Type I and II Interferon Signatures Can Predict the Response to Anti-TNF Agents in Inflammatory Bowel Disease Patients: Involvement of the Microbiota

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Background: Anti-TNF agents have been a cornerstone of IBD therapy; however, response to treatment has been variable, and clinically applicable biomarkers are urgently needed. We hypothesized that the type I and type II interferon (IFN) signatures may be a confounding factor for response to antitumor necrosis factor (TNF) treatment via interactions with the host and its gut microbiota.

Methods: Peripheral blood from 30 IBD patients and 10 healthy controls was subjected to real-time quantitative real-time polymerase chain reaction for type I and type II IFN genes (IFNGs), both at baseline and after treatment with anti-TNF. Correlation between IFN signatures and microbiota composition was also determined for a subgroup of patients and controls.

Results: At baseline, type I IFN score was significantly higher in IBD patients ($P = 0.04$ vs controls). Responders to subsequent anti-TNF treatment had significantly lower baseline scores for both type I and II IFN signatures ($P < 0.005$ vs nonresponders for both comparisons). During treatment with anti-TNF, the expression of type I and II IFNGs was significantly elevated in responders and decreased in nonresponders. In addition, changes in IFN signatures correlated to specific alterations in the abundance of several microbial taxa of the gut microbiome.

Conclusions: Baseline expression of type I and II IFN signatures and their kinetics during anti-TNF administration significantly correlate to treatment responses in IBD patients. Peripheral blood IFN signatures may serve as clinically meaningful biomarkers for the identification of subgroups of patients with favorable response to anti-TNF treatment. Additionally, the distinct synergies between different IFN types and microbiota might help drive therapeutic intervention.

Key Words: IBD, CD, UC, anti-TNF, INF; microbiota

INTRODUCTION

Inflammatory bowel disease (IBDs), Crohn's disease (CD) and ulcerative colitis (UC), are gastrointestinal (GI) inflammatory diseases that mainly affect the bowel as a result of an aberrant immune response to gut microbiota in genetically predisposed individuals.[1] The progression of IBD can be heterogeneous and unpredictable. Patients with severe disease or disease refractory to immunomodulation will usually be prescribed antitumor necrosis (TNF)-α therapy. Indeed, the use of anti-TNF agents such as infliximab (IFX), adalimumab (ADA), golimumab (GOL), and certolizumab pegol (CER) for inducing and maintaining clinical remission in IBD patients is widely accepted.[2–4] Regardless of their effectiveness, literature data support that primary nonresponse to anti-TNF induction therapy occurs approximately in 30% of patients, and longer-term constant response rates are estimated between 21%–48%.[2, 4–7] Therefore, the availability of predictive biomarkers of effectiveness for anti-TNF therapy would be very useful in clinical practice to optimize treatments and eliminate side effects and costs.

Growing evidence recently supports an important role of the type I and II interferon (IFN) system in the pathogenesis of systemic and organ-specific diseases including IBD.[8–14] Type I IFNs (IFNα/β) mainly confer immunity against viral and microbial infections, whereas type II IFN (IFNγ) promotes antibacterial immunity, inflammation, and tissue destruction[12] through the induction of a number of genes, the so-called "type I IFN or type II IFN signature," respectively.[15] Recently, it has been shown that gut microbiota can induce type I IFN via activation of the STING pathway.[16]

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Taking into consideration the high cost of the anti-TNF therapy, the potential treatment failure, and the adverse effects, it is important to identify predictors of response to anti-TNF agents, which could be easily used in clinical practice. Because type IFN I and IFN II signatures have been associated with antimicrobial and anti-inflammatory activities in several immune-related diseases including IBD and previous reports imply a predictive role of type I IFN activity in peripheral blood in patients with rheumatoid arthritis,[17, 18] we hypothesized that type IFN I and/or IFN II signatures might be predictors of response to anti-TNF therapy in IBD patients. Thus in the present study, we aimed to explore if IFN signature could affect anti-TNF treatment response via interactions with the host and its microbiota. Additionally, our goal was to identify differences in type I and II IFN signatures in responders and nonresponders of anti-TNF treatment, along with microbiota associations.

MATERIAL AND METHODS

Patients

The study population included 2 subject cohorts: 30 IBD patients and 10 healthy controls (HCs). The patient cohort included 30 anti-TNF-naïve IBD patients (22 CD patients, 8 UC patients) who required treatment with a TNF antagonist (infliximab, n = 24; adalimumab, n = 4; golimumab, n = 2). These patients were allowed to receive in parallel other disease-related drugs if there was no dose change 8 weeks before enrollment. Age younger than 18 years or older than 0 years, the presence of unclassified IBD, and malignancy were criteria of exclusion. Ten healthy volunteers of similar age and sex distribution to the IBD cohort served as controls.

The IBD diagnosis was based on standard criteria.[19] Infliximab was administered intravenously at a dose of 5 mg/kg at weeks 0, 2, 6, and every 8 weeks thereafter. Adalimumab was administered subcutaneously at a dose of 160 mg at week 0, 80 mg at week 2, and 40 mg every 2 weeks thereafter. Golimumab was administrated subcutaneously at a dose of 200 mg at week 0, 100 mg at week 2, and then every 4 weeks according to the patient's weight (100 mg or 50 mg in patients with more or less than 80 kg, respectively). Disease activities were determined using the Mayo scoring system,[20] the Harvey-Bradshaw Index (HBI) and C-reactive serum protein (CRP) levels, respectively, at various time points: baseline (before the first infusion or injection), the day before each subsequent drug administration, and week 12 after treatment, when appropriate. Ileocolonoscopy was performed at baseline and after 12 to 20 weeks of therapy to assess mucosal healing. Changes of clinical and endoscopic image compared with baseline were classified as responders or nonresponders to anti-TNF therapy as previously described.[21] Responders were defined as patients who accomplished a combination of clinical, endoscopical (absence of ulceration), and biological (normalization of serum) CRP levels after anti-TNF treatment. In contrast, patients without changes in clinical, endoscopical, and/or CRP levels after anti-TNF therapy were characterized as nonresponders. The study was approved by the institutional review board of the Medical School, National and Kapodistrian University of Athens in compliance with the Declaration of Helsinki. All participants gave written informed consent.

