic frequencies for all the SNPs showed no significant deviation from Hardy Weinberg equilibrium (HWE) in the control groups. All samples were successfully genotyped for all SNPs except for two samples for the *rs1800795* SNP (99.9% success rate).

Association of Inflammation Susceptibility Genotypes with Symptom IBS Phenotype

Data in Table I show the associations of the 34 SNPs of interest and the overall IBS phenotype. Part of this table has been previously reported elsewhere (15). The SNP rs 5743836 (nearest gene *TLR9*) was borderline univariately associated with IBS ($p=0.09$); in these carriers, the association corresponded to an increased odds ratio for IBS-D versus controls (nominal $p=0.02$). Table II shows the genotype frequency in each sub-phenotype of IBS for SNP rs 5743836.

No other nominally significant associations were detected between IBS subgroups and the 2 *CDH1* and *IL-6* SNPs.

Association of Inflammation Susceptibility Genotypes with Intermediate phenotype of Colonic Transit

Table III shows univariate associations (not corrected for multiple comparisons) of inflammation susceptibility SNPs with colonic transit using the dominant inheritance model (Table IIIA) and the co-dominant or additive model (Table IIIB). Based on the dominant inheritance model, rs7746082 (nearest gene *PRDM1*) is associated with colonic transit at 24 and 48 hours; rs7927894 (nearest gene *c11orf30*) and rs2872507 (nearest gene *ORMDL3*) with colonic transit at 24 hours, and rs5743836 (nearest gene *TLR9*) with colonic transit at 48 hours. The same univariate associations were observed using the co-dominant or additive model.

Quantitative Differences in Motor Endpoints Associated with Significant SNPs

Table IV shows quantitative data in colonic transit at 24 or 48 hours that were univariately associated with SNPs. The magnitudes of differences within each genotype were approximately 15–25%.

Association of Genes in Combination with Colonic Transit

Figure 1 shows the effects of 2-gene combinations of three different pairs. The magnitude of the difference in colonic GC24h (differences in means of up to 1.0 GC unit) in the combined gene pairs and the intermediate effects of the alternative gene pairs suggest a gene-gene interaction.

DISCUSSION

This study suggests there are nominal associations in patients with lower FGID between genetic susceptibility to mucosal immune responses and colonic transit. Few candidate genes have hitherto convincingly demonstrated association with genetic susceptibility to FGID. Variation in *FAAH* C385A (which determines the level of the rate-limiting enzyme for anandamide metabolism) was associated with both IBS-D phenotype and with accelerated colonic transit (29), and *NPSR1* with colonic transit and rectal sensation (35). Other genetic susceptibility factors associated with IBS symptom phenotype (9) have not been confirmed with quantitative traits.

Associations with Colonic Transit

The association of rs7927894 (nearest gene *c11orf30*) with colonic transit at 24 hours shows the most robust association between the CD susceptibility genes and colonic transit with a univariate α of 0.0071 (versus 0.0015 by Bonferroni correction for 34 genes). This rs7927894 SNP has been associated with CD in some populations (12,36) and with atopic dermatitis (37). It is located in an intergenic region, 38 kb downstream of *C11orf30* and 68 kb upstream of *LRRC32* (leucine rich repeat containing 32).

rs2872507 (nearest gene *ORMDL3*) is associated with colonic transit in our cohort. *ORMDL3* is associated with allergy (34) and asthma in several different ethnicities (38,39), in addition to IBD (12,40). *ORMDL3* is also involved in control of sphingolipids (41) which are structural components of membranes, including those in neural tissues which are conceivably relevant to neuromuscular modulation in IBS and lower FGID.

These observations are intriguing, given the primary role of mast cells in atopy and the proximity of mast cells to nerves, in a subset of IBS (42). The argument suggesting that *C11orf30* and *ORMDL3* (tagged by SNPs rs7927894 and rs2872507 respectively in our study) may be susceptibility factors for accelerated colonic transit is strengthened by the 2-gene interactions illustrated in Figure 1. Thus, a specific combination of variants of these 2 genes is associated with considerably higher (0.7GC units) mean colonic transit relative to another gene combination.

