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Observations and Research

The Genetics of Postoperative Recurrence in Crohn Disease: A Systematic Review, Meta-analysis, and Framework for Future Work

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Background: Recurrence following abdominal surgery in Crohn disease is over 50%. The impact of genetics on postoperative recurrence is not well defined.

Methods: A literature search was conducted where inclusion required an assessment, by genotype, of postoperative recurrence. The primary endpoint was odds of surgical recurrence.

Results: Twenty-eight studies identified a total of 6715 patients. Thirteen loci were identified as modifying the risk of recurrence. NOD2 was identified as a risk factor for recurrence by multiple works (cumulative odds ratio: 1.64, $P = 0.003$).

Conclusions: A NOD2 risk allele is associated with recurrence following surgery in Crohn disease. Progress in this area will require standardized reporting in future works.

Lay Summary

Crohn disease often requires surgery, but disease recurs after surgery about half the time. This work identifies a gene *NOD2*, associated with recurrence, and reviews all published literature on the genetics of postoperative recurrence.

Key Words: Crohn disease, inflammatory bowel disease, recurrence, genetics, NOD2, CARD15

Introduction

An individual's genetic makeup is at the root of many complex disease processes.[1] Mapping of the human genome has not been the panacea that many had hoped, but advancements in the field of genomics have increased our understanding of numerous disease processes, including Crohn disease (CD).[2] Several large, multinational genomic cohorts have identified specific loci associated with the disease and revealed the importance of genetic makeup on the development and phenotype of CD.[2] Investigation of these genetic associations has led to significant insights into the pathogenesis of CD. The identification of *NOD2*, the first and most well-studied genetic loci associated with CD, highlights the importance of microbial–immune system interactions in the process of the disease, given the gene's role in bacterial peptide recognition.[3] The heterogeneity of phenotype in CD encourages a more granular approach to genetic analysis, and work has been done identifying specific loci and their association with aggressive phenotypes, and penetrating and fibrostenotic disease.

The need for surgical intervention in CD is common, with over 60% of patients requiring surgery within 10 years of diagnosis.[4] Surgery is not considered curative as postoperatively up to 80% of patients experience endoscopic recurrence within 1 year of resection, and over 50% require repeat surgical intervention within a decade.[5,6] These data demonstrate the importance of the problem clinically but furthering the understanding of postoperative recurrence may also be important for understanding CD in general. Surgical resection offers a microbial and inflammatory clean slate, and understanding the processes leading to recurrence in this setting may help reveal the mechanism leading to de novo disease occurrences. Given that there is a general lack of cohesive genetic information related to postoperative disease recurrence, a thorough and complete systematic review will help to identify any risk loci, and focus further work regarding the etiology and pathogenesis of postoperative recurrence.[7] To date, there has been no comprehensive project aimed at identifying all loci associated with postoperative recurrence of CD. Therefore, we present the first comprehensive up-to-date systematic

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review and meta-analysis of the genetic factors involved in the recurrence of CD following abdominal surgery.

Materials and Methods

Search Strategy

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[8] The protocol was prospectively registered on the international prospective register of systematic reviews, PROSPERO (ID: CRD42017073629).

A systematic search, designed by our research librarian (TC), was performed of Medline, CINAHL, Web of Science, and Embase databases for all studies published between January 1, 1950 and February 12, 2020 examining the genetics of postoperative CD recurrence. The following key words were used, "Crohn's disease or inflammatory bowel disease" and "genetics or genes or polymorphism or mutations or SNP" AND "recurrence" (Appendix 1). We did not limit our search to a language, nor did we exclude unpublished data. Further works were added after review of the reference lists in the publications, and manual searching of PubMed, for relevant articles missed by our search criteria. Abstracts and full-text reviews were evaluated by 2 investigators (ML and JD) in order to evaluate for inclusion and exclusion criteria. Disagreements were resolved by a third reviewer (TD).

Selection Criteria

Inclusion criteria included studies that examined postoperative recurrence of CD and defined 2 or more cohorts by specific genotypes. Both retrospective and prospective study designs were included. Exclusion criteria included studies with a lack of recurrence definition, case series or case studies, review articles, and subsets of subsequently published larger studies.

Data Extraction

Data extraction was completed independently by 2 reviewers (ML and JD) using a standard data abstraction form. The primary outcome of interest was recurrence of CD between genetic cohorts. Data collected also included year of study, study location, numbers of patients, definitions of recurrence, sex, age, tobacco use, follow-up, candidate genes and polymorphisms, genotyping method, allele frequency, and homozygosity/heterozygosity frequency. Authors of studies were contacted for missing data, and these data are presented when successfully obtained.

Risk of Bias Assessment

The Methodological Index for Non-Randomized Studies (MINORS) tool was used to assess the risk of bias for included studies and results are included in Appendix 2.[9]

Statistical Analysis

Descriptive categorical data are expressed as percentages and continuous data are expressed as weighted mean ± SD. Baseline differences between groups were evaluated by univariate analyses using Fisher exact test for categorical data and independent sample *t* test for continuous data. Meta-analysis was performed when data were available from 3 or more studies, using RevMan 5.3 software,[10] and employed a random-effects model. Sensitivity analyses were planned based on the type of recurrence recorded (ie, clinical, endoscopic, or surgical) and type of surgery performed (ie, ileal resection and ileocolectomy alone). An assessment of individual data combined from each study was planned a priori when the data were available. Univariate analysis of genetic loci was performed using a log-rank test, with time to surgical recurrence or to the end of follow-up used at the time variable. Kaplan–Meier curves were created using this analysis.

Included studies were then tested for heterogeneity using the chi-square test with significance set at $P < 0.10$ and the amount of heterogeneity was quantified by the I^2 statistic as follows: (1) low 25%, (2) moderate 50%, and (3) high 75%.[11]

Results

Study Selection

A comprehensive literature search yielded 461 potentially relevant articles (Fig. 1). After title and abstract screening 65 articles remained for full-text review. From these, 28 studies were eligible for inclusion (Table 1). Five of the included studies were published as abstracts only. Both retrospective and prospective studies were identified and included. Attempt to contact all corresponding and first authors were made, 19 of which were unsuccessful and received no response. Four investigators responded but did not possess the necessary data. Five authors were able to provide the requested information.

