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Altered STAT4 isoform expression in patients with inflammatory bowel disease

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Abstract

BACKGROUND & AIMS—Crohn's disease (CD) and ulcerative colitis (UC) are major forms of inflammatory bowel disease (IBD) and pathogenesis involves a complex interplay between genetic, environmental and immunological factors. We evaluated isoform expression of the IL-12-activated transcription factor STAT4 in children with CD and UC.

METHODS—We performed a study where we collected biopsy samples from both newly diagnosed CD and UC patients. We further collected blood samples from newly diagnosed CD and UC patients as well as patients who had a flare-up after being in clinical remission, and examined the ratios of STAT4β/STAT4α mRNA. In addition to STAT4 isoforms we measured the expression of the cytokines TNFα, IFNγ, GM-CSF and IL-17 using PCR of biopsy samples and multiplex analysis of patient serum samples.

RESULTS—Ratios of STAT4β/STAT4α were increased in specific GI tract segments in both CD and UC patients that correlate with location and severity of inflammation. In contrast, we did not observe changes in STAT4β/STAT4α ratios in biopsy specimens from eosinophilic esophagitis patients. We also observed increased STAT4β/STAT4α ratios in the peripheral blood mononuclear cells of UC and CD patients, compared to healthy controls. Ratios were normalized after patient treatment with steroids.

CONCLUSIONS—Collectively, these data indicate that STAT4 isoforms could be an important non-invasive biomarker in the diagnosis and treatment of IBD, and that expression of these isoforms might provide further insight into the pathogenesis of IBD.

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Keywords

STAT4 isoforms; IBD; biomarker

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are two predominant forms of inflammatory bowel disease (IBD) in humans, characterized by chronic inflammation of the gastrointestinal tract. CD mainly affects the ileum and colon but can involve any region of the gastrointestinal tract in a discontinuous fashion. In contrast, inflammation in UC involves colon, typically starting distally at the rectum and extending proximally in a continuous manner for a variable distance. IBD may present at any age although it occurs primarily during the second and third decades of life, especially during adolescence [1–3]. IBD symptoms include diarrhea, severe abdominal pain, gastrointestinal bleeding, weight loss and fatigue, all of which may substantially affect patient's quality of life and account for considerable costs to the health care system [4–6]. The incidence of IBD is increasing worldwide, with the age of presentation progressively decreasing. [7–10].

The pathogenic mechanisms of IBD are unclear although both genetic and environmental factors have major roles. Available evidence suggests that in genetically susceptible individuals, a dysregulated mucosal immune response against antigens from normal gut flora triggers disease. This uncontrolled response results in excessive proinflammatory cytokine accumulation leading to mucosal inflammation. T cells play a crucial role in mediating mucosal inflammation; Th1 and Th17 cells mediate CD whereas UC exhibits Th2 and Th17 characteristics [11–14]. Patients with CD show increased production of IFNγ, a Th1 cell associated cytokine, from lamina propria and lymph node T cells. Furthermore, lamina propria T cells from CD patients exhibit increased expression of IL12Rβ2, a Th1 cell surface receptor [15–17]. In contrast, lamina propria T cells from UC patients have been shown to produce IL-5 and IL-13 [11, 18, 19]. More recently, IL-9, a Th9 cytokine, was shown to be involved in pathogenesis of UC [20, 21].

The involvement of Th17 cells in both forms of disease is complicated by pleiotropic effects of the cytokines they produce. Several groups have reported a role of cytokines associated with the Th17 subset in IBD, including increases of IL-17A, IL-17F, IL-22, IL-26, and CCL20 in samples from patients with active disease [13, 22–27]. However, the effects of IL-17A in disease are multi-faceted. Hueber *et al* showed that blocking IL-17A by secukinumab (anti-IL-17A monoclonal antibody) had no beneficial effects in patients with moderate or severe Crohn's disease [28]. Friedrich *et al* showed that psoriatic skin of anti-TNF treated patients had significant increases in epidermal IL-36γ and IL-17A levels, suggesting a crucial role for this cytokine pair in the pathogenesis of anti-TNF induced skin inflammation [29]. However, in a murine T cell transfer model of colitis, protective effects of IL-17A were demonstrated [30]. Thus, the effects of Th17 cytokines in the development of IBD are complex and likely vary with the amount of inflammation.

STAT4 (Signal transducer and activator of transcription 4) is predominantly expressed in hematopoietic cells, is critical for the development of Th1 cells, promotes IL-17 production

from Th17 cells and also plays an essential role in various immune-mediated diseases [31–36]. STAT4 is predominantly activated by IL-12 and to a lesser extent by type I interferons and IL-23 [31]. Genetic association studies have shown that the *STAT4* gene is significantly associated with IBD in Spanish and Caucasian populations [37–39]. STAT4 is constitutively expressed in intestinal T cells of CD patients and there are increased levels of phosphorylated STAT4 in the mucosal cells of UC patients [35, 36]. Increased mucosal expression of STAT4 has also been reported in pediatric UC but no significant difference was observed in pediatric CD patients [40]. In mouse models of IBD, STAT4 is required for the development of disease, and transgenic expression of STAT4 promotes disease [41–44]. Thus, evidence suggests a mechanistic link between STAT4 and IBD.

STAT4 is expressed as two isoforms, a full-length STAT4α, and a shorter form termed as STAT4β that lacks the C-terminal transactivation domain [45]. The beta isoform is generated from an mRNA where the last intron of the STAT4 mRNA is not spliced out, followed by cleavage and polyadenylation of transcript proximal to the final exon encoding the transactivation domain (Fig. 1). The protein is translated by read-through of the final intron leading to generation of STAT4 lacking the C-terminal domain but containing a unique seven amino acid tag [45]. The beta isoform is generally expressed in lower amounts than the alpha isoform, but is activated for a longer period of time following IL-12 stimulation [44, 45]. Using a transfer colitis model, we demonstrated that although both isoforms mediate IBD, STAT4β promotes more severe colonic inflammation and tissue destruction that correlates with TNF-α and GM-CSF expression [44]. The aim of this study was to explore whether STAT4 isoforms are differentially expressed in the gut and PBMCs of pediatric CD and UC patients and if this differential expression correlates with disease severity.