Quantitative Real-time Polymerase Chain Reaction

Total RNA from peripheral whole blood was extracted using QIAamp RNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). All samples were incubated with DNAse I (Qiagen, Germany) before cDNA synthesis. The RNA quality and concentration were calculated spectrophotometrically. Then 1 μg

TABLE 1. Type I and II Interferon Inducible Genes Primer Sequences for Gene Expression Analysis

Full Name	Primer	Accession Number	Forward Sequence	Reverse Sequence
Homo sapiens glyceraldehyde-3-phosphate dehydrogenase (GAPDH), mRNA	GAPDH	NM_002046	CAACGGATTTGGTCGTATT	GATGGCAACAATATCCACTT
Homo sapiens interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), mRNA	IFIT1	NM_001548	CTCCTTGGGTTCGTCTATAAATTG	AGTCAGCAGCCAGTCTCAG
Homo sapiens interferon-induced protein 44 (IFI44), mRNA	IFI-44	NM_006417	CTCGGTGGTTAGCAATTATTCCTC	AGCCCATAGCATTCGTCTCAG
Homo sapiens chemokine (C-X-C motif) ligand 9 (CXCL9)/ MIG-1, mRNA	CXCL9/ MIG-1	NM_002416	CATCATCTTGCTGGTTCTG	AGGATTGTAGGTGGATAGTC
Homo sapiens guanylate binding protein 1, interferon-inducible (GBP1), mRNA	GBP1	NM_002053	AGAATGAGAATGAGGTTGAGG	GTCCATCTGCTTCCAAGTC

of total RNA from each sample was reverse-transcribed using the Superscript III reverse transcriptase system (Invitrogen, Carlsbad, CA, USA) using oligo-dT primer (0.5 μM). Quantitative real-time polymerase chain reaction was performed using the IQ Bio-Rad SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). Primers specific for amplifying genes preferentially induced by type I IFNs were selected: interferon-induced protein with tetratricopeptide repeats 1 (IFIT-1), and interferon-induced protein 44 (IFI44). Primers specific for amplifying genes preferentially induced by type II INF were also selected: guanylate binding protein 1 (GBP-l) and chemokine (C-X-C motif) ligand 9 (CXCL9) were used as previously described.[8] As an internal control and normalization gene, the glyceraldehyde phosphate dehydrogenase (GAPDH) was used (Table 1). All reactions were performed in duplicate. Type I and II IFN scores were calculated as described by Kirou et al.[22] The

TABLE 2. Demographic and Clinical Characteristics of Patients and Healthy Control Sets Used

	Crohn's disease	Ulcerative colitis	Controls
	(n = 22)	(n = 8)	(n = 10)
Sex (Male/Female)	13/9	6/2	6/4
Age (years)			
Mean ± SD	37.94 ± 15.61	42.33 ± 13.53	41.22 ± 10.12
Disease Location			
Ileal disease	5		
Colonic disease	2		
Ileal and colonic disease	15		
Disease extend			
Orthritis		0	
Orthosigmoid		0	
Pancolitis		8	
Disease behaviour			
Nonstenosing/nonpenetrating	19		
Behaviour (B1)	0		
Fibro-stenosing (B2)	3		
Penetrating (B3)			
Reposnders/Nonresponders	12/10	4/4	
C-reactive protein (mg/dL, mean ± SD)			
Baseline			
Responders	3.63 ± 2.60	2.67 ± 1.88	
Nonresponders	4.75 ± 2.41	5.15 ± 2.34	
After treatment			
Responders	1.03 ± 0.68	0.54 ± 0.42	
Nonresponders	4.65 ± 2.82	3.54 ± 1.49	
Harvey-Bradshaw Index (HBI)			
Baseline			
Responders	7.80 ± 1.99		
Nonresponders	10.2 ± 5.11		
After treatment			
Responders	0.71 ± 0.75		
Nonresponders	9 ± 4.36		
Mayo Score			
Baseline			
Responders		9.20 ± 1.48	
Nonresponders		9 ± 2.83	
After treatment			
Responders		1 ± 1.20	
Nonresponders		10.5 ± 1.73	

mean and SD levels of each IFN inducible gene in the healthy sample group were used to standardize expression levels of each gene for each study subject. The standardized expression levels were then summed for each patient to provide an IFN type I expression score as the sum of each study subject's relative expression for each of 3 genes preferentially induced by type I IFN and for each of 2 genes preferentially induced by IFN type II, respectively, for the 2 groups (IBD and HC). Type I and II IFN scores were considered high if they exceeded the mean + 2 × standard deviation value of the relative control group.

Microbiome Analysis

The 16s rRNA data analysis was performed using QIIME2 version 2019.10[23] in DNA isolated from inflamed colonic mucosa biopsies. The preprocessing steps included demultiplexing with the default parameters of demux, quality control and denoising, and finally feature table and phylogenetic tree construction. Taxonomic classification was done using a classifier trained on the SILVA[24] r132 99% OTU data set, specifically for the 515/806 primers. Downstream analysis was done solely via the Calypso web platform v.8.84.[25] During quality filtering, all taxa with less than 0.01% relative abundance across all samples were removed, the top 3000 taxa based on mean abundance were included, and cyanobacteria and chloroplasts were excluded. Raw feature counts were transformed into relative abundance using total sum normalization (TSS) and SquareRoot (Hellinger) transformation. To identify correlations between microbial taxa and type I and II IFN readings, Spearman correlation and random forest regression were performed using the top 100 most abundant microbial taxa on the genus level.

