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Perianal Crohn's Disease is associated with distal colonic disease, stricturing disease behavior, IBD-associated serologies and genetic variation in the JAK-STAT pathway

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Abstract

BACKGROUND—Perianal Crohn's Disease (pCD) is a particularly severe phenotype associated with poor quality of life with a reported prevalence of 12%–40%. Previous studies investigating the etiology of pCD have been limited in the numbers of subjects and the intensity of genotyping. The aim of this study was to identify clinical, serological and genetic factors associated with pCD.

METHODS—We performed a case-control study comparing patients with (pCD+) and without perianal (pCD−) involvement in CD; defined as the presence of perianal abscesses or fistulae. Data on demographics and clinical features were obtained by chart review. IBD related serology were determined by ELISA. Genetic data were generated using Illumina genotyping platforms

RESULTS—We included 1721 patients with CD of which 524 (30.4%) were pCD+ and 1197 were pPCD−. Perianal CD was associated with distal colonic disease (OR 5.54 [3.23–9.52], $p < 0.001$), stricturing disease behavior (1.44 [1.14–1.81], $p = 0.002$) and family history of inflammatory bowel disease (4.98 [3.30–7.46], $p < 0.001$). Perianal CD was associated with higher ASCA IgA ($p < 0.001$) and OmpC ($p = 0.008$) antibody levels. Perianal CD was associated with known IBD loci including KIF3B; CRTC3; TRAF3IP2; JAZF1; NRIP1; MST1; FUT2; and PTGER (all $p<0.05$). We also identified genetic association with genes involved in autophagy (DAPK1, $p=5.11\times10^{-5}$); TNF alpha pathways (NUCB2, $p=8.68\times10^{-5}$; DAPK1); IFNg pathways (DAPK1; NDFIP2, $p=8.74\times10^{-5}$) and extracellular matrix and scaffolding proteins (USH1C, $p=8.68\times10^{-5}$; NDFIP2; TMC07, $p=8.87\times10^{-5}$). Pathway analyses implicated the JAK/Stat pathway ($p_c = 3.72 \times 10^{-5}$).

CONCLUSION—We have identified associations between perianal Crohn's disease, more distal colonic inflammation, Crohn's disease-associated serologies, and genetic variation in the JAK/Stat pathway

Keywords

Crohn's disease; perianal crohn's disease; inflammatory bowel disease; genetics; serology

INTRODUCTION

Crohn's disease (CD) has a reported prevalence of 319 per 100,000 persons in North America[1] and is a clinically heterogeneous disease with presentation, natural history, and symptoms related to disease location and behavior. Perianal Crohn's disease (pCD) is a particularly disabling and debilitating manifestation of CD that is often associated with poor quality of life[2]. Prior studies have estimated prevalence rates for pCD ranging from 12%–40%[3–7], with higher prevalence rates reported in referral center-based studies as well as studies that include hemorrhoids, skin tags and anal fissures as perianal manifestations of CD. There have been conflicting data regarding the association between perianal CD and internal fistulizing disease behavior[3, 5, 8, 9]. The conflicting results from earlier studies may have been in part due to designation of perianal fistulas as penetrating disease as per the Vienna classification. A modification of the Vienna classification- the Montreal classification, introduced in 2005, identifies perianal disease as a modifier, thereby distinguishing it from internal penetrating or stricturing disease behavior[10]. Despite conflicting data regarding the association with internal penetrating disease, the presence of perianal involvement in CD has been associated with a complicated disease course[8, 11, 12] and an increased need for surgery[13].

Prior studies investigating factors associated with pCD have been limited by both the relatively small number of patients as well as the density of genotyping and to our knowledge, there have been no studies, to date, that have correlated inflammatory bowel disease-associated antibodies with pCD. As researchers begin to understand the complex pathophysiology of CD, there is a need to embrace the clinical heterogeneity and better characterize the various sub-groups within CD. A clearer understanding of the clinical, serologic and genetic associations with pCD may facilitate identification of novel therapeutic approaches or more appropriate targeting of existing therapies in this challenging subset of IBD patients in whom current treatment strategies are inadequate[14]. The aim of the current study was to characterize the clinical, genetic and serologic associations with pCD allowing a better understanding of the pathophysiology of pCD.

METHODS

Study Setting and Patients

The Cedars-Sinai Medical Center IBD Research Repository (MIRIAD) contains clinical, serologic as well as genetic data on several thousand IBD patients followed at Cedars-Sinai Medical Center as previously described[15, 16]. In addition to providing written consent for access to medical records, MIRIAD participants have provided blood samples for genetic

profiling and serologic analyses. Following approval of the study protocol by the Cedars-Sinai Institutional Review Board, the IBD repository was queried to identify patients with a confirmed diagnosis of CD based on clinical, radiographic, endoscopic or histologic evidence. We excluded patients that did not have genetic data generated using the immunochip or GWAS platforms as well as patients with incomplete data pertaining to disease location and behavior.