The likelihood ratio of IBS was significantly higher in patients with seasonal allergic rhinitis (2.67 times) and with allergic eczema (3.85 times) than controls (43). It would be very interesting to explore the strength of association for patients with IBS whose mast cells are close to nerves on rectal mucosal biopsies (42).

The univariate associations of SNP rs7746082 (nearest gene *PRDM1*) with colonic transit is consistent with a nominal association reported with IBS symptom phenotype (16). The SNP rs7746082 maps in a gene desert region with 100 kb of intervening DNA between this marker and the *PRDM1* gene (also called *BLIMP1*). *PRDM1* is a zinc finger transcriptional repressor, that silences β -interferon gene expression, regulates plasma cell differentiation, and controls gene expression in T lymphocytes, macrophages, sebaceous gland and skin epidermis (reviewed in ref. 44). However, the equivalent molecule in zebra fish is essential for neural crest and sensory neuron specification (28,45) suggesting that *PRDM1* polymorphism may impact colorectal functions by influencing neural function. Figure 1 illustrates that colonic transit is associated with markers of the *PRDM1* and *ORMDL3* genes in combination, and is consistent with a biologically plausible impact of the genes in combination.

rs5743836 (nearest gene *TLR9*) is possibly associated with the phenotype of IBS-D ($p=0.02$) and with colonic transit ($p=0.09$ at 24 hours, and $p=0.02$ at 48 hours) in univariate analysis. Antecedent gastrointestinal infections were not formally assessed in our patient cohort. Our observations build on the recently described association between PI-IBS and *TLR9* (14). The 2-gene interaction between rs7746082 (*PRDM1*) and rs5743836 (*TLR9*) is intriguing as both individually influence barrier functions, and increased permeability has been described in IBS (5). We explored the opportunity to evaluate 67 other SNPs near the *TLR9* gene. Sixty-three had MAF lower than 10%, which was the cut-off used for inclusion in this study based on statistical considerations. Three other SNPs with MAF 10% were either synonymous (rs352140), intronic (rs352139) or 5' near gene (rs187084). We did not evaluate rs187084 because its association with PI-IBS was tested by Villani et al. (14) as part of the fine mapping of the *TLR9* gene, and it was not significant ($p=0.114$). Overall, the data regarding rs5743836 (*TLR9*) and rs7746082 (*PRDM1*) support the hypothesis that genetic variation in mucosal permeability, pathogen recognition, and activation of innate immunity might predispose patients to altered colonic transit that is a common intermediate phenotype of IBS.

Associations with Symptom Phenotype

We previously reported (16) significant association of *TNFSF15*, and the nominal association of *IL23R* and *CCR6* with the IBS symptom phenotype; these all point to a pivotal role of Th17 cells in the mucosal immune activation in IBS based on cytokine

expression and minor inflammatory infiltration (7), most prominent in those with diarrhea-predominant IBS (46). Long after an inflammatory insult in animal models, there may be persistent dysregulation of neuromuscular function in the intestine (47). The fact that SNPs near to *TNFSF15*, *IL23R* and *CCR6* were not associated with the colonic transit suggests involvement of other intermediary mechanisms that may be required to induce the motor dysfunction. Given the focus on colonic transit in this paper, we did not explore the association of other genotypes of *IL23R* and *CCR6*.

Strengths and Limitations

The strengths of this study include the use of an extensively validated, intermediate phenotype (scintigraphic colonic transit) that is associated with symptoms of FGID and quantitatively associated with stool consistency and frequency. This objective measurement has known coefficient of variation (COV), providing a trait to quantitate the effect of the associated SNPs with higher statistical power to detect associations of interest, compared to the larger variation of symptom profiles. A second strength is the application of Bonferroni correction to offset the multiple comparisons involving 34 genes tested and the 6 pairwise comparisons in 2-gene interaction studies. Third, the average 0.4 geometric center unit differences in colonic transit attributed to the gene variants shown in Table IV are clinically relevant, and would correspond to ~0.3 unit on the Bristol stool form scale (score ranging from 1 to 7), or a difference of 0.2 bowel movements per day (19).

