Perianal Crohn's Disease: Predictive Factors and Genotype-Phenotype Correlations

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Key Words

Perianal Crohn's disease • Single-nucleotide polymorphism • *OCTN1* • *CARD15* • *NOD2* • *IL-23r*

Abstract

Aim: To investigate genotype-phenotype correlations in patients with perianal Crohn's disease (PCD) in order to determine which factors predispose to development of perianal disease in Crohn's patients. **Methods:** Seven-hundred and ninety-five Caucasian individuals (317 CD patients and 478 controls without inflammatory bowel disease, IBD) were prospectively enrolled into a clinical/genetic database. Demographic and clinical data, as well as peripheral blood leukocyte DNA were obtained from all patients. The following were evaluated: three *NOD2/CARD15* polymorphisms: R702W, G908R, and 1007insC; five *IL-23r* risk alleles: rs1004819, rs10489629, rs2201841, rs11465804, and rs11209026; a well-characterized single-nucleotide polymorphism (SNP) on the *IBD5* risk haplotype (*OCTN1*) and two peripheral tag SNPs (*IGR2060* and *IGR3096*). **Results:** PCD occurred in 147 (46%) of CD patients. There was no significant difference in the age at disease diagnosis between non-PCD and PCD patients (33 vs. 29 years, respectively). PCD patients were more likely to have disease located in the colon and ileocolic regions (79 PCD vs. 57% non-PCD; $n = 116$ vs. $n = 96$; $p < 0.001$), whereas patients with non-PCD were more likely to have Crohn's within the

terminal ileum and upper gastrointestinal tract (43% non-PCD vs. 21% PCD; $n = 73$ vs. $n = 31$; $p < 0.05$). Thirty-four percent of patients with PCD required a permanent ileostomy ($n = 50$) compared to only 4% of non-PCD patients ($n = 6$; $p < 0.05$). Mutations in *CARD15/NOD2* and *IL-23r* were risk factors for CD overall; however, in contrast to prior reports, in this patient population, *OCTN1* and *IGR* variations within the *IBD5* haplotype were not significant predictors of PCD. **Conclusion:** Colon/ileocolic CD location appears to be a significant predictor of perianal manifestations of CD. Patients with PCD are more likely to require permanent fecal diversion. We did not identify any genetic variations or combination of clinical findings and genetic variations within the *CARD15/NOD2*, *IL-23r*, and *OCTN1* genes or *IGR* that were predictive of PCD.

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Introduction

Identification of Crohn's disease (CD) patients who would or would not develop perianal CD (PCD) could broaden surgical options in these patients. Knowing that a patient would not develop PCD could for example perhaps allow them to undergo sphincter-sparing surgery such as ileal pouch anal anastomosis. While there are many reports on the genetics and clinical progression of CD, few offer insight on predisposing factors to PCD, a

phenotype of the disease that is a source of special morbidity in these patients. Perianal disease has been reported in 13–43% of patients with CD [1, 2] and is one of the major factors preventing sphincter-sparing surgery in these patients. Perianal manifestations may include skin tags that pose hygiene problems, anal fissures and ulcers, anal canal strictures, perianal fistulae, and abscesses [3]. Treatment of PCD is complicated both by the manifestations themselves and their location; frequent bowel movements prevent proper healing and increase chances of local sepsis. Uncontrollable fecal or purulent drainage can lead to skin maceration and urinary tract infections, especially in women with rectovaginal fistulae. Patients with PCD endure not only the debilitating complications from their disease, which often fail medical therapy and require permanent fecal diversion, but also the strain it places on their social and sexual lives [4]. As iterated above, identification of CD patients likely to develop PCD would have several clinical advantages. Patients thought not to be at risk for developing PCD could be selected for sphincter-sparing surgery, while patients at higher risk for developing PCD could perhaps be placed on some type of chemoprophylaxis to prevent PCD and its associated tissue and sphincter destruction.

