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Identification of Diagnostic Biomarkers by Bioinformatics Analysis in the Inflamed and Non-inflamed Intestinal Mucosa of Patients With Crohn's Disease

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ABSTRACT

Background: Crohn's Disease (CD) is a type of inflammatory bowel disease that, despite its unknown etiology, is generally associated with genetics, immune system, and environmental factors. In this study, we uncover transcriptional signatures in patients with CD and subsequently explain the putative molecular pathways in the inflamed and non-inflamed intestinal mucosa.

Materials and Methods: We obtain *GSE83448* gene expression profiles from the Omnibus gene expression database. Also, for the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of Differentially Expressed Gene (DEG) pathways, we used DAVID software. DEGs were detected in the inflamed and non-inflamed intestinal mucosa of CD patients compared to the control group using the GEO2R instrument. Significant modules and hub genes were identified after producing protein-protein interaction (PPIs) networks of DEGs using Cytoscape software.

Results: The 10 specific hub genes of CD, including Matrix Metallopeptidase 2 (*MMP2*), Cadherin 1 (*CDH1*), Periostin (*POSTN*), Collagen type I alpha 2 chain (*COL1A2*), C-X-C motif chemokine ligand 8 (*CXCL8*), Collagen type III alpha 1 chain (*COL3A1*), JUN, Serine Protease Inhibitor clade E member 1 (*SERPINE1*), Integrin alpha M (*ITGAM*), and Connective Tissue Growth Factor (*CTGF*), were used as biomarkers to discriminate between inflamed and non-inflamed intestinal mucosa groups in patients.

Conclusion: These findings could lead to new molecular targets and diagnostic biomarkers for both inflamed and non-inflamed intestinal mucosa in CD patients.

Introduction

Crohn's Disease (CD) is a chronic inflammatory disorder that is classified as one major type of Inflammatory Bowel Disease (IBD) [1, 2]. In CD, which involves intestinal tissue, chronic complications result in inflammation and lesions [3]. CD can lead to serious scenarios and life-threatening problems, and because of socioeconomic development and lifestyle alteration, its prevalence is increasing in different countries [4, 5]. Since there are no fully effective medications and targets for

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treating the disorder, the CD is still not completely curable. Despite the enormous progress in understanding the etiology of CD, early detection, clinical intervention, and the exact molecular mechanisms of CD are still unknown [6].

In CD, any region of the gastrointestinal tract may be affected, but it mostly affects the distal ileum and the colon [7]. One of the most critical complications of CD is inflamed sites of the colon [8]. This inflammatory area is one of the most critical areas studied in various research studies [9]. In addition, non-inflammatory areas in the intestine of patients are also evaluated in some studies [10]. Since inflamed and non-inflamed regions have similar genetic and pathophysiologic characteristics, it is somewhat difficult to diagnose and differentiate them [11]. Hence, to establish more accurate diagnostic and efficient therapeutic strategies for improving patient outcomes, the elucidation of the specific features of inflamed and non-inflamed regions seems to be necessary [12].

In recent years, many efforts, especially in the field of multidisciplinary sciences using bioinformatics and microarray technology, have been made to identify the molecular mechanisms of this disease as much as possible [13, 14]. Various genes and predictive biomarkers have been identified by bioinformatics analyses for inflamed and non-inflamed sites of the colon in CD patients, and Differentially Expressed Genes (DEGs) have been investigated, as well.

In this study, with the focus on CD, the inflammatory and non-inflammatory regions with different DEGs are evaluated. Furthermore, by analyzing the biological activities and pathways of CD, the current study enriches our understanding of the pathogenesis of CD at the molecular level, thus easing diagnosis and identifying possible molecular targets for CD.

Materials and Methods

Microarray data

The *GSE83448* gene expression dataset was obtained from the Gene Expression Omnibus (GEO) database. In addition to storing the original submitter-supplied records, the GEO database (https://www.ncbi.nlm.nih.gov/geo/) stores the control datasets. Adrià Aterido et al. [15] contributed the GSE83448 gene expression dataset to the GEO database, which was generated using the CodeLink Human Whole Genome Array (DISCOVERY probe type) platform. The dataset included 194 samples, including the colon tissue of 53 patients with 20 non-inflamed mucosa, 19 patients with inflamed mucosa, and 14 control samples.

DEGs identification

Data pre-processing was conducted by GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/), an interactive web tool, to sift DEGs between the inflamed and the non-inflamed intestinal mucosa group versus the control group. In addition to comparing two groups of samples under similar laboratory situations, GEO2R can evaluate almost any GEO series. For each group, the adjusted P value 0.01 and | log2 Fold Change (FC) | >1 (i.e., FC>2) thresholds were chosen.

Investigating the enrichment of gene ontology and kyoto encyclopedia of genes and genomes pathways

DAVID online software, which deals with the interpretation, visualization, and integration of information (DAVID 6.8, http://david.ncifcrf.gov/summary.jsp), is a program that extracts biological ideas from gene or protein lists in a systematic manner. This software was utilized to assess the screened DEGs at the functional level, Gene Ontology (GO) enrichment, and Kyoto Encyclopedia of Genes and Genomes KEGG pathway evaluation. The cut-off criterion was established to be P0.05.

