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Genome Wide Association (GWA) Predictors Of Anti-TNF α Therapeutic Responsiveness In Pediatric Inflammatory Bowel Disease (IBD)

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Abstract

Background—Inter-individual variation in response to anti-TNF α therapy may be explained by genetic variability in disease pathogenesis or mechanism of action. Recent genome wide association studies (GWAS) in IBD have increased our understanding of the genetic susceptibility to IBD.

Aim—Test associations of known IBD susceptibility loci and novel “pharmacogenetic” GWAS identified loci with primary non-response to anti-TNF α in pediatric IBD patients and develop a predictive model of primary non-response.

Methods—Primary non response was defined using the HBI for CD and partial Mayo score for UC. Genotyping was performed using the Illumina Infinium platform. Chi square analysis tested associations of phenotype and genotype with primary non-response. Genetic associations were identified by testing known IBD susceptibility loci and by performing a GWAS for primary non-response. Step-wise multiple logistic regression was performed to build predictive models.

Results—Non-response occurred in 22 of 94 subjects. Six known susceptibility loci were associated with primary non-response ($p < 0.05$). Only the *21q22.2/BRWD1* loci remained significant in the predictive model. The most predictive model included 3 novel “pharmacogenetic” GWAS loci, the previously identified BRWD1, pANCA and a UC diagnosis ($R^2 = 0.82$ and $AUC = 0.98\%$). The relative risk of non-response increased 15 fold when number of risk factors increased from 0–2 to ≥ 3 .

Conclusion—The combination of phenotype and genotype is most predictive of primary non response to anti-TNF α in pediatric IBD. Defining predictors of response to anti-TNF α may allow the identification of patients who will not benefit from this class of therapy.

Introduction

Natural history observations in both early and later onset inflammatory bowel disease (IBD) have prompted the increasing use of anti-TNF α therapy for IBD patients (1,2). Both the REACH (3) pediatric study and the ACCENT 1 (4) study demonstrate that infliximab is

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effective for the induction and maintenance response and remission in a subset of CD patients. However, the clinical trial data for all anti-TNF α therapies among adult CD patients report that 40% of patients do not respond to the induction phase (primary non-responder) and that approximately 40% of those patients who do enter the maintenance phase of the trial lose response over time (4,5,6). The pediatric REACH trial reported that close to 90% of children responded to induction, suggesting a more robust acute response to anti-TNF α therapy in children as compared to adults with CD (1,2). This primary response outcome did not, however, require children to have weaned corticosteroids to meet response criteria. This would be a more clinically robust outcome definition given that the importance of steroid sparing in the induction and maintenance phase of these therapies. Moreover, approximately 40% of children, like their adult counterparts, who entered the maintenance phase lost response and were no longer in remission and off steroids at 12 months. Primary non-response appears to be less of an issue in children than adults based on the REACH trial, but more studies are needed to assess the true incidence of primary non-response in children in a non clinical trial setting. The adult UC trials (ACT 1 and ACT 2) reported similar response rates among adult UC patients receiving infliximab as the CD trials (7). Infliximab is being used off label in children with UC and the official clinical trial for indication is currently underway. These data do suggest that children respond better to induction therapy as compared to adult CD patients, but there are many differences in the patient population and outcome measures making a comparison across trials difficult and hard to interpret. One variable that may be an important predictor of response is duration of disease at initiation of therapy, which has been suggested based on post hoc analysis in both the certolizumab and adalimumab trials conducted in the adult CD population (5,6).

