Genetic Markers Predict Primary Non-Response and Durable Response to Anti-TNF Biologic Therapies in Crohn's Disease

Genetic markers predict primary non-response and durable response to anti-TNF biologic therapies in Crohn's disease

Grant E Barber[1], Vijay Yajnik[1], Hamed Khalili[1], Cosmas Giallourakis[1], John Garber[1], Ramnik Xavier[1], Ashwin N Ananthakrishnan[1]

[1] Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School

Word Count: 2,804

Short title: Genetics of anti-TNF response in Crohn's disease

Keywords: anti-TNF; pharmacogenomics; loss of response; infliximab; Crohn's; genetics

Source of funding: Ananthakrishnan is supported in part by a grant from the National Institutes of Health (K23 DK097142). This work is also supported by the National Institutes of Health (NIH) (P30 DK043351) to the Center for Study of Inflammatory Bowel Diseases.

Conflicts of Interest: Ananthakrishnan has served on the scientific advisory boards for Abbvie, Exact Sciences, and Cubist pharmaceuticals and has received grant support from Cubist and Amgen.

Corresponding Author:
Ashwin N Ananthakrishnan, MD, MPH
Massachusetts General Hospital Crohn's and Colitis Center
165 Cambridge Street, 9th Floor, Boston, MA 02114
Email: aananthakrishnan@mgh.harvard.edu
Tel 617-724-9953 Fax 617-726-3080

Disclosures:

Grant Barber	– no conflict of interest exists
Vijay Yajnik	– no conflict of interest exists
Hamed Khalili	– Received consulting fees from Abbvie, Inc.
Cosmas Giallourakis	– no conflict of interest exists
John Garber	- no conflict of interest exists
Ramnik Xavier	- no conflict of interest exists
Ashwin Ananthakrishnan	- Scientific advisory board for Cubist pharmaceuticals, Abbvie, Exact Sciences

Author Contributions:

Barber: study design, analysis and interpretation of data, drafting of manuscript, critical revision of the manuscript for important intellectual content

Yajnik, Khalili, Giallourakis, Garber, Xavier: data collection, drafting of the manuscript, critical revision of the manuscript for important intellectual content

Ananthakrishnan: study design, data collection, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content

ABSTRACT

Background:

One-fifth of patients with Crohn's Disease (CD) are primary non-responders (PNR) to anti-tumor necrosis factor (anti-TNF) therapy, and an estimated 10-15% will fail therapy annually. Little is known about the genetics of response to anti-TNF therapy. The aim of our study was to identify genetic factors associated with PNR and loss of response to anti-TNFs in CD.

Methods:

From a prospective registry, we characterized the response of 427 CD patients to their first anti-TNF therapy. Patients were designated as achieving primary response, durable response, and non-durable response based on clinical, endoscopic, and radiologic criteria. Genotyping was performed on the Illumina Immunochip. Separate genetic scores based on presence of predictive genetic alleles were calculated for PNR and durable response and performance of clinical and genetics models were compared.

Results:

From 359 patients, 36 were adjudged to have PNR (10%), 200 had durable response, and 74 had non-durable response. Primary non-responders had longer disease duration and were more likely to be smokers. Fifteen risk alleles were associated with PNR. Patients with PNR had a significantly higher genetic risk score ($p=8 \times 10^{-12}$). A combined clinical-genetic model more accurately predicted PNR when compared to a clinical only model (0.93 vs. 0.70, $p < 0.001$). Sixteen distinct SNPs predicted durable response with a higher genetic risk score ($p=7 \times 10^{-13}$). The genetic risk scores for PNR and durable response were not mutually correlated suggesting distinct mech-

anisms.

Conclusion:

Genetic risk alleles can predict primary non-response and durable response to anti-TNF therapy in Crohn's disease.

Keywords: Infliximab; adalimumab; non-response

INTRODUCTION

Crohn's disease (CD) is a chronic relapsing inflammatory bowel disease (IBD) resulting in progressive bowel damage and disability[1]. Monoclonal anti-tumor necrosis factor α (anti-TNF) antibodies have revolutionized the care of these patients, enabling achievement of clinical and endoscopic remission and preventing surgery[2]. Infliximab (IFX), adalimumab (ADA), and certolizumab pegol (CZP) have all demonstrated efficacy in inducing and maintaining remission in clinical trials and are approved for the treatment of moderate-to-severe CD. However, despite their established efficacy, one-fifth of patients will have no response at all to these agents (primary non-response, PNR) and an additional one-third will eventually fail therapy (secondary loss of response), requiring addition or change to another medication or surgery[3-5].