Materials and Methods

Study Subjects and Sample collection

Our study focused on four groups of patients; new CD patients, new UC patients, and established CD or UC patients that had disease recurrence. Inclusion criteria for patients were a new diagnosis of CD or UC where the physician had not initiated yet any treatment (for new patients) or a recurrence of disease (for established disease). Patients were above age 7 and under age 18 and had received a clinical diagnosis in the past 21 days. Approximately 4 ml blood was drawn from newly diagnosed CD or UC patients who had yet to receive any treatment on their first visit to the clinic and also on their 2nd visit, two weeks following corticosteroid treatment. For established CD or UC patients, blood was drawn on their initial visit to the clinic as well as two weeks after their initial infliximab treatment. Infliximab is a recombinant chimeric immunoglobulin G monoclonal antibody that neutralizes the biologic activity of TNFα and is approved for treatment of pediatric CD and UC patients[46]. Infliximab dosage given to these pediatric patients was 5 mg/kg/dose. We also enrolled 12 healthy volunteers in our study that did not suffer from any chronic or acute inflammatory disease and the control patients indicated in Figures 2 and 3 are the same population. The clinical profiles of patients are listed in Table 1 and more detailed clinical descriptions including Paris classifications is shown in Supplementary Table 1 [47].

Mucosal biopsy samples from new patients were obtained from esophagus, stomach, duodenum, terminal ileum, colon, cecum and rectum in newly diagnosed UC (N=5) and CD (N=9). Biopsies from control subjects were obtained from individuals undergoing colonoscopy but had no disease upon pathological examination (N=5) and were a separate group from the PBMC controls described above. Biopsies were immediately stored at −80 °C until processing.

Esophageal biopsies were obtained from children with EoE (diagnosed as ≥15 eosinophils per hpf on esophageal biopsies in absence of gastroesophageal reflux and/or lack of response to proton-pump inhibitor therapy). EoE patients (n=14) had an average eosinophil count per hpf of 55; were 45.5% male and had an average age of 12.8 years (range, 4–17.5). Matched controls (n=13) did not have eosinophils in biopsies as part of a clinical diagnosis, were 50% male, and had an average age of 11.9 (range 4–17.5). Esophageal biopsies from children with EoE and corresponding age-matched healthy controls were processed as described below.

Ethical considerations

The IRB of Indiana University approved the study and informed consent for participation in this study was obtained from parents of all the children enrolled in the study.

Isolation of peripheral blood mononuclear cells

Blood samples were obtained from new patients and patients with recurrent disease before and after treatment. Peripheral blood mononuclear cells (PBMCs) were isolated from blood using a Ficoll-paque Plus (GE Healthcare). PBMCs were washed by gently mixing the white interphase layer with sterile phosphate buffered saline. Cells were counted by trypan blue staining and were stored in DMSO-freezing media in liquid nitrogen.

Cytokine measurement

To detect multiple cytokine levels in serum samples from children with IBD, we performed the Multiplex Bead Immunoassay as per manufacturer’s instructions (Millipore, Saint Charles, Mo) using a Luminex 200 instrument (Luminex orporation, Austin, Texas). Cytokine concentrations were calculated using Bio-plex manager 2.3 software with a five parameter curve-fitting algorithm applied for standard curve calculations. The minimal cytokine detection limit is 3.2 pg/ml; the frequency of non-detection was zero for all cytokines.

RNA Extraction, cDNA synthesis and Quantitative RT-PCR

RNA was extracted from frozen tissue samples of CD, UC patients or normal controls as well as from PBMCs using Trizol reagent according to manufacturer’s instructions (Invitrogen, CA). 2ug of RNA per sample were reverse-transcribed into cDNA. Expression of STAT4 isoforms and GM-CSF was evaluated using Sybr Green based RT-PCR. Primer sequences were as follows: Stat4 alpha forward 5`- TAT CCT GAC ATT CCC AAA GAC -3` reverse 5`- CTC TCA ACA CCG CAT ACA CAC -3`; Stat4 beta forward 5` - TAT CCT GAC ATT CCC AAA GAC -3` reverse 58 GAC TTA CTA TGT CAG GAA CTC -3`. TNFα, IFNγ, IL-17 and STAT4 were analyzed using commercially available TaqMan

probes with an ABI PRISM 7500 Sequence Detection System (Applied Biosystems). Expression levels of each gene were normalized to *GAPDH*.

Statistical Analysis

Statistical analyses were performed using the nonparametric Kruskal-Wallis test and the Mann-Whitney U-test in the GraphPad Prism. Results were considered as statistically significant when p-values were ≤ 0.05.

Results

STAT4β/α mRNA ratios are significantly increased in CD patient biopsies

To determine if the expression of STAT4 isoforms is altered in patients with IBD, we used a quantitative PCR approach to distinguish between the alpha and beta transcripts (Fig. 1). We first analyzed biopsy tissue from newly diagnosed pediatric CD patients and observed increased expression levels of *STAT4* mRNA in ileum and colon regions of the gastrointestinal tract when compared with healthy controls and other regions of the GI tract (Fig. 2A). We also found that STAT4β/α mRNA ratios were significantly increased in CD patients when compared with healthy controls, specifically in the duodenum, ileum, cecum and colon, regions of the gut that are most affected by inflammation (Fig. 2B). The smaller magnitude of increase of STAT4β/α mRNA ratios in the cecum corresponded to lower amounts of total STAT4 in that segment (Fig. 2A–B). We further examined the expression of T cell-produced cytokines that have been linked to CD. *TNFA* showed a trend towards increased expression in the colon but was not significantly increased (Fig. 2C). *IFNG* expression was elevated in ileum and cecum regions of the gut, but was only significantly increased in colon and rectum (Fig. 2D). *IL17A* expression was significantly increased in the cecum and colon, and surprisingly in the esophagus (Fig. 2E). In contrast, although there was increased expression in some patient samples, we did not observe a statistically significant increase in the expression of granulocyte macrophage-colony stimulating factor (*CSF2*) (Fig. 2F).