Clinical and Serologic Phenotyping

Demographic data (age, gender, ethnicity and race), family history of IBD, disease duration, disease phenotype (disease location and behavior), presence of extra-intestinal manifestations and surgical history were collected by chart review. Perianal Crohn's disease was defined as the presence of perianal abscesses, perianal fistulae or recto-vaginal fistulae. Hemorrhoids, skin tags and anal fissures were not considered as perianal manifestations of CD in this study. Disease location and extent were determined by endoscopic, histologic, radiographic and operative reports. Disease location was characterized as involving the small bowel, colon or both. Upper gastrointestinal (GI) involvement was defined as endoscopic or histologic evidence of inflammation involving the esophagus, gastric or duodenal mucosa in a pattern consistent with CD involvement. Small bowel disease included inflammation in the ileum as well as jejunum. In cases where microscopic disease extent exceeded the macroscopically visible disease; the former was used to determine disease extent and location. Similarly, in cases with variable extent of disease the maximum extent identified at any time was used to determine disease location.

Sera from patients were analyzed for expression of anti-sacharomyces cerevisae antibodies (ASCA IgG and IgA), perinuclear anti-nuclear cytoplasmic antibody (pANCA), anti-flagellin (CBir1) and anti-outer membrane porin C (anti-OmpC) and anti-Pseudomonas fluorescence associated sequence I2 (anti-I2); measured by ELISA, as previously described[17]. Antibody levels were measured and results expressed as ELISA units (EU/ml) relative to a Cedars-Sinai laboratory (immunoglobulin [Ig]A-I2, IgA OmpC) or a Prometheus laboratory standard (San Diego CA; ASCA IgA and ASCA IgG); having been derived from a pool of patient sera with well characterized disease found to exhibit reactivity to these antigens.

Genotyping

Genotyping was performed at Cedars-Sinai Medical Center using Illumina whole genome arrays (Human610-Quad; HumanOmniExpress) and the Immuno-BeadChip array as previously described[16, 18]. For the Immuno-BeadChip, average genotyping call rate for samples that passed quality control (QC) was 99.98%. Average concordance rate across 83 samples genotyped in replicate was 99.99%. Single-nucleotide polymorphisms (SNPs) underwent methodological review and were evaluated using several SNP statistic parameters, including SNP call frequency, cluster separation, replicate and heritability error rates, heterozygous excess, theta mean and deviation, and R intensity mean. A total of 135,252 autosomal SNPs passed genotyping QC measures and were common across datasets. For the Human OmniExpress and Human610-Quad, average genotyping call rates for samples that passed QC were 99.85% and 99.83%, respectively. Average concordance

rate across 19 samples genotyped in replicate was >99.99%. Genotyping of three control trios yielded a heritability frequency of 99.53%. Optimal allele-calling was verified by manual review of top associated SNPs.

Statistical Analysis

Clinical, demographic and serological parameters—Univariate analyses were performed to test for differences between patients with perianal CD disease (pCD+) and CD patients without perianal involvement (pCD−). Clinical variables were analyzed using the chi-square test for categorical variables and the student t-test for continuous variables. Serologic data were analyzed as categorical (percent seropositive) as well as continuous (antibody level) variables using the Fisher's exact test or Chi-square test and the Wilcoxon rank sum test, respectively. A p value <0.05 was considered statistically significant. We also calculated a quartile sum score, as previously described[17]; combining all the serologies except pANCA and a linear trend test was performed to evaluate the whether there is a higher pCD prevalence rate in high score individuals.

Genetic analyses—Genetic analyses were performed separately on data generated using immunochip (404 pCD+ and 890 pCD− cases) and on data generated using two different genome-wide platforms (610K chip and Omniexpress chip). There were 208 pCD+ cases and 590 pCD− controls with 610K data, and 179 pCD+ cases and 386 pCD− controls with Omniexpress data. Logistic regression was performed to investigate the association of pCD with genotypes after intensive post-genotyping QC. The post-genotyping QC measures include variant and individual call rate, Hardy-Weinberg Equilibrium test, gender check, population-stratification analysis and minor allele frequency filtering, as previously described [15]. Non-Caucasian patients were excluded from the genetic analysis and top four principal components were included in the logistic regression model to control for potential inflation due to population stratification. For the immunochip analyses genetic associations were first assessed at previously identified IBD susceptibility loci[15] using an *a priori* level of significance of $p < 0.05$. Putative genetic associations at novel loci were assessed as well, using an *a priori* level of significance of $p < 0.001$. Results are reported as odds ratios (OR) with 95% confidence intervals (95% CI).

