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Clinical, serologic, and genetic factors associated with pyoderma gangrenosum and erythema nodosum in inflammatory bowel disease patients

Adam Weizman, MD^{*1}, Brian Huang, MD^{*1}, Dror Berel, PhD¹, Stephan R. Targan, MD¹, Marla Dubinsky, MD², Phillip Fleshner, MD¹, Andrew Ippoliti, MD¹, Manreet Kaur, MD¹, Deepa Panikkath, MD¹, Steve Brant, MD³, Ioannis Oikonomou, MD⁴, Rick Duerr, MD⁵, John Rioux, PhD⁶, Mark Silverberg, MD, PhD⁷, Jerome I Rotter, MD⁸, Eric Vasilias, MD¹, Talin Haritunians, PhD⁹, David Shih, MD, PhD¹, Dalin Li, PhD⁹, Gil Y. Melmed, MD, MPH¹, and Dermot P.B. McGovern, MD, PhD^{1,9}

¹F. Widjaja Foundation Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA

²Departments of Pediatrics, Pediatric Inflammatory Bowel Disease Center, Cedars Sinai Medical Center, Los Angeles, California, USA

³Meyerhoff Inflammatory Bowel Disease Center, Department of Medicine, School of Medicine, and Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA

⁴Department of Internal Medicine, Section of Digestive Diseases, Yale School of Medicine, New Haven, Connecticut, USA

⁵Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

⁶Universite de Montreal and the Montreal Heart Institute, Research Center, Montreal, Quebec, Canada

⁷Mount Sinai Inflammatory Bowel Disease Center, University of Toronto, Toronto, Ontario, Canada

⁸Institute for Translational Genomics and Population Sciences, Los Angeles BioMedical Research Institute and Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, California, USA

⁹Medical Genetics Research Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA

Abstract

Correspondence: Dr. Dermot McGovern, F. Widjaja Foundation Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, 8797 Beverly Blvd – suite 300, Los Angeles, CA 90048, USA. Dermot.mcGovern@cshs.org.

^{*}These authors contributed equally to this study

AW and BH performed the chart review and wrote the first draft of the manuscript. DB performed the non-genetic and modeling associated statistical analyses. SRT recruited patients and obtained funding for the study. DPM, MD, PF, AI, MK, SB, IO, RD, JR, MS, and EV recruited and phenotyped subjects. DP phenotyped study subjects. JIR and TH performed and supervised genotyping and QCing of genotype data. DS, GYM, and DPM conceived and supervised the study.

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Objective—Pyoderma gangrenosum (PG) and erythema nodosum (EN) are the most common cutaneous manifestations of inflammatory bowel disease (IBD) but little is known regarding their etiopathogenesis.

Design—We performed a case control study comparing characteristics between IBD patients with a documented episode of PG (PG+) and/or EN (EN+) with those without PG (PG-) and EN (EN-). Data on clinical features were obtained by chart review. IBD related serology was determined using ELISA and genome-wide data generated using Illumina technology. Standard statistical tests for association were used.

Results—We identified a total 92 cases of PG and 103 cases of EN with genetic and clinical characteristics, of which 64 PG and 55 EN were available for serological analyses. Fewer male subjects were identified in the PG(+) (OR 0.6, $p=0.009$) and EN(+) groups (OR 0.31, $p=0<0.0001$). Colonic disease, previous IBD related surgery, and non-cutaneous extra-intestinal manifestations were more common among both PG(+) and EN(+) patients compared to controls. PG(+) was associated with ANCA seropositivity ($p=0.03$) and higher ANCA level ($p=0.02$) in CD. Genetic associations with PG included known IBD loci (*IL8RA* ($p=0.00003$), and *PRDM1* (0.03)) as well as with *USP15* (4.8×10^{-6}) and *TIMP3* (5.6×10^{-7}). Genetic associations with EN included known IBD susceptibility genes (*PTGER4* ($p=8.8\times10^{-4}$), *ITGAL* (0.03)) as well as *SOCS5* (9.64×10^{-6}), *CD207* (3.14×10^{-6}), *ITGB3* (7.56×10^{-6}) and *rs6828740* (4q26) ($p<5.0\times10^{-8}$). Multivariable models using clinical, serologic, and genetic parameters predicted PG (AUC 0.8) and EN (AUC 0.97).