The protein-protein interaction network analysis

A Protein-Protein Interaction (PPI) network, generated by Cytoscape software (https://cytoscape.org/; version 3.6.1), was used to evaluate functional interactions between DEGs. Then, DEGs were mapped to retrieve interacting genes (STRING; version 11.0; http://string-db.org/cgi/input.pl), and only interaction pairs with a total PPI score of greater than 0.7 were selected as acceptable pairs. The Cytoscape CytoHubba plugin was used to calculate the 10 key hub genes that ranked in the relevant DEG networks.

Module analysis

To identify dense areas of the Cytoscape's PPI network, MCODE (Molecular Complex Detection) plugin was used. Then, the following parameters were used to apply MCODE to PPI network screen modules: degree cut-off equal to 2, node score cut-off equal to 0.2, k-score equal to 2, and the maximum depth equal to 100. Also, ClueGo was used to evaluate the functions and pathways of DEGs in each module, and for this purpose, P<0.05 was considered to show the significance.

Statistical analysis

The obtained data were presented as Mean±SD and analyzed by the t-test. P<0.05 was considered significant.

Results

Identification of DEGs

We got the *GSE83448* gene expression dataset using the GEO database. DEGs were identified using GEO2R tools between groups of patients with CD and control. As shown in Figure 1, a total number of 920 DEGs were detected in the inflamed intestinal mucosa at the significance of P<0.05 and |log2FC| >1, including 496 upregulated and 424 downregulated genes in the inflamed intestinal mucosa vs control samples. As shown in Table 1, for the inflamed intestinal mucosa group vs the control group, the top 10 upregulated and downregulated genes are reported. Similarly, when the non-inflamed intestinal mucosa samples were compared to healthy control samples, 526 DEGs were discovered, with 295 upregulated and 231 downregulated genes (Figure 1). Table 1 lists the top 10 DEGs for non-inflamed intestinal mucosa vs control samples. Most of the DEGs found in the inflamed intestinal mucosa group were also differentially expressed in the non-inflamed intestinal mucosa group, which is noteworthy. A total of 297 overlapping DEGs were identified between both inflamed and non-inflamed intestinal mucosa groups vs the controls (Figure 1). Thus, for the inflamed and non-inflamed intestinal mucosa groups, a total of 457 and 85 unique DEGs remained, respectively (Figure 1).

GO and pathway enrichment analysis of DEGs

We used DAVID software to systematically explain and compare inflamed and non-inflamed intestinal mucosa groups in terms of biological functions around detected DEGs, functional annotation, and path analysis, including GO and KEGG and Reactome analyses. The top 10 GO keywords in the biological process classification are displayed in Figure 2. According to the findings of the GO analysis, it was demonstrated that several DEGs in the inflamed intestinal mucosa group were enriched in anatomical structure morphogenesis, cell differentiation, macromolecule metabolic process, cellular component organization, cellular developmental process, system development, multicellular organism development, nitrogen compound metabolic process, cellular macromolecule metabolic process, and cellular component organization or biogenesis (Figure 2). Similarly, certain DEGs

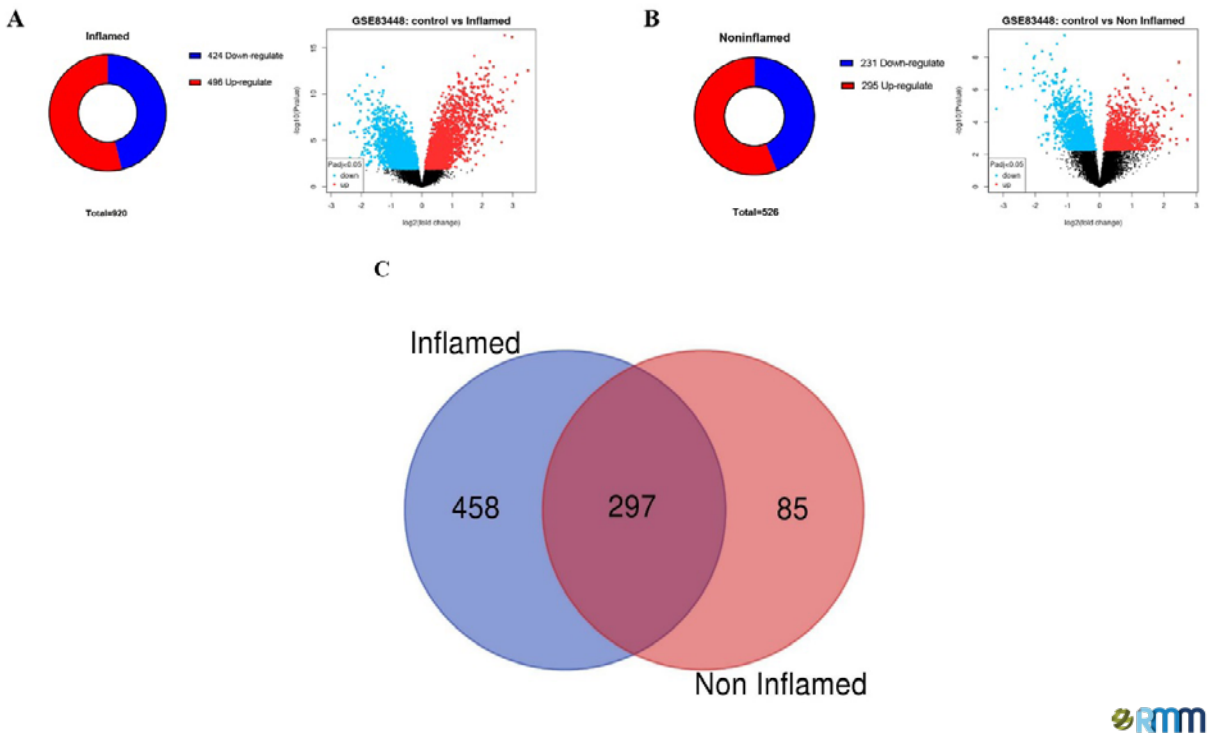

Figure 1. Identification of DEGs in the inflamed and non-inflamed intestinal mucosa