Statistical Analysis

Statistical analysis on the type I and II IFN raw values was performed by Graph Pad PRISM. Pairwise comparisons were assessed using *t* tests (1-way ANOVA). We also performed multiple comparison procedures using the Tukey test. A statistical prediction model was employed by considering the IBD patients at baseline (n = 30) and dividing them into 2 groups: "controls" were responders to therapy (n = 16), and "cases" were nonresponders (n = 14). Using the IFN I and II scores as validators and the known outcome of response or nonresponse to treatment, we implemented a general linear regression model in R (glm function). This allowed us to establish the predictions of the effects of each IFN1 and IFN2 value but also their combination. Receiver operating characteristic (ROC) curves and the subsequent statistics from them were calculated using the reportROC package (https://cran.r-project.org/web/packages/reportROC/index.html).

RESULTS

Demographic and clinical characteristics of the patients are presented in Table 2. We included peripheral blood samples from 30 IBD patients and 10 HCs of similar age and sex distribution. To gain insight into the role of type I and II IFN pathways in IBD, composite type I and II IFN scores were quantified, as described previously. As shown in Figure 1, type I IFN score at baseline (before anti-TNF treatment) was higher in IBD patients compared with HCs (1.57 ± 0.2 vs 0.84 ± 0.06; $P = 0.04$), whereas type II IFN score did not considerably differ between the 2 groups (1.11 ± 0.24 vs 0.83 ± 0.11; $P = 0.49$). Of note, considerable heterogeneity among IBD patients was observed, with no differences detected between CD and UC patients.

Consequently, we aimed to analyze whether type I and/or II IFN scores could serve as predictive biomarkers of response to anti-TNF drugs among IBD patients. With this goal in mind, patients were classified into 2 groups: 16 IBD patients who responded to anti-TNF treatment and 14

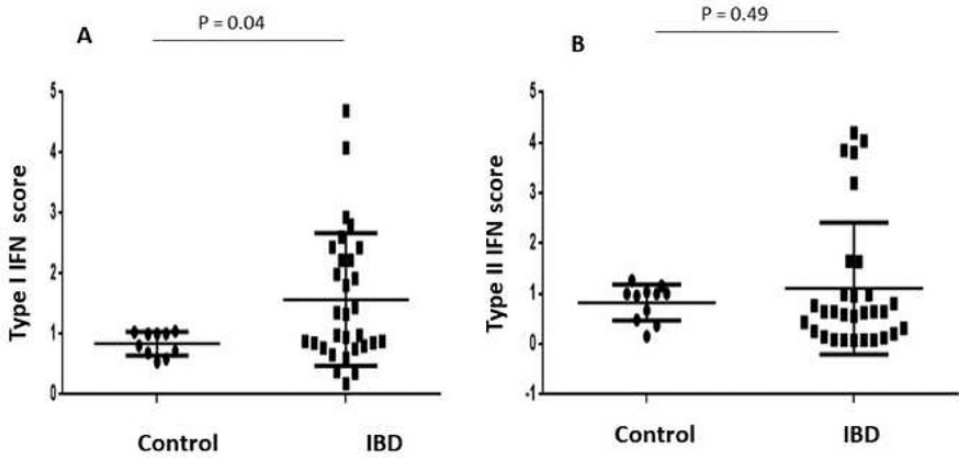

FIGURE 1. A, The levels of the IFN I score were compared between controls (n = 10) and IBD patients (n = 30) before anti-TNF therapy. B, The levels of the IFN II score were compared between controls (n = 10), and IBD patients (n = 30) before anti-TNF therapy. One-way ANOVA analysis was used for pairwise comparisons.

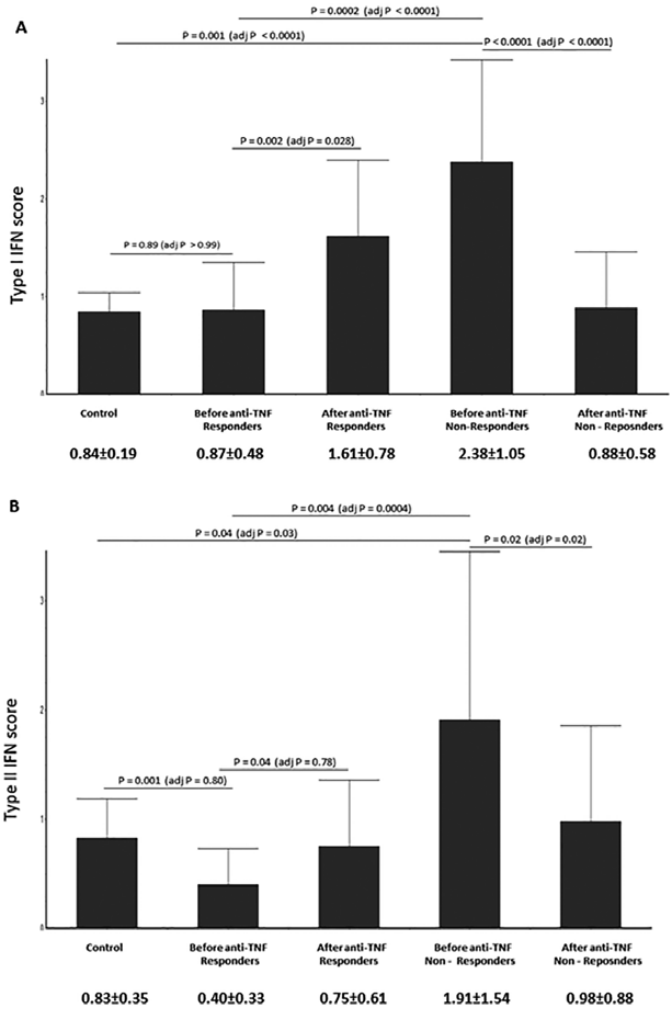

FIGURE 2. A, The levels of the IFN I score before and after anti-TNF therapy. B, The levels of the IFN II score and after anti-TNF therapy. Each column represents the mean values of each group (controls n = 10), anti-TNF responders before and after therapy (n = 16), anti-TNF nonresponder before and after therapy (n = 14) and the error bars the standard deviations. Actual levels ± SD are also presented. One-way ANOVA analysis was used for pairwise comparisons. Adjusted *P* values (adj P) after correction for multiple comparison by Tukey test are indicated in parenthesis.