The exact mechanisms of PNR and secondary LOR remain inadequately defined[3, 5]. Several studies have attempted to identify patient-related or drug-related factors but have yielded heterogeneous results[3, 4]. Consequently, there is considerable interest and an unmet need for use of genetic markers to predict response to these therapies. Most prior studies examining this question studied only single or a few candidate genes, had small sample sizes, and did not yield definitive results[6-15]. Polymorphisms in *TNFα*[10], IBD5 locus[14], Immunoglobulin G (IgG) Fc receptor IIIa[9], autophagy (ATG16L1)[16] and apoptosis-related genes[7] have also been variably associated with a response to IFX or ADA. A limitation of exclusively studying a few candidate loci or IBD-risk alleles alone is the possibility of missing potentially relevant associations across loci that more broadly influence immune function across a spectrum of diseases. Such an analysis may be particularly pertinent given the efficacy of anti-TNF agents across a range of immune-mediated diseases that only modestly share risk loci and pathogenic pathways.

The need for identification of genetic predictors of response to therapy achieves additional urgency with the growing availability of drugs with distinct mechanisms of action. Identifying relevant predictors would allow stratification of patients by likelihood of response to anti-TNF therapy, thereby directing them to other drugs if there is a low pre-test probability of response. Using a large, prospective cohort with detailed genotype information, we performed this study with the following aims: (1) to identify SNPs predicting PNR and durable clinical response (DR) in CD patients initiating anti-TNF therapy; and (2) to compare the utility of clinical and genetic factors in predicting PNR and DR.

METHODS

Study Population

The Prospective Registry in IBD Study at Massachusetts General Hospital (PRISM) is a prospective cohort of patients with IBD receiving care at the MGH Crohn's and Colitis center; details of the cohort have been previously described[17]. Upon providing informed consent, information on demographics, disease characteristics including date of diagnosis, IBD type, disease location and phenotype, and treatment characteristics are obtained for each patient. A total of 427 patients identified from this database met our criteria for inclusion in this study (1) CD diagnosed using standard criteria; (2) received anti-TNF therapy with IFX, ADA, or CZP, and (3) genotyping performed on the Illumina Immunochip. For patients who had received more than one anti-TNF therapy, only data from their first anti-TNF exposure was included.

Outcomes

Using chart review by study investigators (GEB, ANA), we characterized the patients' re-

sponse to their first anti-TNF therapy using clinical, radiologic, endoscopic, and laboratory data where available. PNR was defined as non-response by 12 weeks after starting therapy accompanied by an alteration of therapeutic approach (addition or escalation of corticosteroids, switch to a different agent, or surgery). DR was defined as maintenance of response to anti-TNF therapy for at least 24 months after initiation. Only patients who achieved at least partial initial response were included in analysis of DR. For patients who ceased treatment due to adverse effects prior to the 24 month time point, we classified them as non-responders if the adverse events were related to loss of response (for example, infusion reactions due to immunogenicity) or excluded them from the analyses for those that were unrelated to response (non-CD related infections, insurance reasons). Patients requiring dose escalation who responded could be classified as having DR if they remained on therapy for 24 months. To perform internal validation of our findings, we also calculated time to cessation of therapy irrespective of reason for stopping therapy.

Clinical Covariates

Information was extracted on age, gender, smoking status, duration of disease at anti-TNF initiation, location and phenotype of IBD according to the Montreal classification, and presence of perianal involvement. Information was also obtained on prior therapies and whether index anti-TNF agent was used as monotherapy or in conjunction with an immunomodulator (azathioprine, 6-mercaptopurine, methotrexate) (combination IS).

Genotyping

All patients provided 10mL of blood for extraction of buffy coat from which genomic DNA was isolated. Genotyping was performed on the Illumina Immunochip at the Broad Insti-

tute (Cambridge, MA). The Immunochip is a custom-designed platform to perform deep replication of inflammatory and autoimmune loci covering 196,524 polymorphisms putatively associated with immune function or autoimmune diseases.

Statistical analysis

The study was approved by the Institutional Review Board of Partners Healthcare. Clinical covariates were summarized using means and standard deviations. Categorical variables were summarized using proportions. Comparison between those with PNR or DR to those without was done using the t-test for continuous and the chi-square test for categorical variables. Univariate logistic regression was performed to identify variables significant at $p < 0.10$ for inclusion in the multivariable model.

Genetic analysis was performed using Plink v1.07[18]. All SNPs met the Hardy-Weinberg equilibrium threshold of $p > 0.001$, genotyping call rate > 99% and genotyping success rate > 80%. Genotype-phenotype analysis was performed in two steps. First, candidate polymorphisms associated with PNR or DR among the 163 IBD risk alleles[19] were selected as significant at a p-value ≤ 0.05. A more rigorous threshold of $p < 1 \times 10^{-4}$ was used for the other immunochip loci. Next, a genetic risk score (GRS) was calculated based on the allele burden ($\sum$risk allele haplotypes) with separate scores for PNR and DR. Then, the GRS was entered into the multivariable model along with relevant clinical covariates. We compared the performance of a combined model including clinical and genetic data to those including clinical variables alone and GRS alone using the area under the receiver operating characteristics curve (AUROC) and the likelihood ratio test. To test the consistency of our findings, we performed internal validation in two

steps. First, we included the SNPs identified using logistic regression and genetic association analysis in a Cox proportional hazards models with time to cessation of anti-TNF therapy. Second, we repeated the analysis using bootstrapping with 10,000 replications. All statistical analysis was performed using Stata 13.2 (StataCorp, College Station, TX).