A: Differentially Expressed Gene (DEGs) detected in the inflamed intestinal mucosa; B: DEGs detected in the non-inflamed intestinal mucosa; C: Overlapping DEGs for the inflamed and non-inflamed intestinal mucosa versus control samples.

in the non-inflamed intestinal mucosa group were also involved in cell development, positive regulation of nitrogen compound metabolic process, cellular component organization, cellular macromolecule metabolic process, macromolecule metabolic process, positive regulation of macromolecule metabolic process, positive regulation of the metabolic process, positive regulation of the cellular process, biogenesis of cellular components, and positive control of biological processes (Figure 2).

Subsequently, the results of the KEGG and Reactome pathway analysis represented the existence of DEGs in the inflamed and non-inflamed intestinal mucosa groups that were primarily enriched. Also, certain KEGG and Reactome pathways, including the fatty acid digestion and metabolic pathway, were commonly joined in developing the inflamed intestinal mucosa group. In contrast, other pathways (fatty acid degradation and biological oxidations pathways) were mainly involved in the non-inflamed intestinal mucosa group (Figure 2). Thus, several GO (gene ontology) terms in the biological process category show a significant difference between the inflamed and the non-inflamed intestinal mucosa groups. As a result, we found that the inflamed and non-inflamed intestinal mucosa groups have different pathological features.

PPI network production and identification of hub genes

To analyze the association between DEGs at the protein level, PPI networks were built based on DEG interactions. Using Cytoscape software, specific DEGs for inflamed and non-inflamed intestinal mucosa groups, as well as their overlapping DEGs, were mapped and then visualized into PPI networks. The PPI network of the overlapped DEGs has 350 nodes and 809 edges, with a predetermined standard of a combination score> 0.7. Table 2 lists the top 10 hub nodes in this network with the highest degree of interaction. These genes that function as a hub, known as candidate genes, were as follows: Cadherin 1 (*CDH1*), JUN, Matrix Metallopeptidase 2 (*MMP2*), Collagen type I alpha 1 chain (*COL1A1*), C-

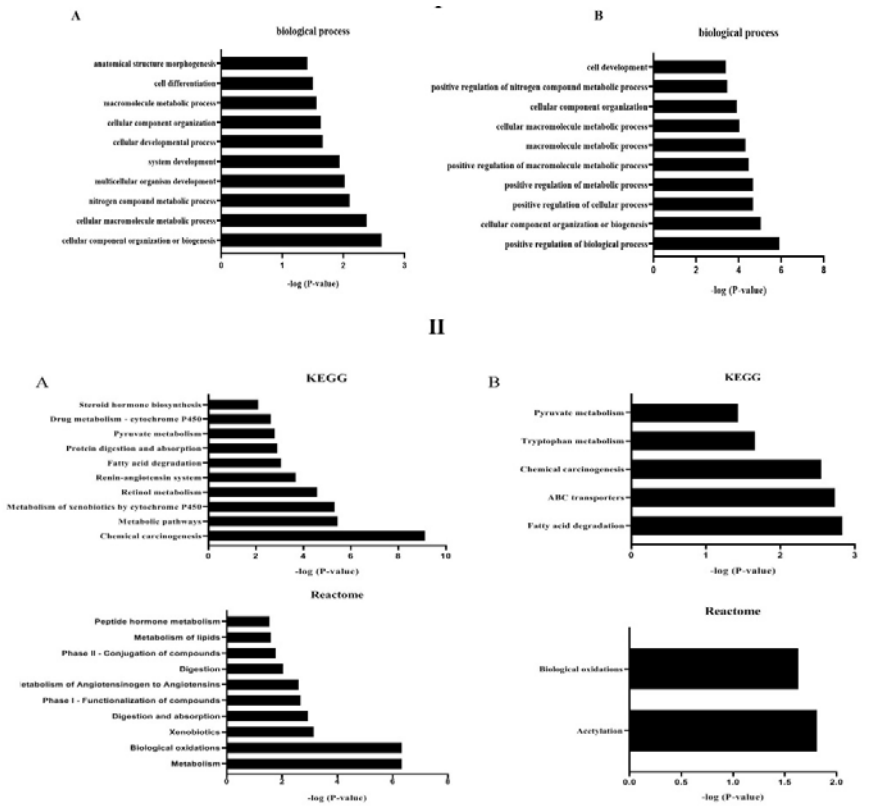

Figure 2. Gene ontology in the inflamed and non-inflamed intestinal mucosa

I) A: Top 10 Gene Ontology (GO) terms in the classification of biological processes for the inflamed intestinal mucosa; B: Top 10 GO terms for the non-inflamed intestinal mucosa in the classification of biological processes; II) A: KEGG (Kyoto Encyclopedia of Genes and Genomes) and reactome pathway analysis representing the existence of Differentially Expressed Gene (DEGs) in the inflamed intestinal mucosa groups; B: KEGG and reactome pathway analysis representing the existence of DEGs in the non-inflamed intestinal mucosa groups.