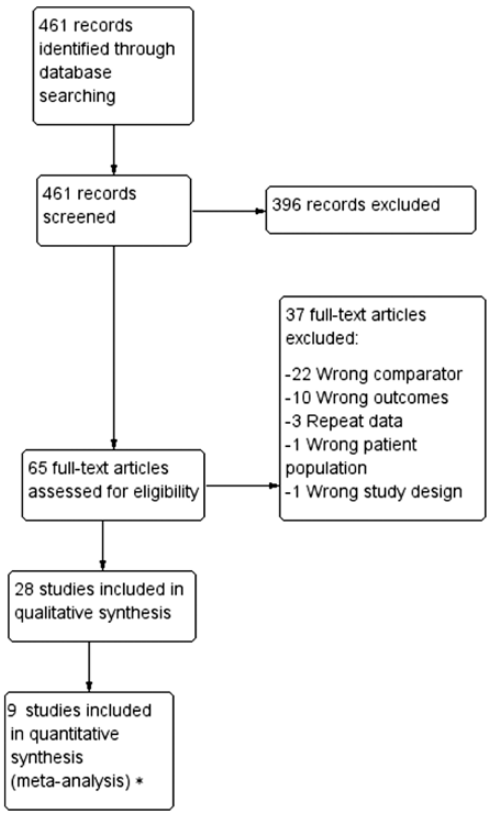

Figure 1. PRISMA flow diagram. *Nine studies were appropriate for meta-analysis based on the presence of a risk allele at *NOD2*. Five studies were appropriate for meta-analysis based upon the presence of separate *NOD2* subtypes. Eight studies were appropriate for meta-analysis based on the presence of a risk allele at *NOD2* specifically for surgical recurrences. Four studies were appropriate for meta-analysis based on the presence of a risk allele at *NOD2* specifically for surgical recurrences after small bowel or ileocolonic resection. Three studies were appropriate for a time-to-event analysis based on the presence of a risk allele at *NOD2*.

Table 1. Study Characteristics[12–39]

Author	Year	Location	Clinical Data Source	Journal	n	Genotyping Method	Genes Examined	No. Loci Examined	Abstract or Full Article
Ahmad	2002	United Kingdom	Prospective database	Gastroenterology	158	PCR sequence-specific primers	NOD2	3	Article
Alvarez-Lobos	2005	Spain	Prospective database	Annals of Surgery	170	PCR sequence-specific primers	NOD2	3	Article
Bhullar	2014	Australia	Retrospective	World Journal of Gastroenterology	30	dHPLC and direct sequencing	NOD2	4	Article
Buning	2004	Germany	Retrospective	Alimentary Pharmacology and Therapeutics	180	PCR sequence-specific primers	NOD2	3	Article
Burke	2013	Ireland	Prospective database	Annals of Surgery	147	SNP Assay	SMAD3 CTGF	5	Article
Chen	2011	NR	NR	—	285	SNP Assay	NOD2 ATG16L1	4	Abstract
Fowler	2014	United States	Prospective database	Journal of Crohn's and Colitis	194	Sequenom genotyping platform	NOD2, CARD9, ITLN1, ATG16L1, IRGM, LRRK2-MUC19, IL23R, JAK2, STAT3, TYK2, SMAD2, TNFSF15	25	Article
Gathungu	2018	United States	Prospective database	World Journal of Gastroenterology	412	Illumina Golden Gate custom Immunochip array	NOD2, ATG16L1, IRGM, CARD9, XBP1, ORMDL, Others NR	NR	Article
Gerich	2013	NR	NR	—	589	200k Immunochip platform	Multiple	NR	Abstract
Germain	2016	France	Prospective database	Surgery	280	SNPlex technology	CARD8 NAT2, Others NR	200	Article
Ghaly	2016	Australia	Prospective database	Inflammatory Bowel Disease	309	Taqman PCR	VDBP	1	Article
Laffin	2018	Canada	Prospective database	Inflammatory Bowel Disease	191	Illumina Goldengate assay platform	SMAD3, IL10RB, IL15RA, BACH2, IL12B, IL18RAP, IFNGR2, JAK2	8	Article
Li	2019	United States	Prospective	PLoS One	106	Illumina Immunochip or Taqman Genotyping Assays	NOD2, ATG16L1, IRGM, CARD9, XBP1, ORMDL3	6	Article
Liu	2014	United States	Retrospective	—	178	NR	NOD2, ATG16L1, Others NR	NR	Abstract
Liu	2017	United States	Retrospective	—	98	Japonica array	Multiple	659,253	Article
Maconi	2009	Italy	Prospective database	The American Journal of Gastroenterology	253	NR	NOD2	3	Article

Table 1. Continued

Author	Year	Location	Clinical Data Source	Journal	n	Genotyping Method	Genes Examined	No. Loci Examined	Abstract or Full Article
Martinek	2015	NR	Retrospective	Rozhledy v chirurgii	76	Primer-specific PCR	NOD2	3	Article
Meijer	2009	Netherlands	Retrospective	Inflammatory Bowel Disease	87	PCR-RFLP and tetra primer ARMS PCR	MMP-TIMP	1	Article
Meresse	2002	NR	NR	Gut	79	Primer-specific PCR	IL10 promoter	1	Article
Naito	2016	Japan	Retrospective	—	113	Japonica array	Multiple	659,253	Abstract
Onnie	2007	United Kingdom	NR	European Journal of Gastroenterology & Hepatology	630	Taqman PCR	NOD2, IBD5	4	Article
Potdar	2019	United States	Prospective database	Journal of Crohn's and Colitis	139	Illumina Immuno-BeadChip	Multiple	NR	Article
Renda	2008	Italy	Prospective	American Journal of Gastroenterology	182	Allele-specific reverse dot-blot hybridization	NOD2	3	Article
Sehgal	2012	United States	Retrospective	Diseases of the Colon & Rectum	66	DNA microarray	Multiple	66	Article
Seiderer	2006	Germany	Retrospective	Scandinavian Journal of Gastroenterology	303	Primer-specific PCR	NOD2	3	Article
Siegel	2011	NR	Prospective	—	376	NR	Multiple	NR	Abstract
Van Dussen	2014	United States	Retrospective	Gastroenterology	178	Human OmniQuad SNP genotyping arrays and Immunochip	NOD2, Others NR	NR	Article
Yang	2014	Korea	Prospective database	Journal of Crohn's and Colitis	906	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based system	TNFSF15	5	Article

dHPLC, denaturing high-performance liquid chromatography; NR, not reported; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

Figure 2. Postoperative recurrence by *NOD2* risk allele.

Quality Assessment of Included Studies

Studies were assessed for bias and methodological quality using the MINORS criteria (Appendix 2).[9] None of the included articles met the global ideal score for noncomparative studies with 9 studies having a score less than 10. This was due to limitations with the inclusion of consecutive patients, the prospective collection of data, and reporting of follow-up periods. Furthermore, none of the included studies performed a prospective calculation of sample sizes.