STAT4β/α mRNA ratios are significantly increased in UC patient biopsies

We next assessed the biopsies of UC patients and healthy controls by quantitative RT-PCR for total STAT4 and STAT4β/α mRNA ratios. Although there was no statistically significant difference in the total STAT4 mRNA expression, we did observe significantly increased STAT4β/α mRNA ratios in the cecum, colon and rectum (Fig. 3A–B). *TNFA*, *CSF2*, and *IFNG* expression were not significantly increased in UC patients, consistent with limited Th1 cell involvement in UC (Fig. 3C, D, F). In contrast, *IL17A* expression was significantly increased in the rectum of the UC patients when compared with healthy controls, and as in the CD samples, *IL17A* was also significantly increased in esophagus (Fig. 3E).

STAT4β/α mRNA ratios are not increased in EoE patient biopsies

To determine if increased STAT4β/α mRNA ratios were a common feature to any inflammatory disease in the GI tract, we examined biopsy samples from children with eosinophilic esophagitis (EoE) and controls that were examined for reflux symptoms but did

not have esophageal inflammation. In contrast to CD and UC, EoE is characterized by allergic inflammation and increased expression of Th2 cytokines [48]. There was no significant difference in the STAT4β/α mRNA ratios between controls and patients with EoE (Supplementary Figure 1). This suggests that there is specificity for increases in STAT4β/α mRNA ratios among types of gastrointestinal inflammation.

Human peripheral blood mononuclear cells express increased STAT4 isoform ratios

Having observed increased STAT4β/α mRNA ratios in biopsy samples from CD and UC patients, we next wanted to determine if altered ratios were also observed in immune cells in the periphery. To test this, we purified PBMC RNA isolated from newly diagnosed CD and UC patients, and age-matched control groups. Newly diagnosed CD patients had modestly but significantly increased expression of total *STAT4* as well as had significantly more STAT4β/α mRNA ratios compared to PBMCs from age-matched controls without any diagnosed autoimmune disease (Fig. 4A–B). However, newly diagnosed UC patients had only significantly elevated STAT4β/α mRNA ratios and had no significant difference in the levels of total *STAT4* mRNA (Fig. 4A–B). Importantly, when newly diagnosed CD or UC patients were treated with steroids for a two-week period, STAT4β/α mRNA ratios were reduced in parallel to a decreased Pediatric Crohn's Disease Activity Index (PCDAI) or UC index (Fig. 4B–D).

In patients with established disease tested before and after infliximab treatment following a recurrence in inflammation, we observed that total *STAT4* levels were elevated at the first visit in CD patients though this was not correlated with an increase in STAT4β/α mRNA ratios, and that treatment did not significantly alter ratios (Fig. 4E–F). In contrast, STAT4 mRNA in UC patients was not altered, but STAT4β/α mRNA ratios were elevated and treatment with infliximab significantly decreased ratios to levels observed in control samples (Fig. 4E–F).

Serum pro-inflammatory cytokine levels in CD and UC patients

To determine if altered STAT4β/α mRNA ratios correlated with changes in serum cytokine concentrations, we analyzed serum from control, CD and UC patients. Newly diagnosed CD patients had significant increases in TNF-α (Fig. 5A). Recurrent CD patients had increases in TNF-α, IFN-γ, GM-CSF, and although the groups were not different, some recurrent CD patients had increased IL-17A concentrations (Fig. 5A–D). Newly diagnosed UC patients had increased TNF-α and IFN-γ, whereas recurrent UC patients did not have significant increases in any cytokine concentrations (Fig. 5). We did not observe any significant differences in IL-12 levels among the various groups, although some individual patients clearly had increased IL-12 compared to controls (Fig. 5E).

Correlation of the STAT4β/α ratio with disease severity

To determine if there was a relationship between the STAT4β/α mRNA ratios and disease score, we tested the correlation in data from biopsy samples. We observed that STAT4β/α mRNA ratios from the colon (Fig. 2) were positively correlated with patient scores for the PCDAI (r^2=0.53, p=0.027) (Fig. 6). However, we did not observe a significant association of STAT4β/α mRNA ratios in tissue from duodenum, ileum, or cecum, despite the

significant elevation of STAT4β/α mRNA ratios at those sites (Fig. 2). We saw a similar relationship between STAT4β/α mRNA ratios determined from PBMCs (Fig. 4) and PCDAI (r^2=0.41, p=0.05).

We did not observe any significant association between any of the cytokine mRNA amounts and PCDAI, nor was there an association between STAT4β/α mRNA ratios and cytokine mRNA. Despite there being increased STAT4β/α mRNA ratios in UC patient biopsies and PBMCs, we did not observe any significant correlation between STAT4β/α mRNA ratios and UC disease severity.

DISCUSSION

Crohn's disease (CD) and ulcerative colitis (UC) are the two most common immune-mediated gastrointestinal diseases and share overlapping genetic risk variants. Despite significant progress in the field, much of the etiology of IBD still remains unresolved. Furthermore not all patients respond to the current therapeutic molecules. Incidence of IBD, particularly pediatric IBD is increasing; hence there is an urgent need for new therapeutic approaches as well as new diagnostic markers. In the current study, we investigated the expression of STAT4 isoforms in CD and UC. We have previously demonstrated in our murine model that although both STAT4α and STAT4β were able to promote inflammation, STAT4β was more efficient in promoting inflammation[44]. Our results here demonstrate that STAT4β/STAT4α mRNA ratios are significantly increased in biopsies and PBMCs from both CD and UC and use of ratios in PBMCs suggests a novel non-invasive marker for diagnosis of disease severity.