For the data from the genome-wide platforms the imputation and association tests were performed separately on the two different data sets (610K chip and Omniexpress chip). After extensive post-genotyping QC, 610K data with 511,991 SNPs and Omniexpress data with 583,561 SNPs were retained for imputation. Both genotype datasets were imputed to ~2.5 million markers with the public Hapmap II (Release 22) reference panel by IMPUTE2[19] after post-imputation QC. Following the separate association tests, only common SNPs with a minor allele frequency > 0.05 (~2.0 million SNPs in total) were included for the meta-analyses that was performed using METAL software[20].

Network analyses—Networks were constructed by combining scores of logistic regression for each gene and the human PPI network. To construct a network with scores of logistic regression from the identified SNPs at gene level, we first calculated the Pearson pairwise correlation coefficient between the scores of every two genes with control samples

only, resulting in a correlation coefficient matrix and the corresponding P-values. Then, the network (adjacency matrix) was determined by the correlation coefficients with a cutoff P–value of $P < 0.00001$. The same lists of genes were also uploaded to STRING (http://string-db.org/). Final network and modules were constructed from genes with common edges from both our calculation and STRING. The nodes on the network represent genes, and edges indicate known and predicted functional interactions. Stronger biological associations between genes are depicted with a thicker edge. The identified genes on the network were uploaded to DAVID (david.abcc.ncifcrf.gov/). Top five KEGG pathways with the unadjusted P values less than 0.005 were selected. Information reported from DAVID includes KEGG pathway names, number of identified genes on the pathway, and the unadjusted and adjusted P values.

Model development—Multivariable analyses were performed using step-wise logistic regression analyses, using a backward elimination approach to generate clinical, serologic and genetic models associated with pCD. The area under the Receiver Operative Characteristic (ROC) curve (AUC [95%CI]) was used to measure the performance of these models for association with pCD. The clinical, serologic and genetic models were studied individually as well as in combination to identify the model with the highest AUC.

RESULTS

Baseline Characteristics

One thousand seven hundred and twenty one CD patients were identified with mean disease duration of 10.2 years (range, 4 months – 51.5 years). Nine hundred and twenty six (53.8%) patients were male (Table. 1). A majority of patients were Caucasian (93.1%) and 39% of the Caucasian patients were Jewish.

Perianal Crohn's disease was identified in 524 patients, constituting approximately one-third of the study cohort. When Montreal disease classification was applied; more than half the patients (54.2%) exhibited complicated disease behavior; characterized by stricturing and/or internal penetrating disease. Among patients with perianal disease, fistulae (perianal as well as recto-vaginal) were present in 75.4% patients (Table. 2). Perianal abscesses were seen in 24.4% patients. There was only one patient with isolated pCD, without any concomitant colonic or small bowel involvement. For the vast majority of patients (96.7%) with pCD, perianal involvement occurred following the diagnosis of CD (median duration 3.3 years). Perianal manifestations preceded the diagnosis of luminal CD in 3.3% of patients (range 6 months-9 years).

Clinical Associations with pCD

Perianal Crohn's disease was associated with a younger age at diagnosis and non-Jewish ancestry (OR 1.29). A family history of IBD among first-degree relatives (OR 4.98) was also associated with pCD, suggesting additional hereditary influences in the development of pCD (Table 3). Isolated small bowel disease conferred a protective effect on the development of pCD (OR 0.38) whereas colonic involvement was significantly associated with pCD (OR 1.35). To further clarify the association between pCD and colonic and small bowel disease,

we identified patients with any colonic or any small bowel disease and observed the same pattern of a protective effect with any small bowel disease (OR 0.74 [0.57–0.96]; p =0.03) and a significant association with any colonic disease (OR 2.55 [1.89–3.49]; $p < 0.001$) for the presence of pCD. Within the colon, distal colonic inflammation showed a strong association with pCD with a higher OR for pCD the more distal the site of disease involvement (Table. 3).

Patients with non-stricturing and non-penetrating behavior were less likely to develop pCD (OR 0.69); whereas stricturing disease behavior was associated with pCD (OR 1.44). We did not find an association between internal penetrating disease and pCD (Table 3). There was a statistically significant association between pCD and dermatologic manifestations of CD, namely pyoderma gangrenosum and erythema nodosum (OR 2.03) as well as a higher number of abdominal surgeries 1.14 versus 0.74) in patients with pCD (Table. 3).