However inter-individual variability in therapeutic response may be better explained by genetic variability as it relates to disease pathogenesis and mechanism of action of this class of therapies. To date, limited candidate gene association studies with anti-TNF α have been reported (8–11). Other than NOD2 (12) and IBD5 (13), IBD susceptibility genes identified via genome wide linkage approach or Genome Wide Association Studies (GWAS) (14–18) have not been evaluated as predictors of response to anti-TNF α therapies. It would be logical as an initial step to test the known IBD susceptibility genes, under the hypothesis that anti-TNF therapy interrupts the pathophysiologic pathway these genes represent. Thus far, NOD2 was not found to be associated with therapeutic response to infliximab in these limited studies. It is conceivable that disease susceptibility genes do not influence the ultimate response to therapeutic targets given the multifactorial influences on disease and the relatively unknown functionality of these susceptibility genes. However, the GWAS approach, which identifies portions of the genome that contain genetic variants associated with specific phenotypes, may identify novel variants that contribute to therapeutic outcome i.e. discovery of genetic loci that are potentially responsible for the mechanism of altered drug response, in this case to anti-TNF α . There may also be important non genetic factors that influence or modify primary response to anti-TNF α . Among the serologic immune responses, pANCA (11,19) has been shown to be negatively associated with primary response in both CD and UC patients. PANCA is present in both CD and UC and defines a specific colitis phenotype suggesting a degree of overlap in the underlying pathway biology of these two disease subtypes (20). The results of recent genetic studies have also suggested this overlap (21). Other than duration of disease at initiation of therapy, there are likely important clinical and demographic variables that also influence therapeutic outcomes. To date, however, it remains unknown whether these are independent of genetic variability. Thus, the aims of this study were to test associations of known IBD susceptibility loci as well as novel loci identified by our pharmacogenetic GWAS with anti-TNF α response in pediatric IBD patients, and to develop a predictive model of anti-TNF α primary non-response using clinical phenotype, serologic and genetic variables.

Methods

Patient Population

A total of 94 pediatric CD and UC patients (age at diagnosis < 21 years) followed at Cedars-Sinai Medical Center (CSMC) by one clinician (MD) were enrolled in this study. All subjects must have received at least 2 doses of (weeks 0 and 2) of infliximab to be eligible. Infliximab was chosen as the first line anti-TNF α used in both CD and UC for children. This study was approved by the IRB.

Phenotyping

All data was collected by chart review and stored in a secured database. For the purpose of this study phenotype was defined as all variables that were not genetic.

Clinical Phenotype—These included demographic and clinical variables: age, gender, IBD subtype (CD vs. UC), disease duration, age at diagnosis, age at initiation of infliximab, immunomodulator history, steroid history, Harvey Bradshaw Index (HBI) activity scores, Partial Mayo scores and reason for infliximab discontinuation.

Immune Phenotype—Serum was collected on all patients and analyzed at CSMC. Serum immune responses: 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 analyzed blinded to therapeutic responsiveness by ELISA as previously described (22).

Genotype

Genotyping was performed at Children's Hospital of Philadelphia (CHOP) using the Illumina Human550 platform (n=70) and the Medical Genetics Institute at CSMC using the Illumina Human610 platform for CD samples (n=17) and HumanCNV370 platform for UC samples (n=11) (23). First, genotype data were tested for association between previously reported IBD susceptibility SNPs and anti-TNF α response. Table 1 illustrates the 28 SNPs included in this part of the analysis and references the GWAS that first reported these associations with disease (14–18). Twenty-one (21) SNPs from the Barrett CD meta-analysis GWAS (14), 5 SNPs from the various UC GWAS (15–17) and 2 SNPs from the Kugathasan pediatric IBD GWAS (18) were analyzed. Second, the genome wide data were tested for association with anti-TNF α response (“pharmacogenetic” GWAS, see below).

Outcomes and Definitions

The primary outcome of this study was to identify genetic loci associated with primary non-response defined for the purpose of this study as:

CD: Failure to decrease HBI ≥ 3 points (24) or increase from baseline at week 10 or 4 weeks after their last infusion if they did not receive the 3rd induction dose

UC: Failure to decrease ≥ 2 points or increase from baseline in the sum of stool frequency and rectal bleeding subscores of the partial Mayo score (7) and no improvement in the physician's global assessment subscore at week 10 or 4 weeks after their last infusion if they did not receive the 3rd induction dose

Statistical Analysis

Univariate Analysis

Association between clinical and demographic variables and primary non-response:

The Chi-square test was used to check the association of primary non-response with the following categorical variables: CD vs. UC, male vs. female, serum immune response positivity, percentage of immunomodulator use at the start of infliximab and primary non-response. The Student t test was used for associations of continuous variables; age of diagnosis, disease duration and duration of immunomodulator use at the start of infliximab.