RESULTS

Study Population

Among 359 patients with sufficient data to assess primary non-response, 36 had PNR (10%). The mean age was 25.7 years and mean disease duration prior to first anti-TNF therapy was 10.6 years. Just over half were women (59%). Ileocolonic involvement was the most common site (57%) and an equal proportion had inflammatory or penetrating disease (38% each). The most common first anti-TNF therapy was infliximab (82%), and about half were on combination IS. Among 274 patients in whom we could define durable response, 73% met this endpoint.

Predictors of Primary Non-response

Patients with and without PNR were similar in gender, disease behavior, perianal involvement, type of anti-TNF therapy, use of combination IS, or history of prior resection (**Table 1**). However, primary non-responders were more likely to have had longer disease duration prior to initiating anti-TNF therapy (15 vs. 10 years), were older at diagnosis (29 vs. 25 years), and more likely to be smokers (53% vs. 33%). Isolated colonic involvement was also more common among primary non-responders (42%) compared to responders (22%) (p=0.03).

On genetic association analysis, 11 IBD-risk alleles met a p-value threshold < 0.05 and 4 additional SNPs on the immunochip met a threshold of $p < 1 \times 10^{-4}$ and were incorporated into the GRS (**Supplemental Table 1**). Primary non-responders had a significantly higher GRS than patients without PNR (16.4 vs. 11.2, $p=8 \times 10^{-12}$) (**Figure 1**). On multivariable analysis including relevant clinical covariates, the PNR GRS was the only significant independent predictor of primary non-response (Odds ratio (OR) 2.65, 95% confidence interval (CI) 1.94—3.61, $p=6 \times 10^{-10}$). Two clinical variables, disease duration at anti-TNF initiation (OR 1.04, 95%CI 1.00 – 1.09, p=0.07) and ileocolonic (vs. ileal) location (OR 0.30, 95% CI 0.08 – 1.18, p=0.09) demonstrated a trend towards statistical significance (**Table 2**). A model combining genetic and clinical variables was superior to a model including only clinical variables (AUROC 0.93 vs. 0.70, p < 0.0001). Indeed, a genetics only model performed almost as well as the combined model with only modest incremental benefit to including clinical covariates (AUROC 0.934 vs. 0.929, p = 0.02).

Predictors of durable response

A history of prior resection was more common in patients without DR compared to those with DR (66% vs. 42%, p < 0.001) with none of the other clinical covariates meeting statistical significance (**Table 3**). On genetic association analysis, 16 SNPs (11 IBD risk alleles, 5 other loci on the immunochip) predicted DR, including those at the IL2RA and ATG16L1 loci (**Supplemental Table 2**). Combining the risk alleles, patients achieving DR had a higher DR GRS than those who did not achieve DR (15.0 vs. 11.2, $p = 7 \times 10^{-13}$). On multivariable analysis, only the GRS and a lack of prior resections were independently predictive of durable response (**Table 4**). Each 1 point increase in the GRS was associated with a 60% increase in likelihood of DR (OR

1.60, 95% CI 1.41 – 1.83, p=2 x 10^{-12}). Combination IS use demonstrated a trend towards a higher likelihood of DR (OR 1.89, 95% CI 0.94 – 3.83). A combined clinical-genetic model (AUROC 0.85) was superior to a clinical only (AUROC 0.66, p < 0.001) or genetics only model (AUROC 0.83, p < 0.001). Our findings were robust on internal validation on bootstrapping with 10,000 replications.

Time to anti-TNF therapy cessation

In a Cox proportional hazards analysis, a lower GRS (fewer alleles predicting DR) predicted earlier cessation of anti-TNF therapy on univariate (hazard ratio (HR) 0.90, 95% CI 0.86—0.94, p=2 x 10^{-7}) and multivariable analysis (hazard ratio 0.89, 95% CI 0.85—0.93, p=4 x 10^{-8}). A total of 47% of patients in the lowest quartile of the GRS achieved DR compared to 97% of patients in the highest quartile (HR 0.40, 95% CI 0.27 – 0.59, p=3 x 10^{-6}) (**Figure 2**).

Polymorphisms associated with PNR and DR are exclusive to their respective outcome

Genetic risk scores for PNR could not predict DR (p=0.71) and vice versa (p=0.72, *rho*=0.02), suggesting that the mechanisms underlying the genetic predisposition to PNR and DR are distinct. The overall burden of CD genes as calculated previously[17] also did not predict either PNR or DR (p=0.97 and 0.77, respectively). The presence of a *NOD2* mutation was not associated with PNR (OR 0.32, 95% CI 0.08-1.25) or DR (OR 2.11, 95% CI 0.91—4.90).