It is still unclear whether the alteration in STAT4β/α mRNA ratios is a result of the IBD, or if altered ratios actually play a role in promoting inflammation. From mouse models of colitis and experimental autoimmune encephalomyelitis, STAT4β is more proinflammatory than the STAT4α isoform [44, 49]. Although T cells expressing either STAT4α or STAT4β had similar IFN-γ production, T cells expressing only STAT4β had greater production of GM-CSF and TNF-α [44]. T cells expressing only STAT4α had increased IL-10 production, correlating with diminished inflammatory potential [49]. The functional link between STAT4 isoform expression and altered inflammatory potential would suggest that altered ratios would lead to altered function of cells in vivo. A causative link between increased STAT4β/α mRNA ratios in biopsies, or from PBMC RNA, and disease is further supported by the correlation of ratios with disease severity, at least in CD patients upon initial diagnosis.

The ability of steroid or infliximab treatment to decrease the STAT4β/α mRNA ratios in PBMCs suggests that the inflammatory environment increases STAT4β/α mRNA ratios. In some preliminary experiments, we observed that stimulation of PBMCs with various cytokines present in patients with autoimmune disease leads to altered STAT4β/α mRNA ratios. This might suggest a feed-forward loop where increased STAT4β/α mRNA ratios result in more pro-inflammatory cytokine production, and increased concentrations of pro-inflammatory cytokines reinforce changes in STAT4β/α mRNA ratios. It is also possible that there are genetic predispositions to altered STAT4β/α mRNA ratios. Although there are

no disease-associated SNPs in the region spanned by exons 18–19, the rs925847 SNP, which is associated with some symptoms of CD and UC[50], is located in intron 17. Whether this might affect isoform generation is not clear. However, as processing of the hnRNA occurs where multiple steps occur simultaneously, it is possible that even distant changes in nucleotide sequence might affect isoform production. These possibilities will be interesting to pursue.

The correlation of STAT4β/α mRNA ratios with PCDAI is somewhat unique in that correlations with PCDAI are not found with any of the cytokines examined, despite the observation that some cytokines are significantly increased in IBD patients. This suggests that measuring STAT4β/α mRNA ratios, especially in the peripheral blood, might be a useful approach for assessing disease, more so than cytokines that are among the classic indicators of inflammation. Measuring STAT4β/α mRNA ratios in the GI tract is limited by location. Although ratios in the colon correlated with disease, no correlation was seen with STAT4β/α mRNA ratios in other segments. This might be linked to the type of cells that are present or recruited to particular segments, or to differing cytokine milieu among the segments examined. These possibilities can be explored further.

The observation of increased *IL17A* mRNA in the esophagus of both CD and UC patients is both striking and puzzling. Increased *IL17A* mRNA was also seen in the colon and rectum of CD and UC patients, respectively. However, there are no reports of increased esophageal pathology or cytokine expression in patients that do not have esophageal symptoms. It is not clear how bowel inflammation would result in altered cytokine expression in the esophagus, or why that would specifically be *IL17A*. *IL17A* is also increased in samples from patients with eosinophilic gastritis or esophageal cancer[51, 52], and it is possible that IBD patients have some underlying pathology in the esophagus. Importantly, when we examined 14 patients diagnosed with EoE there was a decrease, not an increase, in *IL17A* mRNA (data not shown). Whether this parameter might be a useful diagnostic tool is not clear.

There are a number of limitations to this study, primarily the relatively small population examined. Yet, the statistical significance of the changes observed was clearly apparent. Moreover, we were able to detect significant differences in the concentration of serum cytokines and in the relative amounts of cytokine mRNA in biopsy tissue, each consistent with previous observations in patients with IBD [53]. Furthermore, we were able to detect changes in STAT4β/α mRNA ratios following treatment, and changes followed the decrease in disease. This study focused on a pediatric population because disease onset is more uniform in children, and because treatment recommendations, particularly with anti-TNFα therapies for recurrent disease, are more limited. However, it is likely that these observations would be consistent in adult patients, and it will be interesting to test that hypothesis in future studies.

This study highlights a novel biomarker of inflammation in IBD. Measurement of STAT4β/α mRNA ratios in PBMCs might be a less invasive approach to monitoring inflammation, at least for initial diagnosis. Analyzing STAT4β/α mRNA ratios in biopsies or PBMCs might provide some additional prognostic capabilities, perhaps distinguishing

how well patients respond to specific therapies. Future analysis of larger patient cohorts will determine if STAT4β/α mRNA ratios will have utility in a clinical setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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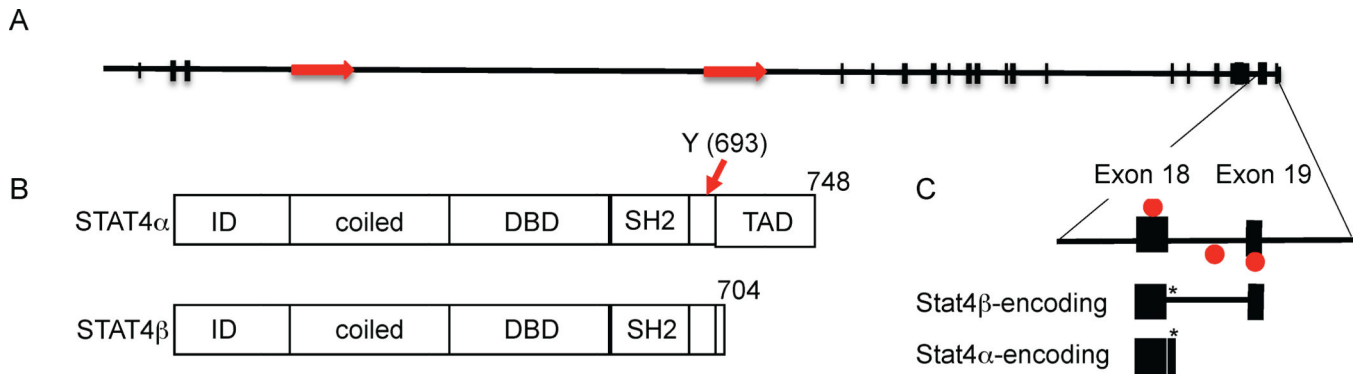

Figure 1.