The major weaknesses of the study are that the univariate associations are to be regarded as hypothesis-generating, and candidate gene approaches may not identify the specific variation(s) that are actually responsible for the intermediate phenotypes of FGIDs. However, our analysis narrows the number of inflammatory susceptibility loci that deserve to be included in future studies aimed at replicating our findings.

In conclusion, our preliminary studies support the hypothesis that susceptibility loci that have the potential to control mucosal immune function including mast cells, inflammatory mediators, barrier function and cytokines associated with Th17 lymphocyte lineage, appear to be associated with colonic transit in IBS. These data also support a role of inflammation with motility disturbances in the etiopathogenesis of lower FGID.

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Abbreviations

C11orf30	chromosome 11 open reading frame 30
CDH1	Cadherin-1
IBS-D	diarrhea-predominant irritable bowel syndrome
IBS-C	constipation-predominant irritable bowel syndrome
IBS-Alt	irritable bowel syndrome with alternating bowel function
IL-6	interleukin 6
MUC 20	mucin 20, cell surface associated
OR	odds ratio

ORMDL3	orosomucoid1-like 3
PCR	polymerase chain reaction
PRDM1	positive regulatory domain zinc finger protein 1
SNP	single nucleotide polymorphism
TLR9	Toll-like receptor 9
TNFSF15	tumor necrosis factor super family 15

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Figure 1. Genetic interaction at selected loci and association with colonic transit

In each pair, there were overall differences between genetic variants (Kruskal-Wallis test), and one set of combinations was significantly different (corrected for 6 comparisons) from one other set of gene variations within that pair of genes. rs7746082 is closest to *PRDM1*, rs5743836 to *TLR 9*, rs2872507 to *ORMDL3*, and rs7927894 to *c11orf30*.

Table 1
Association between Candidate Risk Loci and IBS in Population of Patients from the Midwestern Region of the USA

HWE is calculated based on the entire sample in the study. Data on SNPs in italics are reproduced from ref. 16 (submitted). Data on *CDH1*_rs16260, *TLR9*_rs5743836, *CDH1*_rs1728785, *IL-6*_rs1800795, *PARM1*_rs3822109, are previously unreported.