Patient Characteristics

A total of 6715 patients were included from North America, Europe, Asia, and Australia (Table 1). The weighted mean age was 28.8 years at diagnosis or time of surgery and weighted sex was 52.9% male. The rates of active smokers ranged from 12.9% to 45.6%. The most common index surgical procedures included were primarily ileocolectomy or ileal resection (10 studies), and unspecified bowel resection (9 studies) (Table 2). Index surgical procedure was not reported in 2 studies. Median follow-up ranged from 3.4 to 16.2 years postoperatively. Postoperative recurrence occurred in 36.3% of patients. The majority of studies defined recurrence as surgical (67.9%), with fewer studies using clinical (17.9%), endoscopic (10.7%), and radiologic (7.1%) definitions. Three studies[12,13,28] defined endoscopic recurrence as a Rutgeerts score of i2 or more. Four studies did not report on their definition of recurrence.

Genetic Data

The total number of loci interrogated across all included studies was over 650,000. Available data are summarized in Table 2. In total, complete data were obtained from 13 out of 28 studies. *NOD2* was analyzed in 14 studies, while other genes examined in single studies included *BACH2*, *CTGF*, *CARD8*, *SMAD3*, *ATG16L1*, *TIMP-1*, *IL-10*, *VDBP*, *IBD5*, and *TNFSF15*. Only 3 loci were appropriate for meta-analysis, all belonging to *NOD2*. These loci were G908R, L1007fs, R702W and were analyzed in a combined manner, as well as by subtype.

Genes Associated With Postoperative Recurrence

NOD2 was the identified loci with the most available data, as 9 studies presented data suitable for meta-analysis at this risk allele[14–20] (Fig. 2). These studies included 1204 patients of which 411 (34.1%) had a *NOD2* mutation. The overall recurrence rate was 43.8% in the *NOD2* group compared to 32.4% for wild-type patients. A meta-analysis found the presence of a risk allele at *NOD2* to be a significant risk factor for postoperative recurrence [odds ratio (OR) 1.64, 95% confidence interval (CI) 1.18–2.27, $P = 0.003$]. There was moderate heterogeneity in the included studies ($I^2 = 31\%$, $P = 0.17$). A separate meta-analysis was performed on prominent variants (R702W, G908R, and L1007fs) at the *NOD2* loci with data included from 5 studies (Fig. 3).[17,19–22] The R702W SNP was found to be significantly associated with recurrence (OR 1.59, 95% CI 1.06–2.40, $P = 0.02$), with a low degree of heterogeneity ($I^2 = 0\%$, $P = 0.91$) (Fig. 3). The mutations L1007fs and G908R were not significantly associated with postoperative recurrence. Two additional sensitivity analyses were performed to similar results, examining first only studies including surgical recurrence (Fig. 4), and second, examining only studies including surgical recurrence following small bowel or ileocolonic resection (Supplementary Material 1).

Data on time to recurrence for individual subjects were obtained for 3 works, containing 521 subjects.[17,19,21] No differences in survival were noted for *NOD2* risk alleles combined, or individually (Supplementary Material 2).

Several genes were analyzed by other papers, none of which were appropriate for meta-analysis (Table 3). Genetic factors that were found to be associated (ie, $P < 0.05$) with postoperative recurrence in single work included *BACH2* homozygosity (hazard ratio [HR]: 1.54),[34] *CARD8* homozygosity (OR: 7.56),[40] *TNFSF15* (with the development of strictures) (HR: 1.71),[23] and IRGM (HR: not reported),[24] *IRF8* (HR: 0.6), *LSP1/TNNI2* (HR: 1.4), *PTGER4* (HR: 1.3), *DAP* (HR: 1.4), *FAM49B* (HR: 3.2), *PELI3* (HR: 1.8), *CHL1* (HR: 2.6), *PARVB* (HR: 0.5), and *STK24* (HR: 1.7), though many of these findings were either not analyzed in a multivariable manner or corrected for multiple testing.

Discussion

This work represents the first systematic review comprehensive for all known genetic loci associated with the postoperative recurrence of CD. It is also the first meta-analysis to find an association between any specific gene, namely *NOD2*, and disease recurrence. This effect was impressive, with an OR of 1.64 for postoperative recurrence in the *NOD2* risk allele cohort. The inability to perform additional meta-analysis on the numerous other studied highlights a major issue with the study of postoperative recurrence in CD, namely the lack of standardized reporting and data transparency.

Table 2. Study Outcomes

Study	Genetic Loci	Associated Gene	Gene Carriers	Index Surgical Procedures Included	Definition of Recurrence	Total Recurrence	Recurrence With SNP	Recurrence Without SNP	*P*
			n (%)			n (%)	n (%)	n (%)	
Ahmad 2002	G908R, L1007fs, R702W	NOD2	NR	ICR for stenotic disease	Surgical	66 (41.8%)	NR	NR	NR
Alvarez-Lobos 2005	G908R, L1007fs, R702W	NOD2	28 (40.0)	Any bowel resection or strictureplasty	Clinical and surgical	23 (32.9%)	14 (50.0)	9 (21.4)	0.019
Bhullar 2014	G908R, L1007fs, R702W, P268S	NOD2	8 (26.7)	ICR, small bowel resection and strictureplasty	Surgical	16 (53.3)	6 (75.0)	10 (45.5)	0.226
Buning 2004	G908R, L1007fs, R702W	NOD2	29 (34.5)	ICR	Surgical	14 (27.5)	12 (41.4)	2 (9.1)	<0.001
Burke 2013	rs9402373 (GC)	CTGF	43 (29.7)	ICR	Surgical	32 (22.1)	26 (25.5)	6 (14.0)	0.187
	rs17293632 (TC)	SMAD3	50 (36.8)			32 (22.1)	17 (19.8)	15 (30.0)	0.210
Chen 2011	G908R, L1007fs, R702W	NOD2	117 (41.1)	ICR	Surgical	NR	NR	NR	NR
	T300A	ATG16L1	234 (82.1)			NR	NR	NR	NR
Fowler 2014	Multiple	Multiple	NR	Any bowel resection	Surgical	69 (35.6)	NR	NR	NR
Gathungu 2018	rs2066843, rs2066844, rs2066845, rs2076756 and rs2066847, rs2241880, rs13361189	NOD2 CARD9 IRGM ATG16L1	NR	ICR or isolated ileal resection	Surgical	NR	NR	NR	NR
Gerich 2013	NR	IRF8	NR	Any bowel resection	Surgical	210 (35.7)	NR	NR	0.015
		LSP1/ TNNI2							0.04
									0.04
		PTGER4							0.04
		DAP							<0.001
		FAM49B							<0.001
		PELI3							<0.001
		CHL1							<0.001
		PARVB							<0.001
		STK24							
Germain 2016	Multiple	Multiple	NR	Any bowel resection	Surgical	38 (27.7)	NR	NR	NR
Ghaly 2016	rs7041	VDBP	NR	NR	Clinical	80 (79.2)	NR	NR	NR
Laffin 2018	rs1847472	BACH2	88 (46.1)	Any bowel resection	Surgical	87 (45.5)	47 (53.4)	40 (38.8)	0.04
	rs17293632	SMAD3							
	rs2284553	IL10RB							
	rs12722515	IL15RA							
	rs6871626	IL12B							
	rs917997	IL18RAP, IL18R1							
	rs2284553	IFNGR2							
	rs10758669	JAK2							