Serologic Associations with pCD

One thousand six hundred and sixteen patients had serology data available for analyses. Patients with pCD were more likely to exhibit positive serology for ASCA IgA ($p < 0.001$), ASCA IgG (p= 0.001), I2 (p= 0.001), OmpC ($p < 0.001$) and ANCA (p= 0.006) antibodies when compared to patients without perianal involvement (supplementary material). The median antibody levels for ASCA IgG, ASCA Ig A, I2, Omp C and CBir-1 antibodies were higher among patients with pCD after adjusting for stricturing and penetrating disease behavior as a co-variate(Figure 1). The quartile sum score combining ASCA IgG, ASCA Ig A, I2, Omp C and CBir-1 was strongly associated with pCD, with higher pCD prevalence rate in high score individuals ($p=7.18 \times 10^{-9}$) (supplementary material).

Genetic Associations with pCD

Data from four hundred and four pCD+ and 890 pCD− subjects genotyped using the immunochip survived QC procedures. Following correction for population structure genetic associations with pCD were examined at the previously described IBD susceptibility loci (supplementary material).[15]. We also report some putative novel pCD associations from the immunochip. Known IBD genes implicated included *MST1* and *JAZF1* and associations with pCD at novel loci included: cadherin (*CHD1*); genes involved in encoding extra-cellular matrix and scaffolding proteins (*USH1C, HAS3, NDFIP2, TMCO7*); TNF pathways (*NUCB2* and *DAPK1*); autophagy (*DAPK1*); endoplasmic reticulum stress (*DAPK1*); interferon gamma pathways (*DAPK1, NDFIP2*); and multi-drug resistance (*ABCC8*) (supplementary material).

The whole genome approach also revealed novel putative associations in genes associated with other gastrointestinal immune condition immune-related conditions including HLA class II (ulcerative colitis), and *FOXP1* (Behcet's disease). Furthermore these associations implicate processes such as regulation of T cell differentiation (*FOXP1*), and antigen presentation (HLA Class II) in the development of perianal disease(supplementary material).

The logistic regression/human PPI network and pathway annotation analyses performed on the Immunochip data implicated the JAK-STAT (Janus Kinase-signal transducer and activator of transcription) signaling pathway. The pathway annotation analyses also revealed

association between pCD and, Leishmaniasis, phagosome antigen processing and presentation, and ABC transporte (table 4). After False Discovery Rate (FDR) correction the association with pCD and the JAK-STAT pathway remained highly statistically significant ($p_c = 3.72 \times 10^{-5}$).

Multivariable Logistic Regression Analyses

Multiple logistic regression analyses were performed using the clinical, serologic and Immunochip data to create models for association with pCD. The performance of the models is measured with leave-one-out cross validation and the predicted area under ROC curve (AUC). The clinical only model showed a significant association between family history of IBD, rectal inflammation, stricturing disease behavior and pCD (supplementary material) with a predicted AUC of 0.74 [0.69–0.80]. In the serology only model ASCA IgA ($p < 0.001$) and OmpC ($p = 0.008$) levels remained significantly associated with pCD with a predicted AUC of 0.55 [0.52–0.59]. The genetic model (supplementary material)showed significant associations with pCD at known as well as novel loci with predicted AUC of 0.65 [0.61–0.68]. A model combining clinical and genetic data (figure 2) achieved the best performance with a predicted AUC of 0.80 [0.74–0.86]. The clinical-genetic model demonstrated statistically significant improvement over the clinical only model ($p < 0.01$). Interestingly, a model combining clinical, serologic, and genetic data did not have the best performance; with a predicted AUC of 0.78[0.72–0.84], indicating high correlation between genetic and serologic variables.

DISCUSSION

Increased understanding of the underlying pathophysiologic mechanisms in subsets of CD, such as pCD, may allow clinicians to individualize their approach to disease management as well as identify potential novel therapeutic targets in an area of unmet medical need. The current study is, to our knowledge, the largest study specifically studying pCD patients, and the only one that we are aware of that includes clinical, serologic and genetic variables associated with pCD.

The prevalence of pCD in this cohort is approximately 30%, which is similar to prevalence rates reported in population-based cohorts[8, 12]. We observed an association between pCD and colonic involvement as well as a ‘protective’ effect of concomitant small bowel disease, consistent with findings previously reported by Tang et al in a Canadian population based cohort[3]. However, Tang et al also reported an association between pCD and internal penetrating disease; a finding that was not replicated in our cohort. There have been conflicting data regarding the association between pCD and internal penetrating disease. In a multi-center retrospective study (5491 patients across the USA and Europe) the association between pCD and internal fistulizing disease was observed consistently across all centers among patients with colonic disease only[5]. We observed a significant association between pCD and stricturing disease. At least two population-based studies have identified an association between pCD and complicated luminal disease behavior[8, 12]. While both studies demonstrated an association between pCD and complicated disease behavior, neither study distinguished between penetrating or stricturing complications. We observed an increased

number of abdominal surgeries among patients with pCD as has been previously reported in a smaller population based cohort[13].