SNP	Nearest gene	Chromosome position	MAF	HWE	P	OR	(95% CI)
<i>rs7746082(G/c)</i>	<i>PRDM1</i>	106435269	0.23	0.6082	0.031	1.34	(1.02–1.75)
<i>rs5743836(A/g)</i>	<i>TLR9</i>	52260782	0.15	1	0.09 *	1.39	(0.95–2.04)
<i>rs7927894(C/t)</i>	<i>C11orf30</i>	76301316	0.41	0.2417	0.63	0.94	(0.74–1.20)
<i>rs2872507(G/a)</i>	<i>ORMDL3</i>	38040763	0.44	0.3731	0.84	0.98	(0.77–1.24)
<i>rs744166(A/g)</i>	<i>STAT3</i>	40514201	0.41	0.9778	0.76	0.96	(0.76–1.22)
<i>rs762421(A/g)</i>	<i>ICOSLG</i>	45615561	0.35	0.4407	0.069	1.24	(0.98–1.58)
<i>rs1456893(A/g)</i>	<i>IKZF1</i>	50269672	0.26	0.814	0.15	1.21	(0.93–1.57)
<i>rs1551398(A/g)</i>	<i>TRIB1</i>	126540051	0.40	0.5731	0.92	0.99	(0.78–1.25)
<i>rs1736135(T/c)</i>	<i>USP25</i>	16805220	0.44	0.703	0.54	0.93	(0.74–1.17)
<i>rs2066844(C/t)</i>	<i>NOD2</i>	50745926	0.04	0.6587	0.92	0.97	(0.53–1.79)
<i>rs2188962(C/t)</i>	<i>C5orf56</i>	131770805	0.38	1	0.16	1.18	(0.93–1.50)
<i>rs2274910(C/t)</i>	<i>ITLN1</i>	160852046	0.35	1	0.26	0.87	(0.68–1.11)
<i>rs2301436(C/t)</i>	<i>CCR6</i>	167437988	0.49	0.6466	0.20	0.86	(0.68–1.08)
<i>rs2476601(G/a)</i>	<i>PTPN22</i>	114377568	0.10	0.7987	0.73	1.07	(0.73–1.58)
<i>rs2542151(T/g)</i>	<i>PTPN2</i>	12779947	0.18	0.1876	0.67	0.94	(0.69–1.27)
<i>rs3197999(G/a)</i>	<i>MST1</i>	49721532	0.26	0.4389	0.92	1.01	(0.78–1.31)
<i>rs3764147(A/g)</i>	<i>C13orf31</i>	44457925	0.23	0.4126	0.94	1.01	(0.77–1.33)
<i>rs3828309(G/a)</i>	<i>ATG16L1</i>	234180410	0.48	0.2175	0.54	0.93	(0.74–1.17)
<i>rs4263839(G/a)</i>	<i>TNFSF15</i>	117566440	0.37	0.3977	0.0027	0.69	(0.54–0.88)
<i>rs4613763(T/c)</i>	<i>PTGER4</i>	40392728	0.14	0.7555	0.64	1.08	(0.77–1.51)
<i>rs6908425(C/t)</i>	<i>CDKALI</i>	20728731	0.22	0.1785	0.92	0.99	(0.74–1.32)
<i>rs9286879(A/g)</i>	<i>TNFSF18</i>	172862234	0.26	0.7344	0.94	0.99	(0.75–1.30)
<i>rs10045431(C/a)</i>	<i>IL12B</i>	158814533	0.30	0.1459	0.42	0.90	(0.69–1.17)
<i>rs10758669(A/c)</i>	<i>JAK2</i>	4981602	0.35	0.7205	0.75	1.04	(0.81–1.33)
<i>rs10995271(G/c)</i>	<i>ZNF365</i>	64438486	0.35	0.326	0.42	1.11	(0.86–1.42)
<i>rs11775593(C/t)</i>	<i>LRRK2</i>	40601940	0.03	0.5151	0.059	0.48	(0.23–1.02)
<i>rs11190140(C/t)</i>	<i>NKX2-3</i>	101291593	0.49	0.6768	0.36	0.90	(0.71–1.13)

SNP	Nearest gene	Chromosome position	MAF	HWE	P	OR	(95% CI)
rs11465804(T/g)	IL23R	67702526	0.03	1	0.0060	2.21	(1.21–4.04)
rs11584383(T/c)	C1orf81	200935866	0.32	0.3613	0.33	0.88	(0.69–1.13)
rs11747270(A/g)	IRGM	150258867	0.10	0.9377	0.050	0.66	(0.44–1.00)
rs17582416(T/g)	CUL2	35287650	0.38	0.9377	0.80	0.97	(0.76–1.24)
rs16260(C/a)	CDH1	68771034	0.29	0.3779	0.35	1.17	(0.84–1.62)
rs1728785(C/a)	CDH1	68591230	0.21	0.0679	0.47	1.14	(0.81–1.60)
rs1800795 (G/c)	IL6	22766645	0.42	0.9409	0.22	1.24	(0.88–1.74)
rs3822109 (C/t)	PARM1	75937971	0.44	1	0.31	1.20	(0.84–1.70)

* There is an increased odds for IBS-D vs healthy control status (p=0.02)

Table II
Percentages of Genotypes in Sub-phenotypes of IBS and Healthy Controls in All Postulated Susceptibility Loci Investigated