Conclusion—Cutaneous manifestations in IBD are associated with distinctive genetic characteristics as well as with the similar clinical characteristics including the development of other extra-intestinal manifestations suggesting shared and distinct etiologies.

Keywords

Crohn's disease; extra-intestinal manifestations; inflammatory bowel disease; pyoderma gangrenosum; erythema nodosum; ulcerative colitis; genetics; serology

Introduction

In addition to gastrointestinal tract pathology, the inflammatory bowel diseases are associated with extra-intestinal manifestations (EIMs), including cutaneous EIMs such as pyoderma gangrenosum (PG) and erythema nodosum (EN). While troublesome in their own right, EIMs may 'tag' more homogenous sub-groups within IBD with unique pathogenic characteristics.

PG, observed in 0.5-5% of IBD patients, is reportedly more commonly associated with colonic disease and is a significant source of morbidity requiring intensive, in-patient multi-disciplinary approaches to management [2, 3] [4-7]. PG is a neutrophilic dermatosis and usually begins as a papule or pustule at a site of trauma with a surrounding violaceous, undermined border [8]. PG can significantly affect activities of daily living, quality of life and can be cosmetically disfiguring leading to permanent scarring, significant pain, bacterial super-infection, and may require skin grafting [8, 9]. Moreover, potent immunosuppression with therapeutic agents such as intravenous cyclosporine or infliximab may be needed [10,

11]. There is limited data on genetic associations with PG, although small studies have suggested association with *PSTPIP1*, *PTPN6*, and *TRAF3IP2* [12-14].

EN is the most common dermatologic manifestation in IBD and affects 3-15% of IBD patients [15-17]. Typically diagnosed clinically and rarely before the onset of IBD, lesions most commonly affect the anterior surface of the lower extremities in the form of tender, erythematous, and raised nodules with histology revealing a neutrophilic inflammatory infiltrate and panniculitis [7, 18-20] [1]. Previous studies have noted that EN is also associated with colonic disease [16] and with a more benign IBD natural history [2, 16]. Prior studies have suggested an association between the *HLA* and EN in IBD [14, 23].

As researchers begin to understand the molecular characteristics of IBD, there is a need to better characterize sub-groups of these complex and heterogeneous conditions. An increased understanding of the etiology of both PG and EN in IBD may highlight potential novel therapeutic pathways for the sub-set of patients who are at risk for skin manifestations. The aim of this study was to better characterize IBD disease behavior, serologic profiles, and genetic associations among patients with PG or EN.

Methods

Subjects

We reviewed two large IBD databases for cases of PG and EN. The Cedars-Sinai Medical Center IBD Research Repository contains clinical (demographics, disease phenotype, disease history, EIMs, treatment records), serologic, and genetic data on over 5000 consented IBD patients followed at our center. From this database, we identified all subjects with genome wide association (GWA) genotyping for inclusion in this study [24, 25]. Those subjects with at least one documented episode of PG [PG(+)] or EN [EN(+)] were identified and underwent a detailed confirmatory medical record review. Subjects without a history of PG [PG(-)] or EN [EN(-)] formed the control group. An episode of PG was confirmed on record review based on either dermatology consultant impression or typical features, as defined elsewhere as having three or more of the following: (i) leg or peristomal location (ii) pathergy (iii) initial pustular lesion (iv) purulent discharge (v) violaceous or undermined borders (vi) crater like holes/cribiform scarring [26]. Histology was reviewed, when available, to exclude alternative diagnoses. An episode of EN was confirmed via chart review by dermatology consultation or typical features including erythematous, non-ulcerative, raised, tender bruise-like lesions usually on the legs.