Generation of STAT4 isoforms. (A) Schematic of the *STAT4* gene. Exons are denoted as vertical lines. Red arrows indicate direction of transcription. (B) Schematic of *STAT4* isoforms showing domain structures. ID, interaction domain; coiled, coiled-coil domain; DBD, DNA binding domain; TAD, transactivation domain. (C) Schematic of exons 18 and 19 showing sites of primers (red circles) used for analysis of STAT4 isoforms. Asterisk indicates the stop codon for each isoform.

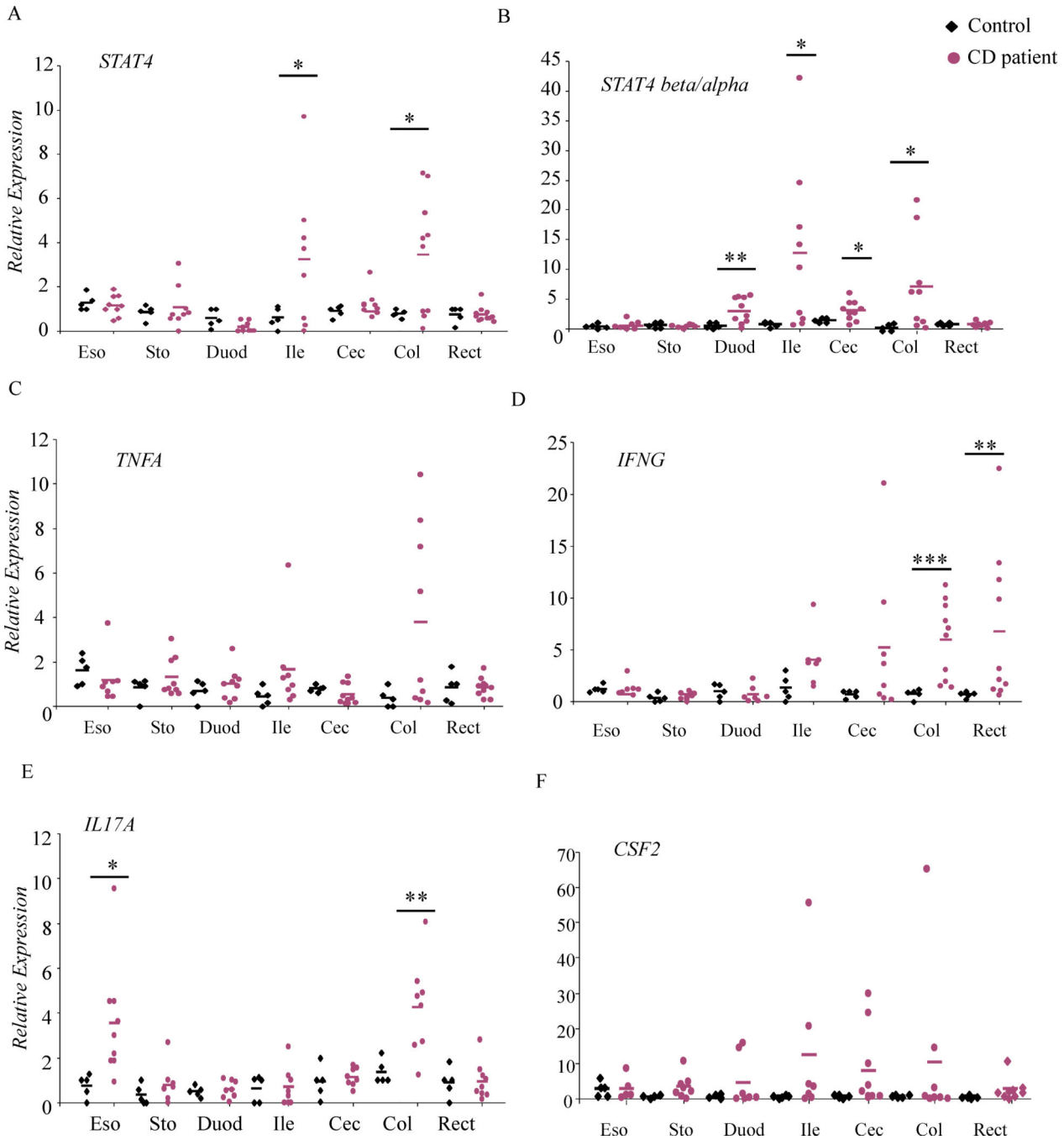

Figure 2.

STAT4β/α mRNA expression in biopsies from patients with CD. (A-B) Quantitative assessment of total STAT4 and STAT4β/α mRNA expression in regions of gastrointestinal tract of newly diagnosed CD patients (n =10) and healthy controls (n =5). (C-F) Quantitative assessment of *TNFA*, *IFNG*, *CSF2* and *IL17A* mRNA expression in mucosal tissue biopsies from regions of the gut of CD patients (n =10) and healthy controls (n =5). Eso, esophagus; Sto, stomach; Duod, duodenum; Ile, ileum; Cec, cecum; Col, colon; Rect, rectum. Statistical comparisons versus control. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