nonresponders. For both responders and nonresponders, there were available samples at baseline and after treatment. Interestingly, anti-TNF responders exhibited baseline type I IFN scores compared with nonresponders (0.87 ± 0.48 vs 2.38 ± 1.05; $P = 0.0002$ [adjusted $P < 0.0001$]), which are similar scores in comparison with the HC group. Moreover, a significant increase in type I IFN score in anti-TNF responders was observed at 12 weeks after treatment (1.61 ± 0.78 vs 0.87 ± 0.48; $P = 0.002$ [adjusted $P = 0.028$]), together with a significant decrease in type IFN I score in nonresponders (2.38 ± 1.05 vs 0.88 ± 0.58; $P < 0.001$ [adjusted $P < 0.0001$]; Fig. 2A).

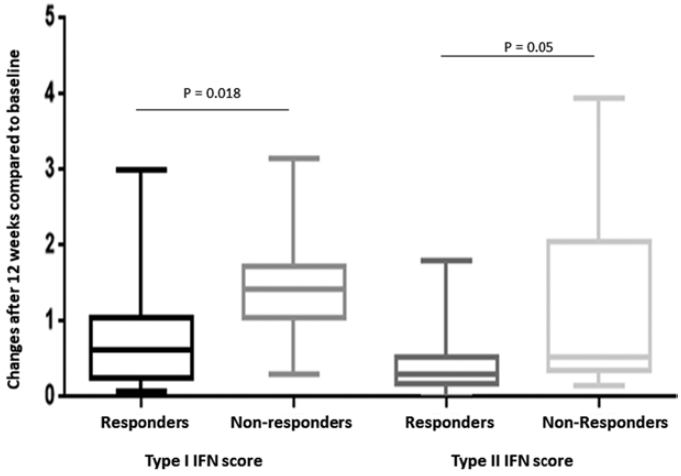

FIGURE 3. Differences at type I and II IFN scores between responders (n = 16) and nonresponders (n = 14; scores after 1 week of anti-TNF therapy, baseline). One-way ANOVA analysis was used for pairwise comparisons.

Regarding type II IFN scores as illustrated in Figure 2B, anti-TNF responders exhibited a lower baseline type II IFN score (0.40 ± 0.33) compared with both HC (0.83 ± 0.35) and nonresponders (1.91 ± 1.54; $P = 0.001$ [adjusted $P = 0.80$]; and $P = 0.004$ [adjusted $P = 0.0004$]), respectively. The latter displayed a significantly higher type II IFN score compared with HCs, ($P = 0.04$ [adjusted $P = 0.03$]). As observed for IFN I scores, an increase in type II IFN score in anti-TNF responders was observed 12 weeks after treatment compared with baseline (0.40± 0.33 vs 0.75 ± 0.61; $P = 0.04$ [adjusted $P = 0.03$]), with a significant decrease in nonresponders (1.91 ± 1.54 vs 0.98 ± 0.88; $P = 0.02$ [adjusted $P = 0.02$]).

Comparing the changes in type I and II IFN scores between responders and nonresponders after therapy, we observed that in nonresponders changes in both type I and II IFN scores were increased compared with responders ($P = 0.018$ and $P = 0.05$, respectively; Fig. 3).

When patients were divided into distinct groups according to low and high type I and II IFN scores at baseline, using as cutoff value the median of controls (0.9 for type I, 0.83 for type II IFN), increased rates of nonresponders displayed high type I and II IFN scores compared with nonresponders (100% vs 25% for type I IFN scores, $P < 0.0001$; and 50% vs 12.5% for type II IFN scores, $P = 0.054$, respectively).

The generalized linear model employed to test the predictive power of type I and II IFN scores regarding response to treatment returned ROC curves with a 80%–93% accuracy (Fig. 4). Regarding IFN type I, the area under the curve (AUC) is 0.95, sensitivity (SEN) of 93%, specificity (SPE) of 88%, a positive predictive value (PPV) of 87%, and a negative predictive value of 93%. Interferon type II shows an AUC of 87%, with 85.7% SEN, 75% SPE, a PPV of 75%, and an NPV of 85.7%. Finally, their combination yields a ROC curve with an

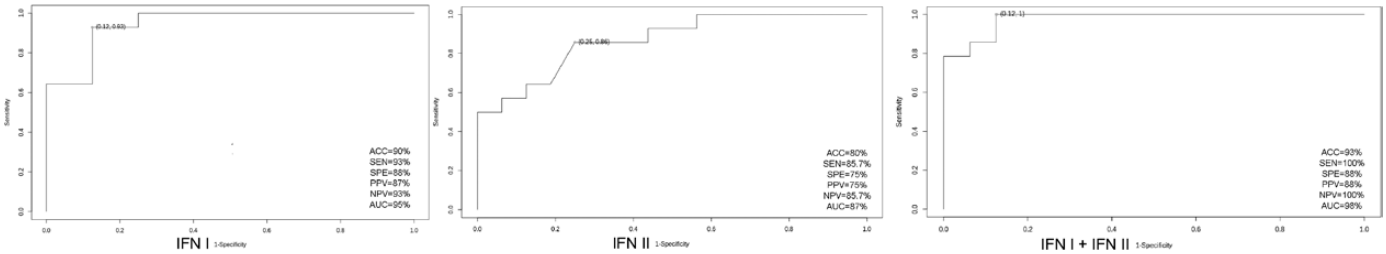

FIGURE 4. ROC curves and their respective statistics when using the generalized linear models using as predictor of response to treatment the IFN1 score (Prediction~IFN1), the IFN2 score (Prediction~IFN2) and their combination (Prediction~IFN1+IFN2). These tests were performed using patient data at baseline (responders n = 16, nonresponders n = 14).