We also included IBD cases with GWA data available from the National Institute for Diabetes and Digestive and Kidney Diseases IBD Genetics Consortium (NIDDK-IBDGC) (Cedars-Sinai Medical Center, Johns Hopkins University, University of Montreal, University of Pittsburgh, University of Toronto, Yale University, and University of Chicago (Data Coordinating Center)). The validity and reliability of the clinical features collected in this database has been previously documented [28]. The clinical and genetic data on IBD patients (care was taken to exclude any CSMC patients in the NIDDK cohort in order to avoid double 'reporting') were combined with the samples from the CSMC database (Supplementary Figures 1a and 1b). The serologic analysis was limited to the subjects

identified in the CSMC IBD Research Repository. The CSMC IBD Research Repository contained 4137 IBD subjects with GWA data. Of these, 64 were PG(+), 55 EN(+), and 4073 PG/EN(−). The NIDDK IBDGC database contained 1619 IBD subjects with GWA data (28 PG(+), 48 EN(+), and 1591 PG/EN(−)). The genetic and clinical analyses were performed on the combined datasets. Therefore, this analysis consisted of 5756 subjects (92 PG(+), 103 EN(+), and 5664 PG/EN(−)). The serologic analysis was limited to the subjects in the CSMC IBD Research Repository (total 4137 subjects: 64 PG(+), 55 EN(+); and 4073 PG/EN(−)). A total of 14 patients with both EN and PG were identified and were included in both EN and PG analyses.

Clinical and Serologic Phenotyping

Clinical data were collected at all contributing centers by chart review and included patient demographics (age, gender, ethnicity, race), family history, disease duration, disease phenotype (IBD subtype and disease location/behavior according to the Montreal Classification), surgical history, treatment record, smoking status, and presence or absence of other extra-intestinal manifestations [29]. IBD-associated serologies (anti-saccharomyces cerevisiae antibodies (ASCA IgG and IgA), perinuclear anti-nuclear cytoplasmic antibody (pANCA), anti-flagellin (anti-CBir1), anti-outer membrane porin C (anti-OmpC) and anti-Pseudomonas fluorescens-associated sequence I2 (anti-I2) were measured by ELISA, as previously described [30]. Antibody levels were determined and results expressed as ELISA units (EU/mL) that are relative to CSMC laboratory (immunoglobulin [Ig] A-I2, IgA-OmpC) or a Prometheus Laboratory standard (San Diego, CA; IgA and IgG ASCA) having been derived from a pool of patient sera with well-characterized disease found to have reactivity to these antigens. Quantitation of IgG anti-CBir1 reactivity was expressed in ELISA units derived based on a proportion of reactivity relative to a standardized positive control. Quantitative serologic results were converted to binary variables (i.e. positive or negative). Qualitative positivity to any antibody was defined as being greater than cut-off values 2 standard deviations above mean control titers for each assay. All assays were performed blind to any knowledge of patient clinical characteristics.

Genotyping and genotype quality control

Genotyping was performed at Cedars Sinai Medical Center using Illumina Human610 platforms for CD and HumanCNV370 platforms for UC [23, 24]. The NIDDK IBDGC samples were genotyped using the HumanHap300 and HumanHap550 platforms as previously described [31, 32]. In the genotyped datasets individual and genotype missingness, allele frequencies, and deviations from Hardy-Weinberg Equilibrium were calculated using the PLINK software package (<http://pngu.mgh.harvard.edu/~purcell/plink>). Of the 5756 IBD cases, 21 failed quality control (QC) metrics, in which we required a genotyping call rate of > 95%, inbreeding coefficient of < 0.05, and lack of cryptic relatedness which was defined as unexpected relatedness not reflected by known family structure and can sometimes be caused by sample contamination. Analysis of population substructure with Eigenstrat identified 134 subjects that were outside of the main Caucasian cluster on principle components analysis and those subjects were subsequently removed. SNPs with a call rate of < 0.95, with minor allele frequency (MAF) of < 0.01, and that strongly deviated from Hardy-Weinberg equilibrium ($P < 1 \times 10^{-7}$) were removed. After the