X-C motif chemokine ligand 8 (*CXCL8*), Erythroblastic oncogene B2 (*ERBB2*), Insulin-like Growth Factor 1 (*IGF-1*), Periostin (*POSTN*), C-X-C motif chemokine ligand 12 (*CXCL12*), and Collagen type I alpha 2 chain (*COL1A2*). Similarly, Most of the hub genes in the non-inflamed group were associated with the chemokine signaling pathway, leukocyte migration, and intestinal fibrosis pathways. The hub genes identified in non-inflamed tissues that may be important in distinguishing non-inflamed groups from inflamed include *MMP2*, *CDH1*, *POSTN*, *COL1A2*, *CXCL8*, Collagen type III alpha 1 chain (*COL3A1*), *JUN*, Serine Protease Inhibitor clade E member 1 (*SERPINE1*), Integrin alpha M (*ITGAM*), and Connective Tissue Growth Factor (*CTGF*) (Table 2). However, specific candidate genes, including *CDH1*, *JUN*, *MMP2*, *CXCL8*, *POSTN*, and *COL1A2*, that were identified in inflamed tissues may not discriminate inflamed from non-inflamed tissues. Individual PPI networks were created for inflamed and non-inflamed groups, with 933 and 650 nodes and 3321 and 2282 edges, respectively. Table 2 lists the top 10 hub genes with the highest degree of interaction among these nodes. It is remarkable that *COL1A1*, *ERBB2*, *IGF-1*, and *CXCL12*, unlike the non-inflamed group, were highly expressed in the inflamed group. These specific hub genes may be marker genes for the inflamed group. In addition, *COL3A1*, *SERPINE1*, *ITGAM*, and *CTGF*, unlike the inflamed group, were highly expressed in the non-inflamed group.

In addition, several DEGs in the inflamed intestinal mucosa group were enriched in antigen processing: ubiquitination and proteasome degradation, class I MHC mediated antigen processing and presentation, adaptive immune system, neddylation, post-translational protein modification, the response of mycobacterium tuberculosis to phagocytosis, and APC/C: Cdc20 mediated degradation of cyclin B. Similarly, certain DEGs in the non-inflamed intestinal mucosa group were also ubiquitin-mediated proteolysis, antigen processing: ubiquitination and proteasome degradation, class I MHC mediated antigen processing and presentation, neddylation, adaptive immune system, and post-translational protein modification.

Module analysis of the PPI network

Using the MCODE plugin from Cytoscape, the top modules of PPI networks were selected for inflamed and non-inflamed regions, and then functional enrichment of DEGs analysis was done in the relevant functional modules (Figure 3). DEGs in inflamed and non-inflamed sections of the modules were predominantly associated with endocrine resistance, immunological system, adaptive immune system, and disorders of signal transduction by growth factor receptor, according to KEGG and Reactome pathway enrichment analyses.

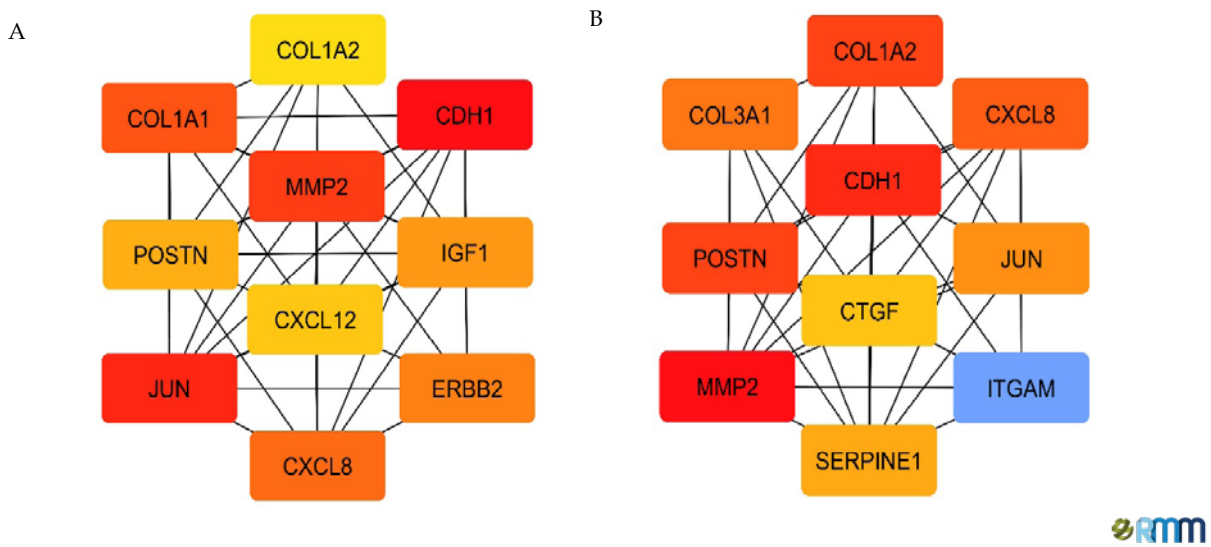

Figure 3. Protein-Protein Interaction (PPI) in the inflamed and non-inflamed intestinal mucosa