Association between known IBD susceptibility loci and primary non-response: The Chi-square test was applied to test the association between each SNP (Table 1) and primary non-response. A dominant model based on the presence of the rare allele was assumed. Relative risk was calculated by comparing the risk of non-response in the patients with a specific genotype versus those without the genotype.

Pharmacogenetic genome wide association study (GWAS)

- a. Principal components (PC) analysis (using Eigenstrat) was conducted to examine population stratification (25). All the subjects formed one cluster with no significant outliers. We did not need to correct for population stratification during the association analysis, as the first ten PC evaluated were not significantly associated with primary non-response.
- b. For the purpose of quality control, SNPs with a minor allele frequency (MAF) <0.01, genotype failure rate >0.10, HWE P value <0.001 were excluded from the analysis. Allelic association between an individual SNP and primary non-response was carried out by chi-square test in PLINK (26). The first 10 SNPs with the most significant results were then retained for modeling. Following quality control, 301,742 SNPs were available in all data sets for analysis.

Multivariate Analysis

Predictive models of primary non-response: Models to predict non-response were built using step-wise multiple logistic regression, combining a) IBD susceptibility SNPs, b) the top 10 hits from the pharmacogenetic GWAS analysis, c) serology status and d) IBD subtypes. Since rs5975493 and rs7059861 are in high linkage disequilibrium, only rs7059861 was kept in the model. Exact logistic regression was used if the estimate from a regular regression model was not available. The significance level for a variable to enter and stay in the model was 10%. The likelihood based pseudo-R squared from the logistic regression model was used to measure the strength of association as well as the proportion of variance of the outcome accounted by the model's independent variables (27). Models were built at five different levels:

- I. demographic variables, serology status and IBD subtype only
- II. known IBD susceptibility SNPs only
- III. model I and II combined
- IV. pharmacogenetic GWAS SNPs (dominant model assumed) only
- V. model III and IV combined (final model). (known IBD susceptibility SNPs, pharmacogenetic GWAS top hits, serologic status and clinical variables)

Clinical Utility Measures: The area under the Receiver Operating Characteristic (ROC) curve (AUC) was used as a measure of the predictive performance of the final model. The

risk score was calculated based on the final model by assigning each risk phenotype or genotype as 1 point. Sensitivity [$\# \text{ true positives} / (\# \text{ of true positives} + \# \text{ of false negatives})$], specificity [$\# \text{ of true negatives} / (\# \text{ of true negatives} + \# \text{ of false positives})$], accuracy [$(\text{sensitivity} + \text{specificity}) / 2$] and positive likelihood ratio test ($\text{sensitivity} / (1 - \text{specificity})$) for primary non response were also calculated for the final model (28). All statistical analysis was conducted by SAS software v9.1 (SAS Institute; Cary, NC.).

Results

Patient population and phenotype associations

Of the 94 patients evaluated, 22 patients (23 %) met the criteria of primary non-response. Table 2 illustrates the key demographic data for both responders and non responders. A diagnosis of UC ($p < 0.0001$) and pANCA positivity ($p = 0.0001$) were associated with primary non-response. Gender, mean age at diagnosis, disease duration at initiation of infliximab, percentage of immunomodulator use and duration of use at start of infliximab did not differ between the two groups.