Table 2. Continued

Study	Genetic Loci	Associated Gene	Gene Carriers	Index Surgical Procedures Included	Definition of Recurrence	Total Recurrence	Recurrence With SNP	Recurrence Without SNP	*P*
			n (%)			n (%)	n (%)	n (%)	
Li 2019	rs2066847, rs2066884, rs2066845, rs5743289	NOD2	37 (34.9)	ICR, total abdominal colectomy, right hemicolectomy	Endoscopic	27 (44.2)*	NR	NR	NR
							NR	NR	NR
	rs2241880	ATG16L1	91 (85.8)				NR	NR	NR
	rs13361189	IRGM	30 (28.3)				NR	NR	NR
	rs10870077	CARD9	101 (95.3)				NR	NR	NR
	rs35873774	XBP1	14 (13.2)				NR	NR	NR
	rs2872507	ORMDL3	79 (74.5)						
Liu 2014	NR	NOD2	NR	Any bowel resection	NR	NR	NR	NR	NR
	T300A	ATG16L1							
	NR	Others NR							
Liu 2017	NR	Multiple	NR	Ileal resection	Endoscopic and radiologic	NR	NR	NR	NR
Maconi 2009	G908R, L1007fs, R702W	NOD2	91 (36.0)	Any bowel resection or strictureplasty	Surgical	89 (35.2)	31 (34.1)	58 (35.8)	0.891
Martinek 2015	G908R, L1007fs, R702W	NOD2	24	Any bowel resection or strictureplasty	Surgical	25 (48.1)	10 (41.7)	15 (28.8)	0.471
Meijer 2009	372 T/C	TIMP-1	60 (69.8)	ICR, small bowel resection, subtotal colectomy	Clinical, endoscopic, radiologic and surgical	NR	NR	NR	NR
Meresse 2002	IL-10	G7-8 and G10-13	17 (47.2)	ICR	Endoscopic	19 (52.8)	7 (41.2)	12 (63.2)	0.316
Naito 2016	Multiple	Multiple	NR	Ileal resection	NR	NR	NR	NR	NR
Onnie 2007	G908R, L1007fs, R702W	NOD2	90 (24.9)	Any bowel resection	Surgical	111 (30.7)	37 (41.1)	74 (27.3)	0.017
	IGR2063	IBD5				111 (30.7)	58 (35.2)	53 (27.0)	0.109
Potdar 2019	Multiple	Multiple	54 (38.9)	Small bowel resection	Clinical and surgical	NR	NR	NR	NR
Renda 2008	R702W	NOD2	35 (31.8)	Any bowel resection	Surgical	32 (29.1)	12 (34.3)	20 (26.7)	0.500
Sehgal 2012	Multiple	Multiple	NR	ICR	Surgical	NR	NR	NR	NR
Seiderer 2006	G908R, L1007FS, R702W	NOD2	NR	NR	NR	NR	NR	NR	NR
Siegel 2011	NR	Multiple	NR	Any bowel resection	Clinical	NR	NR	NR	NR
VanDussen 2014	G908R, L1007fs, R702W	NOD2	39 (41.1)	ICR or ileal resection	NR	50 (52.6)	21 (53.8)	29 (51.8)	1.000
	T300A	ATG16L1	42 (44.2)			50 (52.6)	24 (57.1)	26 (49.1)	0.536
Yang 2014	Multiple	TNFSF15	NR	Any bowel resection or intestinal bypass	Surgical	87 (25.9)	NR	NR	NR

Only most significant results reported from each study. *P* values calculated using Fisher exact test.
*Only 61 of 106 patients were assessed endoscopically for recurrence.
ICR, ileocolonic resection; NR, not reported; SNP, single nucleotide polymorphism.

CD is a chronic inflammatory bowel disease that follows a relapsing and remitting course. Longstanding, active inflammation results in progression of disease and development of penetrating or stricturing complications, often requiring surgical management. Both the incidence and prevalence of CD are rising in Western and newly industrialized countries,[41] especially in younger patients.[42] Despite advances in medical therapy, postoperative recurrence of CD still occurs endoscopically in 85%–100% and clinically in 34%–86% of patients within 3 years of surgery.[43] It is estimated that up to 60% of patients will require a second major abdominal surgery 20 years from their initial surgery.[44] Postoperative recurrence of Crohn, especially in younger individuals, can predispose patients to multiple surgeries over their lifetime, resulting in significantly lower health-related quality of life, increased medical care costs, and lower earnings.[45] Thus, identifying factors leading to postoperative CD recurrence is paramount in both understanding and treating CD.

If high-risk loci sufficiently predict recurrence, they may 1 day help guide clinical postoperative therapy choices. Thiopurines and anti-TNF therapies have been identified as effective for the prevention of postoperative recurrence, though the data here do not demonstrate a clear relationship between time of publication and rate of recurrence. The use of postsurgical medical therapies results in serious adverse events in over 15% of enrolled subjects. Therefore, to balance the risk and benefits of postoperative therapy, clinicians must be able to identify patients at a high risk of recurrence. A number of high-risk clinical features have been identified that predict postoperative recurrence including a younger patient (age <30 years old), disease duration greater than 10 years, tobacco usage, previous surgery, and family history.[46,47] Less well understood are the nonclinical, patient-specific factors that predispose patients to postoperative recurrence such a patient's individual microbiome, genetic makeup, and the complex interplay between these factors and the environmental exposures that drive inflammation in CD. The gut microbiome has long been thought to play a causative role in the high rates of CD recurrence following surgical resection.[48] Several studies[48–51] have shown that the mucosal microbial composition in CD patients at the time of surgery is predictive of future disease relapse.[52] Work in this area is ongoing and may lead to identification a microbial signature predicting recurrence, which could assist clinicians in predicting recurrence, or altering the microbiome in a way that prevents disease progression. The addition of genetic information to these other factors, may improve the utility of any method of disease prognostication.

