Predicting complicated Crohn's disease and surgery: phenotypes, genetics, serology and psychological characteristics of a population based cohort.

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Abstract

Background: Predictors of complicated Crohn's disease (CD), defined as stricturing or penetrating behaviour, and surgery have largely been derived from referral center populations. We investigate whether serological markers, susceptibility genes, or psychological characteristics are associated with complicated CD or surgery in a population-based cohort.

Methods: 182 members of the Manitoba IBD Cohort with CD phenotyped using the Montreal classification underwent genetic and serologic analysis at enrolment and after 5 years. 127 had paired sera at baseline and 5 years later and their data were used to predict outcomes at a median of 9.3 years.. Serologic analysis consisted of a seven antibody panel, and DNA was tested for CD-associated NOD2 variants (rs2066845,rs2076756,rs2066847), ATG16L1 (rs3828309, rs2241880) and IL23R (rs11465804). Psychological characteristics were assessed using semi-structured interviews and validated survey measures.

Results: 65% had complicated CD and 42% underwent surgery. Multivariate analysis indicated that only ASCA IgG positive serology was predictive of stricturing/penetrating behavior (OR=3.01; 95%CI:1.28-7.09; p=0.01) and ileal CD (OR=2.2; 95%CI:1.07-4.54, p=0.03). Complicated CD behavior was strongly associated with surgery (OR=5.6; 95%CI:2.43-12.91; p<0.0001) while in multivariate analysis, only ASCA IgG was associated (OR=2.66; 95% CI, 1.40-5.06, p=0.003). ASCA titre results were similar at baseline and follow-up. Psychological characteristics were not significantly associated with disease behavior, serologic profile, or genotype.

Conclusions: ASCA IgG at baseline was significantly associated with stricturing/penetrating disease at 9 to 10 years from diagnosis. Stricturing/penetrating disease was significantly associated with surgery. In a model including serology, the genotypes assessed did not significantly associate with complicated disease or surgery.

Keywords: Crohn's disease, phenotype, serological markers, psychological characteristics, surgery, cohort study

INTRODUCTION

Crohn's disease (CD) is an idiopathic disorder that is classically characterized byrelapsing and remitting inflammation that may affect any region of the gastrointestinal tract. The natural history of CD is often characterized by progression to complications such as strictures, abscesses and fistulas that may result in surgery for as many as half of all patients(1). Distinct clinical patterns of disease have long been recognized and remain the basis upon which CD phenotypes are defined using the Montreal classification(2). Several large referral centre studies have identified phenotypic characteristics such as younger age at diagnosis, ileal involvement, and perianal fistulas as risk factors for progression to complicated CD, defined as stricturing or penetrating behavior, and the need for surgical intervention(3, 4, 5).No consensus has emerged, however, on when or how phenotyping should be undertaken and early identification of those individuals at risk of CD complications remains elusive using the Montreal classification alone. This has limited the widespread adoption of formal phenotyping into everyday clinical practice. It has also bred enthusiasm for the potential that a serological marker or genotype or combination of both might be predictive of disease outcomes**.** In fact, a recent pediatric study assessing CD outcomes suggested that clinical parameters at diagnosis were insufficient to predict a disabling course of pediatric CD, and that more complex models including serological and genetic biomarkers should be tested (6).

As knowledge of the complex interaction between the immune system, gut microflora and genetic susceptibility to CD grows, so too does the number of potential biological markers of disease. This has prompted the authors of the Montreal classification to call for further studies on

the integration of new biomarkers of CD to better characterize those individuals at risk of disease complications(2,7). In recent years, numerous genome wide association studies (GWAS) have identified a wide array of single nucleotide polymorphisms (SNPs) and novel susceptibility loci for CD, including oligomerization domain protein 2 (NOD2), lymphocyte signalling, including IL23 receptor (IL23R), and autophagy involving autophagy-related 16-like 1 gene (ATG16L1)(8). Furthermore, NOD2 mutations have been associated with early age of onset, ileal involvement, and penetrating and stricturing disease(9-18). Serologic markers including anti-*Saccharomyces cerevisiae* antibody (ASCA), as well as antibodies to *Escherichia coli* outer-membrane porin C (anti-OmpC), *Pseudomonas fluorescens*-related protein (anti-I2), bacterial flagellin CBir1 (anti-CBir1) and perinuclear staining anti-neutrophil cytoplasmic antibody (p-ANCA) have also been associated with increased disease severity and complications. However, it is unclear if serologic and/or genetic analyses augment the ability to predict the development of complicated CD over and above the knowledge of the disease phenotype at baseline, or whether these factors are associated with a stricturing/penetrating phenotype. Population-based studies are needed to confirm that the same phenotypic risk factors as well as predictive genetic or serological markers present in referral center cohorts are relevant when characterizing CD in the general population.