cleaning of the genotype datasets, for comparison purpose, we imputed SNPs separately for each genotyping platform using IMPUTE2 (mathgen.stats.ox.ac.uk/impute/impute_v2.html) with the HapMap Phase 2 (www.hapmap.org, all population groups combined) serving as the reference. After the imputation, a further stringent cleaning procedure was applied thereby removing any imputed SNP with a certainty score below 0.8, quality score below 0.5 and MAF < 0.01 in any of the cohorts, leaving 2,017,629 SNPs available for analysis.

Statistical Analysis

Univariate analysis—Univariate analyses were conducted to test for differences between PG(+) and PG(−) subjects and between EN(+) and EN(−) subjects. Fisher Exact Test was used to test for associations between PG/EN status and clinical characteristics. Serology was assessed both qualitatively (i.e. percent seropositive) and quantitatively (i.e. median antibody level) using the Fisher's Exact Test and the Wilcoxon Rank Sum Test, respectively. A p-value <0.05 was considered statistically significant.

Genetic analysis—In the imputed datasets, the association between PG and EN status and each single SNP were evaluated using logistic regression with SNPTTEST(http://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html) in the Cedars and NIDDK cohorts separately. Top 4 principle components calculated using Eigenstrat (<http://genepath.med.harvard.edu/~reich/Software.htm>) were included in the analysis to control for potential confounding effects due to population structure. We then performed a meta-analysis combining the Cedars and NIDDK cohorts to obtain combined estimates using an inverse variance weighting of cohort-specific estimates. Genetic associations with PG and EN were assessed using both a genome wide approach using, given that the skin EIMs are rare conditions, a nominal level of significance of $p < 5 \times 10^{-5}$, and at 99 previously identified IBD susceptibility loci using a level of significance of $p < 0.05$ [33, 34].

Multivariable analysis—Models to predict PG/EN development were created using step-wise multiple logistic regression, combining IBD clinical features, serology, significant IBD susceptibility SNP's, and top GWA hits. The models built for PG analysis included: (1) clinical features alone, (2) serology alone, (3) genetics alone, (4) clinical features + serology, and (5) clinical features + serology + genetic associations. The models build for EN analysis included (1) genetics alone, (2) clinical features alone, and (3) clinical + genetics. The area under the Receiver Operating Characteristic (ROC) curve (AUC [95% CI]) was used to measure the predictive performance of these models for PG and EN.

Ethical Considerations

The Institutional Review Board at Cedars-Sinai Medical Center and the GRCs of the NIDDK IBDGC approved this study and enrolled subjects had provided written informed consent.

Results

Clinical Associations

Pyoderma Gangrenosum—Overall, 92 PG(+) cases were identified among 5756 subjects for the genetic and clinical analysis while the serologic analysis was limited to 64 PG(+) among 4137 samples (see Methods section above and supplementary figures 1a and 1b).

Table 1a shows the demographic and disease features stratified according to PG status. PG was more common in women and patients with PG were diagnosed with IBD at an older age. Among those with CD, fewer subjects had small bowel disease in the PG(+) group but perianal disease and colonic disease were more common compared to the PG(−) group. Eye EIMs (i.e. iritis, uveitis), IBD associated arthritis and ankylosing spondylitis were more prevalent among those with PG.

Erythema Nodosum—Overall, 103 EN(+) cases were identified among 5756 subjects for the genetic and clinical analysis while the serologic analysis was limited to 55 EN(+) among 4137 samples (see Methods section and supplementary figures 1a and 1b).

Demographic and clinical characteristics associated with EN are shown in table 1b. Similar to the findings seen in PG, EN co-occurred with other EIMs and was more common in women and patients with colonic disease.