Single-nucleotide polymorphisms (SNPs) in the interleukin-23 receptor (*IL-23r*) gene and the caspase recruitment domain 15 (*CARD15/NOD2*) gene correlate with increased susceptibility of acquiring CD [5, 6]. Additional mutations within the organic cation/carnitine transporter (*OCTN*) gene and intergenic region (*IGR*) of the *IBD5* gene cluster have been found to predict CD [7, 8]. In addition, several reports have identified clinical factors that may predict a more aggressive course of CD including a family history of inflammatory bowel disease (IBD) and active cigarette smoking, a young age at onset, and a diagnosis of CD in the ileum [9–11].

Few reports have studied genotype-phenotype correlations that may specifically predispose CD patients to PCD. An association was previously identified between a genetic variation in the *IBD5* haplotype and CD; furthermore, *IBD5* variants were most strongly associated with perianal complications of CD [12, 13]. Two years later, Vermiere et al. [14] proposed that variants in the *OCTN1* gene, which is located within the *IBD5* gene cluster, are involved in the phenotypic expression of PCD, but not in CD susceptibility itself. Freire et al. [15] noted that *CARD15/NOD2* mutations did not predispose to perianal fistulization. Similarly, Karban et al. [16] did not find *CARD15/NOD2* or *OCTN* to be a risk factor for PCD in a cohort of non-Ashkenazi Jews with CD.

Table 1. The four studied genes (*NOD2*, *IL-23r*, *IGR*, and *OCTN1*) with the corresponding SNPs

Genetic marker	Variants
<i>NOD2</i>	
rs1004819	3020insC/1007fs
rs2066844	R702W
rs2066845	G908R
<i>IL-23r</i>	
rs1004819	
rs10489629	
rs2201841	
rs11465804	
rs11209026	R381Q
<i>IGR 2060</i>	
rs2522057	
<i>IGR 3096</i>	
rs7705189	
<i>OCTN1</i>	
rs1050152	SLC22A4

Based on the above, we examined genotype-phenotype correlations as associated with the occurrence of PCD with the hope of determining predictive criteria that could be used prospectively in clinical patient care.

Materials and Methods

Patients

This study was approved by the University of Louisville Institutional Review Board, and written informed consent was obtained from all subjects. Clinical and genetic data were prospectively entered into a password-protected Microsoft Access relational database containing data on over 3,000 medically and/or surgically treated digestive disease patients treated between 1997 and 2008 [5].

The diagnosis of CD was made via conventional endoscopic, radiologic, and histological findings. In cases of colonic CD, the diagnosis was verified by a single gastrointestinal pathologist with a special interest in IBD. For purposes of our study, PCD was defined as presence of at least one of the following: perianal fistulae and/or abscesses, anal canal fissures or ulcers, and anal canal stenosis. Controls were comparison patients seen in the same practice who presented for evaluation of noninflammatory, non-malignant conditions (internal hemorrhoids, constipation, etc.).

Pertinent clinical information for each patient was carefully reviewed, including cigarette smoking status using the Behavioral Risk Factor Surveillance Survey [17]; family history of IBD; family history of colorectal cancer; number of operations for CD; operations for PCD, including the need for permanent fecal diversion; and last date of follow-up. Patients for whom long-term fol-

Table 2. Patient characteristics of non-PCD, PCD and controls

Characteristics	Non-PCD patients ¹	PCD patients	Controls
Total patients	170 (21)	147 (19)	478 (60)
Mean age at study entry, years	51 (21–100)	48 (23–90)	56 (17–99)
Mean age at CD diagnosis, years	33 (9–90)	29 (6–82)	no disease
Sex			
Female	103 (61)	94 (64)	325 (68)
Male	67 (39)	53 (36)	153 (32)
Smoking status			
Current	50 (30)	34 (23)	92 (22)
Former	53 (31)	50 (34)	138 (34)
Never	67 (39)	63 (43)	182 (44)
Family history of IBD ²	77 (45)	64 (44)	42 (9)
Family history of cancer	55 (32)	31 (21)	161 (34)
Patients with permanent stoma	6 (4)	50 (34)	not applicable
Operations per patient ³			
0	30 (18)	13 (9)	not applicable
1–2	97 (57)	58 (39)	not applicable
≥3	43 (25)	76 (52)	not applicable

Figures in parentheses indicate percentages or range.

¹ Patients diagnosed with CD without perianal disease manifestations.