In addition, there are emerging data indicating an effect of psychological factors, including mood disorders and stress, on disease course in IBD(19-21). For this study, in addition to previously identified psychological factors, we were particularly interested in whether childhood trauma was related to complicated disease in CD, given prior research that has shown this chronic early life stressor can predispose to later health issues(22, 23). Rates of childhood trauma and

adversity are surprisingly high, with two-thirds of the general community reporting this type of experience (24) . Briere et al reported that as many as one in three girls and one in seven boys will be sexually abused at some point in their childhood(25). While the mechanisms are unclear, experimental research has suggested that those with early abuse experiences may have cortisol suppression as an adaptation to an exaggerated stress response at the pituitary and/or hypothalamus level, which may impact on immune function(26-28). As well, prospective work by Danese et al found a relationship between early life stress and later life inflammation(29). They tested the life-course association between childhood maltreatment and adult inflammation in a birth cohort followed to age 32 years as part of the Dunedin Multidisciplinary Health and Development Study(29). Maltreated children showed a significant and graded increase in the risk for clinically relevant C-reactive protein levels 20 years later, in adulthood (relative risk (RR) 1.80, 95% confidence interval, (CI) 1.26–2.58). Finally, in a meta-analysis of over 300 empirical articles describing a relationship between psychological stress and parameters of the immune system in human, chronic stressors were associated with suppression of both cellular and humoral measures(30).

Therefore, we aimed to assess predictors of developing complicated CD and the need for abdominal surgery after several years of disease using clinical phenotype, serological markers, and susceptibility genes in a population-based cohort of CD participants. We also performed a novel analysis of the association between psychological characteristics, CD phenotype and clinical outcomes.

METHODS

Cohort Description:

The Manitoba IBD Cohort Study is a prospective, longitudinal study that aims to identify determinants of IBD outcomes. This population-based sample of adults was recruited within seven years (median four years) of their diagnosis of IBD. They have been tracked prospectively through semi-annual surveys using standardized and validated self-report instruments, and annual clinical interviews and blood draws completed by research nurses. The participants were originally recruited from a validated population-based research registry that has been described elsewhere(31-34). Briefly, all adults with IBD in the province are included in a population-based database (the University of Manitoba IBD Epidemiology Database), drawn from the comprehensive provincial health administrative database using a validated definition of IBD. All those in the University of Manitoba IBD Epidemiology Database were invited to participate in a research registry. The University of Manitoba IBD Research Registry has subsequently been updated periodically with individuals identified from the administrative health database. Just over half of all the provincial cases of IBD (n=3192) were enrolled in the Research Registry at the initiation of this study(31).

Of those enrolled in the Registry, 606 were eligible for this study, based on the criteria of adult age and recent disease onset. The final Cohort was comprised of 388 who completed the baseline survey and interview, after those who declined (14%) or could not be reached (17%) were excluded. The Cohort has been established as having excellent representativeness of the

provincial IBD population, with comparative age distribution, sex distribution, and rural/urban residence(35). Of the final, Cohort 182 had CD. Of these 182, 127 had paired serum from both time points of this substudy. In comparing the 127 participants in this study with the 182 population-representative participants that are in the overall Cohort Study, the current study sample had comparative age and gender distribution, rates of smoking, family history of IBD, and marital status distribution. Their CD behavior and location, and surgery frequency are also similar.

While we have used the Cohort Study to explore a variety of psychological issues in relation to IBD it provides a unique population-based opportunity to explore whether psychological variables as well as serological and genotypic variables are associated with specific CD phenotypes and the need for surgery. Cohort participants were prospectively followed with semi-annual surveys assessing disease activity and psychological functioning. A detailed description of the study population has previously been published(32). As our cohort was drawn from the general population there was significant variability in the diagnostic investigations undertaken before inclusion. For the purpose of this study, baseline CD phenotype was determined using the Montreal classification within 3 years from diagnosis (T1)and repeated 5 years after enrollment in the Cohort Study which equated to a median of 9.3 years from diagnosis (T2). The 3 years from diagnosis allowed time for adequate diagnostic investigations to occur in order to effectively classify a baseline phenotype. Bloodwork for serologic and genetic analysis was obtained at enrollment in the Cohort Study and at T2.