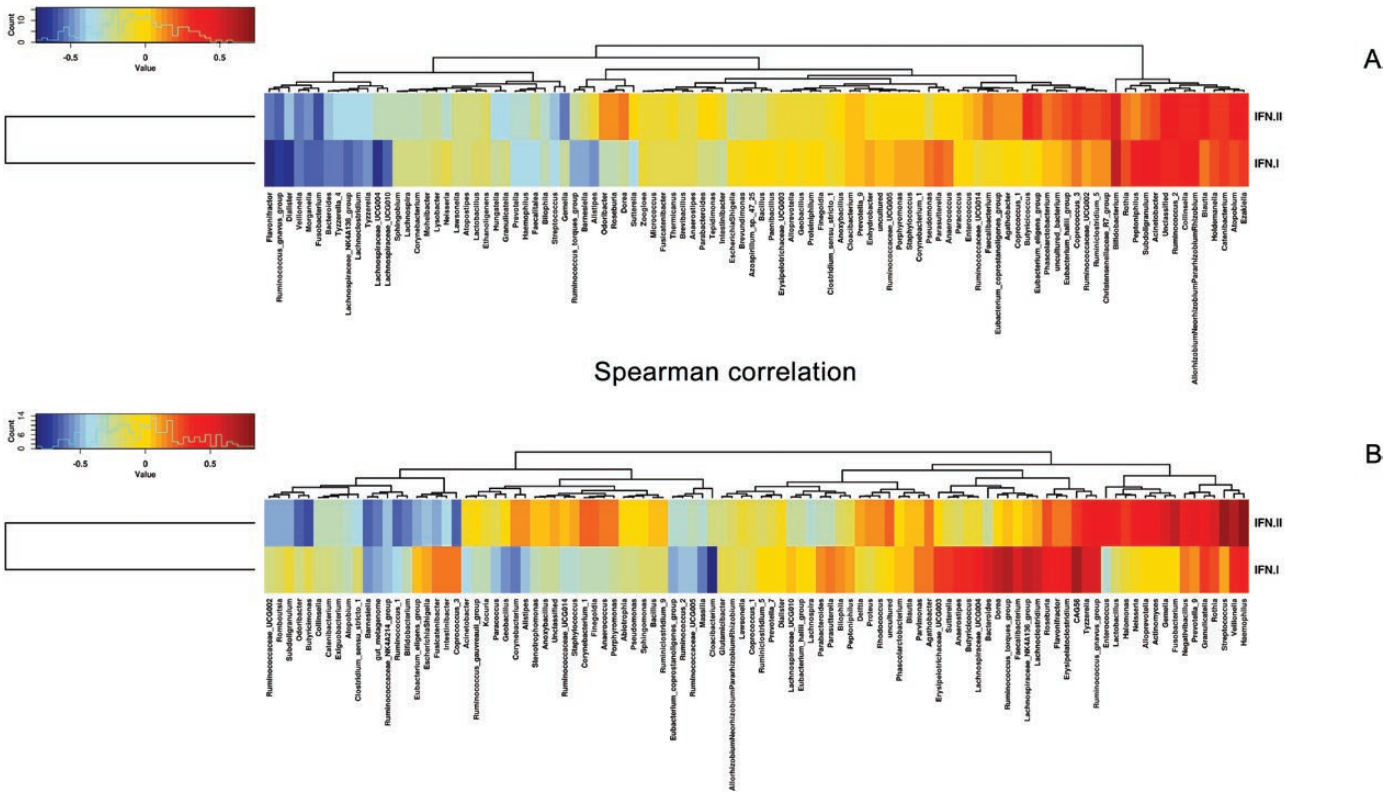

FIGURE 5. Spearman correlation between IFN I and II levels (responders n = 7, nonresponders n = 7) and microbial genera in (A) before and (B) after treatment

AUC of 98%, 100% SEN, 88% SPE, 88% PPV, and an NPV of 100%.

Given that microbiota can regulate IFNGs, we used the microbiota data that we had from our previous study[26] for 14 of the IBD patients (10 CD and 4 UC) and 9 controls to identify IFN type I and II differences in responders and nonresponders of anti-TNF treatment, along with their associated microbial taxa. The microbiome analysis was performed on IBD patients before and after treatment but also between responders and nonresponders at baseline. Spearman correlation heatmaps on patients before (Fig. 5A) and after (Fig. 5B) treatment showcase several microbial genera and their inverse or direct correlations to IFN type I and II gene expression levels. Notably before treatment, most taxa seem to have the same correlation (positive or negative) to both types of IFN, perhaps with *Odoribacter, Roseburia*, and *Dorea* showing an inverse pattern (negative correlation with IFN I and positive with IFN II). After treatment though, taxa like *Coprococcus, Enterococcus, Corynebacterium, Alistipes Parabacteroides, Bacteroides*, and *Intestinibacter* seem to have inverse correlations to IFN I and II, whereas, *Roseburia, CAG56, Tyzerella, Ruminococcus gnavus, Negativibacillus*, and *Veillonella* seem to be in sync in both IFN types. Regarding responders and nonresponders to treatment, Figure 6 illustrates an inverse pattern of 2 main clusters highlighted by hierarchical clustering. The first cluster contains nonresponders and IFN