² Denotes family history in first- or second-degree relative.

³ Denotes all operations including intestinal resections as well as perianal procedures.

low-up was not achieved (defined as not having been seen for >1 year; n = 158) were excluded from this study. Each patient was classified according to the Vienna classification with respect to age at onset of disease (≤ 40 years, >40 years), location of disease [terminal ileum (L1), colon (L2), ileocolonic (L3), upper GI (L4)], and disease behavior [inflammatory (B1), stricturing (B2), penetrating (B3)] [18].

Genotyping

Genomic DNA was extracted from peripheral whole blood with a Puregene® DNA extraction kit (Gentra Systems, Inc., Minneapolis, Minn., USA). Polymerase chain reaction (PCR) amplification and genotyping were performed using an ABI prism 7300 sequence detection system (Applied Biosystems®, Foster City, Calif., USA). SNP-specific TaqMan® PCR primers and fluorogenic probes were obtained from Applied Biosystems. The fluorogenic minor groove binder TaqMan probes were labeled with a reporter dye specific for the wild-type and variant alleles of eleven IBD-associated SNPs.

Marker Selection

Eleven SNPs (table 1) chosen for their reported association with CD and/or PCD were genotyped for all CD and PCD patients as well as controls.

Statistical Analysis

Descriptive statistics related to genetic and clinical characteristics were produced for all patients. Categorical variables were compared using the Pearson χ^2 test (or Fisher's exact test) for contingency tables [19]. The t test or Wilcoxon rank sum test was used

to examine the cohort for continuous variables. We also fit the univariable and multivariable logistic regression models for the probabilities of patients in PCD or CD groups regarding their possible genetic and clinical predictors [20].

Hardy-Weinberg equilibrium and genotype and allelic association for 11 SNPs were assessed using the Pearson χ^2 test. For allelic association analysis, we also used logistic regression to estimate odds ratios and 95% confidence intervals for patients with PCD or non-PCD as compared to the control group, or for patients with PCD as compared to patients with non-PCD. Logistic regression was also used to determine the interaction effects with inclusion of the main effects of each gene in the model. Finally, haplotype analysis was conducted and *NOD-2* (3 SNPs), *IL-23r* (5 SNPs) and *IGR* (2 SNPs) were compared between PCD and non-PCD patients. All calculations were performed with SAS statistical software [21].

Results

Cohort Demographics

The study cohort consisted of a well-defined patient population originating from a small, largely rural geographic area in the Midwest United States (Kentucky and southern Indiana). For purposes of genetic homogeneity, only Caucasian patients were included in this analysis. The Caucasian cohort contains 795 individuals: 317 patients with CD (170 of whom have CD without perianal

disease [non-PCD] 39% male) and 147 with PCD (36% male), and 478 controls (32% male; table 2). The mean patient age was 51 years for non-PCD, 48 years for PCD, and 56 years for controls ($p > 0.05$). Forty-four percent ($n = 141$) of all CD patients in our cohort, both PCD and non-PCD, had a positive family history of IBD, defined as a first- or second-degree relative with IBD, as compared to only 9% of controls ($n = 42$).

Of the non-PCD patients, 30% were current smokers, 31% are former smokers, and 39% have never smoked ($n = 50, 53$, and 67 , respectively). Of the PCD patients, 23% were current smokers, 34% former smokers, and 43% have never smoked ($n = 34, 50$, and 63 , respectively). In our control group, 22% were current smokers, 34% formerly smoked, and the remaining 44% have never smoked ($n = 92, 138$, and 182 , respectively; $p > 0.05$).

Clinical Characteristics of Non-PCD and PCD Patients

There was no significant difference in the age at diagnosis between the PCD and the non-PCD group, 29 versus 33 years, respectively ($p > 0.05$). Only 25% of non-PCD patients underwent more than three operations for their disease at the time of last follow-up ($n = 43$), while 52% of the PCD group had had more than three operations for their disease at the time of last follow-up ($n = 76$; $p < 0.05$).