The clinical importance of postoperative recurrence of CD is well established, and an understanding of the pathophysiology of this disease process may play a role in the broader context of CD. Our understanding of the inciting events in the development of CD remains underdeveloped. This is in large part due to the difficulty in surveying and identifying patients at risk of disease development over the years they are most at risk, as well as the tendency for the disease to progress for many years prior to presentation to medical personnel. The postoperative period offers a unique opportunity to surveil patients with a high risk of disease development and progression. The bowel, postresection, should offer a relative clean slate in terms of inflammation, disease activity, and microbial composition. Therefore, factors identified to be associated with the development of recurrent disease, free from the confounding influence of an established inflammatory milieu and entrenched microbial communities, may also play a disproportionally large role in the development of de novo disease. The specific genotypes implicated in postoperative recurrence may be worth investigation as loci of interest in terms of initial disease development.

The *NOD2* gene was first linked to genetic predisposition to developing CD in 2001.[53,54] *NOD2*, located within the IBD1 susceptible region on chromosome 16, allows intracellular recognition of gut microbes through detection of released peptidoglycans[55] and is important in regulating innate and adaptive immunity in the gut through NF-κB.[56] Autophagic pathways that act through both *NOD2* and *ATG16L1* can also be impaired with comutations in both genes.[57] Since the association was first identified, *NOD2* mutation has been shown not only to increase the susceptibility to developing CD, but also predict younger age of disease onset, more severe stricturing and penetrating phenotype, and need for surgical intervention.[58] A previous systematic review and meta-analysis demonstrated no association between *NOD2* and postoperative recurrence.[7] However, in our study, which is the first comprehensive review of all genetics involved in postoperative CD, we found that there was a strong correlation of presence of *NOD2* and recurrence of disease. We also identified that the G908R genotype was significantly associated with disease recurrence, while the other common 2 genotypes were not. This phenomenon has not been described prior to this work. The exact functional impact of G908R is not known. The lack of association seen between the L1007fs mutation and postoperative recurrence may be related to the recent discovery that those patients with a L100fs variant are less likely to smoke,[59] as smoking is the most well-established risk factor in the recurrence of CD following surgery. The G908R mutation is not protective from smoking, and therefore those individuals with the G908R mutation may be more likely to smoke, increasing their risk compared to the L100fs cohort.

In addition to *NOD2*, 7 additional genes have been implicated in postoperative recurrence, namely *BACH2*, *CARD8*, *TNFSF15*, *IRGM*, *IRF8*, *LSP1/TNNI2*, *DAP*, *PTGER4*, *PELI3*, *CHL1*, *PARVB*, and *STK24*. However, these genes have only been found in individual cohort studies, and their discovery is not unified by 1 biologic process or mechanism. The heterogeneity of included index surgical procedures and indications makes meta-analysis more challenging. This work employed multiple sensitivity analyses to address these concerns, including focusing on specific index surgical procedures and types of recurrence. The findings regarding the role of *NOD2* in recurrence postoperatively were robust across these comparisons. Additionally, some studies employed multi-SNP arrays, leading to a high risk of false discovery due to the measurement of up to hundreds of thousands of gene loci.[60] Finally, the defined endpoint of recurrence varied between studies. Attempts to replicate these exploratory studies have not been published and the lack of consistency in loci examined across published works greatly limits the util-

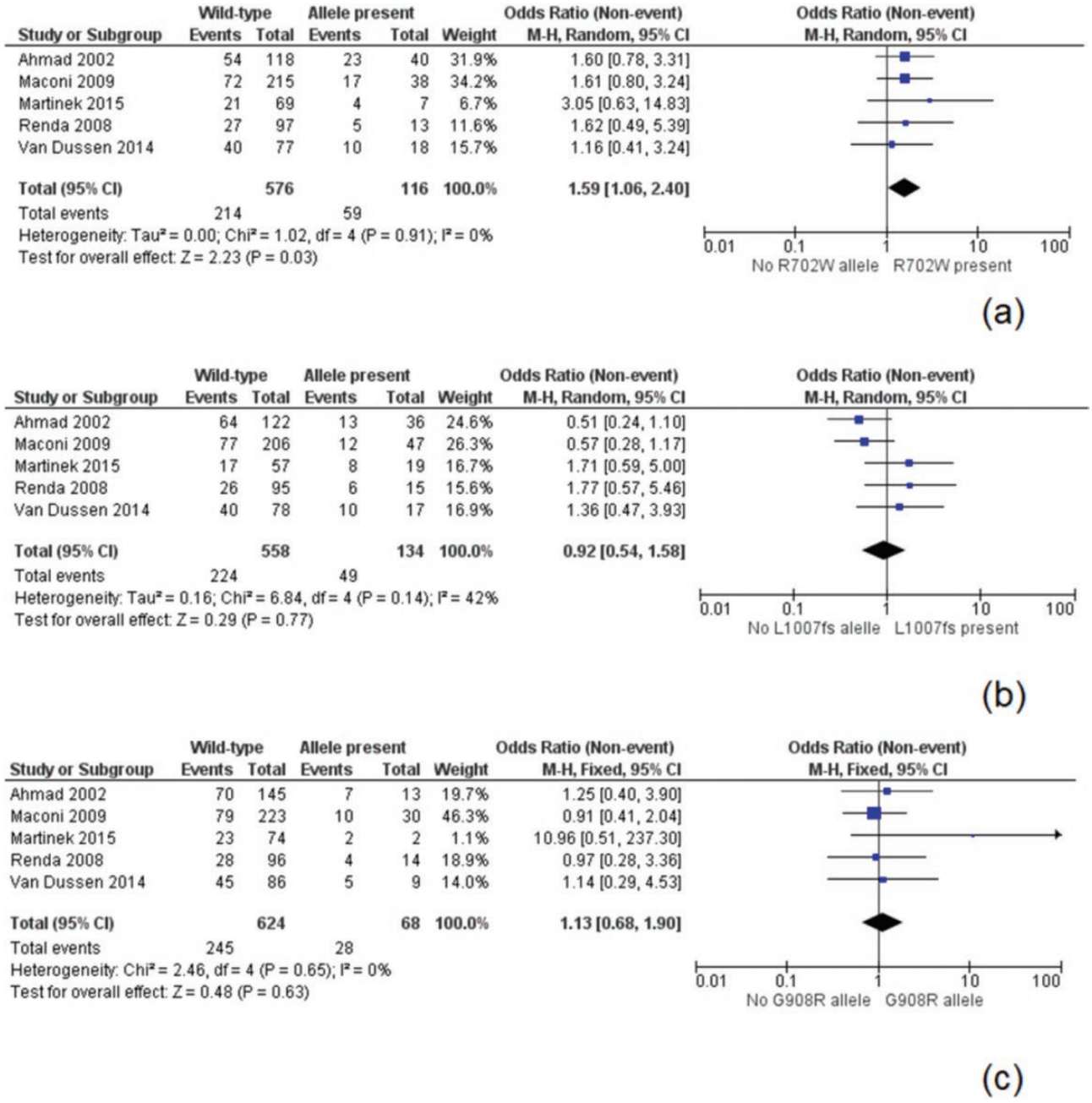

Figure 3. Postoperative recurrence by *NOD2* subtype: Panel A, R702W subtype; Panel B, L1007fs subtype; Panel C, G908W subtype.