Assessment of Disease Characteristics: We assessed 3 main outcomes at T1 and T2. These outcomes were:1) disease location as defined by the Montreal classification (2); 2) complicated disease, defined as stricturing (B2) or penetrating (B3) behavior;and 3) abdominal surgery for CD, excluding perianal surgery. Other characteristics recorded at baseline included, extra-intestinal manifestations of CD, family history of IBD and smoking status. Personality characteristics were assessed using the Neuroticism, Extroversion, Openness (*NEO) Five-Factor Inventory* (NEO-FFI)(36). The NEO-FFI is a well-validated 60-item scale designed to give quick and reliable measures of the five major domains of adult personality. The domains include neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness. Psychiatric diagnoses and adverse childhood experiences including sexual and physical abuse were determined through a semi-structured clinical interview, the *Comprehensive International Diagnostic Interview* (CIDI), which identified lifetime prevalence of anxiety, mood disorders and phobias(37, 38). Self-description of usual medication adherence was assessed with a validated self-report measure, the *Medication Adherence Report Scale*(39, 40).

DNA samples were obtained from peripheral whole blood samples collected from 167 subjects at T1. Samples were genotyped using the Illumina Golden Gate custom SNP assay on Illumina BeadStation500G. Genetic analysis focused on SNPs rs2066845, rs2076756 and rs2066847 in NOD2, rs3828309 and rs2241880 in ATG16L1, and rs11465804 in IL23R which had previously been determined to be associated with CD in a separate population based sample of Manitobans(41).There was insufficient sample for adequate DNA isolation for 15 participants, and 40 had insufficient serum either at baseline or at 5 years, such that paired serum was not available. This left 127 participants with complete data to be assessed including paired sera at T1

and T2. All assays were performed at Prometheus Laboratories using a commercial assay (IBD-Serology 7, Prometheus Laboratories) that included pANCA, ASCA IgA and IgG, anti-I2, anti-OmpC, and anti-CBir1. Serology results at T1 and T2 were then compared.

Statistical analysis: Descriptive statistics of demographic variables were generated using SAS version 9.2. WilcoxonRank-Sum Test and Chi-Square testing was used to compare phenotype variables between sub-groups. PLINK version 1.06 was used to obtain summary statistics of the SNPs such as the allele frequency and genotype distribution and to test for Hardy-Weinberg equilibrium (HWE) for each marker based on chi-square test. Association analyses were applied to detect the associations between the SNPs, serological markers, psychological variables, phenotype and surgical endpoints. Multivariate analysis using a logistic regression model was then applied for association analysis. The following variables were included the multivariate logistic regression model; sex, age at diagnosis, marital status, history of smoking, pANCA, Anti-I2, Anti-OmpC, ASCA IgA, ASCA IgG, CBir1, SNP rs2066845, SNP rs2076756, and SNP rs2066847. Two-sided statistical tests were applied. Odds ratios (OR) and 95% confidence intervals (CI) were estimated. We used a genetic additive model for primary analysis and set a p-value = 0.01 as cut point for nominal associations. For the T2 or final outcomes we assessed all subjects with the outcome of interest (ie disease location, disease behavior, surgery) by that timepoint regardless of when that outcome was achieved. There was too small a sample size to analyze for predictors of developing those outcomes between T1 and T2 (if not already present by T1).

Ethical considerations: This study and the Manitoba IBD Cohort Study overall has been approved by the University of Manitoba Research Ethics Board

RESULTS

Participantdemographic and psychological characteristics and Montreal Classification are reported in Table 1 and Table 2 for all 182 members of the Manitoba IBD Cohort with CD. The baseline assessment for disease phenotype includes the first 3 years from disease diagnosis (T1). The baseline demographic and psychological assessment took place at Cohort Study enrolment, at a median of 4.3 years after diagnosis, and the 5 year follow up measurement was done at a median of 9.3 years following diagnosis (T2). Sixty-two percent of participants were females. Seventy-eight percent had ileal involvement at study enrolment (either isolated or combined with colon involvement). Fifty five percent of study enrollees had stricturing and/or penetrating disease at enrollment, and 65% had developed complications by the final assessment. By a median 9 years post-diagnosis 42% had undergone surgery. Persons diagnosed under the age of 40 were more likely to have perianal disease ($p=0.004$), but age at diagnosis was not associated otherwise with phenotypic expression, or development of complicated CD. A history of smoking was associated with a lower incidence of complicated CD and perianal disease ($p=0.05$), but was not associated with need for surgery.