Serologic Associations

CD patients with PG were more likely to be ASCA negative, CBir1 negative and ANCA positive as well as having lower median ASCA and higher median ANCA levels in keeping with colonic disease location (supplementary figure 2a and 2b). No significant associations were seen between IBD-associated serologies and EN apart from a borderline association with median anti-CBir1 level (supplementary figures 2c and 2d) in CD cases. No significant serological differences were seen in UC between the two groups (data not shown).

Genetic Associations

Pyoderma Gangrenosum—A number of known IBD susceptibility loci showed significant association with PG including the loci containing *IL8RA* ($p=0.00003$), *MUC17* (0.01), *MMP24* (0.01), *WNK2* (0.01), *DOCK9* (0.02), *PRDM1* ($p=0.03$) and *NDIFI1* (0.04) (table 3a). While no genetic associations with PG reached a genome wide level of significance, several SNP's met the nominal level of significance of $p<5\times10^{-5}$ (table 2a and supplementary figure 2a).

Erythema Nodosum—A number of known IBD susceptibility loci showed significant association with EN including *PTGER4* ($p=8.7\times10^{-4}$), *ITGAL* (0.03), and *IKZF1* (0.03) (table 3b). There were several SNP's that met the nominal level of genome-wide significance of $p<5\times10^{-5}$ (table 2b, supplementary figure 2b). A SNP on chromosome 4q26 reached genome wide level ($p=4.94\times10^{-8}$).

Multivariable Logistic Regression Analysis

Pyoderma Gangrenosum—Multiple logistic multiple regression was performed to create predictive models for PG development (figure 1a). Model 1 examined the importance of genetic associations in predicting PG. The SNP's rs2382817 (*IL8RA*) and rs130555 (*TIMP3*) remained significant in the model, with an AUC of 0.73 [95% CI 0.7-0.76]. Model II examined clinical features in predicting PG and a history of perianal disease and eye manifestations remained significant. Model III examined the significance of serology on PG, and p-ANCA status remained significant in this model while Model IV involved the combination of clinical features and serology. Finally, Model V examined the combination of clinical, serologic, and genetic data for predicting PG, and performed the best with an AUC of 0.80 [0.73-0.87].

Erythema Nodosum—Similar models were created for EN (figure 1b). Model 1 examined the predictive value of genetic associations in EN (AUC, 0.76 [95% CI 0.71-0.80]) and it appeared that using genetics alone was modestly predictive of EN development. Model II employed clinical features including gender, small bowel involvement, IBD related arthritis, and eye involvement with significantly greater success in predicting EN (AUC, 0.95 [95% CI 0.94-0.97]). Model III, a combination of clinical and genetic parameters, showed only a modest improvement than model II (AUC 0.97).

Discussion

Extra-intestinal manifestations (EIMs) can often be more debilitating and difficult to treat than the underlying IBD and the ability to predict the development of these lesions could have implications on management. For example, patients at high-risk of PG may be educated regarding the importance of early detection and reporting of skin lesions. An increased understanding of the pathogenesis of EIMs may define sub-sets of disease and potentially identify novel therapeutic targets. The current study is among the first and largest in the IBD literature to characterize genetic, clinical and serologic associations of both PG and EN.

In keeping with previous findings, skin EIMs occur more commonly in women[23,24]. We observed that patients with PG were diagnosed with IBD at an older age and developed PG much later into IBD disease course, with a mean onset of 15 years after IBD diagnosis (data not shown), which is longer than the 2-7 years previously reported in the literature [36, 37]. Earlier studies have suggested that PG was more common in UC, however more recent studies have shown stronger associations with CD [6, 36, 38]. In a population-based study, Bernstein et al. showed a prevalence of PG of 1.3% among CD patients compared to 0.8% among those with UC [6]. However, we found no difference in PG episodes among IBD disease type i.e. CD or UC in our cohort of patients largely collected from tertiary centers. While there are conflicting data, colonic disease has been more consistently demonstrated to be associated with EIM's [17, 36, 39] and our findings support this in both EN and PG. It has been proposed that the predominance of colonic disease in a subject with EIM's may be related to interactions between colonic disease and the microbiome with subsequent cross-reacting of skin, joint, eye, etc. antigens with gut antigens [40]. A number of studies have