ity of any meta-analysis. Further, the reporting on the rates of recurrence for the vast majority of interrogated genetic loci in the identified works was incomplete. This prevented our study from including data in our analysis and certainly skews our findings based on reporting bias. These concerns highlight many established issues in genetics research. For the genetics of any disease to be studied in coordinated manner, a framework must be established, clearly identifying important data that must be reported. In general, for genetic studies, a number of factors should be documented, including geographic area in which the study takes place, population age, population gender, loci studied, alleles analyzed, method of genotyping and the minor allele and homozygote frequency in the population. Specific disease factors must be described as well, and in the case of postoperative recurrence of CD, type of intraabdominal surgery performed (specifically resection vs strictureplasty), method of assessing disease recurrence, and prevalence of smoking in the cohort. We also encourage the reporting of deidentified individual patient data, including time to recurrence, Montreal disease classification, and postoperative medical therapy, as this information will add granularity to future study. Here, we include a standardized reporting (Supplementary Materials 4 and 5) form for genetic associations in postoperative recurrence of CD, which

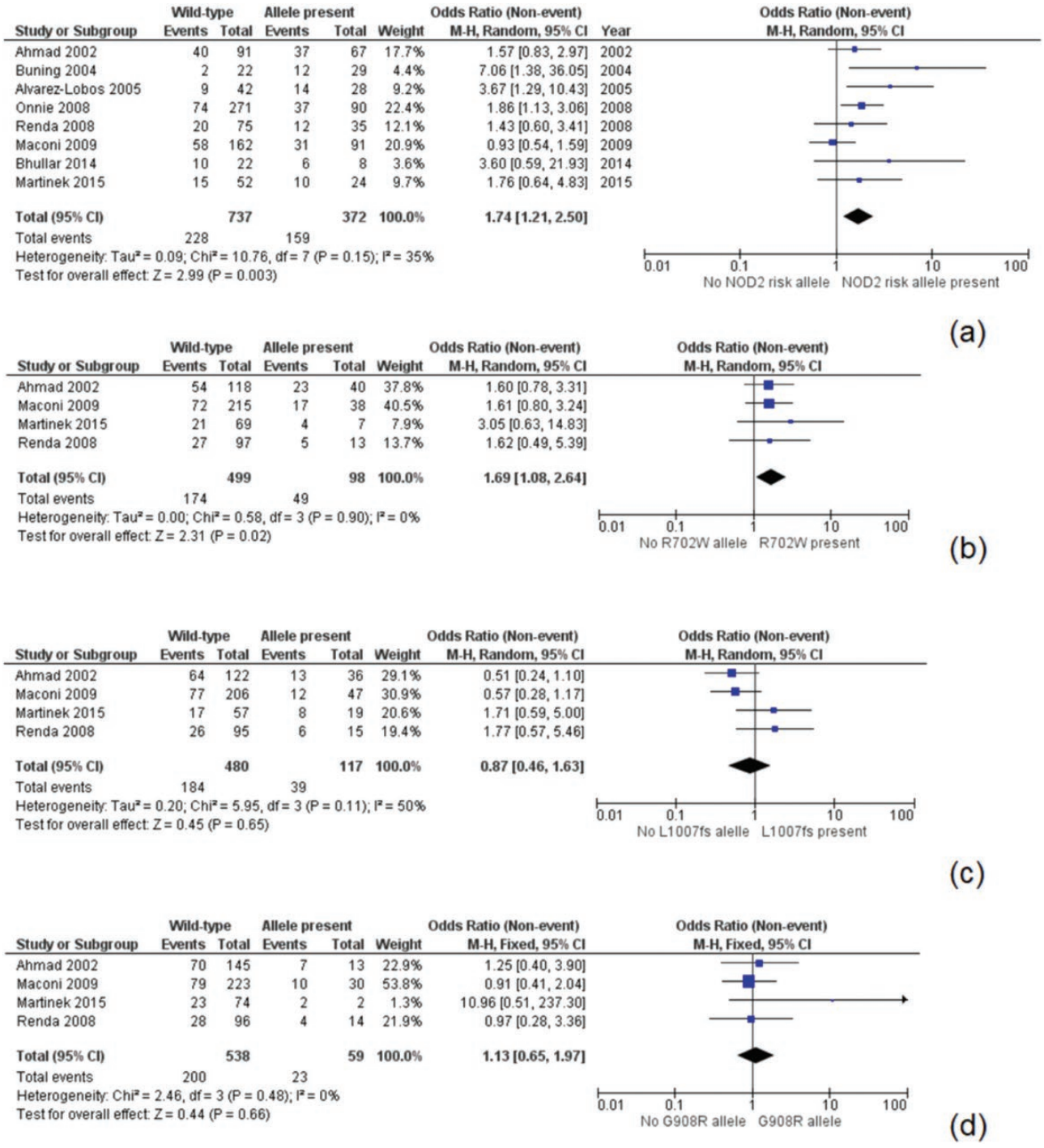

Figure 4. Postoperative surgical recurrence by *NOD2* and subtype: Panel A, grouped *NOD2* risk alleles; Panel B, R702W subtype; Panel C, L1007fs subtype; Panel D, G908W subtype.

represents the minimum we suggest be reported in future published works on this topic.

The presence of the *NOD2* risk allele increased the odds of surgical recurrence following intestinal resection in CD. Other genes identified as associated with recurrence were *BACH2*, *CARD8*, *TIMP-1*, *TNFSF15*, and *IRGM*, *IRF8*, *LSP1/TNNI2*, *DAP*, *PTGER4*, *PELI3*, *CHL1*, *PARVB*, and *STK24*, however these were only represented in single studies and future research must be conducted in the context of a standardized framework examining the genetics of postoperative recurrence in CD.