A: Using the Molecular Complex Detection (MCODE) plugin from cytoscape, the top 10 modules of Protein-Protein Interaction (PPI) networks for inflamed tissues; B: Using the MCODE plugin from cytoscape, the top 10 modules of PPI networks for non-inflamed tissues

CDH1, Cadherin 1; MMP2, Matrix Metallopeptidase 2; COL1A1, Collagen type I alpha 1 chain; CXCL8, C-X-C motif chemokine ligand 8; ERBB2, Erythroblastic oncogene B2; IGF-1, Insulin-like Growth Factor 1; POSTN, Periostin; CXCL12, C-X-C motif chemokine ligand 12; COL1A2, Collagen type I alpha 2 chain; COL3A1, Collagen type III alpha 1 chain; SERPINE1, Serine Protease Inhibitor clade E member 1; ITGAM, Integrin alpha M; CTGF, Connective tissue growth factor.

Table 1. Top 10 upregulated and downregulated genes for inflamed intestinal mucosa and non-inflamed intestinal mucosa versus control

Inflamed Intestinal Mucosa		Non-Inflamed Intestinal Mucosa	
Downregulated Gene Expression	**Upregulated Gene Expression**	**Downregulated Gene Expression**	**Upregulated Gene Expression**
Familial Adenomatous Polyposis (*FAP*)	Guanylate Cyclase Activator 2B (*GUCA2B*)	Retinol Binding Protein 4 (*RBP4*)	ER Degradation Enhancing alpha-Mannosidase like protein 2 (*EDEM2*)
Chordin Like 2 (*CHRDL2*)	Zymogen Granule protein 16 (*ZG16*)	Ciliary Rootlet Coiled-Coil, rootletin (*CROCC*)	Solute Carrier family 39 member 5 (*SLC39A5*)
Cytochrome P450 family 1 subfamily B member 1 (*CYP1B1*)	Scinderin (*SCIN*)	RAB9B, Member RAS oncogene family (*RAB9B*)	BMP2 inducible Kinase (*BMP2K*)
Cysteine Dioxygenase type 1 (*CDO1*)	Transmembrane and Immunoglobulin Domain containing 1 (*TMIGD1*)	Cysteine Dioxygenase type 1 (*CDO1*)	N-Acyl Phosphatidylethanolamine Phospholipase D (*NAPEPLD*)
Ficolin 2 (*FCN2*)	Solute Carrier family 15 member 1 (*SLC15A1*)	Familial Adenomatous Polyposis (*FAP*)	ATP Binding Cassette subfamily B member 1 (*ABCB1*)
TSC complex subunit 2 (*TSD2*)	ATP Binding Cassette subfamily B member 1 (*ABCB1*)	Chordin Like 2 (*CHRDL2*)	Myosin Light chain 4 (*MYL4*)
Serine Protease Inhibitor clade E member 1 (*SERPINE1*)	Cytochrome P450 family 3 subfamily a member 4 (*CYP3A4*)	Retinol Binding Protein 7 (*RBP7*)	ArfGAP with Coiled-coil, Ankyrin repeat and PH domains 1 (*ACAP1*)
Cysteine-rich angiogenic inducer 61 (*CYR61*)	Guanine deaminase (*GDA*)	Ficolin 2 (*FCN2*)	Endothelin 2 (*EDN2*)
Collagen type IV alpha 1 chain (*COL4A1*)	Hepatocyte Nuclear Factor 4 Gamma (*HNF4G*)	TSC complex subunit 2 (*TSD2*)	N-Acetyltransferase 8 (*NAT8*)
Tissue Factor Pathway Inhibitor 2 (*TFPI2*)	Endothelin 2 (*EDN2*)	Cytochrome P450 family 1 subfamily B member 1 (*CYP1B1*)	Chromatin Assembly Factor 1 subunit A (*CHAF1A*)

Since inflammation of non-inflamed tissues is a different disease, the two groups have different pathological features in common. As a result, the biological roles and pathways shared by inflamed and non-inflamed regions were investigated further. For the inflamed and non-inflamed area groups, a total of 297 DEGs overlapped. Following that, the PPI network of the overlapped DEGs yielded 10 important modules (Figure 3). The top module's genes were mainly associated with ubiquitin-mediated proteolysis and cell cycle, according to the KEGG study. The DEGs included in the top module were mainly associated with antigen processing. According to a Reactome pathway enrichment study, it comprises ubiquitination and proteasome degradation, class I MHC mediated antigen processing and presentation, adaptive immune system, phosphorylation of the APC/C, conversion from APC/C:Cdc20 to APC/C:Cdh1 in late anaphase, inhibition of the proteolytic activity of APC/C required for the onset of anaphase by mitotic spindle checkpoint components, inactivation of APC/C via direct inhibition of the APC/C complex, immune system, neddylation, APC/C:Cdc20 mediated degradation of cyclin B, APC-Cdc20 mediated degradation of Nek2A, transcriptional Regulation by VENTX, autodegradation of Cdh1 by Cdh1:APC/C, APC/C:Cdc20 mediated degradation of securin, Cdc20:Phospho-APC/C mediated degradation of cyclin A, CDK-mediated phosphorylation and removal of Cdc6, APC/C:Cdh1 mediated degradation of Cdc20 and others, APC/C:Cdh1 targeted proteins in late mitosis/early G1, APC:Cdc20 mediated degradation of cell cycle proteins prior to satisfaction of the cell cycle, checkpoint Senescence-Associated Secretory Phenotype (SASP), APC/C:Cdc20 mediated degradation of mitotic proteins, activation of APC/C and APC/C:Cdc20 mediated degradation of mitotic proteins, regulation of APC/C activators between G1/S and early anaphase, APC/C-mediated degradation of cell cycle proteins, regulation of mitotic cell cycle, switching of origins to a post-replicative state, mitotic spindle checkpoint, synthesis of DNA and DNA replication.