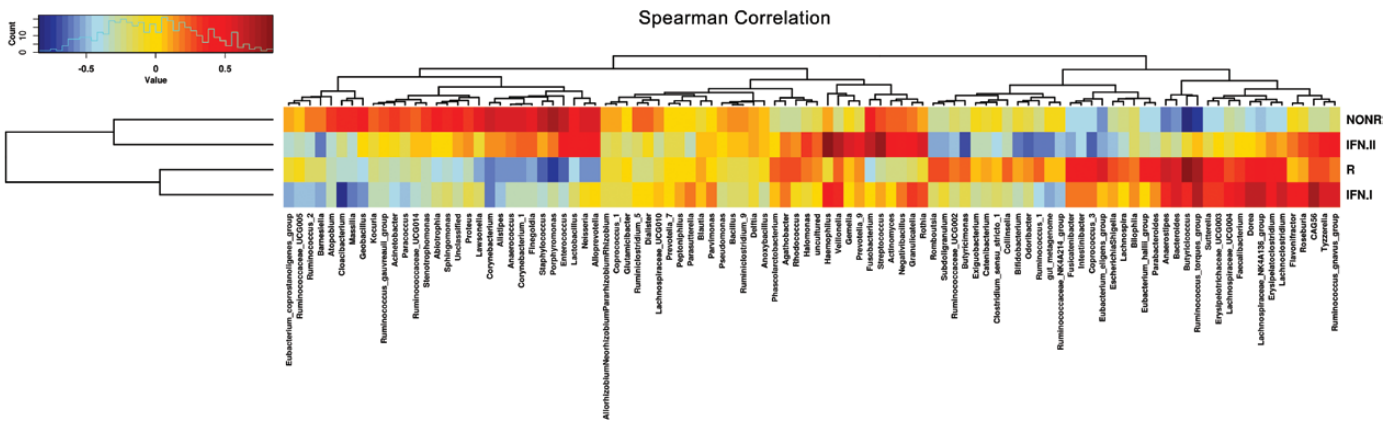

FIGURE 6. Spearman correlation of IFN II responders (n = 7) and nonresponders (n = 7) highlights clusters of similar microbial composition between IFN I responders and IFN II nonresponders.

II levels, whereas the second includes responders and IFN I levels. Overall, these data support the idea that genera that directly correlate to changes in IFN II also correlate with nonresponders, but the same genera show inverse correlations to IFN I and responders.

When the 2 types of IFN were used as study variables in random forest regression analysis both before and after treatment and in responders and nonresponders, several genera are highlighted as highly important for the variable. Figure 7 shows the microbial genera that can best explain type I and II IFN scores before and after treatment; however, Figure 8 shows the microbial genera that can best explain type I and II IFN scores in responders and nonresponders. In all cases the bacterial genera associated with IFN levels seems to change significantly. For IFN I before treatment, *Lachnospira*, *Alloprevotella*, *Lachnoclostridium*, *Fusicatenibacter*, *Bacteroides*, and others seem to explain variation. But after treatment, *Escherichia/Shigella* (either one or the other genus, there is a classification dispute here[27]), *Atopobium*, *Ruminococcaceae_UCG014*, *Romboutsia*, *Anaerococcus*, and others explain the IFN I levels better. As for genera that best explain variation of IFN II levels, regarding treatment, the analysis highlights *Parasutterella*, *Moheibacter*, *Granulicatella*, *Brevundimonas*, and *Coprococcus_3* among others before treatment and *Kocuria*, *Faecalibacterium*, *Parasuterella*, *Ruminococcaceae_UCG005*, and *Finegoldia* after treatment. Between responders and nonresponders to treatment, *Micrococcus*, *Dialister*, *Glutamicibacter*, *Coprococcus_3*, *Geobacillus*, and *Negativibacillus* seem to factor in IFN I levels, whereas *Barnesiella*, *Bifidobacterium*, *Ruminococcus_gnavus*, *Ruminococcaceae_UCG005*, and *Clostridium_senso_stricto* seem to better explain IFN II levels in responders. For the nonresponders group, IFN I can be best predicted by *Gardnerella*, *Exiguobacterium*, *Lachnospira*, *Parabacteroides*, and *Subdoligranulum* and IFN II by *Kocuria, Ruminoclostridium_9, Haemophilus, Coprococcus_1*, and *Bifidobacterium.*

DISCUSSION

Undoubtedly, biological therapies based on anti-TNF agents have revolutionized the treatment of IBD. Nevertheless, the responsiveness to therapy is greatly variable among IBD patients. The failure of treatment in nonresponders coupled with the substantial cost that associates with biological therapies necessitate the identification of predictors of IFX response.[2, 3] It has been suggested that type I and II IFN IFNGs are upregulated in pretreatment peripheral blood samples of patients with autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus[17, 28, 29] and that anti-TNF therapeutic agents modulate the expression of IFNGs in a heterogeneous way.[30] In the present study, we found that type I IFNGs—but not type II—are upregulated in IBD peripheral blood compared with levels observed in HCs. Even if it has been supported that type I IFN inhibits inflammatory responses and possibly confer protection against DSS colitis in mice,[11, 15] our results are in agreement with the recent finding by Samie et al[31] supporting a higher expression of type I IFNGs in inflamed colonic biopsies of IBD patients compared with uninflamed areas from those patients or HCs.