Table 3. Summary of Genes Other Than *NOD2* With a Significant Impact on Postoperative Recurrence of CD

Study	Identified Genetic Factor Associated With Recurrence	Statistical Analysis	Definition of Recurrence	Change in Risk	*P*
Gerich 2013	*IRF8*	Univariate survival analysis. No correction for multiple testing.	Repeat surgery	HR: 0.6	0.015
	LSP1/TNNI2			HR: 1.4	0.04
	PTGER4			HR: 1.3	0.04
	DAP			HR: 1.4	0.04
	FAM49B			HR: 3.2	<0.001
	PELI3			HR: 1.8	<0.001
	CHL1			HR: 2.6	<0.001
	PARVB			HR: 0.5	<0.001
	STK24			HR: 1.7	<0.001
Germain 2016	Homozygosity for the variant at *CARD8* snp rs2043211	Multivariable survival analysis. No correction for multiple testing.	Repeat surgery	OR: 7.56	0.04
Laffin 2018	Homozygosity for the variant at *BACH2* snp rs1847472	Multivariable survival analysis. No correction for multiple testing.	Repeat surgery	HR: 1.54	<0.05
Sehgal 2012	Presence of the variant at *IRGM* rs4958847	Multivariable survival analysis. Corrected for multiple testing.	Repeat surgery	NR	0.007
Yang 2014	Homozygosity for the variant at *TNFSF15* snp rs6478108	Multivariable survival analysis. No correction for multiple testing.	Development of stricture	HR: 1.7	0.005

NR, not reported.

Supplementary Material

Supplementary data are available at *Crohn's & Colitis 360* online.

Data Availability

No new data were created for this study.

References

1. Lees CW, Barrett JC, Parkes M, *et al*. New IBD genetics: common pathways with other diseases. *Gut*. 2011;60:1739–1753.
2. Mirkov MU, Verstockt B, Cleynen I. Genetics of inflammatory bowel disease: beyond NOD2. *Lancet Gastroenterol Hepatol*. 2017;2:224–234.
3. Sidiq T, Yoshihama S, Downs I, *et al*. Nod2: a critical regulator of ileal microbiota and Crohn's disease. *Front Immunol*. 2016;7:367.
4. Burisch J, Jess T, Martinato M, *et al*.; ECCO-EpiCom. The burden of inflammatory bowel disease in Europe. *J Crohns Colitis*. 2013;7:322–337.
5. Rutgeerts P. Strategies in the prevention of post-operative recurrence in Crohn's disease. *Best Pract Res Clin Gastroenterol*. 2003;17:63–73.
6. Landsend E, Johnson E, Johannessen HO, *et al*. Long-term outcome after intestinal resection for Crohn's disease. *Scand J Gastroenterol*. 2006;41:1204–1208.
7. Solon JG, Burke JP, Walsh SR, *et al*. The effect of NOD2 polymorphism on postsurgical recurrence in Crohn's disease: a systematic review and meta-analysis of available literature. *Inflamm Bowel Dis*. 2013;19:1099–1105.
8. Moher D, Liberati A, Tetzlaff J, *et al*.; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009;151:264–269, W64.
9. Slim K, Nini E, Forestier D, *et al*. Methodological index for non-randomized studies (minors): development and validation of a new instrument. *ANZ J Surg*. 2003;73:712–716.
10. Collaboration C. *Review Manager (Version 5.3) [Computer Software]*. Copenhagen, Denmark: Nord Cochrane Cent; 2014.
11. Higgins JP, Thompson SG, Deeks JJ, *et al*. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327:557–560.
12. Li E, Zhang Y, Tian X, *et al*. Influence of Crohn's disease related polymorphisms in innate immune function on ileal microbiome. *PLoS One*. 2019;14:e0213108.
13. Meresse B, Rutgeerts P, Malchow H, *et al*. Low ileal interleukin 10 concentrations are predictive of endoscopic recurrence in patients with Crohn's disease. *Gut*. 2002;50:25–28.
14. Alvarez-Lobos M, Arostegui JI, Sans M, *et al*. Crohn's disease patients carrying Nod2/CARD15 gene variants have an increased and early need for first surgery due to stricturing disease and higher rate of surgical recurrence. *Ann Surg*. 2005;242:693–700.
15. Bhullar M, Macrae F, Brown G, *et al*. Prediction of Crohn's disease aggression through NOD2/CARD15 gene sequencing in an Australian cohort. *World J Gastroenterol*. 2014;20:5008–5016.
16. Büning C, Genschel J, Bühner S, *et al*. Mutations in the NOD2/CARD15 gene in Crohn's disease are associated with ileocecal resection and are a risk factor for reoperation. *Aliment Pharmacol Ther*. 2004;19:1073–1078.
17. Maconi G, Colombo E, Sampietro GM, *et al*. CARD15 gene variants and risk of reoperation in Crohn's disease patients. *Am J Gastroenterol*. 2009;104:2483–2491.