DISCUSSION

Given the significant likelihood of primary or secondary non-response to anti-TNF agents and with growing availability of therapies targeting CD through diverse pathways, there is an

important unmet need to define predictors and mechanisms of response to each therapeutic class. Using a large prospective cohort of CD patients, we demonstrate several SNPs to be associated with PNR and maintenance of DR to anti-TNF therapy. Additionally, prediction models incorporating genetics were significantly more accurate in predicting PNR and DR than clinical covariates alone.

There are several novel hypothesis-generating observations from our study. First, we demonstrated that 31 distinct SNPs could be used to predict response to anti-TNF therapy in CD while clinical covariates alone had only modest value. A few prior studies have examined the utility of genetics to predict response to anti-TNF therapy, though they often analyzed only a target set of candidate genes[6-15, 20]. While Niess *et al*. reported an association between *NOD2* mutations and response to IFX[21], other studies including ours failed to identify such an association[15, 22]. Hlavaty *et al*. identified an association between apoptosis related-genes including *Fas* ligand-843, *Fas*-670, and caspase 9 and response to IFX therapy[7]. Consistent with this, we found a SNP at the *CARD11* locus, a caspase recruitment domain-containing protein to be associated with non-response. The *CARD11* protein interacts with *BCL10*, a signaling protein that regulates apoptosis and nF-kB mediated signalling[23]. Our study also replicated the previously described association between the ATG16L1 polymorphism and response to anti-TNF therapy[16].

Several other loci offer mechanistic plausibility by virtue of their importance in regulating TNFα-mediated inflammatory responses. The TNF receptor super family 9 (TNFRSF 9) is an nf-kB dependent transcript induced by TNFα in regulatory T-cells that down regulates T-cell mediated suppression of inflammation[24]. The retinoic-acid related orphan receptor (RORC) is

downregulated on treatment with IFX and plays a role in mucosal healing through down-regulation of Th1/Th-17 associated inflammatory cytokine production[25]. IFX administration also down-regulates IL1R1, IL1R2, and IL18 receptor complex[26, 27]; polymorphisms at all these sites were associated with primary non-response in our study. The T-Cell Activation RhoGTPase Activating Protein (TAGAP), associated with loss of response in our cohort, was also shown to be differentially down-regulated on colon biopsies of responders to IFX when compared to non-responders[28].

Similar to previously published studies, we found that patients with long-standing disease prior to initial TNF therapy and smoking were associated with PNR[12]. Curiously, isolated colonic involvement had previously been associated with better response to anti-TNF (albeit not focusing on PNR), whereas in our cohort colonic disease was more common in PNR[29]. Combination IS demonstrated a trend towards DR consistent with recent literature demonstrating reduce immunogenicity and higher rates of response with dual immunosuppression[2, 30]. Results have been variable in prior clinical studies were potentially relevant parameters identified including gender and disease location, smoking, longer disease duration, and stricturing disease[12].

Another interesting finding from our study is that although genetic factors predicted both PNR and DR, no alleles were common to both analyses. In addition, the PNR GRS was not predictive of DR and vice versa, suggesting that the mechanisms behind PNR and DR are distinct. Mechanistically, a significant proportion of patients with secondary LOR have sub-therapeutic trough levels often driven by anti-drug antibodies. This is supported by the trend towards an inverse association between combination IS use and DR in our cohort and in prior studies[30, 31]. In

contrast, PNR is often seen despite adequate level of drug during induction therapy supporting the hypothesis of a distinct inflammatory pathway in that subset of patients. Further in evidence of this is that in patients with prior anti-TNF exposure, those with PNR demonstrate low rates of response to subsequent anti-TNF agents[32].

There are several implications to our findings. First, the association between genetics and response to anti-TNF therapy offers the potential to better understand the biological mechanisms for heterogeneity in response. This may also help identify novel pathways to serve as target for future therapies. Second, the significant improvement in predictive value with genetics, particularly for PNR, offers the potential to tailor therapy to individuals based *a priori* on likelihood of response. This allows us to accurately balance risks of therapy with likelihood of benefit. Additionally, with growing availability of therapies with distinct mechanisms of action, this approach allows for potentially matching the patient to the drug.

We readily acknowledge several limitations to this study. First, our definitions of PNR and DR were by chart review rather than prospectively collected disease activity indices. However, this is also a strength as we used comprehensive clinical, endoscopic, and radiologic evidence to adjudicate response status rather than relying on symptom based activity indices alone that notoriously correlate poorly with objective disease activity. Future studies should prospectively include endoscopic, fecal and serologic evaluations to define response. In addition, our findings were consistent in analyses using time to cessation of therapy as an outcome which is a hard outcome not influenced by the retrospective design of our study. Second, we could not assess adherence or episodic use which may affect efficacy. Third, in this hypothesis generating

study, we selected a less rigorous p-value threshold for genetic association analysis than has been used in genome-wide associated studies. Thus, there is a need for external validation in other cohorts. Fourth, we did not have information on anti-drug antibody in the vast majority of our patients and so could not explore the genetics of this phenomenon. Finally, our enrollees may have more severe CD than the general population by virtue of seeking care at a referral center.