Patients with pCD demonstrated a higher prevalence as well as higher median levels of antibodies typically associated with complicated small bowel involvement in CD (ASCA IgG, ASCA IgA, I2, Omp C, C-Bir) despite a lower prevalence of small bowel disease in this group. This novel finding remained significant after adjusting for stricturing and penetrating luminal disease behavior as covariates, suggesting that distinct immune processes may contribute to complicated disease pathogenesis in both small bowel and perianal pathology.

There have been several studies aimed at identifying genetic associations with pCD, mostly limited by both small sample sizes and genotyping density. While *NOD2/CARD15* and the IBD5 locus (5q31) have previously been associated with pCD; other studies, including this one, have not confirmed these findings [16–21]. A study from the Netherlands reported an association between *ATG16L1* and perianal disease[21]. While we could not confirm this finding, we did observe nominal association with another autophagy-related gene-*DAPK1* (death associated protein kinase). It is important to acknowledge that none of the associations at the known loci are significant after correction for multiple testing.

In addition to nominal genetic associations with pCD at known IBD susceptibility loci, our study also identified putative genetic associations at novel loci, although, again, none of these reached a level consistent with genome-wide significance. While these genetic associations will need to be confirmed in larger cohorts, they hint at clues to the pathogenesis of perianal involvement in CD. The putative novel association between pCD and genes encoding extra cellular matrix and scaffolding proteins (*USH1C, HAS3, NDFIP2, TMCO7*), TNF pathways (*NUCB2* and *DAPK1*), and autophagy (*DAPK1*) suggest related, yet distinct pathogenic mechanisms between pCD and more-proximal CD. The association between pCD and CHD1 (E-Cadherin) is particularly intriguing. *CHD1* encodes epithelial cadherin, a calcium dependent adhesion glycoprotein that has been implicated in cancer progression. E-cadherin, in conjunction with alpha E beta 7 (αEβ7) integrin plays a central role in lymphocyte retention in the intestinal mucosal epithelium[22]. Selective inhibition of intestinal leucocyte trafficking remains a promising therapeutic approach in IBD[23, 24] and more specifically a molecule that inhibits the interaction between E-Cadherin and αEβ7, etrolizumab, is currently under investigation for use in IBD[25]. Furthermore our network analysis strongly implicated the Janus Kinase-signal transducer and activator of transcription (JAK-STAT) pathway, a finding that did remain highly significant after correcting for multiple testing. Recent studies have suggested benefit in CD with oral JAK inhibitors (Tofacitinib).[26] Collectively, these findings suggest targeted trials of etrolizumab and particularly tofacitinib in pCD may be warranted.

The aim of our study was to identify clinical, serologic and genetic associations with pCD. Multivariable models incorporating each of these variables were studied independently as well as in combination to identify a model most ‘associated’ with pCD. The clinical model, comprising of presence of rectal inflammation, stricturing disease behavior and family history of IBD demonstrated an AUC of 0.74 and outperformed the genetic only and

serology only models. The model combining clinical and genetic variables demonstrated the best AUC of 0.80, a statistically significant improvement over the clinical only model. The compelling contribution of family history of IBD among first-degree relatives in the clinical model alludes to a significant genetic component in conferring susceptibility to pCD and further studies looking for additional genetic associations with pCD should be encouraged.