demonstrated the co-occurrence of EIMs and this phenomenon is present in the cohorts from this study [17, 39]. In one study from Switzerland, patients with IBD-associated arthritis had an OR of 2.9 for subsequent PG development [17]. Moreover, certain EIM's tended to cluster, with EN and musculoskeletal manifestations commonly occurring together and PG occurring together with inflammatory eye manifestations. In this study, subjects with PG had a higher prevalence of iritis, uveitis, IBD associated arthritis, and ankylosing spondylitis (AS) and EN was associated with oral ulcers, IBD related arthritis, and ophthalmologic involvement providing additional evidence of common underlying pathophysiology to the IBD EIMs.

The genetic contributions to the EIMs remain unclear, however, we have identified several putative genetic associations (from the GWA approach) between IBD and PG including *GPBAR1* which encodes a G-protein involved in bile salt absorption, highly expressed in the ileum and colon, and interestingly, associated with PSC [44, 45] providing additional evidence of shared etiology for the EIMs. PG was also associated with *TIMP3*, an important inhibitor of metalloproteinase involved in the degradation of the extracellular matrix [46]. Given the large contribution of the extracellular matrix to skin morphology, this may be a particularly pertinent finding [47]. We also identified associations between known IBD susceptibility genes and PG including *IL8RA*, a known mediator of neutrophil migration [33, 34, 48] which may be highly relevant given the role of neutrophils in PG pathogenesis. We identified several SNP's that met our nominal level of for genome wide significance ($p < 5 \times 10^{-5}$) and one SNP (*rs6828740*) reached the highly stringent level for genome wide level of significance ($p < 5 \times 10^{-8}$) for association with EN. This SNP, *rs6828740*, is located on chromosome 4q26 in a gene desert highly conserved across species that contains a binding site for *PRDM1*, a known IBD and autoimmune disease 'gene' also associated with PG development in this study (table 1b). Other putatively associated genes of interest include: *SOCS5* (2p21), a negative regulator of cytokine signaling [59]; *CD207* (2p13), a non-classical antigen-processing pathway gene that facilitates uptake of antigens for presentation to T-cells [60]; and *ITGB3* (17q21), an integrin involved with cell adhesion and cells-surface mediated signaling that is associated with asthma and diabetes susceptibility [61]. Many of the genes that we have identified play a role in antigen presentation and cytokine signaling/production that contribute both, to the inflammation of the intestinal tract during IBD flares, as well as to the lympho-histiocytic infiltrate of the dermis and panniculitis seen with EN lesions [1]. Our multivariable model for PG showed that the combination of clinical features, serology, and genetic associations performed better in predicting PG development than any variable alone. Our results add to the growing number of published examples of predictive multiplatform models. [49, 50].

We recognize that this study, despite being the largest of its type performed to date, has a number of limitations including the sample size. Furthermore, there is no definitive definition or definitive diagnostic test for PG or EN and all studies to date have been based on clinical impression from typical dermatologic features [8]. The diagnoses of PG and EN, in this study, were made by experienced IBD clinicians or dermatologists based at large tertiary referral IBD centers in North America. Reassuringly, our clinical associations with skin manifestations replicate results from other studies as well as identifying novel

demographic, clinical, serological, and genetic associations. These findings should be replicated both independently and through combination with this existing cohort. The multivariable model represent an interesting and potentially powerful example of multiplatform predictive tools.