Genetic Associations: Univariate Analysis

Known IBD Susceptibility Loci—Of the 28 previously identified genetic loci (Table 1), 6 were found to be significantly associated with primary non-response. Figure 1 illustrates the frequency of primary non-response for the different genotypes of these 6 SNPs. Four of the 6 SNPs are from the CD meta-analysis (14), 1 from the UC GWAS (15–17) and 1 from pediatric IBD GWAS (18). For this analysis, the dominant model of the rare allele was assumed. The common allelic variant was associated with non-response in 4 of the 6 SNPs. Table 3 compares the reported IBD risk allele with the allele found to be associated with non-response in this study for all 6 SNPs. In only 2 of the SNPs was the disease risk allele the same as that found to be associated with non response to anti-TNF α .

Pharmacogenetic GWAS—Table 4 lists the results of the chi square analyses for the pharmacogenetic GWAS. Only those SNPs with a p value $< 10^{-4}$ are listed.

Multivariate Analysis

Predictive models of non-response—Logistic multiple regression was employed to develop models of primary non-response. Five different models were developed. Model 1 examined the significance of pANCA and IBD subtype (UC vs. CD). Both pANCA (OR 5.4; $p = 0.01$) and the diagnosis of UC (OR 15.0; $p = 0.0001$) remained significant in model I, with an R^2 of 0.48. Model II ($R^2 = 0.30$) examined the 6 SNPs from the univariate analysis. Four (4) of the 6 known IBD susceptibility SNPs remained significant; rs2188962 (5q31) (OR 3.3; $p = 0.04$), rs6908425 (6p22/CDKAL1) (OR 3.2; $p = 0.04$), rs2836878 (21q22/BRWD1) (OR 3.3; $p = 0.05$) and rs2395185 (6p21/HLA-DAQ1) (OR 4.6; $p = 0.01$). Table 5 shows the results of Model III which included serology, IBD subtype and known susceptibility SNPs (combining the variables of models I and II). Only 3 SNPs survived the model when combined with these other independent variables. Model IV analyzed the top 10 SNPs from our pharmacogenetic GWAS and only 4 SNPs remained significant; rs975664 (TACR1) (OR 17.6, $p = 0.0006$), rs4855535 (FAM19A4) (OR 8.8, $p = 0.006$), rs4796606 (KRT32 KRT35 KRT36 KRT13) (OR 13.4, $p = 0.01$) and rs765132 (OR 30.1, $p = 0.03$). The R^2 for this particular model was 0.67. The results of our final model (V) are shown in Table 6. Model V ($R^2 = 0.82$, including all variables) examined the associations of pANCA, diagnosis of UC, the 6 known susceptibility SNPs and the top 10 SNPs from the pharmacogenetic GWAS. UC, pANCA, 3 SNPs from the pharmacogenetic GWAS, and rs2836878 (21q22/BRWD1), a known susceptibility SNP originally reported in the pediatric IBD GWAS (18), remained significant.

Clinical Utility Measures

The potential clinical utility of the final model (V) was calculated. Table 7 lists the AUC, sensitivity, specificity, accuracy and positive likelihood ratio of non response in a patient who had at least 3 of the 6 risk factors of non response based on model V. In addition, the negative likelihood ratio, i.e. the likelihood that a patient will not be a non-responder if at least 3 risk factors are absent, was calculated at 0.06.

The relative risk of non-response was calculated based on the number of risk factors (model V) carried by an individual patient. Both the frequency of non-response and the relative risk increase with increasing number of risk factors ($p < 0.0001$) (Figure 2).

Discussion

Anti-TNF α is an important and effective class of therapies for the management of both adult and pediatric IBD patients. Clinical experience suggests inter-individual variation in efficacy, both induction and maintenance, and in the occurrence of side effects. There are likely multiple host factors that influence these variations such as disease and immune phenotype as well as genetic background. The ability to predict which patient would have a lower likelihood of response before treatment is initiated in order to minimize exposure to potentially ineffective therapies may be an important consideration in our IBD patients. In the current era of risk; benefit balance; this concept may be very timely.