In conclusion; we have identified distinct clinical, serologic and genetic variables associated with perianal CD in our referral center based cohort. While these findings need to be confirmed in a larger prospective cohort; they may help identify novel therapeutic targets as well as facilitate identifying patients at increased risk of developing perianal disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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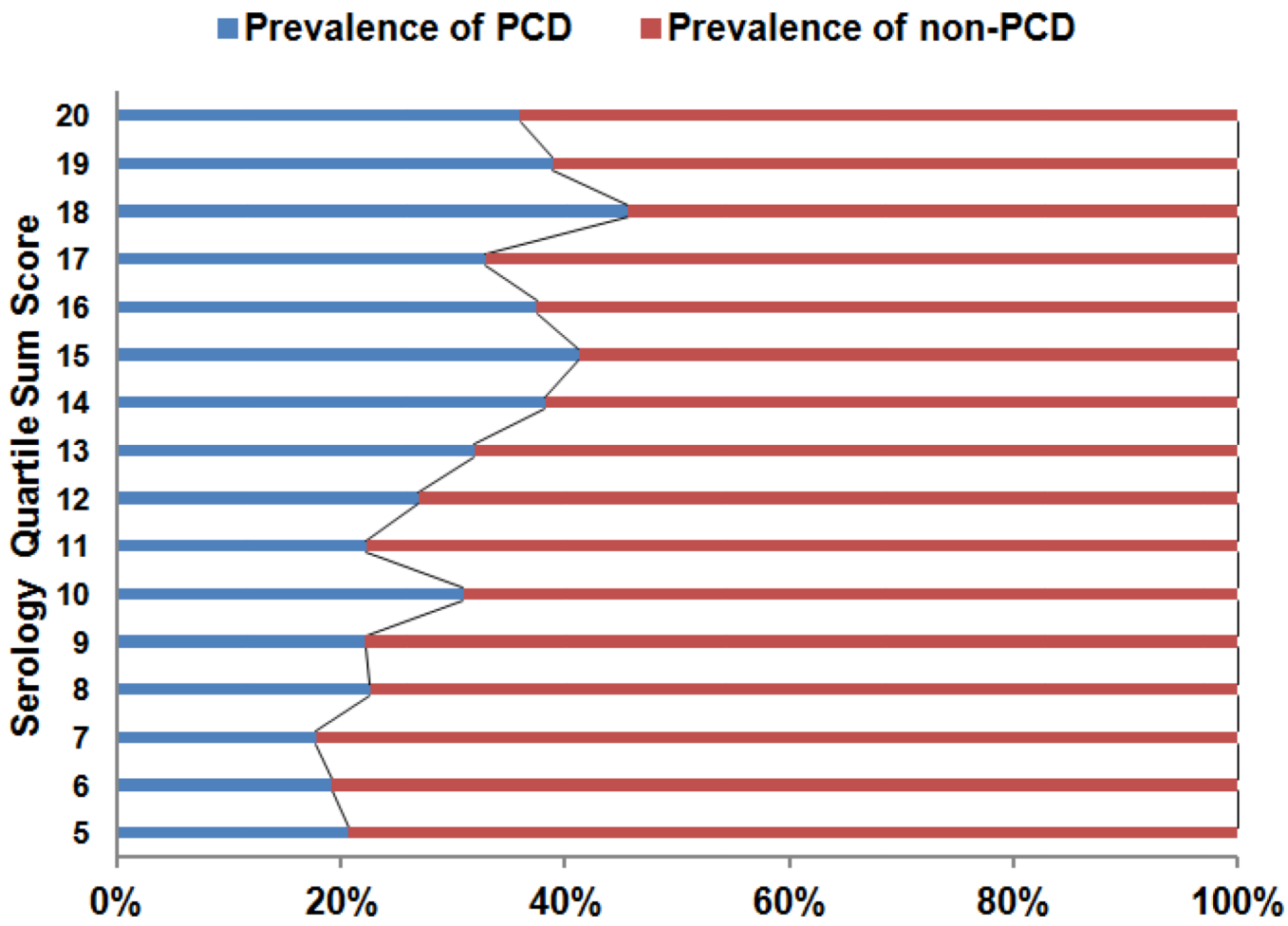

Figure 1.

Perianal CD prevalence rate in patients with different serology quartile sum scores*

pCD, perianal Crohn's disease;

* Quartile sum score calculated based on ASCA IgG, ASCA Ig A, I2, Omp C and CBir-1; P-value calculated using linear trend test.