Several studies support that type I and II IFN scores are associated with responses to anti-TNF treatment; however, the results are contradictory among the studies. A number of studies on rheumatoid arthritis reported that increased levels of plasma type I IFN activity are linked with favorable responses to anti-TNF therapy,[17, 18] but other studies supported the opposite.[30, 32] In the present study on IBD patients, we found that low type I IFN score at baseline is related with response to anti-TNF therapy, whereas patients with high type I IFN score failed to respond to anti-TNF drugs. The same association was also observed for type II IFN scores.

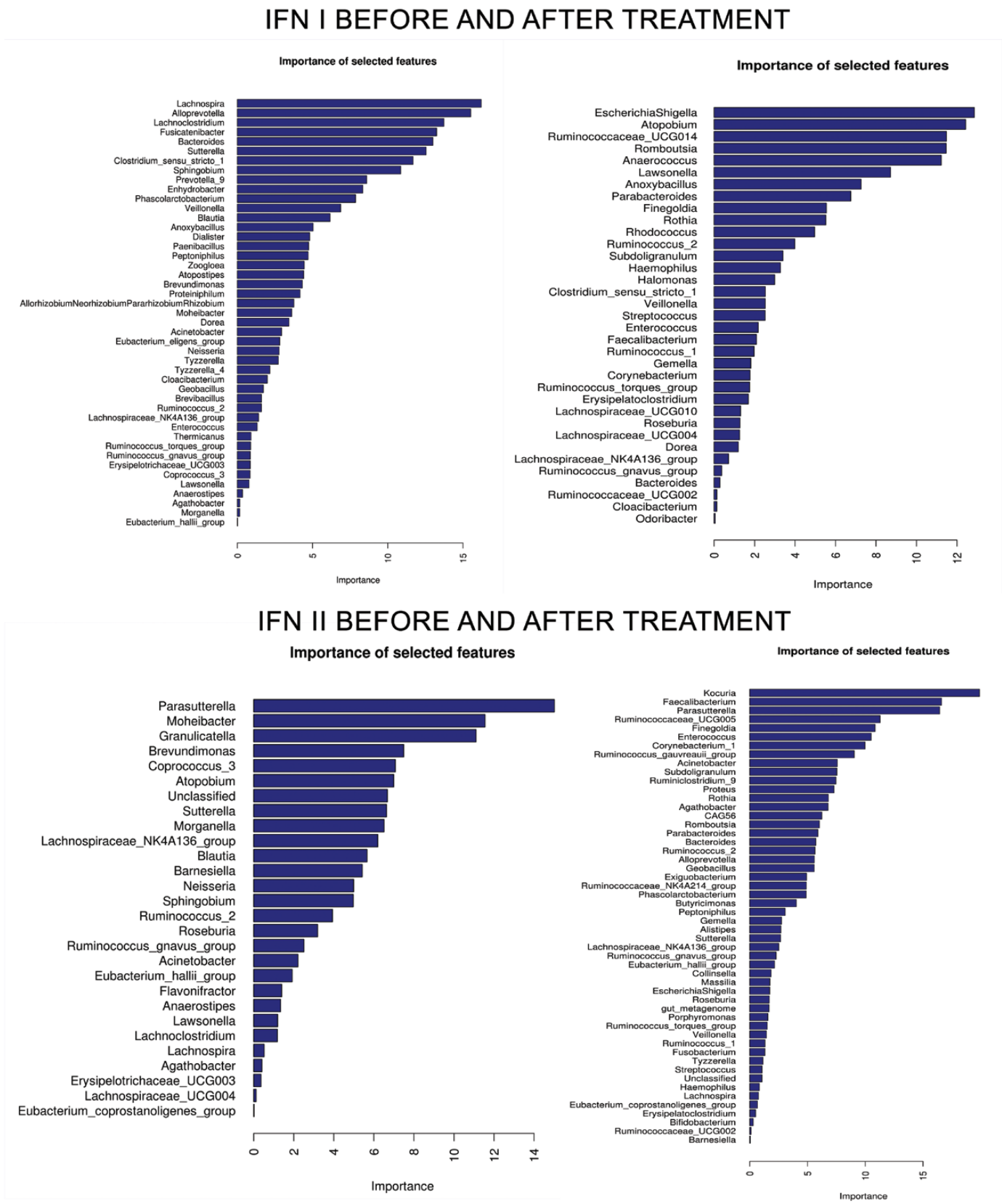

FIGURE 7. Random forest regression analysis highlights microbial genera that better explain IFN I and II levels before (n = 14) and after treatment (n = 14).