Relationship of Psychological Factors to Disease Characteristics

Most subjects reported some level of adverse childhood experience (73%) and high childhood adversity impact tended to be more common in those who underwent surgery (p=0.06). Subjects who underwent surgery were also less likely to report being married or in common-law relationships (p=0.02). No other psychological variables were associated with disease location, complicated CD or surgery. High adherence to medications was modest (57%) and was not associated with outcomes of interest. Subjects with a family history of IBD (46%) did not have any significant differences in phenotype or rate of surgery.

Relationship of Serologic and Genetic Markers and Disease Location

Persons with disease confined to the ileum (L1) versus those with colonic (L2) and ileocolonic (L3) combined were more likely to be CBir1 positive at T1 (p= 0.02) and ASCA IgG positive at T2 (p=0.04) (Supplemental Table 1). No genetic markers were associated with disease location, though the presence of NOD2 rs2076756 genotype tended to associate with isolated ileal location (odds ratio (OR) 1.54; 95% CI: 0.99-2.42, p=0.06) (Supplemental Table 2). Multivariate analysis indicated that only ASCA IgG at T2 remained a significant predictor of CD location and specifically disease of the terminal ileum (L1) (OR 2.2; 95%CI:1.07-4.54,p=0.03) (Table 3). No further correlations between psychological, phenotypic, serological and genetic factors were present in our population. Upper GI (L4) disease and extra-intestinal manifestations were uncommon in our cohort.

Predictors of Complicated Disease

Disease location did not predict the development of complications. NOD2 rs2066847 was the only SNP associated with complicated behavior (OR 3.25; 95% CI: 1.32-8.01, p=0.01). Subjects

at T1 and T2 who were ASCA IgA (p = 0.01; p = 0.002, respectively) and ASCA IgG positive (p=0.0001, p=0.0006, respectively) were more likely to have stricturing or penetrating disease. Multivariate analysis of complicated behavior (stricturing/penetrating disease) indicated that ASCA IgG remained the only significant predictor (OR = 3.01; 95%CI:1.28-7.09; p=0.01), while NOD2 rs2066847 trended toward an association (OR 3.08; 95% CI:0.87-10.89, p=0.08). Sex, age at diagnosis, perianal disease behavior, family history, adherence to medications and all other psychological traits were not correlated with complicated disease behavior. Conversely, those subjects with a positive pANCA at T2 (p=0.04) and with a history of smoking (p=0.05) appeared to be protected from stricturing/penetrating complications; however, these associations were not significant when included in the multivariate analysis. Antibody testing using a quartile sum score analysis (42) was not predictive of any outcomes (data not shown). The positive predictive value ASCA IgG for complicated disease is 61.4%, and the negative predictive value of a negative ASCA IgG is 66.2%.

Predictors of surgery

Stricturing/penetrating behavior was significantly associated with need for surgery (p<0.0001). Perianal disease activity was marginally with surgery (p=0.06). NOD2 rs2066847 was the only SNP predictive of surgery (OR 2.39; 95% CI:1.06-5.38) and was weakly associated with early surgery (OR 1.96, 95% CI:0.95-4.07, p=0.07). Surgery was associated with ASCA IgA at T1 (p=0.02) and T2 (p=0.03) and ASCA IgG at T1 (p=0.002) and at T2 (p=0.006). While stricturing/penetrating disease was predictive of surgery, we excluded complicated disease from our multivariate analysis of surgery since it was one of our independent outcomes. On

multivariate analysis of predictors of surgery there was an association with ASCA IgG (OR 2.66 , 95% CI, 1.40-5.06, p=0.003). Sex, age at diagnosis, disease location, family history, smoking, adherence to medications and all other psychological variables were not significant in either model for early surgery or surgery overall.

DISCUSSION

This study confirms that a significant relationship exists between stricturing and penetrating disease behavior and the need for surgical intervention. At 5 years from inclusion in the Manitoba IBD Cohort Study and a median of 9 years from diagnosis, 65% of our subjects had complicated disease and 42% had undergone surgery. Disease location remained quite stable over this time span and disease behavior progressed in a minority of subjects (Table 2). Subjects with stricturing or penetrating disease were on average 6 times more likely to undergo surgery, even after accounting for all other variables in our analysis.