In this study we tested the associations of known and novel genetic loci with primary response outcome and developed a predictive model of primary non response using clinical phenotype, serologic and genetic variables. Six of the 28 known susceptibility loci tested were found to be associated with primary non response in the univariate analysis. The relative risk of primary non response ranged from 2.1–2.9. When tested in the multivariate analysis, only 4 of these loci remained significant. However when combined with the novel pharmacogenetic GWAS loci, only 1 loci remained significant. This one locus was initially reported in the pediatric IBD GWAS reported by Kugathasan et al (18). The functional significance of this locus remains unknown. Of the 10 novel pharmacogenetic GWAS loci tested in our predictive model, 3 remained significant in the final model. TACR1 is a receptor for substance P a known pro-inflammatory molecule. PHACTR3 (phosphatase and actin regulator 3) is associated with the nuclear scaffold in proliferating cells. While there is little known about FAM19A4 it is thought to be structurally related to MIP1 α and function as a chemokine. The pharmacogenetic GWAS identified top loci did substantially improve the strength of the prediction of non-response compared to known susceptibility loci. In addition, a diagnosis of UC and pANCA positivity was independently associated with primary non-response. Table 8 compares the r-squared and AUC values for all 5 models. The combination of genotype, phenotype and serotype was the best predictive model of non response to anti-TNF α with an r-squared of 0.82 and an AUC of 0.98, and substantially better than the models that included only known IBD SNPs. (models II or III).

Our findings suggest that the majority of the known IBD susceptibility loci do not appear to greatly modify or influence primary response outcomes to anti-TNF α in pediatric IBD patients. This raises the possibility that the majority of genes that are associated with risk of disease may not influence the immune pathways that should be targeted to control or modify disease activity. The results of previously reported candidate gene association studies with anti-TNF α response have not been translated into the clinic and the functional significance of the genes tested remain unknown (8–13). The functionality of the cytokines and/or receptor renders them of interest as it relates to therapeutic outcome. With the GWAS approach, however, no a priori assumptions need to be set, as there is not a prior focus on a particular protein or target or enzyme as it relates to drug response. This hypothesis

generating approach allows us to identify genetic variants that are associated with response and non-response and thus potentially identify pathways that are responsible and may well be apparent from a functional and mechanistic perspective. In this study we have analyzed the associations with primary non-response only, as we believed this was of the greatest clinical relevance. In future studies, the genetic, immunogenic and phenotypic influences on loss of response also need to be explored in order to determine those patients who may quickly lose their initial response.

Despite our significant findings, this study was limited by its relatively small sample size and lack of replication cohort, hence it is essentially a pilot study. It should be noted that previous important genetic discoveries were made using similarly small discovery cohorts for GWAS including the original publication of TNFSF15 (n=94) (29) as well as the genetic associations with statin induced myopathy (85 cases, 90 controls) (30) and with Kawasaki disease (119 cases, 135 controls) (31). In this study we included all pediatric IBD subjects receiving anti-TNF α therapy. Currently clinicians treat both CD and UC with anti-TNF therapy and the clinical trial data suggest similar primary non response and steroid free remission outcomes for both disease subtypes. Moreover there is genetic and serologic evidence that there is pathway biology overlap within the spectrum of CD and UC phenotype (20,21). In this study we did analyze UC, CD and shared susceptibility loci and the pharmacogenetic GWAS would identify loci that are independent of disease phenotype.

Defining predictors of response to anti-TNF α will assist clinicians in choosing the appropriate therapy for the appropriate IBD patient, with the goal of maximizing efficacy and minimizing toxicity. As research progresses in defining the characteristics of patients who require biologics, of equal importance will be the research as proposed herein to individualize therapy based on who will or will not respond to different classes of IBD therapeutic interventions. The development of adverse events to anti-TNF therapies such as lymphoma, and sepsis naturally induce caution in clinicians who would like the ability to appropriately select patients who are most likely to respond to these therapies.. It is important that the findings from this study be replicated in an independent cohort and also tested in adult IBD patients. We are conducting a large prospective study with predefined outcomes in an independent pediatric cohort, which, in addition to primary non-response, will also examine loss of response and sustained response as an outcome. If replicated, the novel pharmacogenetic information gained from this pilot study has the potential to improve the management of patients in the clinic with more targeted use of the existing anti-TNF α agents. Such approaches may change the way we conduct large scale clinical trials, such that only patients with a higher probability of response to specific therapies will be enrolled in an attempt to minimize exposure to ineffective therapies and thus protect patients from treatment related serious and potentially fatal adverse events. The ultimate goal of this approach is the right therapy for the right patient.