Genotype	Nearest gene	A-IBS	Chr. Abd. Pain	C-IBS	D-IBS
Total N=		84	19	156	175
rs7746082 CC/CG/GG	<i>PRDM1</i>	7.1/42.9/50.0	10.5/36.8/52.6	7.7/37.8/54.5	8.6/41.1/50.3
rs5743836 GG/AG/AA	<i>TLR9</i>	77.4/20.2/2.4	63.2/36.8/0	74.3/21.2/4.5	67.4/31.4/1.2
rs7927894 CC/CT/TT	<i>C11orf30</i>	45.2/41.7/13.1	31.6/36.8/31.6	29.5/53.9/16.7	38.3/48.0/13.7
rs2872507 AA/AG/GG	<i>ORMDL3</i>	21.4/45.2/33.3	21.1/31.6/47.4	14.1/55.1/30.8	19.4/54.3/26.3
rs744166 AA/AG/GG	<i>STAT3</i>	38.1/45.2/16.7	21.1/58.9/21.0	34.6/49.4/16.0	38.3/46.9/14.8
rs762421 AA/AG/GG	<i>ICOSLG</i>	35.7/47.6/16.7	26.3/36.8/36.8	37.8/46.8/15.4	36.6/48.6/14.9
rs1456893 AA/AG/GG	<i>IKZF1</i>	50.0/40.5/9.5	52.6/42.1/5.3	51.3/36.5/12.2	48.0/42.9/9.1
rs1551398 AA/AG/GG	<i>TRIB1</i>	40.5/46.4/13.1	26.3/52.6/21.1	43.6/43.0/13.4	36.0/44.0/20.0
rs1736135 CC/CT/TT	<i>USP25</i>	14.2/50.0/35.7	26.2/42.1/31.6	20.5/46.8/32.7	17.7/47.4/34.9
rs2066844 CC/CT/TT	<i>NOD2</i>	97.6/2.4/0.0	84.2/15.8/0.0	91.0/9.0/0.0	92.0/8.0/0.0
rs2188962 CC/CT/TT	<i>LOC441108</i>	34.5/47.6/17.9	36.8/52.6/10.5	37.8/41.0/21.2	33.1/46.3/20.1
rs2274910 CC/CT/TT	<i>ITLN1</i>	42.9/39.3/17.9	36.8/47.4/15.8	44.9/46.2/8.9	49.1/40.6/10.3
rs2301436 CC/CT/TT	<i>CCR6</i>	31.0/47.6/21.4	31.6/36.8/31.6	23.7/48.7/27.6	30.3/50.3/19.4
rs2476601 AA/AG/GG	<i>PTPN22</i>	0.0/19.1/80.9	0.0/5.3/94.7	1.9/18.0/80.1	1.1/18.9/80.0
rs2542151 GG/GT/TT	<i>PTPN2</i>	8.3/26.2/65.5	5.3/15.8/78.9	0.6/25.6/73.7	3.4/26.3/70.3
rs3197999 AA/AG/GG	<i>APEH_MST1</i>	3.6/38.1/58.3	15.8/26.3/57.9	8.3/40.4/51.3	8.6/36.6/54.9
rs3764147 AA/AG/GG	<i>CCDC122(C13orf31)</i>	69.1/25.0/5.9	57.9/36.8/5.3	58.3/35.3/6.4	56.6/36.6/6.9
rs3828309 AA/AG/GG	<i>ATG16L1</i>	26.2/50.0/23.8	10.5/31.6/57.9	17.6/52.6/29.5	28.6/42.3/29.1
rs4263839 AA/AG/GG	<i>TNFSF15</i>	13.1/36.9/50.0	15.8/42.1/42.1	6.4/37.8/55.8	7.4/47.4/45.1
rs4613763 CC/CT/TT	<i>PTGER4</i>	1.2/29.8/69.1	0.0/42.1/57.9	0.0/26.3/73.7	2.9/25.1/72.0
rs6908425 CC/CT/TT	<i>CDKALI</i>	59.5/38.1/2.9	79.0/21.0/0.0	61.5/34.0/4.5	58.3/36.0/5.7
rs9286879 AA/AG/GG	<i>TNFSF18</i>	53.6/38.1/8.3	57.9/36.8/5.3	58.3/35.9/5.8	58.9/36.6/4.6
rs10045431 AA/AC/CC	<i>IL12B</i>	10.7/35.7/53.8	10.5/47.4/42.1	5.8/41.7/52.6	6.9/46.9/46.3
rs10758669 AA/AC/CC	<i>JAK2</i>	34.5/56.0/9.5	36.8/52.6/10.5	40.4/46.2/13.4	44.6/44.6/10.8
rs10995271 CC/CG/GG	<i>ZNF365</i>	14.3/54.8/30.9	15.8/42.1/42.1	11.5/46.8/41.7	15.4/42.9/41.7
rs11175593 CC/CT/TT	<i>LRRK2(MUC19)</i>	97.7/2.4/0.0	100.0/0.0/0.0	96.8/3.2/0.0	96.0/4.0/0.0