The evolution of CD in our cohort is consistent with the majority of population-based studies to date. However, there is considerable methodological variation as well as differences in the incidence of surgery and disease complications reported in the literature(1). At a median of 10 years from diagnosis, stricturing or penetrating complications occurred in 39% of subjects in Olmsted County(43), 53% of a Norwegian inception cohort(44), and 56% of a New Zealand cohort(45). In Olmsted county 58% of subjects underwent surgery by a median of 13.2 years from diagnosis(46). At 10 years from diagnosis, 38% of subjects in Norway (44) and 71% from Sweden(47) had undergone surgery. At five years from diagnosis surgery had been performed in 39% of subjects from Wales(48) and 21% from Hungary(49). Where reported, stricturing and

penetrating disease behavior has been a key factor associated with surgery(43, 44, 48, 49). While some studies found ileal location to be associated with complicated disease or surgery(43-45, 49) others found an association with disease involving the colon(48, 49). Age at diagnosis was predictive of surgery in Norway(44) and Sweden(47) although the associated ages were different in these two cohorts. Similar to our findings, perianal disease was associated with surgery in Sweden(47) and stricturing and penetrating complications in New Zealand(45). In contrast to other population-based studies, we did not find any significant correlations between age at diagnosis and disease location with complicated CD or risk of surgery. However, as noted above, there are conflicting data as to which specific phenotypes are key predictors. Whether the absence of such correlations reflects our relatively small sample size or actual systematic differences between our population and others remains unclear. Nevertheless, CD characterized by stricturing and/or penetrating behavior has consistently been associated with higher rates of surgery across populations and is likely the most important, independent phenotypic risk factor. We excluded complicated disease from our multivariate analysis of predictors of surgery since it was an independent outcome as well.

Previously established markers for CD analyzed in our study cohort included positive ASCA IgA and IgG, mutations in NOD2, ATG16L1, IL23R, family history of IBD, Jewish ethnicity and tobacco use(41, 50, 51). Both the number and magnitude of antibodies present in the sera of CD subjects have been shown to correlate with disease location, behavior and the need for surgery independent of the NOD2 genotype(52, 53). However, some studies have suggested that CD subjects with NOD2 variants have a hypersensitive adaptive immunologic response leading to increased antibody formation(42, 54). Therefore, it remains unclear whether the presence of

specific antibodies or susceptibility genes are indeed independent risk factors for more complicated disease. The presence of ASCA IgG in our study was on average associated with a 3-fold increase in the risk of complicated CD and a 2-fold risk of surgery even after inclusion in the multivariate model. ASCA-IgG was also associated with increased risk of disease confined to the terminal ileum. Another important finding of our study was that in general serological antibody testing for ASCA remained stable over the 5-year interval suggesting that serological status may be determined at any time with similar prognostic implications. Few population-based studies have so far confirmed a significant association between ASCA and complicated CD and our results represent an important contribution to this literature(55).

No other antibodies proved to be significant predictors of complicated CD or surgery in our cohort. To date, the remainder of the antibodies tested in our study have been associated with complicated CD or surgery in at least one study but results are conflicting(56). Other studies have used quartile sum scores to demonstrate higher amalgamated antibody response in subjects with more complicated CD and higher rates of surgery(52, 53, 57-59). Whether quartile sum scores provide additional prognostic information beyond basic ASCA serology remains unclear and requires further population-based testing. Our data suggests that knowledge of ASCA IgG status alone was sufficient in our population to predict worse disease outcomes.

At least 27 variants of NOD2 have been identified but three mutations, R702W, G908R, and 1007fs, appear to account for the majority of variation seen in the CD population(60). A recent analysis of 71 CD loci estimated that when combined they accounted for less than 25% of predicted heritability(61). Hence, with our relatively small sample size we focused on the more

commonly associated SNPs. Other than the 3 NOD2 SNPs, we analyzed for SNPs in IL23R and ATG16L1 which may be correlated with disease severity (10-17). The only SNP to emerge as a suggestive predictor of complicated CD or surgery in our study was NOD2 rs2066847, however, this association did not remain significant after inclusion in the multivariate model. Elsewhere NOD2 variants have been associated with complicated disease and surgery(62, 63). In a recent meta-analysis of the association between NOD2 mutations with CD outcomes there was significant heterogeneity across all studies and the majority were referral-center based(63). Therefore, there remains a need for larger population-based studies to confirm the association between NOD2 variants and more complicated CD and surgery. We performed an additional analysis to look for correlations between SNPs and serological markers and found a trend toward an association between NOD2 rs2066847 and ASCA IgG ($p=0.1$) and IgA ($p=0.06$). Hence, NOD2 and ASCA may not be independently associated with disease activityand further studies are required to determine the degree of correlation between genetic and serological markers.