Discussion

Although studies on Crohn's Disease (CD) have positive results, the CD's molecular mechanisms at the gene expression level have remained unknown because of the complexity of gene interaction and genes up- and down-regulation [15]. So, additional research in this field is needed. As a result, advances in understanding the molecular mechanisms of CD using the microarray technique may provide new diagnostic and therapeutic targets in both the inflamed and non-inflamed regions of CD [16].

The gene expression profile of *GSE83448* used in this investigation was supplied by Zabana et al. [15]. This

Table 2. Top 10 hub genes for inflamed intestinal mucosa, non-inflamed intestinal mucosa, and inflamed and non-inflamed intestinal mucosa versus control

Inflamed Intestinal Mucosa		Non-Inflamed Intestinal Mucosa		Inflamed and Non-Inflamed Intestinal Mucosa	
Genes	**Degree**	**Genes**	**Degree**	**Genes**	**Degree**
Cadherin 1 (*CDH1*)	76	Matrix Metallopeptidase 2 (*MMP2*)	38	Matrix Metallopeptidase 2 (*MMP2*)	37
JUN	68	Cadherin 1 (*CDH1*)	37	Cadherin 1 (*CDH1*)	33
Matrix Metallopeptidase 2 (*MMP2*)	66	Periostin (*POSTN*)	33	Collagen type I alpha 2 chain (*COL1A2*)	33
Collagen type I alpha 1 chain (*COL1A1*)	61	Collagen type I alpha 2 chain (*COL1A2*)	33	Periostin (*POSTN*)	33
C-X-C motif chemokine ligand 8 (*CXCL8*)	60	C-X-C motif chemokine ligand 8 (*CXCL8*)	32	Collagen type III alpha 1 chain (*COL3A1*)	31
Erythroblastic oncogene B 2 (*ERBB2*)	58	Collagen type III alpha 1 chain (*COL3A1*)	31	Connective Tissue Growth Factor (*CTGF*)	27
Insulin-like Growth Factor 1 (*IGF-1*)	56	*JUN*	30	*JUN*	26
Periostin (*POSTN*)	55	Serine Protease Inhibitor clade E member 1 (*SERPINE1*)	29	C-X-C motif chemokine ligand 8 (*CXCL8*)	26
C-X-C motif chemokine ligand 12 (*CXCL12*)	53	Integrin alpha M (*ITGAM*)	25	Serine Protease Inhibitor clade E member 1 (*SERPINE1*)	25
Collagen type I alpha 2 chain (*COL1A2*)	52	Connective Tissue Growth Factor (*CTGF*)	28	Fibrillin 1 (*FBN1*)	20

study, on the contrary, aimed to identify genetic signatures in the inflamed and non-inflamed regions of CD patients, as well as to elucidate possible associated molecular mechanisms underlying these regions. So, to obtain general genetic modifications in the inflamed and non-inflamed regions of CD patients vs controls, the microarray dataset was used.

In the present study, 920 DEGs were found in the inflamed group (496 upregulated and 424 down-regulated genes), and 526 DEGs were found in the non-inflamed group (295 upregulated and 231 down-regulated genes). The inflamed and non-inflamed groups shared 297 DEGs, suggesting that the two regions have genetic components that are significant and overlap.

Previously, the reduced serum level of IGF-1 in IBD patients has been shown [17, 18]. IGF-1 plays a role in regulating cellular process anabolism and the metabolism of protein, carbohydrate, and lipid [19, 20]. In a study investigating the role of the stanniocalcin-2/PAPP-A/IGFBP-4 axis on the IGF-1 system of IBD patients, the concentration of IGF-1 and Insulin-like Growth Factor-Binding Protein 4 (IGFBP-4) were significantly changed in IBD patients and restored to near-normal after treatment with prednisolone or infliximab as anti-inflammatory treatment [21]. The endocrine and paracrine effects of IGF-1 on the intestinal epithelium are exerted from circulating IGF-1 mainly derived from hepatocytes and locally expressed IGF-1 synthesized by intestinal mesenchyme, respectively. The amount of IGF-1 in circulation is affected by Growth Hormone (GH), caloric and protein intake, and insulin. However, local intestinal IGF-1 is less regulated by GH, particularly in severe GH insufficiency or excess, but it is positively regulated by luminal nutrients [22-24] and transiently upregulated in response to ileocecal resection [22, 25]. Ion IBD chronic increase of pro-inflammatory cytokines is associated with lower levels of circulating IGF-1, which may indicate GH resistance in hepatocytes and the effect of malabsorption or malnutrition [26]. In contrast, higher local IGF-1 expression was identified, leading to different levels of circulating and locally expressed IGF-1 in response to intestinal inflammation.