In conclusion, in a large CD cohort, we identified risk alleles that predicted primary non-response and durable response to anti-TNF therapies. A composite genetic score of risk alleles was successful in predicting response to therapy and had greater accuracy and performance than clinical covariates. Further work is required to validate our findings in other cohorts, following which such genetic markers may be useful in personalizing therapy in CD to ensure optimal outcomes for our patients.

REFERENCES

1. Cosnes J, Gower-Rousseau C, Seksik P, et al. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology 2011;140:1785-94.
2. D'Haens GR, Panaccione R, Higgins PD, et al. The London Position Statement of the World Congress of Gastroenterology on Biological Therapy for IBD With the European Crohn's and Colitis Organization: When to Start, When to Stop, Which Drug to Choose, and How to Predict Response? Am J Gastroenterol 2011.
3. Allez M, Karmiris K, Louis E, et al. Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: Definitions, frequency and pharmacological aspects. Journal of Crohns and Colitis 2010;4:355-366.
4. Chowers A, Sturm A, Sans M, et al. Report of the ECCO workshop on anti-TNF therapy failures in inflammatory bowel diseases: Biological roles and effects of TNF and TNF antagonists. Journal of Crohns and Colitis 2010;4:367-376.
5. Gisbert JP, Panes J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. Am J Gastroenterol 2009;104:760-7.
6. Dideberg V, Louis E, Farnir F, et al. Lymphotoxin alpha gene in Crohn's disease patients: absence of implication in the response to infliximab in a large cohort study. Pharmacogenet Genomics 2006;16:369-73.
7. Hlavaty T, Pierik M, Henckaerts L, et al. Polymorphisms in apoptosis genes predict response to infliximab therapy in luminal and fistulizing Crohn's disease. Aliment Pharmacol Ther 2005;22:613-26.
8. Jurgens M, Laubender RP, Hartl F, et al. Disease activity, ANCA, and IL23R genotype status determine early response to infliximab in patients with ulcerative colitis. Am J Gastroenterol 2010;105:1811-9.
9. Louis E, El Ghoul Z, Vermeire S, et al. Association between polymorphism in IgG Fc receptor IIIa coding gene and biological response to infliximab in Crohn's disease. Aliment Pharmacol Ther 2004;19:511-9.
10. Mascheretti S, Hampe J, Kuhbacher T, et al. Pharmacogenetic investigation of the TNF/TNF-receptor system in patients with chronic active Crohn's disease treated with infliximab. Pharmacogenomics J 2002;2:127-36.
11. Pierik M, Vermeire S, Steen KV, et al. Tumour necrosis factor-alpha receptor 1 and 2 polymorphisms in inflammatory bowel disease and their association with response to infliximab. Aliment Pharmacol Ther 2004;20:303-10.
12. Siegel CA, Melmed GY. Predicting response to Anti-TNF Agents for the treatment of crohn's disease. Therap Adv Gastroenterol 2009;2:245-51.
13. Taylor KD, Plevy SE, Yang H, et al. ANCA pattern and LTA haplotype relationship to clinical responses to anti-TNF antibody treatment in Crohn's disease. Gastroenterology 2001;120:1347-55.
14. Urcelay E, Mendoza JL, Martinez A, et al. IBD5 polymorphisms in inflammatory bowel disease: association with response to infliximab. World J Gastroenterol 2005;11:1187-92.
15. Vermeire S, Louis E, Rutgeerts P, et al. NOD2/CARD15 does not influence response to infliximab in Crohn's disease. Gastroenterology 2002;123:106-11.
16. Koder S, Repnik K, Ferkolj I, et al. Genetic polymorphism in ATG16L1 gene influences the response to adalimumab in Crohn's disease patients. Pharmacogenomics 2015;16:191-204.