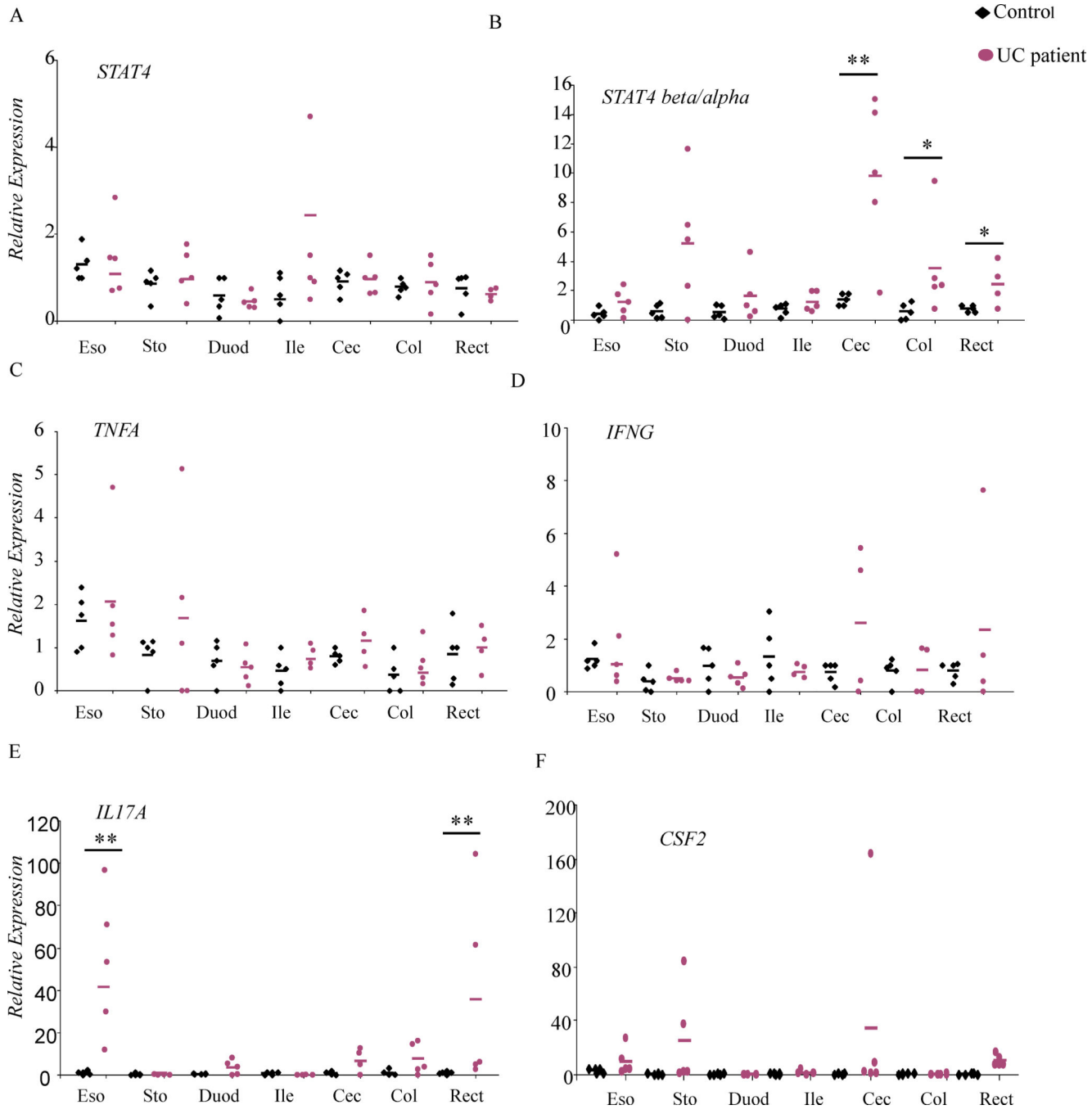

Figure 3.

STAT4β/α mRNA expression in biopsies from patients with UC. (A-B) Quantitative RT-PCR analysis of Total STAT4 and STAT4β/α mRNA expression in regions of the gastrointestinal tract of newly diagnosed UC patients (n =5) and healthy individuals (n =5). (C-F) mRNA expression levels of *TNFA*, *IFNG*, *CSF2* and *IL17A* in the mucosal tissue biopsies from various regions of the gut of UC patients (n =5) and healthy controls (n =5). Eso, esophagus; Sto, stomach; Duod, duodenum; Ile, ileum; Cec, cecum; Col, colon; Rect, rectum. Statistical comparisons versus control. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