Genotype	Nearest gene	A-IBS	Chr. Abd. Pain	C-IBS	D-IBS
rs11190140 CC/CT/TT	NKX2_3	33.3/47.6/19.1	31.6/42.1/26.3	26.9/51.3/21.8	28.0/48.6/23.4
rs11465804 GG/GT/TT	IL23R	0.0/11.9/88.1	0.0/15.8/84.2	0.0/10.3/89.7	1.1/12.0/86.9
rs11584383 CC/CT/TT	C1orf81(KIF21B)	16.7/36.9/46.4	10.5/26.3/63.2	7.1/43.6/49.5	9.1/38.9/52.0
rs11747270 AA/AG/GG	IRGM	85.7/13.1/1.2	100/0/0/0	86.5/12.9/0.6	86.3/13.7/0.0
rs17582416 GG/GT/TT	CUL2	6.0/52.4/41.6	10.5/68.4/21.1	11.5/50.0/38.5	13.1/45.7/41.2
rs16260 AA/AC/CC	CDH1	11.9/35.7/52.4	5.3/57.9/36.8	7.1/46.8/46.2	10.3/37.7/52.0
rs1728785 AA/AC/CC	CDH1	6.0/33.3/60.7	0.0/42.1/57.9	2.6/34.6/62.8	6.9/27.4/65.8
rs1800795 CC/CG/GG	IL6	17.9/47.6/34.5	21.1/63.2/15.8	14.1/49.4/36.5	18.9/50.9/30.2
rs3822109 CC/CT/TT	PARM1	29.8/59.5/10.7	31.6/47.4/21.1	23.7/50.6/25.6	33.7/46.3/20.0

Table IIIA
Association of Inflammation Susceptibility Genotypes and Quantitative Traits of Colonic
Function using the Dominant Genetic Model which Combined the Minor Allele Subgroup
with the Heterozygote Subgroup (table lists p values from Wilcoxon rank sum test).[†]

GENE/SNP	Colonic transit GC24h N=172	Colonic transit GC48h N=171
PRDM1_rs7746082	0.011	0.011
TLR9_rs5743836	0.093	0.010
C11orf30_rs7927894	0.007	0.058
ORMDL3_rs2872507	0.014	0.086
STAT3_rs744166	0.799	0.540
ICOSLG_rs762421	0.085	0.316
IKZF1_rs1456893	0.960	0.461
TRIB1_rs1551398	0.087	0.210
USP25_rs1736135	0.387	0.162
LOC441108_rs2188962	0.131	0.564
ITLN1_rs2274910	0.170	0.169
CCR6_rs2301436	0.663	0.571
PTPN22_rs2476601	0.426	0.274
PTPN2_rs2542151	0.917	0.497
APEH_MST1_rs3197999	0.501	0.154
CCDC122_C13orf31_rs3764147	0.348	0.658
ATG16L1_rs3828309	0.455	0.211
TNFSF15_rs4263839	0.174	0.256
PTGER4_rs4613763	0.750	0.223
CDKAL1_rs6908425	0.638	0.310
TNFSF18_rs9286879	0.728	0.636
IL12B_rs10045431	0.681	0.839
JAK2_rs10758669	0.287	0.312
ZNF365_rs10995271	0.608	0.894
LRRK2_MUC19_rs11175593	0.314	0.985
NKX2_3_rs11190140	0.087	0.072
IL23R_rs11465804	0.669	0.778
C1orf81_KIF21B_rs11584383	0.717	0.535
IRGM_rs11747270	0.774	0.679
CUL2_rs17582416	0.277	0.359
CDH1_rs16260	0.710	0.856
CDH1_rs1728785	0.305	0.579
IL6_rs1800795	0.058	0.900
PARM1_rs3822109	0.587	0.687