17. Ananthakrishnan AN, Huang H, Nguyen DD, et al. Differential effect of genetic burden on disease phenotypes in Crohn's disease and ulcerative colitis: analysis of a North American cohort. Am J Gastroenterol 2014;109:395-400.
18. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-75.
19. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature (in press) 2012.
20. Dubinsky MC, Mei L, Friedman M, et al. Genome wide association (GWA) predictors of anti-TNFalpha therapeutic responsiveness in pediatric inflammatory bowel disease. Inflamm Bowel Dis 2010;16:1357-66.
21. Niess JH, Klaus J, Stephani J, et al. NOD2 polymorphism predicts response to treatment in Crohn's disease--first steps to a personalized therapy. Dig Dis Sci 2012;57:879-86.
22. Mascheretti S, Hampe J, Croucher PJ, et al. Response to infliximab treatment in Crohn's disease is not associated with mutations in the CARD15 (NOD2) gene: an analysis in 534 patients from two multicenter, prospective GCP-level trials. Pharmacogenetics 2002;12:509-15.
23. Bertin J, Wang L, Guo Y, et al. CARD11 and CARD14 are novel caspase recruitment domain (CARD)/membrane-associated guanylate kinase (MAGUK) family members that interact with BCL10 and activate NF-kappa B. J Biol Chem 2001;276:11877-82.
24. Nagar M, Jacob-Hirsch J, Vernitsky H, et al. TNF activates a NF-kappaB-regulated cellular program in human CD45RA- regulatory T cells that modulates their suppressive function. J Immunol 2010;184:3570-81.
25. Liu C, Xia X, Wu W, et al. Anti-tumour necrosis factor therapy enhances mucosal healing through down-regulation of interleukin-21 expression and T helper type 17 cell infiltration in Crohn's disease. Clin Exp Immunol 2013;173:102-11.
26. Keino H, Watanabe T, Taki W, et al. Effect of infliximab on gene expression profiling in Behcet's disease. Invest Ophthalmol Vis Sci 2011;52:7681-6.
27. Pittoni V, Bombardieri M, Spinelli FR, et al. Anti-tumour necrosis factor (TNF) alpha treatment of rheumatoid arthritis (infliximab) selectively down regulates the production of interleukin (IL) 18 but not of IL12 and IL13. Ann Rheum Dis 2002;61:723-5.
28. Toedter G, Li K, Marano C, et al. Gene expression profiling and response signatures associated with differential responses to infliximab treatment in ulcerative colitis. Am J Gastroenterol 2011;106:1272-80.
29. Vermeire S, Louis E, Carbonez A, et al. Demographic and clinical parameters influencing the short-term outcome of anti-tumor necrosis factor (infliximab) treatment in Crohn's disease. Am J Gastroenterol 2002;97:2357-63.
30. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med 2010;362:1383-95.
31. Teshima CW, Thompson A, Dhanoa L, et al. Long-term response rates to infliximab therapy for Crohn's disease in an outpatient cohort. Can J Gastroenterol 2009;23:348-52.
32. Sandborn WJ, Rutgeerts P, Enns R, et al. Adalimumab induction therapy for Crohn disease previously treated with infliximab: a randomized trial. Ann Intern Med 2007;146:829-38.

Figure 1: Comparison of primary non-response (PNR) genetic risk score in CD patients with and without PNR to anti-TNF therapy

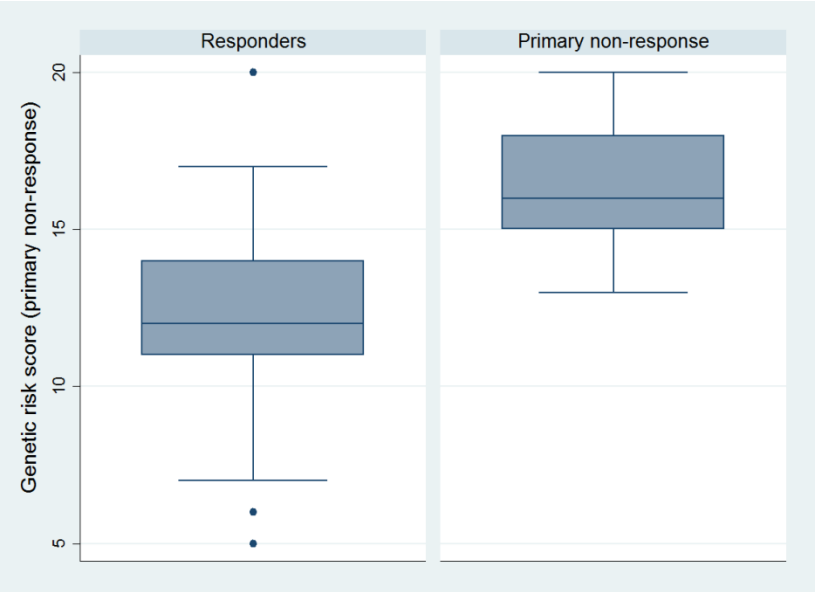

Figure 2: Kaplan-Meier curve of quartiles of durable response (DR) genetic risk score in predicting time to cessation of anti-TNF therapy