Patients with PCD were more likely to have disease in the colon and ileocolic regions (Vienna L2, L3; 116 PCD

vs. 96 non-PCD; 79 vs. 57%; $p < 0.001$; tables 3, 4), whereas patients with non-PCD were more likely to have inflammation in the terminal ileum and upper GI tract (Vienna L1, L4; 73 non-PCD vs. 31 PCD; 43 vs. 21%). Fifty patients with PCD required a permanent ileostomy (34%) as compared to only 6 non-PCD patients (4%).

On multivariate regression analysis, the number of operations and obviously the need for an ileostomy were

Table 3. Vienna classification for studied patients

Characteristics	Non-PCD patients ($n = 170$)	PCD patients ($n = 147$)
Age at CD onset		
A1 (<40 years)	119 (70)	121 (82)
A2 (>40 years)	51 (30)	26 (18)
CD location – Vienna		
L1 terminal ileum	58 (34)	23 (16)
L2 colon	49 (29)	46 (31)
L3 ileocolonic	47 (28)	70 (48)
L4 upper GI	16 (9)	8 (5)
Behavior – Vienna		
B1 inflammatory	38 (22)	0
B2 stricturing	75 (44)	0
B3 penetrating	57 (34)	147 (100)

Figures in parentheses indicate percentages.

Table 4. Multivariable logistic regression model for factors affecting PCD versus non-PCD

Predictor	PCD versus non-PCD		
	estimate ¹	p value	OR (95% CI)
Surgery (number)	0.20	0.005	1.22 (1.06–1.40)
Family history of colorectal cancer			
Yes	–0.84	0.015	0.43 (0.22–0.85)
No	0		
Vienna location			
L1 + L4 (upper GI and terminal ileum)	–0.67	0.036	0.51 (0.28–0.96)
L2 + L3 (colonic and ileocolonic)	0		
Vienna behavior			
B1 + B2 (inflammatory and stricturing)	–1.55	<0.0001	0.21 (0.12–0.39)
B3 (penetrating)	0		
Permanent ileostomy			
Yes	2.79	<0.0001	16.36 (4.50–59.50)
No	0		

¹ A positive estimate indicates more susceptibility to PCD. For example, the presence of a permanent ileostomy is predictive of PCD. A negative estimate indicates more susceptibility to non-PCD, i.e., a positive family history, L1 + L4 CD location and a B1 + B2 CD behavior are predictive of non-PCD.

Table 5. Allelic association test for SNPs

SNP	Control (n = 320)	Non-PCD (n = 170)	PCD (n = 147)	p value
<i>NOD2</i>				
NOD2 1007insC				
N	602 (98.4)	295 (92.2)	266 (94.3)	<0.001 ¹
C	10 (1.6)	25 (7.8)	16 (5.7)	<0.001 ² 0.299 ³
NOD2 R702W				
C	517 (97.2)	288 (88.9)	254 (90.1)	<0.001 ¹
T	15 (2.8)	36 (11.1)	28 (9.9)	<0.001 ² 0.637 ³
NOD2 G908R				
C	618 (99.0)	319 (95.5)	272 (96.5)	0.002 ¹
G	6 (1.0)	15 (4.5)	10 (3.5)	<0.001 ² 0.554 ³
<i>IL-23r</i>				
rs1004819				
G	381 (72.7)	203 (64.6)	164 (60.3)	<0.001 ¹
A	143 (27.3)	111 (35.4)	108 (39.7)	0.014 ² 0.277 ³
rs10489629				
T	335 (56.0)	180 (58.8)	177 (63.2)	0.129 ¹
C	263 (44.0)	126 (41.2)	103 (36.8)	0.420 ² 0.277 ³
rs2201841				
C	433 (71.7)	213 (66.1)	169 (61.7)	0.010 ¹
G	171 (28.3)	109 (33.9)	105 (38.3)	0.080 ² 0.257 ³
rs11465804				
T	586 (94.5)	329 (97.9)	285 (97.6)	0.011 ¹
G	34 (5.5)	7 (2.1)	7 (2.4)	0.013 ² 0.790 ³
rs11209026				
G	544 (96.5)	310 (97.5)	266 (98.5)	0.224 ¹
A	20 (3.5)	8 (2.5)	4 (1.5)	0.402 ² 0.377 ³
<i>IGR</i>				
rs2522057-2060				
C	300 (55.6)	137 (49.3)	118 (49.2)	0.120 ¹
G	240 (44.4)	141 (50.7)	122 (50.8)	0.088 ² 0.979 ³
rs7705189-3096				
A	319 (52.0)	159 (48.8)	130 (48.1)	0.478 ¹
G	295 (48.0)	167 (51.2)	140 (51.9)	0.353 ² 0.879 ³
<i>OCTN1</i>				
rs1050152				
C	364 (56.9)	166 (51.2)	132 (48.9)	0.052 ¹
T	276 (43.1)	158 (48.8)	138 (51.1)	0.096 ² 0.569 ³