18. Onnie CM, Fisher SA, Prescott NJ, *et al.* Diverse effects of the CARD15 and IBD5 loci on clinical phenotype in 630 patients with Crohn's disease. *Eur J Gastroenterol Hepatol.* 2008;20:37–45.
19. Renda MC, Orlando A, Civitavecchia G, *et al.* The role of CARD15 mutations and smoking in the course of Crohn's disease in a Mediterranean area. *Am J Gastroenterol.* 2008;103:649–655.
20. VanDussen KL, Liu TC, Li D, *et al.* Genetic variants synthesize to produce paneth cell phenotypes that define subtypes of Crohn's disease. *Gastroenterology.* 2014;146:200–209.
21. Ahmad T, Armuzzi A, Bunce M, *et al.* The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology.* 2002;122:854–866.
22. Martinek L, Kupka T, Simova J, *et al.* Original article: NOD2/CARD15 mutations and the risk of reoperation in patients with Crohn' s disease. *Rozhl. Chir.* 2015;94:242–46.
23. Yang DH, Yang SK, Song K, *et al.* TNFSF15 is an independent predictor for the development of Crohn's disease-related complications in Koreans. *J Crohns Colitis.* 2014;8:1315–1326.
24. Sehgal R, Berg A, Polinski JI, *et al.* Mutations in IRGM are associated with more frequent need for surgery in patients with ileocolonic Crohn's disease. *Dis Colon Rectum.* 2012;55:115–121.
25. Ta-Chiang L, Feng G, Thaddeus S. P-070 an integrated risk stratification system incorporating paneth cell phenotype and clinical parameter predicts outcome in post-operative Crohn's disease patients. *Inflamm Bowel Dis.* 2014;20.
26. Chen C, Zhang T, Li E, *et al.* The effects of NOD2 genotype, smoking and immunomodulators on postoperative recurrence of ileal Crohn's disease Chien-Huan. *Gastroenterology.* 2011;6:s29.
27. Fowler SA, Ananthakrishnan AN, Gardet A, *et al.* SMAD3 gene variant is a risk factor for recurrent surgery in patients with Crohn's disease. *J Crohns Colitis.* 2014;8:845–851.
28. Liu TC, Naito T, Liu Z, *et al.* LRRK2 but not ATG16L1 is associated with Paneth cell defect in Japanese Crohn's disease patients. *JCI Insight.* 2017;2:e91917.
29. Siegel CA, Fleshner P, Siegel LS, *et al.* Predicting Crohn's disease post-operative recurrence using clinical, endoscopic, serologic and genetic factors. *Gastroenterology.* 2011;140:S-153.
30. Naito T, Liu T-C, Kakuta Y, *et al.* 321 Paneth cell phenotype is associated with novel genetic determinants and clinical outcome in Japanese Crohn's disease patients. *Gastroenterology.* 2016;150:S75.
31. Seiderer J, Schnitzler F, Brand S, *et al.* Homozygosity for the CARD15 frameshift mutation 1007fs is predictive of early onset of Crohn's disease with ileal stenosis, entero-enteral fistulas, and frequent need for surgical intervention with high risk of re-stenosis. *Scand J Gastroenterol.* 2006;41:1421–1432.
32. Potdar AA, Li D, Haritunians T, *et al.* Ileal gene expression data from Crohn's disease small bowel resections indicate distinct clinical subgroups. *J Crohns Colitis.* 2019;13:1055–1066.
33. Germain A, Guéant RM, Chamaillard M, *et al.* NOD2 gene variant is a risk factor for postoperative complications in patients with Crohn's disease: a genetic association study. *Surgery.* 2016;160:74–80.
34. Laffin MR, Fedorak RN, Wine E, *et al.* A BACH2 gene variant is associated with postoperative recurrence of Crohn's disease. *J Am Coll Surg.* 2018;226:902–908.
35. Gathungu G, Zhang Y, Tian X, *et al.* Impaired granulocyte-macrophage colony-stimulating factor bioactivity accelerates surgical recurrence in ileal Crohn's disease. *World J Gastroenterol.* 2018;24:623–630.
36. Gerich ME, Fleshner P, Panikkath D, *et al.* Mo1299 genotype and post-operative immunosuppression impact surgical recurrence in Crohn's disease. *Gastroenterology.* 2013;144:S-630.
37. Ghaly S, Murray K, Baird A, *et al.* High vitamin D-binding protein concentration, low albumin, and mode of remission predict relapse in Crohn's disease. *Inflamm Bowel Dis.* 2016;22:2456–2464.
38. Burke JP, O'Connell RM, Lennon G, *et al.* The influence of CTGF single-nucleotide polymorphisms on outcomes in Crohn's disease. *Ann Surg.* 2013;258:767–773; discussion 773.
39. Meijer MJ, Mieremet-Ooms MA, Sier CF, *et al.* Matrix metalloproteinases and their tissue inhibitors as prognostic indicators for diagnostic and surgical recurrence in Crohn's disease. *Inflamm Bowel Dis.* 2009;15:84–92.
40. Germain A, Guéant RM, Chamaillard M, *et al.* CARD8 gene variant is a risk factor for recurrent surgery in patients with Crohn's disease. *Dig Liver Dis.* 2015;47:938–942.
41. Molodecky NA, Soon IS, Rabi DM, *et al.* Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology.* 2012;142:46–54.e42; quiz e30.
42. Benchimol EI, Fortinsky KJ, Gozdyra P, *et al.* Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. *Inflamm Bowel Dis.* 2011;17:423–439.
43. Buisson A, Chevaux JB, Allen PB, *et al.* Review article: the natural history of postoperative Crohn's disease recurrence. *Aliment Pharmacol Ther.* 2012;35:625–633.
44. Peyrin-Biroulet L, Harmsen WS, Tremaine WJ, *et al.* Surgery in a population-based cohort of Crohn's disease from Olmsted County, Minnesota (1970–2004). *Am J Gastroenterol.* 2012;107:1693–1701.
45. Ganz ML, Sugarman R, Wang R, *et al.* The economic and health-related impact of Crohn's disease in the united states: evidence from a nationally representative survey. *Inflamm Bowel Dis.* 2016;22:1032–1041.
46. Regueiro M, Velayos F, Greer JB, *et al.* American gastroenterological association institute technical review on the management of Crohn's disease after surgical resection. *Gastroenterology.* 2017;152:277–295.e3.
47. Unkart JT, Anderson L, Li E, *et al.* Risk factors for surgical recurrence after ileocolic resection of Crohn's disease. *Dis Colon Rectum.* 2008;51:1211–1216.
48. Wright EK, Kamm MA, Wagner J, *et al.* Microbial factors associated with postoperative Crohn's disease recurrence. *J Crohns Colitis.* 2017;11:191–203.
49. Mondot S, Lepage P, Seksik P, *et al.* Structural robustness of the gut mucosal microbiota is associated with Crohn's disease remission after surgery. *Gut.* 2016;65:954–962.
50. De Cruz P, Kang S, Wagner J, *et al.* Association between specific mucosa-associated microbiota in Crohn's disease at the time of resection and subsequent disease recurrence: A pilot study. *J Gastroenterol Hepatol.* 2015;30:268–278.
51. Dey N, Soergel DA, Repo S, *et al.* Association of gut microbiota with post-operative clinical course in Crohn's disease. *BMC Gastroenterol.* 2013;13:131.
52. Laffin MR, Perry T, Park H, *et al.* Endospore forming bacteria may be associated with maintenance of surgically-induced remission in Crohn's disease. *Sci Rep.* 2018;8:9734.
53. Hampe J, Cuthbert A, Croucher PJ, *et al.* Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet.* 2001;357:1925–1928.
54. Hugot JP, Chamaillard M, Zouali H, *et al.* Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature.* 2001;411:599–603.
55. Girardin SE, Boneca IG, Viala J, *et al.* Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem.* 2003;278:8869–8872.
56. Kobayashi KS, Chamaillard M, Ogura Y, *et al.* Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science.* 2005;307:731–734.
57. Plantinga TS, Crisan TO, Oosting M, *et al.* Crohn's disease-associated ATG16L1 polymorphism modulates pro-inflammatory cytokine responses selectively upon activation of NOD2. *Gut.* 2011;60:1229–1235.
58. Adler J, Rangwalla SC, Dwamena BA, *et al.* The prognostic power of the NOD2 genotype for complicated Crohn's disease: a meta-analysis. *Am J Gastroenterol.* 2011;106:699–712.
59. Kuenzig ME, Yim J, Coward S, *et al.* The NOD2-smoking interaction in Crohn's disease is likely specific to the 1007fs mutation and may be explained by age at diagnosis: a meta-analysis and case-only study. *Ebiomedicine.* 2017;21:188–196.
60. Brzyski D, Peterson CB, Sobczyk P, *et al.* Controlling the rate of GWAS false discoveries. *Genetics.* 2017;205:61–75.