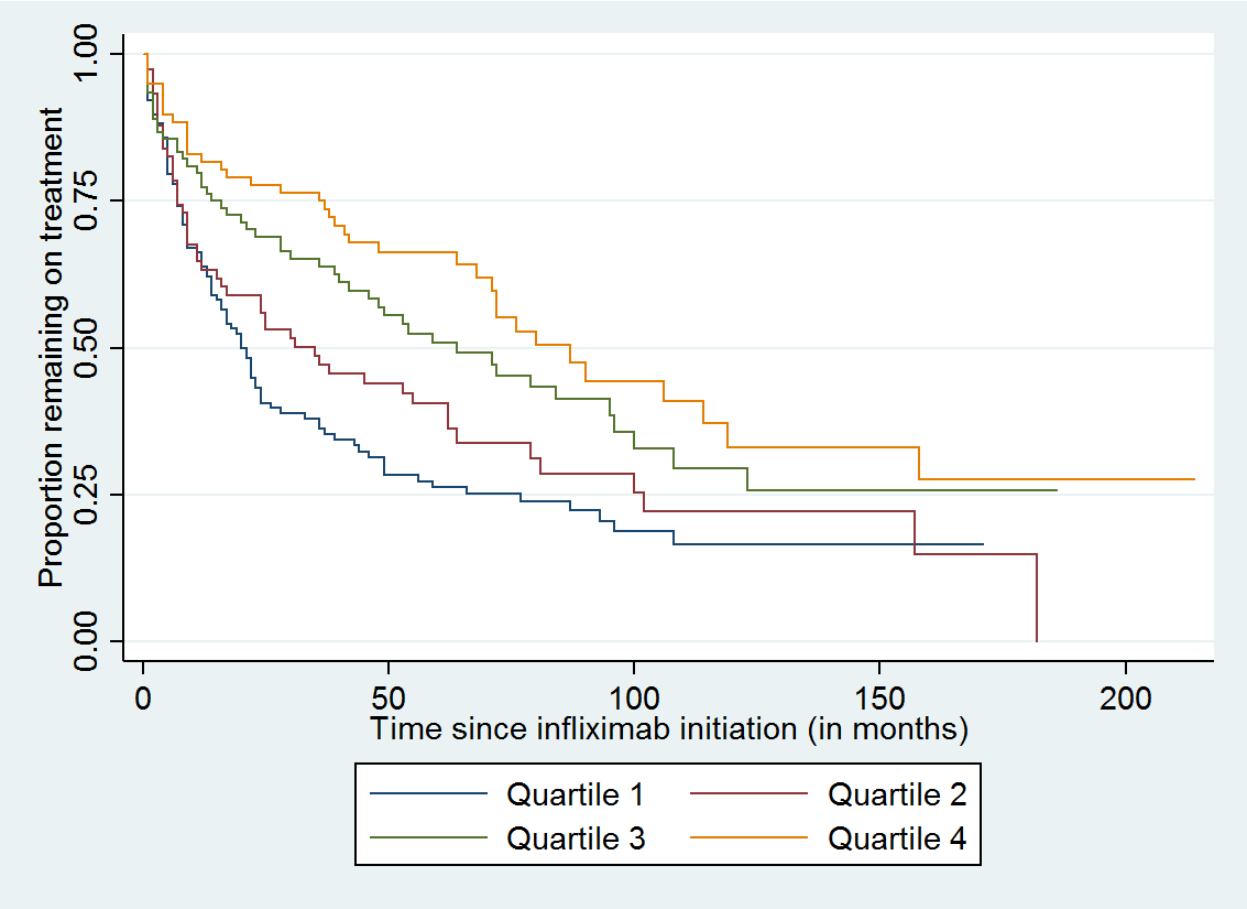

Table 1: Comparison of characteristics of patients with Crohn's disease initiating anti-tumor necrosis factor therapy (anti-TNF) with and without primary non-response

	Primary non-responders (n=36)	Responders (n=323)	P-value
Age at diagnosis (SD) (in years)	29 (14)	25 (12)	0.07
Disease duration (SD) (in years)	15 (14)	10 (10)	0.01
Female (%)	61	59	0.82
Disease location, n (%)			0.03
Ileal	6 (16.7)	62 (19)	
Colonic	15 (41.7)	71 (22)	
Ileocolonic	15 (41.7)	190 (59)	
Disease Behavior, n (%)			0.55
Inflammatory	13 (36)	124 (38)	
Stricturing	11 (31)	73 (23)	
Penetrating	12 (33)	126 (39)	
Perianal disease, n (%)	14 (39)	109 (34)	0.54
First anti-TNF Therapy, n (%)			0.64
Infliximab	28 (78)	265 (82)	
Adalimumab	7 (19)	45 (14)	
Certolizumab	1 (3)	13 (4)	
Combination immunosuppression, n (%)	18 (51)	165 (52)	0.99
Prior resection, n (%)	16 (44)	155 (48)	0.69
History of smoking, n (%)	19 (53)	108 (33)	0.02
PNR Genetic Risk Score (SD)	16.4 (1.9)	12.1 (2.2)	$8x10^{-12}$

Table 2: Multivariable analysis of predictors of primary non-response to anti-TNF therapy in Crohn's disease

	Odds ratio	95% confidence interval	p-value
Age at diagnosis	1.01	0.97-1.06	0.65
Disease duration	1.04	1.00-1.09	0.073
Disease location			
Ileal	1.00	-	-
Colonic	1.05	0.28-4.00	0.94
Ileocolonic	0.30	0.08-1.18	0.85
History of Smoking	2.12	0.75-6.34	0.15
Genetic Risk Score (per 1 unit increase)	2.65	1.95-3.61	<0.001

Table 3: Comparison of characteristics of patients with Crohn's disease initiating anti-tumor necrosis factor therapy (anti-TNF) with and without durable response