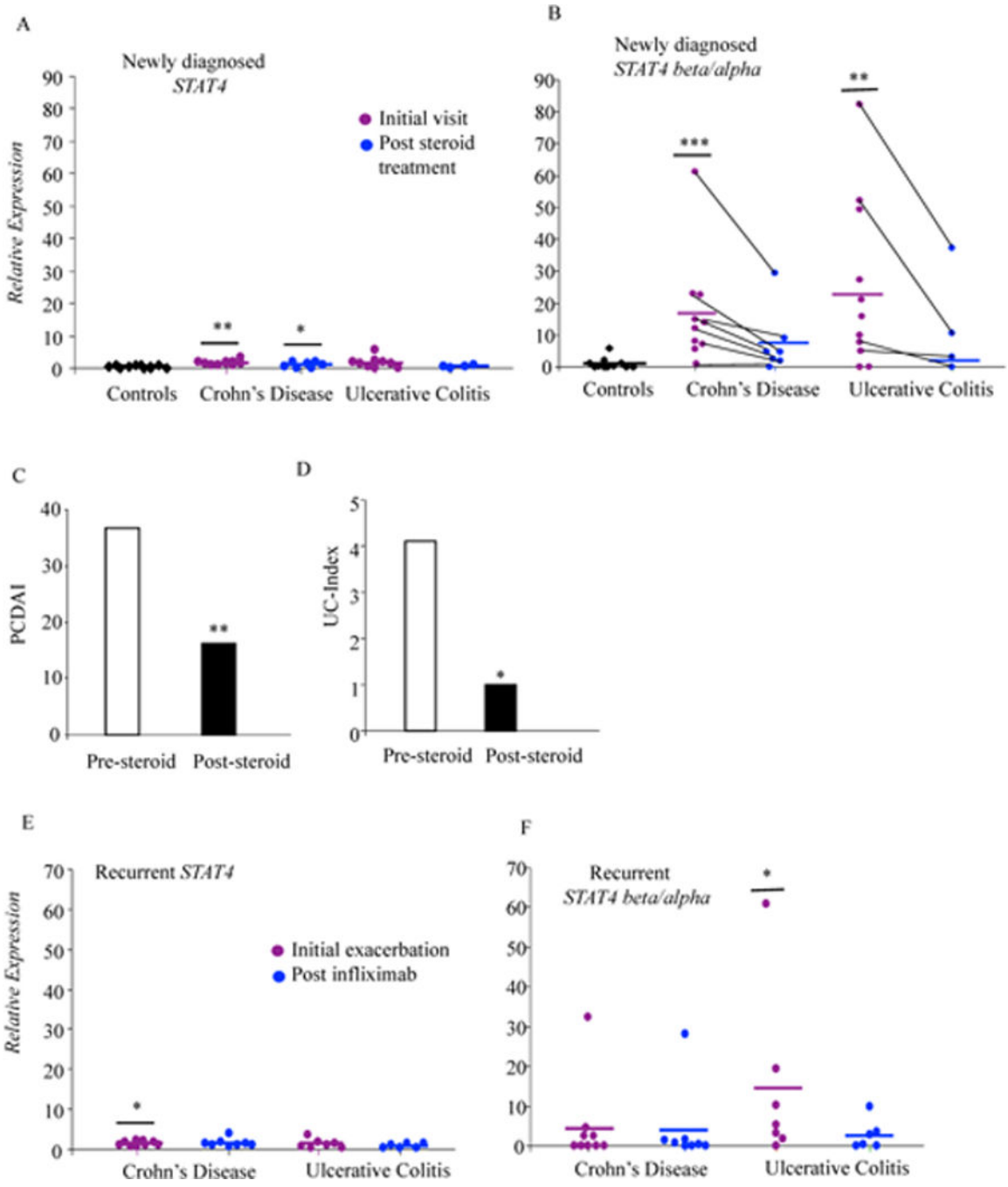

Figure 4.

PBMCs from CD and UC patients have increased STAT4β/α mRNA ratios. (A-B) PBMCs were isolated from both newly diagnosed CD (n =12 and 7 for first and 2nd visit respectively) and UC patients (n =12 and 4 first and 2nd visit respectively) and mRNA expression levels were measured by RT-PCR. Lines link data points for patients examined at both time points (C-D) Average disease scores for CD and UC patients before and after steroid treatment respectively. (E-F) PBMCs from established CD (n =9 and 7) or UC (n =7 and 6, first and 2nd visit respectively) patients were isolated on their first and 2nd visit and

expression of total *STAT4* and STAT4β/α mRNA was measured by RT-PCR. Statistical comparisons versus control. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