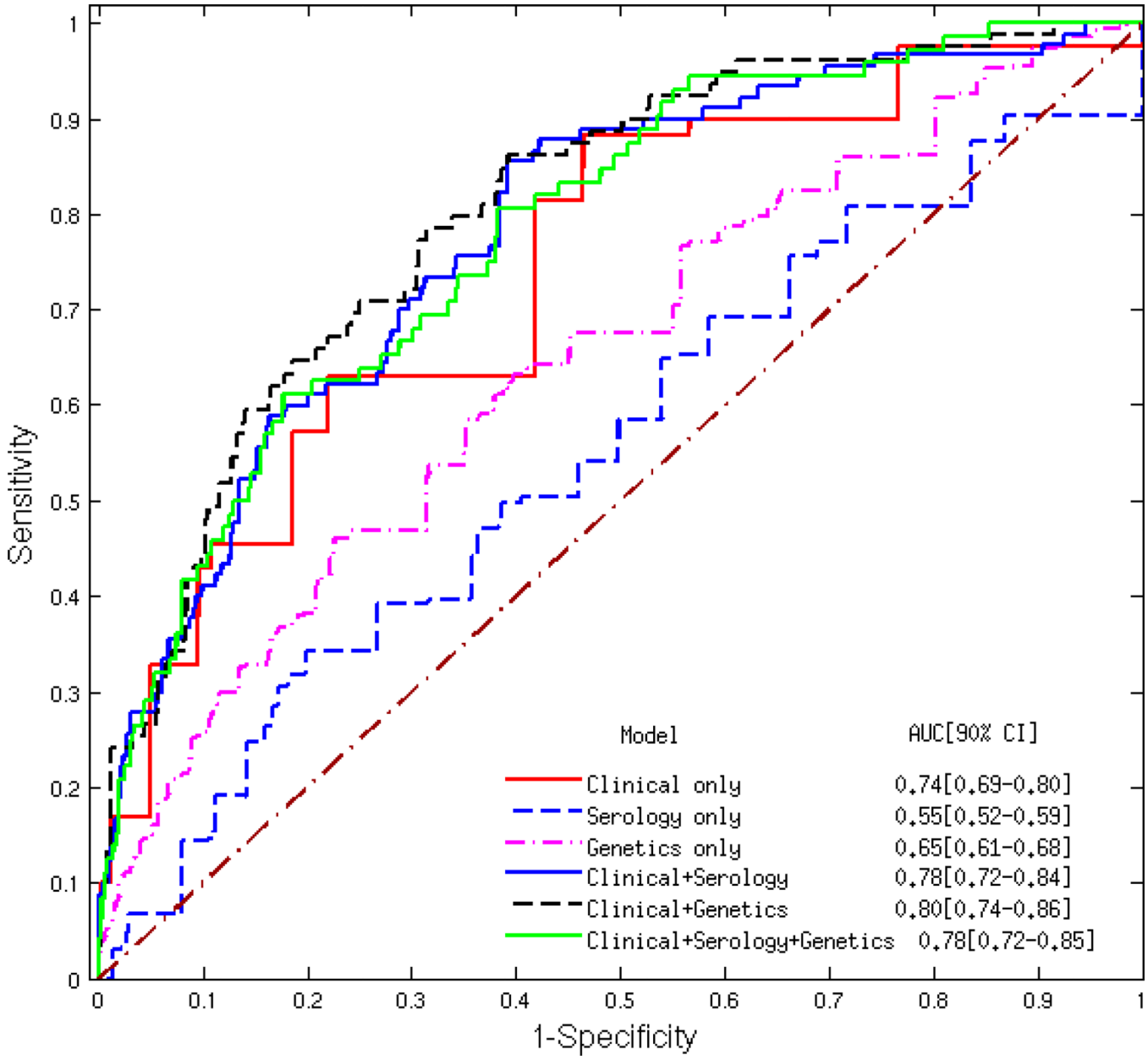

Figure 2.

Multivariable models for association with perianal CD in 1721 CD patients from the Cedars-Sinai Medical Center IBD research repository

Table 1

Baseline demographic and clinical characteristics of 1721 Crohn's disease patients from the Cedars-Sinai Medical Center IBD research repository

Clinical variable	N (%)
Sex	
Male	926 (53.8)
Female	795 (46.2)
Race	
Caucasian	1602 (93.1)
African American	33 (1.9)
Asian	40 (2.3)
Hispanic	32 (1.8)
Other	14 (0.8)
Ethnicity	
Jewish	650 (37.8)
Non-Jewish	1049 (60.9)
Unknown	22 (1.3)
Median disease duration in years (range)	10.2 (0.3–51.5)
Age at diagnosis	
A1 (≤16 years)	530 (30.8)
A2 (17–40 years)	962 (55.9)
A3 (> 40 years)	229 (13.3)
Disease location	
L1 (Ileal)	333 (20.2)
L2 (Colonic)	308 (18.7)
L3 (Ileocolonic)	1001 (60.7)
L4 (Upper GI)	319 (18.5)
Disease Behavior	
B1 (Nonstricturing- non penetrating)	778 (45.8)
B2 (Stricturing)	447 (26.4)
B3 (Penetrating)	170 (9.9)
B2 and B3	305 (17.9)
Perianal Crohn's disease	524 (30.4)
Family history of Crohn's disease	162 (9.4)
Number of abdominal surgeries for complicated CD	
0	869 (50.4)
1–2	690 (40.1)
≥3	162 (9.5)
Mean duration from CD diagnosis to onset of pCD in years (range)	3.5 (0.1–38.3)

CD, Crohn's disease; pCD, perianal crohn's disease

Table 2

Clinical characteristics of 524 perianal Crohn's disease patients relating to type of perianal involvement

Clinical Characteristics	N (%)
Median duration from CD diagnosis to onset of pCD in years (range)	3.3 (0.1–38.3)
Perianal abscess *	128 (24.4)
Perianal fistulae *	368 (70.6)
Recto-vaginal fistulae *	56 (10.7)
Number of procedures for pCD	
0	196 (37.6)
1–2	284 (54.5)
≥3	41 (7.8)

pCD, perianal Crohn's disease;

* Patients could have had more than one perianal clinical characteristic.