	Durable Response (n=200)	No Durable Response (n=74)	P-value
Age at diagnosis (SD)	24 (11)	26 (13)	0.35
Disease duration, yrs (SD)	10 (10)	10 (10)	0.81
Female (%)	58	58	0.99
Disease location, n (%)			0.10
Ileal	34 (17)	16 (22)	
Colonic	51 (25)	10 (13)	
Ileocolonic	115 (58)	48 (65)	
Disease Behavior, n (%)			0.11
Inflammatory	79 (39)	24 (32)	
Stricturing	43 (22)	25 (34)	
Penetrating	78 (39)	25 (34)	
Perianal disease, n (%)	62 (33)	28 (38)	0.29
First anti-TNF Therapy, n (%)			0.77
Infliximab	160 (80)	62 (84)	
Adalimumab	31 (15)	9 (12)	
Certolizumab	9 (5)	3 (4)	
Immunomodulator, n (%)	114 (57)	34 (46)	0.10
Prior resection, n (%)	84 (42)	49 (66)	<0.001
History of smoking, n (%)	63 (32)	26 (35)	0.57
Genetic Risk Score, (SD)	15.0 (3.0)	11.2 (2.7)	$7x10^{-13}$

Table 4: Multivariable analysis of predictors of durable response to anti-TNF therapy in Crohn's disease

	Odds ratio	95% confidence interval	p-value
Disease location			
Ileal (reference)	1.00	-	-
Colonic	1.89	0.60-5.97	0.28
Ileocolonic	1.50	0.63-3.57	0.36
Disease Behavior			
Inflammatory (reference)	1.00	-	-
Stricturing	1.15	0.45-2.92	0.77
Penetrating	1.39	0.58-3.34	0.46
Immunomodulator	1.90	0.94-3.83	0.07
Prior Resection	0.38	0.18-0.83	0.02
History of Smoking	0.73	0.35-1.51	0.39
Genetic Risk Score (per 1 unit increase)	1.60	1.41-1.83	<0.001

Supplemental Table 1: Genetic polymorphisms associated with primary non-response to anti-TNF therapy in Crohn's disease

Chromo-some	SNP	Risk allele	Frequency -PNR	Frequency – re-sponders	p-value	Odds ratio	Potential Associated Genes
1	rs3766606	T	0.0556	0.1563	0.02168	0.317	TNFRSF9
1	rs4845604	A	0.25	0.1192	0.001889	2.463	RORC
2	rs6708413	G	0.3194	0.2152	0.04466	1.712	IL1R2, IL18RAP, IL18R1, IL1R1, IL1RL1, IL1RL2
3	rs3197999	A	0.1944	0.3235	0.02475	0.505	MST1,PFKFB4,MST1R,UCN2,GPX1,IP6K2,BSN,IP6K1,USP4
3	rs9847710	C	0.2639	0.4149	0.01312	0.506	
3	rs17200795	G	0.2639	0.1009	4.60E-05	3.193	
3	rs2045307	C	0.3611	0.1687	7.40E-05	2.785	
6	rs2503322	A	0.3472	0.4737	0.04116	0.591	
7	rs1182188	C	0.4028	0.2663	0.01439	1.859	CARD11,GNA12,TTYH3
8	rs921720	A	0.2778	0.3963	0.04991	0.586	TRIB1
9	rs4246905	T	0.3889	0.2771	0.04698	1.66	TNFSF8,TNFSF15,TNC
10	rs10761659	A	0.5278	0.4025	0.04068	1.659	
12	rs7956809	G	0.2639	0.1006	4.30E-05	3.204	Keratin 4
16	rs1728785	A	0.1389	0.2446	0.0445	0.498	ZFP90
18	rs8083571	A	0.6806	0.4195	2.40E-05	2.948	

Supplemental Table 2: Genetic polymorphisms associated with durable response to anti-TNF therapy in Crohn's disease

Chromosome	SNP	Risk allele	Frequency – DR	Frequency – non-DR	p-value	Odds ratio	Potential Associated Genes
1	rs2651244	A	0.43	0.33	0.04091	1.509	
2	rs1440088	G	0.23	0.14	0.03549	1.737	RFTN2,PLCL1
2	rs12994997	G	0.39	0.49	0.04763	0.682	ATG16L1, INPP5D
5	rs254560	A	0.36	0.47	0.02713	0.651	
6	rs17119	G	0.21	0.14	0.04722	1.701	
6	rs212388	C	0.42	0.54	0.01374	0.6219	TAGAP
7	rs9297145	C	0.30	0.22	0.0456	1.572	SMURF1
9	rs55689715	C	0.24	0.09	6.00E-05	3.324	
10	rs12722515	A	0.16	0.06	0.001984	2.997	IL2RA,IL15RA
11	rs11229555	T	0.22	0.32	0.009972	0.5791	CNTF,LPXN
12	rs2682714	C	0.39	0.21	6.10E-05	2.438	RalGDS/AF-6
14	rs194749	C	0.23	0.15	0.04907	1.663	ZFP36L1
16	rs35725751	T	0.23	0.39	9.30E-05	0.4505	
16	rs7201929	C	0.24	0.41	8.10E-05	0.4505	SH2B1
17	rs9904253	A	0.30	0.47	9.80E-05	0.4663	
20	rs6087990	C	0.46	0.34	0.01333	1.637	DNMT3B