In summary, we have better characterized the clinical, serologic, and genetic associations in IBD patients who develop PG or EN. While they are clearly distinct entities from one another, we demonstrate that there are shared associations suggesting common underlying mechanisms and pathways. These findings may also suggest potential novel therapeutic targets for managing these extra-intestinal manifestations including TIMP inhibitors and anti-cell adhesion strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A, Multivariable model for predicting PG development. B, Multivariable model for predicting EN development.

Table 1
Clinical and Demographic Characteristics of PG Cohort

Clinical Feature	PG(+), n = 92 (%)	PG(-), n = 5664 (%)	P	OR (95% CI)
Male	34 (37)	2825 (51)	0.009	0.6 (0.4–0.9)
Crohn's disease	57 (62)	3580 (63)	NS	—
Ulcerative colitis/IBDU	35 (38)	1851 (37)	NS	—
Mean age of IBD onset (yr)	28.5	24.8	0.026	—
Active smoking	30 (33)	869 (26)	NS	—
Family history of IBD	23 (27)	439 (20)	NS	—
Small bowel involvement	43 (77)	1403 (88)	0.021	0.5 (0.2–0.9)
Perianal CD	33 (54)	470 (29)	<0.0001	2.8 (1.6–4.7)
Colonic disease	55 (89)	1148 (71)	0.001	3.2 (1.5–7.1)
CD disease behavior				
B1	19 (34)	623 (39)	NS	—
B2	17 (30)	659 (41)		
B3	25 (45)	514 (32)		
Previous IBD-related surgery	61 (66)	1629 (48)	<0.0001	2.2 (1.4–3.4)
IBD-associated arthritis	26 (28)	248 (9)	<0.0001	3.8 (2.4–6.1)
Ankylosing spondylitis	4 (4)	31 (1)	0.029	3.8 (1.3–11)
Ophthalmologic (iritis, uveitis)	18 (20)	93 (4)	<0.0001	6.7 (3.8–11.5)
Primary sclerosing cholangitis	2 (2)	51 (2)	NS	—

OR, odds ratio; IBDU, inflammatory bowel disease unclassified.

Table 2
Clinical and Demographic Characteristics of EN Cohort

Clinical Feature	EN(+), n = 103 (%)	EN(-), n = 5664 (%)	P	OR (95% CI)
Male	25 (24)	2823 (51)	<0.0001	0.3 (0.2–0.5)
Crohn's disease	69 (67)	3578 (66)	NS	—
Ulcerative colitis/IBDU	34 (33)	1851 (34)	NS	—
Mean age of IBD onset (yr)	23.5	24.8	NS	—
Active smoking	70 (69)	868 (26)	<0.0001	6.2 (4.0–9.5)
Family history of JBD	31 (30)	439 (20)	0.012	1.8 (1.2–2.7)
Small bowel involvement	36 (49)	1405 (87)	<0.0001	0.1 (0.1–0.2)
Perianal CD	26 (30)	470 (29)	NS	1.0 (0.6–1.6)
Colonic disease	77 (88)	1148 (71)	0.001	2.9 (1.5–5.5)
CD disease behavior				
B1	35 (48)	623 (39)	NS	—
B2	21 (30)	659 (41)		
B3	29 (41)	514 (32)		
Previous IBD-related surgery	68 (66)	1628 (48)	<0.0001	2.1 (1.4–3.2)
IBD-associated arthritis	49 (48)	248 (9)	<0.0001	8.7 (5.8–13.1)
Ankylosing spondylitis	2 (2)	31 (1)	NS	3.8 (1.3–11)
Ophthalmologic (iritis, uveitis)	26 (25)	93 (4)	<0.0001	9.2 (5.6–15)
Primary sclerosing cholangitis	2 (2)	34 (3)	NS	—

OR, odds ratio; TBDU, inflammatory bowel disease unclassified.