Table 3

Univariate associations between baseline clinical variables and presence of perianal Crohn's disease

Clinical Variable	Patients without pCD N=1197 (%)	Patients with pCD N=524 (%)	Odds Ratio [95% CI]	*p* value
Male	632 (52.8)	294 (56.1)	1.14 [0.08–1.58]	0.23
Non-Jewish ancestry	705 (58.9)	344 (66.0)	1.29 [1.04–1.62]	0.02
Mean age at diagnosis (Std. dev)	26 (14.8)	22.7 (12.7)	0.98 [0.97–0.99]	<0.001
Median disease duration in yrs	16.5	11.5	-	<0.001
Family history of IBD	116 (9.7)	46 (34.8)	4.98 [3.30–7.46]	<0.001
Disease location:				
L1, ileum	277 (24.3)	56 (11)	0.38 [0.28–0.52]	<0.001
L2, colon	196 (17.2)	112 (22)	1.35 [1.04–1.75]	0.03
L3, ileocolonic	661 (58.1)	340 (66.7)	1.44 [1.16–1.8]	0.001
L4, upper gastrointestinal	227 (20.3)	92 (19)	92 (0.70–1.20)	0.55
Colonic disease:				
Ascending colon	545 (56.2)	243 (56.8)	1.02 [0.81–1.29]	0.88
Transverse colon	379 (39.2)	2.2 (47.5)	1.4 [1.11–1.77]	0.01
Descending colon	385 (39.6)	227 (51.1)	1.58 [1.26–1.99]	<0.001
Sigmoid colon	453 (46.5)	276 (63.3)	1.99 [1.58–2.51]	<0.001
Rectum	360 (36.4)	325 (71.3)	4.32 [3.4–5.51]	<0.001
Disease behavior:				
B1, inflammatory	571 (48.5)	207 (39.5)	0.69 [0.56–0.85]	0.001
B2, stricturing	283 (24.0)	164 (31.3)	1.44 [1.14–1.81]	0.002
B3, penetrating	123 (10.5)	47 (8.97)	0.85 [0.59–1.2]	0.39
B2 and B3, stricturing and penetrating	200 (17.0)	105 (20.0)	1.22 [0.94–1.59]	0.31
Extra-intestinal manifestations:				
Ankylosing spondylitis	68 (6.7)	39 (8.0)	1.21 [0.80–1.81]	0.43
Skin manifestations (EN+PG)	48 (4.89)	46 (9.45)	2.03 [1.33–3.09]	0.001
Eye symptoms (iritis/uveitis)	51 (5.28)	23 (4.73)	0.89 [0.53–1.47	0.75
Primary sclerosing cholangitis	15 (1.43)	1 (0.21)	0.16 [0.01–0.81]	0.05
Deep vein thrombosis	25 (2.61)	17 (3.49)	1.35 [0.71–2.52]	0.44
Nephrolithiasis	67 (6.94)	46 (9.45)	1.40 [0.64–2.07]	0.115
Mean number of abdominal surgeries (Std. dev)	0.74 (1.04)	1.14 (1.36)	1.33 [1.22–1.45]	<0.001

IBD, inflammatory bowel disease; pCD, perianal crohns disease; CI, confidence interval; Std. dev, standard deviation; Yrs, years; EN, erythema nodosum; PG, pyoderma gangrenosum

Table 4

Pathway analyses from genetic associations with perianal CD from Ichip analyses

	Number of Genes	p-value	p-value (FDR)	p-value (Bonferroni)
JAK-STAT signaling pathway	9	1.76×10^{-07}	3.72×10^{-05}	3.72×10^{-05}
Leishmaniasis	5	0.001	0.13	0.27
Phagosome	6	0.003	0.18	0.64
Antigen processing and presentation	5	0.004	0.18	0.84
ABC transporters	3	0.004	0.18	0.89

CD, Crohn's disease; JAK-STAT, Janus Kinase-Signal Transducer and Activator of Transcription; ABC, ATP-binding cassette transporters; FDR, false discover rate