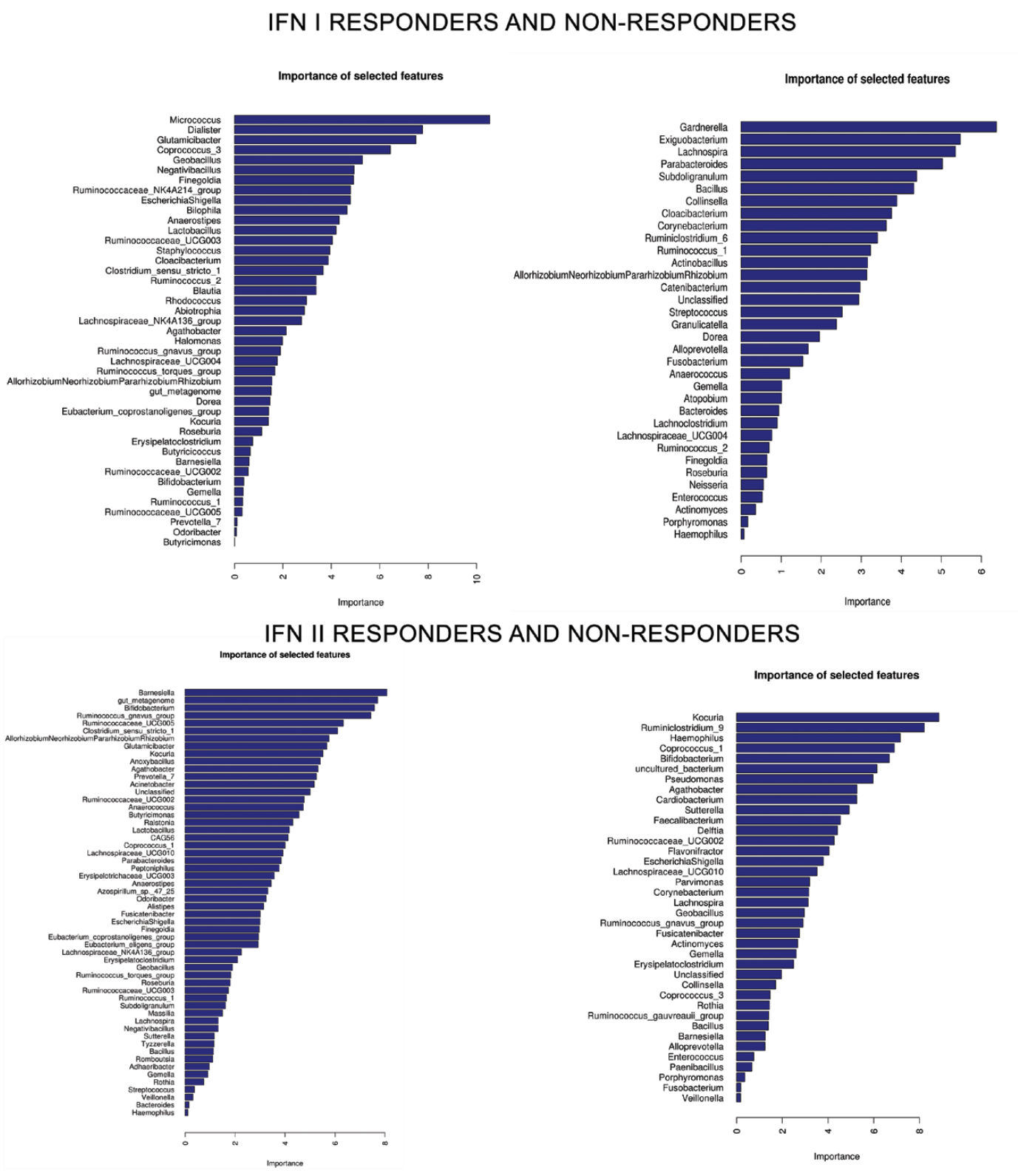

FIGURE 8. Random forest regression analysis highlights microbial genera that better explain IFN I and II levels in responders (n = 7) and nonresponders (n = 7).

Regarding type I IFN signature, our results are in agreement with the recent findings by Samie et al;[31] they also observed higher expression of the IFNGs signature in pretreatment colonic biopsies of patients with IBD who would fail to respond to infliximab and significantly lower expression of the IFNGs signature in responders. Regarding type II IFN score, there are not data available so far. Twelve weeks after treatment, a significant increase in type I and type II IFN scores was found in anti-TNF responders and a decrease in nonresponders. However, Samie et al[31] did not observe a decrease in type I IFN signature after treatment in nonresponders, but they found that nonresponders sustained elevated expression of the IFNGs signature regardless of treatment. This difference might be due to different samples used because Samie et al used colonic biopsies from inflamed areas of the patients, and the cellular immunology that leads to various ratios of type I and II IFNs in circulation is not well defined.[32] Together, our findings reveal that type I and type II IFN scores have good predictive value for the anti-TNF response and can define therapeutically related subsets of patients with IBD. Based on the ROC analysis especially, IFN type I shows greater predictive potential than IFN type II, but their combination seems to be the most accurate biomarker.

Growing bodies of evidence support that type I IFNs contribute to immune defenses against gut pathogens and intestinal inflammation.[15, 33–35] Furthermore, autophagy proteins are necessary in the gut epithelium to prevent a spontaneous type I IFN response to the gut microbiota.[36] Alterations to the gut microbiota and autophagy have been linked to IBD pathogenesis.[26, 38, 39] Furthermore, alterations to the gut microbiota are associated with response of the IBD patients to anti-TNF biological therapy.[26, 40] On this basis, under the hypothesis that gut microbiota might be linked to type I and II signature, we examined type I and II IFN scores in responders and nonresponders of anti-TNF treatment, along with its microbiota associations. Our results reveal that distinct microbiota taxa relate to type I and type II IFN signatures at baseline, whereas after treatment, specific taxa were found to have inverse correlations to type I and type II IFN signatures. The 2 types of IFN have distinct activities and may act complementary to each other as innate host defense factors. Type II IFN can possibly activate macrophages to produce proinflammatory cytokines against bacterial, fungal, and protozoan pathogens. On the other hand, type I IFN has recently been reported in studies of conserved bacterial products, such as lipopolysaccharides, which can activate distinct signal transduction pathways that merge, after pathogen recognition, with the pathways that are activated by viruses and lead to increased levels of type I IFN production.[41] Interestingly, regarding anti-TNF response, we observed that the genera that correlate with type II IFN signature changes are correlated with nonresponders, and the same genera show inverse correlations to type I IFN signature and responders.

We understand that our study has certain limitations. The total number of patients is relatively small, and in relation to this, we analyzed patients with UC and CD together so as to have an adequate sample size for statistical analysis. Finally, our patients were treated with both intravenous and subcutaneous anti-TNF agents. These shortcomings notwithstanding, we believe that our findings provide a significant starting point to understand the importance of IFN signatures as biomarkers in IBD, which need to be further confirmed in larger, prospective studies.

Our data support a role for type I and II IFNs in the pathogenesis of IBD, possibly through complex interactions with host microbiota and the prediction of response to anti-TNF agents, providing a tool for practicing clinicians. Larger multi-effort studies are required to validate these findings.

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