CXCL12, also known as stromal cell-derived factor-1a, is a 7.8 kDa chemokine originally identified from a bone marrow stromal cell line [27]. CXCL12 binds to C-X-C motif chemokine ligand 4 (CXCR4) and C-X-C motif chemokine ligand 7 (CXCR7), with a tenfold higher affinity of CXCL12 to CXCR7 than to CXCR4 [28, 29]. It has been shown that CXCL12 or CXCR4 genetic deletions in mice result in embryonic mortality owing to abnormalities in hematopoiesis and myelopoiesis [30, 31], gastrointestinal vascularization, and ventricular septal malformations, indicating that this axis plays a critical role in the development of these systems. CXCL12 is crucial in the proliferation and migration of stem cells, as well [32-36]. Moreover, it has been suggested that CXCL12 is involved in joint, lung, brain, and intesti-

nal inflammation [37]. For instance, it was discovered that blocking the CXCL12/CXCR4 axis with a CXCR4 antagonist relieved colitis in Dextran Sodium Sulfate (DSS) and Interleukin 10 (IL-10) knockout mice [38].

In a study on the constitutive and inflammatory role of *CXCL12* and *CXCR4* in the gut, the expression of CXCL12 in normal IECs and its up-regulation in IBD IECs along with its differential distribution was shown [37], whereas its most expression was observed in ulcerative colitis following by inflamed and non-inflamed CD, respectively [37]. Therefore, the CXCR4/CXCR7/CXCL12 axis can be considered a potential therapeutic target in managing IBD.

In a variety of developing mammalian tissues, including the intestinal tract, the Erythroblastic oncogene B (ErbB) tyrosine kinases as the cell surface growth factor receptors, including Epidermal Growth Factor Receptor (EGFR), ErbB2/Human Epidermal Growth Factor receptor 2 (HER2), Erythroblastic oncogene B3 (ErbB3), induce signaling pathways to promote epithelial cell growth and survival. IBD, Necrotizing Enterocolitis (NEC), and Total Parenteral Nutrition (TPN) models have been demonstrated to have lower ErbB ligand expression and, as a result, lesser ErbB activity. As a result, ErbB ligands can be used to treat pediatric gastrointestinal disorders such as IBD, NEC, and inflammation associated with TPN, most likely in combination with anti-inflammatory medications to create a double-edged attack on disease [39].

In a study on the biological dysregulation underlying Ulcerative Colitis (UC) UC and CD, through DNA microarray of inflamed colonic tissue, higher expression of *COL1A1* gene in both UC and CD over control was observed [40]. More recently, it has been shown that the expression of genes involved in collagen metabolism in the terminal ileum of CD patients with intestinal fibrosis suggests a plethora of therapeutic targets. In contrast, the mRNA expression of *COL1A1* and *COL3A1* in fibrotic CD was higher than in non-fibrotic CD. In the fibrosis-affected area, mRNA expression of proteins involved in intracellular and extracellular post-translational modification of collagens (prolyl- and lysyl-hydroxylases, lysyl oxidases, chaperones), collagen-degrading enzymes (MMPs and cathepsin-K), and collagen receptors was elevated. So, the inhibition of post-translational modification and alteration of collagen metabolism may reduce the production of fibrosis in the intestine in CD patients [41].

There are only a few serum biomarkers that can be used to identify patients with CD who are at risk of developing strictures. COL3A1 and other extracellular matrix components may have a role in intestinal fibrosis. In 2019, Ballengee et al. discovered that plasma concentrations of COL3A1 were considerably greater in patients with CD who later developed strictures than those without them. They suggested that a combination of COL3A1 and anti-CSF2 concentrations could be utilized to identify children with CD at risk for future strictures [42].

In a study, Serpin family E member 1 (SerpinE1) levels were measured in inflamed and non-inflamed parts of the colon of IBD patients. Their results suggest that serum and mucosal SeprinE1 expression reflects the endoscopic activity of IBD. The discovery of a link between SerpinE1 expression in the blood and the gut mucosa could lead to novel non-invasive IBD disease monitoring options [43]. In 2019, Kaiko et al. used colon samples to undertake global transcriptome analysis to identify IBD-related pathways, and the coagulation gene pathway was found to be one of the most enriched gene sets in IBD patients. They discovered that among the coagulation pathway genes, SerpinE1 (PAI-1) expression was highly enriched in active disease and in patients with IBD who did not respond to anti-TNF biologic therapy and that PAI-1 is a critical link between the epithelium and inflammation, using this gene-network analysis across 14 independent cohorts and 1800 intestinal biopsies. Regarding function, PAI-1 and its direct target, the fibrinolytic protease tissue Plasminogen Activator (tPA), are critical regulators of intestinal inflammation. Intestinal epithelial cells produce tPA, protecting mice against colonic damage caused by chemicals and mechanical forces. By limiting tPA-mediated cleavage and activation of anti-inflammatory TGF-β, PAI-1 worsens mucosal damage, whereas inhibiting PAI-1 reduces both mucosal damage and inflammation. This research finds an immune-coagulation gene axis in IBD that may contribute to more aggressive illness [44]. Eshelman et al. found that the levels of 21 immune transcripts are significantly correlated with age at diagnosis, from which *SERPINE1* was one of the most strongly associated genes with age involved in ECM processes. All genes they discovered were significantly elevated in CD patients diagnosed before 30 years of age compared with those diagnosed later in life [45].