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Figure 1.

Genotype associations of known IBD susceptibility loci with primary non-response. The dominant rare allele model (i.e. presence of the rare allele) was assumed for the statistical analyses). The relative risk (RR) and frequency of non-response are shown for the genotypes for each locus that met p value significance in univariate analysis.

Figure 2.

Relative Risk (RR) of non-response based on number of risk factors derived from Model V (the most general model). Risk of non response was compared between patients with 2 or less risk factors as compared to 3–4 markers and then as compared to at least 5 of the 6 risk factors for non-response.

Table 1

Known Genetic Susceptibility Loci

Chromosome/Loci of interest	GWAS Reference 1 = Adult CD (14) 2 = Adult UC (15–17) 3 = Pediatric IBD (18)	Chromosome/Loci of interest	GWAS Reference 1 = Adult CD (14) 2 = Adult UC (15–17) 3 = Pediatric IBD (18)
1p13 / <i>PTPN22</i>	1	6q27 / <i>CCR6</i>	1
1p36	2	8q24.13 / <i>TRIB1</i>	1
1q23 / <i>ITLN1</i>	1	9p24 / <i>JAK2</i>	1
1q24	1	9q32 / <i>TNFSF15</i>	1
1q21.2 / <i>ECM1</i>	2	10q21 / <i>ZNF365</i>	1
1q32.1 / <i>IL10</i>	2	10q24 / <i>NKX2-3</i>	1
1p31 / <i>IL23R</i>	1	12q15	2
2q37 / <i>ATG16L1</i>	1	13q14 / <i>C13orf31</i>	1
3p21 / <i>MST1</i>	1	16q12 / <i>NOD2</i>	1
5p13 / <i>PTGER4</i>	1	17q12 / <i>CCL2</i>	1
5q31 / <i>IBD5</i>	1	17q21 / <i>ORMDL3/STAT3</i>	1
5q33 / <i>IL12b</i>	1	20q13 / <i>TNFRSR6B</i>	3
6p21 / <i>HLA-DQA1/TNFα, TNFβ</i>	2	21q22 / <i>COSLG</i>	1
6p22 / <i>CDKAL1</i>	1	21q22.2 / <i>BRWD1</i>	3

Table 2Phenotype Associations with Therapeutic Outcomes to Anti-TNF α

CLINICAL VARIABLE	NON-RESPONSE	RESPONSE	P Value
UC vs. CD	14:8	6:66	<0.0001
Mean age at diagnosis (years)	10.1	10.5	0.71
Gender M:F	8:14	40:32	0.12
Mean Disease duration at start of IFX (months)	26	25	0.96
IMM use at start of IFX (%)	79%	92.8%	0.1
Mean Duration of IMM at start of IFX (months)	12.7	15.3	0.57
pANCA +	76.2%	29%	0.0001
ASCA+	0%	46.9%	0.0003

Table 3

Allelic Variants

SNP and Gene/Locus	GWAS Reference 1 = Adult CD (14) 2 = Adult UC (15–17) 3=Pediatric IBD (18)	IBD Risk Allele	Non-Response Allele
rs2241880 2q37/ATG16L1	1	C	T
rs2188962 5q31	1	T	C
rs6908425 6p22/CDKAL1	1	C	T
rs762421 21q22/ICOSLG	1	G	A
rs2395185 6p21/HLA-DAQ1	2	G	G
rs2836878 21q22/BRWD1	3	G	G