[†] NOTE: Univariate p values (<0.05) are reported in bold; no p values were significant after Bonferroni correction for the 34 SNPs tested.

Table IIIB
Association of Inflammation Susceptibility Genotypes and Quantitative Traits of Colonic Function using the General (Additive) Genetic Model which Considers the Number of Minor Allele Copies as the Predictor Variable in a Linear Regression Model.[†]

GENE/SNP	Colonic transit GC24h N=172	Colonic transit GC48h N=171
PRDM1_rs7746082	0.038	0.040
TLR9_rs5743836	0.527	0.044
C11orf30_rs7927894	0.048	0.093
ORMDL3_rs2872507	0.040	0.153
STAT3_rs744166	0.371	0.993
ICOSLG_rs762421	0.194	0.177
IKZF1_rs1456893	0.943	0.519
TRIB1_rs1551398	0.204	0.108
USP25_rs1736135	0.582	0.422
LOC441108_rs2188962	0.132	0.881
ITLN1_rs2274910	0.492	0.368
CCR6_rs2301436	0.660	0.844
PTPN22_rs2476601	0.255	0.192
PTPN2_rs2542151	0.942	0.314
APEH_MST1_rs3197999	0.905	0.237
CCDC122_C13orf31_rs3764147	0.304	0.972
ATG16L1_rs3828309	0.302	0.322
TNFSF15_rs4263839	0.124	0.504
PTGER4_rs4613763	0.488	0.540
CDKAL1_rs6908425	0.861	0.175
TNFSF18_rs9286879	0.896	0.775
IL12B_rs10045431	0.437	0.733
JAK2_rs10758669	0.296	0.548
ZNF365_rs10995271	0.939	0.634
LRRK2_MUC19_rs11175593	0.320	0.988
NKX2_3_rs11190140	0.910	0.396
IL23R_rs11465804	0.691	0.759
C1orf81_KIF21B_rs11584383	0.931	0.624
IRGM_rs11747270	0.693	0.714
CUL2_rs17582416	0.174	0.664
CDH1_rs16260	0.413	0.772
CDH1_rs1728785	0.170	0.871
IL6_rs1800795	0.089	0.859
PARM1_rs3822109	0.447	0.786

[†] NOTE: Univariate p values (<0.05) are reported in bold; no p values were significant after Bonferroni correction for the 34 SNPs tested.

Table IV
Quantitative Effect of SNPs of Interest on Physiological Endpoints

Genotype (SNP)	Allele	GC @ 24 h	GC @ 48 h
<i>PRDMI_rs7746082</i>	GG	94 2.41±1.05	93 3.48±1.17
	GC/CC	78 2.80±1.13	78 3.93±1.07
<i>TLR9_rs5743836</i>	AA	126 2.53±1.11	125 3.55±1.16
	AG/GG	46 2.74±1.06	46 4.04±1.03
<i>C11orf30_rs7927894</i>	CC	67 2.87±1.19	67 3.88±1.12
	CT/TT	105 2.40±1.00	104 3.56±1.15)
<i>ORMDL3_rs2872507</i>	GG	50 2.29±0.98	50 3.48±1.11
	GA/AA	122 2.70±1.13	121 3.77±1.15

Data show N, and Mean ± SD analyzed by dominant genetic model categorization (which combined the minor allele with the heterozygote subgroup); bolded text refers to significant univariate associations.