Similar to the results of a population-based study from New Zealand(64), we failed to demonstrate a significant relationship between SNPs in ATG16L1 and IL23R and any outcomes of interest. This may reflect further differences between subjects with CD presenting to tertiary referral centers and those in the general population. Alternatively, the influence of ATG16L1 and IL23R on phenotype may be modest, requiring much larger sample sizes to reach statistical significance. Our study suggests that identifying common NOD2 variants alone may be helpful in further delineating those subjects at risk of more complicated CD and surgery but that this information may be of little additional benefit in the presence of other phenotypic or serological

risk factors. We also found no association between sex, smoking status or family history and complicated CD or risk of surgery.

Regarding smoking we were unable to demonstrate a significant risk of complicated CD or surgery. Instead we report a weak association between smoking and inflammatory disease behavior without strictures or fistulae. We also found that smokers were less likely to experience perianal disease behavior. Most evidence for smoking as a risk factor comes from referral centre studies(65,66).Whether our results are spurious or underscore an actual difference between subjects attending referral centres and those in general population will require more population-based studies to elucidate.

Finally, we undertook what we believe to be the first study of psychological variables in association with CD phenotype, genetic and serological markers. Psychological variables were included as they have been found by our group and others to be associated with disease activity We have reported on perceived stress and distress in CD and their association with active disease(32, 33). We have also reported that these subjects are more likely to have depression diagnosed years prior to their IBD diagnosis(34). Further, we have reported that a high level of perceived stress was the only statistically significant predictor of a symptomatic flare of CD activity in a subsequent 3 month period(67). So while psychological variables may influence future IBD diagnosis and disease activity it is unknown if they influence CD phenotype or outcome. Although psychological characteristics did not appear to be significant predictors of CD outcomes when included in the multivariate analysis, we did find some associations with potentially important clinical implications that warrant further exploration. Our results suggest

that many of our subjects with CD have experienced adversity in childhood. There were trends of associations between surgery and higher childhood adversity scores, and in particular with childhood physical abuse as one form of adversity. Undoubtedly, adverse childhood experiences occurred long before disease presented in our cohort, who were for the most part diagnosed after the age of 17. This finding may underscore the need for further exploration as to how early childhood stressors may set the stage for later intestinal inflammation. Taken together, these findings suggest that clinicians should anticipate that those subjects undergoing surgery for CD may be particularly vulnerable to increased stress during the course of their illness. Therefore, we feel it is important to consider the potential interaction between psychological characteristics and disease outcomes when considering the longterm prognosis of CD in the general population.

The strength of our study lies in the fact that it is population-based and hence less likely to suffer referral center bias. A referral center bias may lead to overestimates of the effects of risk factors by focusing primarily subjects with more aggressive phenotypes. Conversely, this bias may also underestimate the magnitude of risk factors by failing to include sufficient numbers of subjects with less aggressive disease as comparators. Our study design also attempted to control for lag time bias by using a comprehensive update of our subjects' phenotype and disease course over a 5 year interval. By reporting phenotype at 3 years from diagnosis and again at 5 years from inclusion we allowed time for adequate investigation to be undertaken and for subjects to manifest their CD phenotype. We are reporting associations with the important outcomes of complicated disease or surgery at a median of 9.3 years of disease duration. However, a weakness of our study is that we did not have specific time points at which participants had evaluations of their phenotype or surgery status. Hence a survival analysis showing the evolution

of phenotype, for instance, over time could not be undertaken. The most important weakness of our study is the relatively small sample size, especially for genetic analysis. For this reason we limited our analysis on 6 SNPs in 3 major CD susceptibility genes, namely NOD2, IL23R and ATG16L1, which were previously established correlates of CD in our cohort (41). Nevertheless, our study was underpowered to detect all but the most strongly correlated factors.