Table 4Pharmacogenetic GWAS: $p < 0.0001$

Chromosome	SNP	Position *	Gene db129 *	Other loci **	OR	P
13	rs1155848	78786477		RBM26	35.73	5.35E-07
5	rs1592749	159904599			5.949	1.09E-06
X	rs765132	133150270		347475 402425 644403	35	1.09E-06
6	rs4707930	72463040			6.603	5.55E-06
10	rs7905482	81853944		PLAC9 389988 642506 642521 642538 727879	4.875	1.08E-05
20	rs6100556	57701043	PHACTR3	PHACTR3	4.951	1.23E-05
X	rs7059861	133191565		347475 402425	8.333	1.62E-05
X	rs5975453	133212999		347475 402425	8.333	1.62E-05
10	rs4077511	5568172		CALML3 CALML5 100132159	8.214	1.74E-05
2	rs975664	75222305	TACR1	TACR1	0.1667	1.75E-05
3	rs4855535	69017124	FAM19A4	FAM19A4	5.619	1.77E-05
17	rs4796606	36917613		KRT13 KRT15 KRT32 KRT35 KRT38 KRT37 KRT36	11.73	1.98E-05
8	rs2943177	88121513	CNBD1	CNBD1	6.412	2.06E-05
8	rs11991611	139676572	COL22A1	FAM135B COL22A1	15.29	2.12E-05
10	rs3740543	129140065	DOCK1	DOCK1 NPS	4.932	2.18E-05
21	rs2825673	19888587		100128057	6.438	2.28E-05

Chromosome	SNP	Position*	Gene db129*,	Other loci**,	OR	P
1	rs7521532	70351206	LRRC7	SFRS11 LRRC7	7.143	2.54E-05
3	rs4605505	141323576	CLSTN2	CLSTN2	7.143	2.54E-05
8	rs7003556	5213929			7.143	2.54E-05
14	rs1243519	94370110		GSC	6	3.06E-05
5	rs2044111	22897160		CDH12	4.444	3.54E-05
6	rs2103867	47374655	TNFRSF21	TNFRSF21	12.07	3.6E-05
7	rs17168564	14977349			8.286	3.68E-05
6	rs10485363	13245751	PHACTR1	PHACTR1	5.182	3.87E-05
5	rs7726515	129736249			5.187	4E-05
11	rs835780	44816947		TSPAN18	5.187	4E-05
11	rs835791	44823513		TSPAN18	5.187	4E-05
1	rs3795727	154856074	HAPLN2	GPATCH4 HAPLN2 BCAN IQGAP3 646129	4.5	4.1E-05
6	rs6906890	13069206	PHACTR1	PHACTR1	5.899	4.31E-05
11	rs7124825	44812803		TSPAN18	0.1263	4.31E-05
19	rs302827	61102034	NLRP13	NLRP13 NLRP8 NLRP1	4.545	4.45E-05
12	rs2723829	11819364	ETV6	ETV6	4.333	4.47E-05
2	rs1372256	141070866	LRP1B	LRP1B	4.626	4.65E-05
4	rs13138970	155494510	DCHS2	DCHS2	5.297	4.75E-05
20	rs1205434	36302930	KIAA1755	BPI TGM2 KIAA1755	0.1526	4.75E-05
13	rs9556658	96476252		OXGR1	6.111	4.92E-05
2	rs7588326	75202554	TACR1	TACR1	0.1826	5.07E-05
2	rs3771823	75205456	TACR1	TACR1	0.1826	5.07E-05
20	rs1555901	20762132			0.1826	5.07E-05
X	rs4465121	133249071		PHF6	7	5.26E-05
6	rs12527937	16652564	ATXN1	ATXN1	5.5	5.74E-05