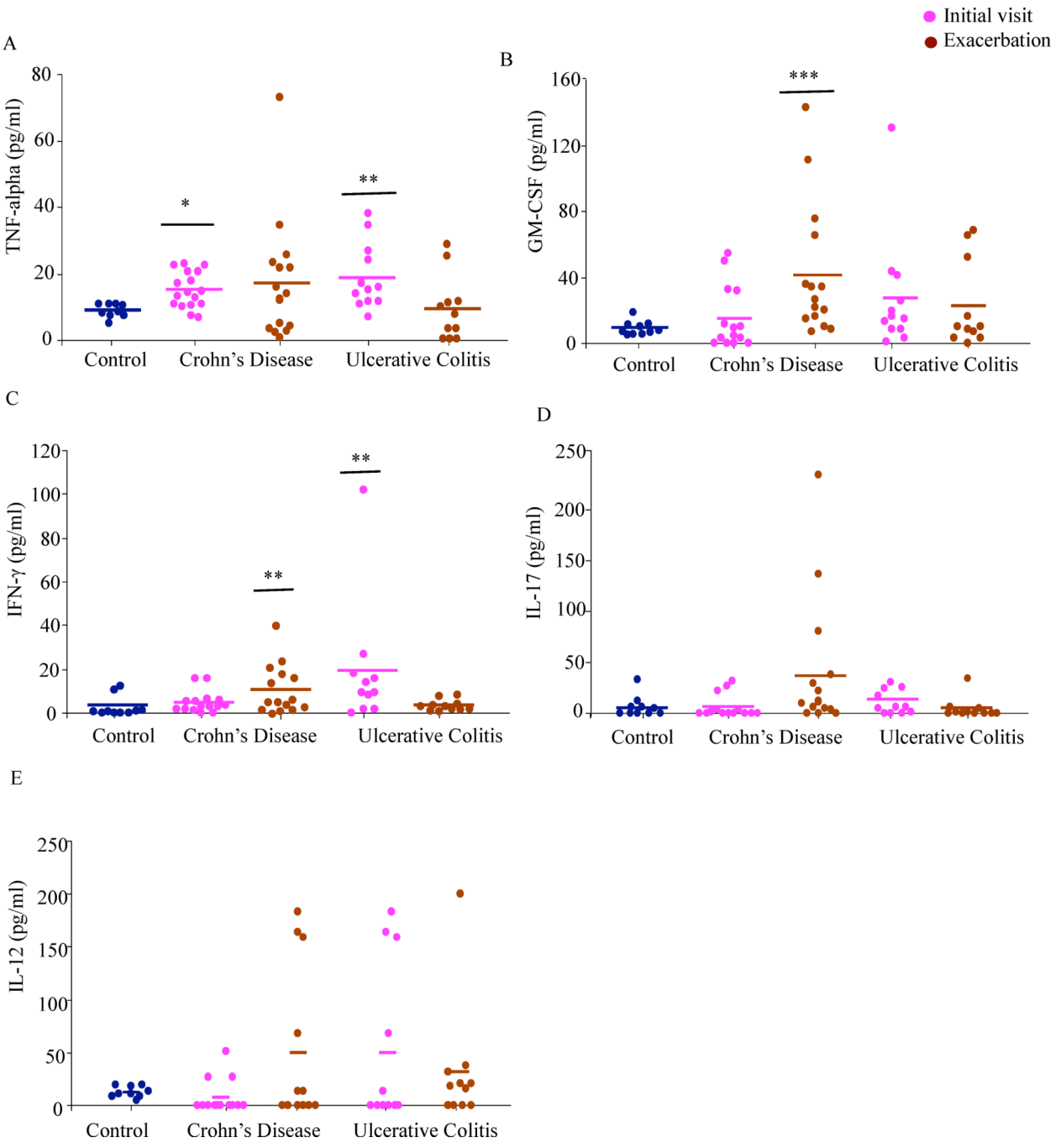

Figure 5.

Serum cytokines in CD and UC patients. (A-E) Concentrations of cytokines in the serum of newly diagnosed CD and UC patients, established CD and UC patients, and healthy controls. Statistical comparisons versus control. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

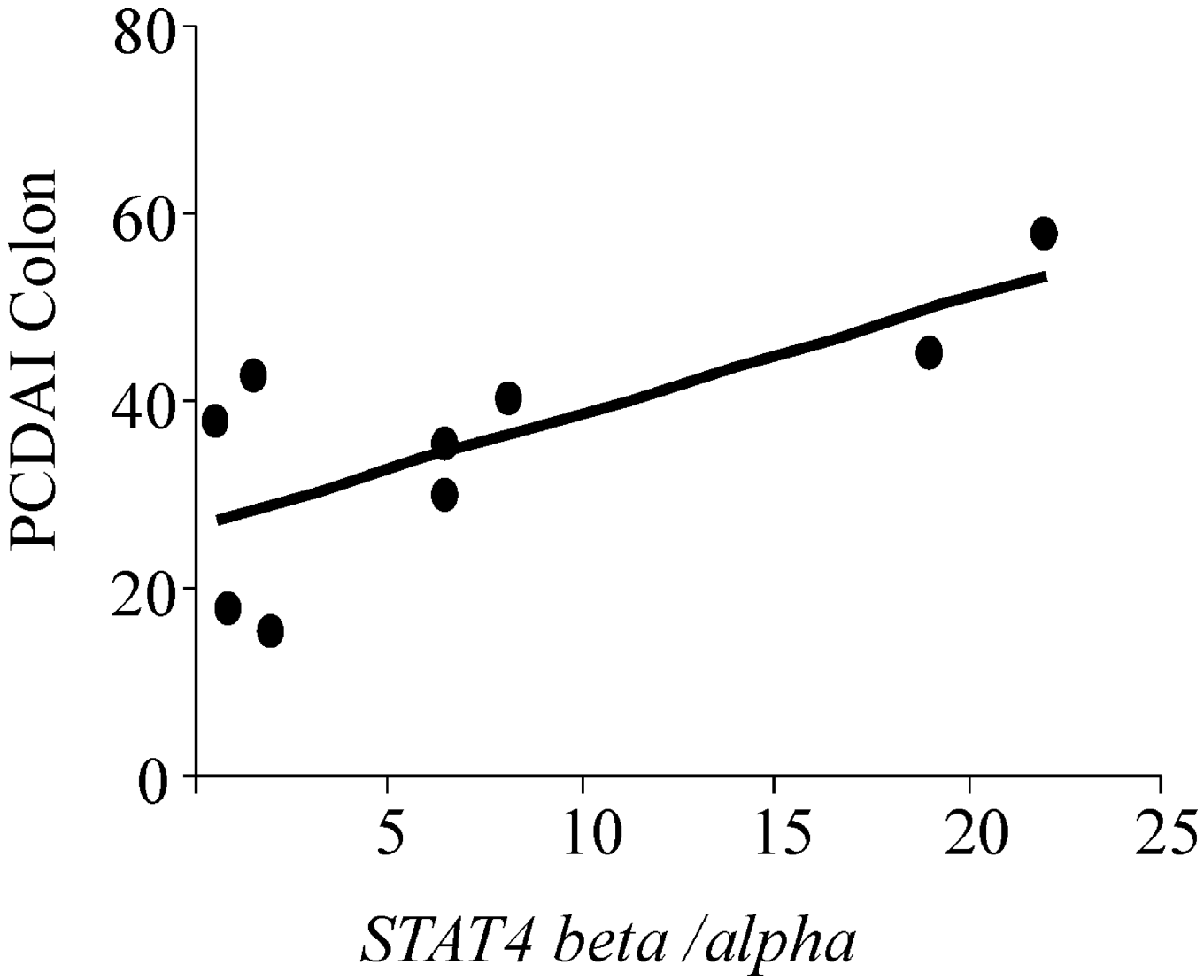

Figure 6.

Correlation of disease score with STAT4β/α mRNA ratios. RNA was isolated from colon biopsies of CD patients as described in the methods section and mRNA levels were measured. Because of the small *n*, the p-values were not corrected for multiple comparisons.

Table 1

Clinical features of CD and UC patients

RCAI/UC		Number of subjects	AGE (Mean)	Male (%)	Race (% Caucasian)	PCDAI	index
Biopsy	Control	5	12	60	100	N/A	N/A
Initial	UC	5	10	75	75	N/A	9
Diagnosis	CD	9	12	78	100	35.5	N/A
PBMC	Control	12	13	83	83	N/A	N/A
Initial	UC	12	12	92	83	N/A	7.8/4.2
Diagnosis	CD	10	13	70	90	36.7	N/A
PBMC	UC	7	15	71	85	N/A	5.5/3.6
Recurrence	CD	10	13	90	80	19.2	N/A

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