Intestinal fibrosis is a significant complication of CD. The profibrotic protein TGF-β has been considered critical for the induction of the fibrotic program. TGF-β can induce not only the expression of Extracellular Matrix (ECM), including collagen but also the production of

PAI-1, preventing enzymatic degradation of the ECM during the onset of fibrotic diseases. In 2020, Imai et al. concluded that the PAI-1-mediated fibrinolytic system facilitates collagen degradation suppression. Hence, the PAI1 inhibitor could be applied as an anti-fibrotic drug in CD treatment [46]. On the other hand, because of the close link between IBD and cancer, some of the anticipated genes are also associated with tumor initiation. Yuan et al. also identified SERPINE1, a serine proteinase inhibitor that encodes the primary inhibitor of tissue Plasminogen Activator (tPA) and urokinase, as a potential gene in another investigation (uPA). Tissue plasminogen activator and urokinase are two enzymes involved in wound healing and inflammation. SERPINE1 and proteins in the downstream pathway of its particular pathway have also been linked to IBD as a functional regulator [3].

Because of a lack of understanding of disease-site, and protein-level processes, anti-TNF medication resistance is a serious therapeutic problem in IBD. Various common Protein-Coding Genes (PCGs) with a potent inflammatory and immunological profile were consistent in mouse-human colon and blood. Several of these genes have previously been linked to IBD, including *ITGAM*. Consistent with previous studies, we discovered *ITGAM* to be highly expressed in the non-inflamed group [47, 48].

A major clinical problem in some patients of CD and UC, as two types of IBD, is to distinguish plainly between them, which is important to design suitable medical and surgical therapy. In an experimental study, Connective Tissue Growth Factor (CTGF), a new peptide linked to fibrotic illnesses, was analyzed in CD and UC patients to see if it could have a role in these two diseases. Their findings suggest that CTGF has a distinct role in IBD, which may be beneficial in distinguishing between UC and CD, particularly in individuals with unique illness presentations. Furthermore, they demonstrated that CTGF has an essential role in CD, where fibrosis and stenosis are expected consequences that necessitate surgery [49]. Also, Song et al. discovered that the severity of UC was associated with an increase in CTGF expression in the intestinal mucosa. CTGF inhibition can enhance the intestinal flora and partially cure colitis-induced damage in UC mice by blocking the Extracellular Signal-Regulated Kinase (ERK) signaling pathway and down-regulating the production of inflammatory factors [50]. Furthermore, CTGF's significance in fibrosis is explained by its increased expression in stricture areas of Crohn's fibroblasts. Tumor Necrosis Factor (TNF) promotes stricture development by suppressing fibrosis by downregulating fibroblast CTGF production, an effect that may be lost with anti-TNF treatment [51].

It is worth noting that the current research has some limitations. For example, the used dataset has an inadequate sample size. As a result, further research with larger sample size is needed to obtain more definitive results. When a comprehensive study is integrated with microarray chips, more efficient results can be obtained. The possibility of uncovering robust candidates for diagnosis and treatment is high when numerous transcriptome datasets are analyzed. As a result, it is suggested that meta-analysis be used to assess the overall effect. However, there are several other aspects to consider. Because of differences in microarray platforms, facilities, and workers, different research may be heterogeneous. Furthermore, data format and class labels should be consistent across datasets. Besides, integrated analysis of multi-chip data may be difficult because of differences in research design, calculation mistakes, and insufficient information (biopsy position [colon and ileum] and disease activity status [inflamed and non-inflamed]). A second limitation is the lack of experimental verification. In this study, the results of a chip expression profile were examined utilizing a bioinformatics system review, but they were not validated using Reverse-Transcription-quantitative (RT-q) PCR. Apart from that, the original study only looked at the differential expression of the epithelial barrier genes Mucin 1 (*MUC1*), Mucin 4 (*MUC4*), Trefoil Factor-1 (*TFF1*), Claudin 1 (*CLDN1*), Claudin 8 (*CLDN8*), and Occludin (*OCLN*), Desmoglein 3 (DSG3), and Membrane-Associated Guanylate kinase with an Inverted repeat member 1 (*MAGI1*) [52]. Therefore, although there are some limitations in the case of experimental validation and the number of clinical samples which should be larger, new information about the underlying mechanisms of IBDs was revealed in the current study.

Conclusion

Using an integrated analysis approach to define DEGs and biological functions and pathways shared by or specific to the inflamed and non-inflamed regions in CD patients enriches the current knowledge of CD pathogenesis and molecular mechanisms of the inflamed and non-inflamed regions. Moreover, these findings may lead to the identification of possible biomarkers for determining CD activity status (inflamed and non-inflamed), as well as therapeutic targets for introducing new CD therapies. The current study, however, only performed bioinformatics analysis and no trials to validate the biomarkers. More tests and analyses of larger datasets are needed to confirm the potential of the above putative genes to differentiate CD.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article.

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Authors contribution's

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interests.

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