In summary, from a clinical perspective the usefulness of any disease marker or classification scheme will be a measure of its ability to predict the natural history of disease and to direct therapy accordingly. On the whole, the results of our study reinforce several important findings with regards to the prognosis of CD. In our subjects, who were followed every 6 months over a 5 year period, we report the following: a) more than half of subjects will experience a significant complication from their disease within the first 9 years from diagnosis; b) disease behavior is the single greatest predictor of surgical risk, especially early surgery; c) ASCA IgG is an independent predictor of complicated CD and surgical risk; and d) while there are some associations with NOD2 risk alleles they may not be independent of risks associated with ASCA, which currently is an easier test to access. For clinicians, our results should emphasize the importance of classifying CD phenotype early in the course of disease when most disease morbidity is likely to be encountered and the potential to identify those subjects at risk of surgical intervention is greatest. Our study also provides a rationale for undertaking serological testing for ASCA IgG as a means of not only discriminating CD from ulcerative colitis but also improving prognostication. This may be of particular importance to clinicians who encounter CD earlier in its natural history and who are increasingly asked to balance the benefits of early, aggressive intervention against the long-term risks and costs associated with this strategy. Our

results suggest that ASCA IgG is the most important marker in serological panels for assessing prognosis in CD. Additionally, the CD-associated risk variants assessed here do not seem to add value as predictors of CD prognosis. Ultimately, it is imperative that any classification scheme and any proposed new marker of CD be tested in population-based cohorts where clinical utility will determine the relevance to everyday practice.

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Dr Charles Bernstein is acting as the submission's guarantor

Specific author contributions:

JD Ryan: P*erformed the research, analysed the data, drafted the original manuscript and edited the final version of the paper, and G contributed to the design of the study.)*

M Silverberg: analyzed the data, contributed to the design of the studyand edited the final version of the manuscript

L Graff: contributed to the design of the study Performed the research, analysed the data, drafted the original manuscript and edited the final version of the paper, and G.)

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TABLE 1. Baseline participant demographics and psychological characteristics

CHARACTERISTIC	**n = 182 (%)**
Mean age	38.5 yrs
Female/male	112/70 (68%/32%)
Married or common law	129 (71%)
Extraintestinal disease	33 (18%)
Surgery	77 (42%)
Early Surgery*	53 (29%)

Family history (first degree)	39 (21%)
Family history (second degree)	45 (25%)
Smoking	35 (19%)
Current smoker	
Ex-smoker	60 (33%)
Never smoker	87 (48%)
Psychological factors	
History of Depression	71 (39%)
Low conscientiousness	102 (57%)
High neuroticism	98 (54%)
Low adherence to medications	43 (24%)
Adverse childhood experience	132 (73%)
Childhood sexual abuse	23 (13%)
Childhood physical abuse	13 (7%)
High childhood adversity score	67 (37%)

- Early surgery refers to surgery within 3 years of diagnosis

TABLE 2. Phenotype within 3 years from diagnosis (T1) and at five years from enrolment in the Manitoba IBD Cohort Study (T2).

	T1 n= 182 (%)	T2 n= 182 (%)
AGE AT DIAGNOSIS (A)		
A1 – 16 years or less	18 (10%)	
A2 – 17 – 40 years	116 (64%)	
A3 – 41 years or greater	48 (26%)	
LOCATION (L)		
L1 – Terminal Ileum	79 (43%)	75 (41%)
L2 – Colon	40 (22%)	34 (19%)
L3 – Ileum and Colon	62 (35%)	72 (40%)
L4 – Upper GI modifier	13 (7%)	13 (7%)

BEHAVIOR (B)		
B1 – Non-stricturing, Non-penetrating	82 (45%)	63 (34%)
B2 – Stricturing	60 (33%)	71 (39%)
B3 – Penetrating	40 (22%)	48 (26%)
P – Perianal modifier	42 (23%)	48 (26%)

Table 3. Multivariate analysis of phenotype, serology, genetics, and surgery.

OUTCOME	RISK FACTOR	P-value	OR (95%CI)
Complicated CD	ASCA IgG	0.0003	3.46 (1.75-6.84)
	NOD2	0.01	3.25 (1.27-8.35)
Surgery	ASCA IgG	0.003	2.66 (1.40-5.06)
Perianal CD	Age at diagnosis	0.004	2.13(1.02-3.94)
Terminal Ileum	ASCA IgG	0.03	2.2 (1.07-4.54)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*)For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable

Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized

		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.