Table 3
Genetic Associations with PG Assessed at Known IBD Susceptibility Loci

SNP	"Risk" Allele	Chromosome	P	OR (95% CI)	Gene
rs2382817	C	2q35	0.00003	0.4 (0.3–0.6)	<i>ILSRB, TMBIM1, TNSI, GPBAR1, ILSRB</i>
rs1734907	A	7q22	0.01	1.8 (1.2–2.7)	<i>MUC17, APIS1, SERPINE1, VGF, ARS2</i>
rs6088765	G	20q11	0.01	1.6 (1.1–2.3)	<i>MMP24, PROCR, EIF6, EDEM2</i>
rs4743820	T	9q22	0.01	0.6 (0.4–0.9)	<i>WNK2, NINJ1, ZNF484, FGD3, SUSP3</i>
rs95S7195	T	13q32	0.02	0.6 (0.3–0.9)	<i>DOCK9, GPR18, UBAC2, EBI2, TM9SF2</i>
rs6568421	G	6q21	0.03	1.5 (1.1–2.2)	<i>PRDM1</i>
rs11230563	T	11q12	0.03	1.5 (1.0–2.1)	—
rs6863411	T	5q31	0.04	0.7 (0.5–0.9)	<i>SPRY4, PCDH12, GNPDA1, NDFIP1</i>

OR, odds ratio.

Table 4
Genetic Associations with PG Using a Genome-Wide Approach

SNP	"Risk" Allele	Chromosome	P	OR (95% CI)	Genes
rs10184704	C	2q14	6.3×10^{-6}	2.3 (1.6–3.3)	<i>GYPC</i>
rs13392177	C	2q35	4.8×10^{-6}	2.3 (1.6–3.4)	<i>APRC2/GPBARI/HMGIL9</i>
rs161857	T	5q23	7.3×10^{-6}	3.0 (1.9–4.9)	<i>TNFAIP8/HSD17B4/FABP5L6</i>
rs11174436	A	12q14	4.6×10^{-6}	2.3 (1.6–3.4)	<i>FAM19A, USP15, MON2</i>
rs12607055	C	18q12	1.7×10^{-6}	3.5 (2.1–5.9)	<i>RIT2/SYT4</i>
rs17770334	C	18q21	1.8×10^{-6}	3.1 (1.9–4.8)	<i>SERPINB3/4/SJ711113, BCL2, VPS4B, KDSR</i>
rs130555	C	22q12	5.6×10^{-7}	2.5 (1.7–3.5)	<i>TIMP3</i>

OR, odds ratio.

Table 5
Genetic Associations with EN Assessed at Known IBD Susceptibility Loci

SNP	"Risk" Allele	Chromosome	P	OR (95% CI)	Gene
rs11742570	C	5p13	8.77×10^{-4}	0.56 (0.4–0.8)	<i>PTGER4</i>
rs11150589	T	16p11	0.027	1.41 (1.0–1.9)	<i>ITGAL</i>
rs1456896	C	7p12	0.03	1.6 (1.1–2.3)	<i>IKZF1</i>

OR, odds ratio.

Table 6
Genetic Associations with EN Using a Genome-Wide Approach

SNP	“Risk” Allele	Chromosome	P	OR (95% CI)	Gene
rs7586612	A	2p21	9.64×10^{-6}	2.01 (1.5–2.7)	<i>SOC55, MCFD2, TTC7A, CALM2</i>
rs390966	G	2p13	3.14×10^{-6}	2.17 (1.6–3.0)	<i>CD207, NAGK, CLEC4F, MCEE</i>
rs6828740	C	4q26	4.94×10^{-8}	4.14 (2.5–6.9)	—
rs9405444	A	6p25	8.07×10^{-6}	2.21 (1.6–3.1)	<i>FOXQ1</i>
rs10138297	G	14q21	7.29×10^{-6}	2.63 (1.7–4.0)	<i>ARF6, METTL21D, SOS2</i>
rs11079757	A	17	7.6×10^{-6}	2.2 (1.6–3.1)	<i>JTGB3, MYL4, CCD27</i>

OR, odds ratio.