Figures in parentheses indicate percentages. Under each studied SNP in the table, the top row correlates with the major allele distribution while the bottom row correlates with the minor allele distribution in the studied group.

¹ Global p value among all groups. ² Control vs. non-PCD. ³ Non-PCD vs. PCD.

both predictive of PCD. In contrast, CD located in the ileum/upper gastrointestinal tract (L1 + L4) or presence of inflammatory or stricturing disease behavior (B1 + B2) was more predictive of non-PCD (table 4).

Genetic Analysis of Total CD Cohort, PCD and Non-PCD Patients

For the genetic analyses, we considered only data from patients who had successful genotyping of at least 7 of the 11 SNPs. Overall, we analyzed data for 637 patients (170 CD, 147 PCD, and 320 controls). The control group was in Hardy-Weinberg equilibrium for all 11 SNPs. Univariate analysis of SNPs revealed the minor allele of each *NOD2* SNP (1007insC, R702W, G908R) and two *IL-23r* SNPs (rs1004819, rs11465804) to be significantly associated with CD ($p = 0.001$). Additionally, two *IL-23R* SNPs (rs10489629, rs2201841) were found to be associated with PCD patients, but not the CD-only patients (table 5).

Minor allele expression in all SNPs was not significantly different between CD and PCD patients. Moreover, haplotype analysis of *NOD2* (3 SNPs), *IL-23r* (5 SNPs) and *IGR* (2 SNPs) genes revealed no significant difference in haplotypes between CD and PCD patients beyond two rare haplotypes of all five *IL-23r* loci.

Discussion

A disease of unknown etiology, Crohn's disease most likely describes several discrete diseases of the GI tract where inflammation arises due to currently unknown environmental antigens in the setting of a genetically susceptible host [22]. Tobacco use is one confirmed environmental factor that increases the severity of CD [22, 23]. The risk for CD is 13-fold higher in first-degree relatives of individuals affected with CD [24], and CD concordance rates are 50–60% in monozygotic twins [25, 26]. Taken together, this suggests that CD progression is most likely coordinated by a combination of genetic and environmental factors.

Since CD is a complex disorder with several distinct phenotypes, it is thought that these different phenotypes might have unique risk factors and genetic identities. We wished to carefully survey clinical factors and genotype-phenotype correlations that might predispose a patient to PCD. Such an association could have important implications for patient treatment in that identification of patients not destined to develop PCD could permit patients to be considered for sphincter-sparing surgery. Identification of patients with Crohn's colitis not likely to devel-

op PCD could permit some of these patients to undergo ileal pouch anal anastomosis. Many patients would choose this option over ileostomy even at the risk of subsequent inflammatory disease. There have been several reports of satisfactory control of inflammatory pouch-related symptomatology with agents such as infliximab [27, 28]. PCD complications such as fistulae associated with stenosis are much more difficult to treat in this manner.

Gender

Studies contrast regarding gender as a risk factor for PCD. In one report of 221 CD patients with anal fistulae, there were 125 men (33%) and 96 women (22%), suggesting that men were more prone to PCD [29]. Conversely, our study and that of Schwartz et al. [30] found no association between gender and PCD. Differences in the overall gender distribution of the population samples studied and varying definitions for PCD may contribute to these conflicting reports on gender as a risk factor for PCD.