Chromosome	SNP	Position*	Gene db129*	Other loci**	OR	P
7	rs10269232	10442565		100128638	6.368	6.33E-05
6	rs3757105	73950398	KCNQ5	KCNQ5	24.2	6.51E-05
18	rs1667216	27383929		DSG2 TTR	4.952	6.75E-05
12	rs278917	39666755	CNTN1	CNTN1	4.172	6.93E-05
6	rs9404502	104605814		100129694	5.409	7.01E-05
X	rs5977968	133151511		347475 402425 644403	7.333	7.03E-05
1	rs12567958	154861280	HAPLN2	GPATCH4 HAPLN2 BCAN IQGAP3 646129	4.167	7.03E-05
8	rs1880473	88079222	CNBD1	CNBD1	5.085	7.09E-05
4	rs7689941	182246739	hCG_2025798	728081	4.244	7.3E-05
17	rs12937472	15194290		PMP22 TEKT3	8.509	7.5E-05
5	rs4301261	159902033			5.433	7.59E-05
X	rs1264379	132893432	GPC3	GPC3	6.092	7.59E-05
X	rs6529954	4194349			0.1781	7.69E-05
22	rs3088103	25250048	TPST2	CRYBA4 TPST2 TFIP11 HPS4 644380 653715 729905 100128401 HMGB1L10	4.308	7.77E-05
6	rs4711716	42375201	TRERF1	TRERF1 387535 653802	8.727	7.82E-05
7	rs10464448	141433796	MGAM	MGAM	0.134	8.32E-05
X	rs12559781	4225952			7.973	8.49E-05
7	rs2540678	36227249	EEPD1	EEPD1	4.6	8.54E-05
4	rs7659755	182246126	hCG_2025798	728081	4.461	8.62E-05
13	rs770389	50043157	LOC730194	730194	5.1	8.96E-05

Chromosome	SNP	Position*	Gene db129*	Other loci**	OR	P
21	rs2825699	19934102			5.1	8.96E-05
12	rs7309734	39564210	CNTN1	CNTN1	4.171	9.7E-05
2	rs11903032	3840992			5.469	9.78E-05
8	rs10808755	68679235	CPA6	CPA6	0.08701	9.87E-05

* Defined using dbSNP Build 129

** Loci include genes that overlap each SNP within 100kb of 5' end and 10kb of 3' end of each gene. Numbers refer to dbGene (NCBI).

Table 5

Model III: known susceptibility SNPs and phenotype

Model	Variable	P value	OR	R squared
III:	UC vs. CD	0.0008	14.7	0.59
	pANCA pos	0.004	15.3	
	rs6908425 6p22.3 CDKA1	0.05	4.6	
	rs2836878 21q22.2 BRWD1	0.02	9.8	
	rs2395185 6p21 HLA-DQA1	0.047	5.4	

Table 6

Model V: Final Model

Model	Variable	P value	OR	R squared
V:	Diagnosis: UC vs. CD	0.008	28.9	0.82
	pANCA pos vs neg	0.03	15.4	
	rs975664 2p12 / TACR1	0.01	26.5	
	rs4855535 3p14 / FAM19A4	0.02	10.8	
	rs6100556 20q13 / PHACTR3	0.02	13.8	
	rs2836878 21q22 / BRWD1	0.07	8.0	

Table 7

Clinical Utility Measures

Model	Variable	AUC	Sensitivity	Specificity	Accuracy	Positive Likelihood Ratio
V:	Diagnosis	0.98	0.95	0.88	0.92	8
	<u>PANCA</u>					
	rs975664					
	<u>2p12 TACR1</u>					
	rs4855535					
	<u>3p14</u>					
	<u>FAM19A4</u>					
	rs6100556					
	<u>20q13</u>					
	<u>PHACTR3</u>					
	rs2836878					
	21q22					
	BRWD1					

Table 8

MODEL	MODEL DESCRIPTOR	R SQUARED	AUC
I	Diagnosis and pANCA	0.48	0.9
II	Known Susceptibility SNPs only	0.3	0.8
III	Diagnosis, pANCA and known susceptibility SNPs	0.59	0.93
IV	Pharmacogenetic GWAS SNPS only	0.67	0.94
V	Diagnosis, pANCA, known susceptibility SNPs AND pharmacogenetic GWAS SNPs	0.82	0.98