Family History

Family history of IBD is a strong predictor of CD behavior [9, 31], but Dorn et al. [32] and Cosnes et al. [23] found no evidence that family history of IBD can be used to predict PCD. In our cohort, 77 non-PCD patients (45%) had a family history of IBD (defined as having a first- or second-degree relative with IBD) as compared to 42 controls (9%). This did not differ between non-PCD and PCD patients [77 patients (45%) and 64 patients (44%), respectively]. We do not have data regarding the incidence of PCD in family members with IBD. It is possible that family history of PCD could be a better predictor of PCD than family history of IBD alone.

Age at Diagnosis

Several reports indicate that patients who are diagnosed with CD at an early age are more predisposed to aggressive disease, including PCD [4, 33]. Schwartz et al. [30] reported that 50% of CD patients who had CD for over 20 years developed fistulae, 26% of which were perianal, emphasizing the progressive nature of CD. Our data showed no difference in age at diagnosis between PCD and non-PCD patients (29 vs. 33 years, respectively, $p > 0.05$).

Vienna Behavior and Location

Multiple reports indicate that CD patients with colitis, ileocolitis, or proctitis are at a higher risk of developing perianal complications [4, 16]. Not surprisingly, our data

support this finding; according to the Vienna classification system [18], CD was located in the colon and ileocolon in 79% of PCD patients (n = 116), as compared to 57% of patients without PCD (n = 96). Colon/ileocolic CD location is a predictor of PCD (p < 0.001). Patients with colonic CD seem to be at higher risk of developing PCD.

Medical Therapy

Information regarding use of biologics was available in 44 CD patients (14%). Infliximab received approval for CD in the United States in 1998, adalimumab in 2007, and certolizumab in 2008. Infliximab was used in approximately 13.5% of CD cases where information regarding treatment was available (n = 23) and 14.3% of PCD cases (n = 21). Anti-TNF therapy, a chemoprophylaxis agent, is often used for long-term symptom control in PCD patients unresponsive to antibiotics and antimegakalins [34, 35]. Prospective randomized controlled trials have shown that infliximab administered as a triple-dose induction regimen at 0, 2, and 6 weeks is associated with at least a 50% decrease in fistula drainage [3, 36]. A previous report from our unit has, however, highlighted the failure even of biologic therapy in the presence of anal canal stenosis and extensive colonic disease [2]. Based on our findings, clinical predictors could be used to identify patients at higher risk for developing PCD. For example, patients with ileocolic or colonic disease would routinely be placed on 5-ASA or other medications as chemoprophylaxis to hopefully deter the development of PCD.

SNPs and SNP Haplotypes

Our study confirms previous reports implicating *CARD15/NOD2* and *IL-23r* SNPs as risk factors for CD [5, 6]; however, we found no relationship between minor alleles in the *CARD15/NOD2* and *IL-23r* genes and the incidence of PCD, coinciding with previous work by Freire et al. [15] and Karban et al. [16].

Also, in agreement with others [37], we did not identify *OCTN1* and *IGR* variation within the *IBD5* haplotype to be predictors of PCD. This is in contrast to the group of Armuzzi and Vermeire et al. [14] who did find *OCTN1* and *IGR* to be predictors of PCD.

In viewing our data, the nature of our patient population should be considered. Unlike many other cohorts, this is largely a group of patients that has failed medical therapy and is referred for surgical management, thus selection bias may play a role. All PCD patients had failed medical therapy and slightly over half had had ≥ 3 operations, while 34% required a permanent ileostomy (n = 50). In addition, there is a profound environmental effect,

as the population is derived from one of the areas in the United States with the highest rates of adult cigarette smoking [38]. Fifty-seven percent of PCD patients (n = 84) and 61% of non-PCD patients (n = 103) were former or current smokers. Our rural geographic area is one in which there is an unusually high rate of IBD. Our results may not be translatable to other populations of Crohn's patients.

Conclusion

Colon/ileocolic CD location is a significant predictor of PCD. We found no genetic variations in the *CARD15/NOD2*, *IL-23r*, and *OCTN1* genes or *IGR* that could help further identify patients at risk for PCD. More genotype-phenotype studies with large and well-characterized patient populations are needed to further explore clinical and genetic factors that predispose CD patients to PCD.

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Disclosure Statement

None of the authors has any financial or other conflicts of interest to disclose.

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