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IDENTIFICAÇÃO DE GENES RELEVANTES E OS PRINCIPAIS CAMINHOS ENTRE AS DOENÇAS CELÍACA E DE CROHN ATRAVÉS DE FERRAMENTAS DE BIOINFORMÁTICA

IDENTIFICATION OF HUB GENES AND KEY PATHWAYS BETWEEN CELIAC AND CROHN'S DISEASES VIA BIOINFORMATICS TOOLS

BIYOINFORMATIK ARAÇLAR ARACILIĞIYLA ÇÖLYAK VE CROHN HASTALIKLARI ARASINDAKI HUB GENLERIN VE ANAHTAR YOLAKLARIN TANIMLANMASI

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RESUMO

Introdução: As doenças inflamatórias crônicas são a resposta de longo prazo do organismo a qualquer estímulo. As doenças de Crohn (CD) e Celíaca (CeD) estão entre as doenças inflamatórias crônicas e ambas causam inflamação crônica nos intestinos. Ambas as doenças são causadas por fatores de risco poligênicos, ambientais e de estilo de vida. A inflamação pode perpetuar a doença e torná-la crônica. Por esta razão, DC e CeD que escolhem o intestino como órgão-alvo podem desencadear um ao outro. Embora a relação entre essas doenças seja amplamente mencionada na literatura, há poucos conhecimentos e pesquisas sobre os mecanismos imunológicos dessas doenças inflamatórias. **Objetivos**: Este estudo teve como objetivo determinar genes hub, fatores de transcrição-miRNAs e redes de interação proteína-química compartilhadas entre CD e CeD. **Metodos**: Os conjuntos de dados NCBI-GEO foram baixados e analisados em GEO2R para identificar genes diferencialmente expressos (DEGs). A ferramenta STRING para interação proteína-proteína (PPI) e a ferramenta NetworkAnalyst foram usadas para análise de enriquecimento de conjunto de genes (GSEA), fator de transcrição (TF) - redes coregulatórias de miRNA e interações proteína-químicas. **Resultados e Discussões**: Os conjuntos de dados GSE11501 e GSE3365 foram utilizados para reconhecer 54 DEGs em CD e CeD. 13 desses genes comumente expressos foram definidos como genes hub. A GSEA indicou que esses genes estão associados a processos do sistema imunológico, resposta de defesa celular, proteólise e apoptose. KAT6A e SPI1 são fatores de transcrição que direcionam a continuidade das células epiteliais intestinais. Agentes antirreumáticos e metotrexato provavelmente serão usados para tratar essas doenças. **Conclusões**: Em conclusão, pensamos que a hipersensibilidade do tipo retardado resultante da propagação de epítopos é um mecanismo imunológico comum de DC e CeD. Dada a crescente prevalência de DC e CeD na população, fica claro que mais estudos são necessários para entender a patogênese compartilhada e os mecanismos imunológicos sobrepostos dessas doenças.

Palavras-chave: *Bioinformática, doença celíaca, doença de Crohn, genes expressos diferencialmente (DEGs), genes hub, inflamação.*

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ABSTRACT

Background: Chronic inflammatory diseases are the long-term response of the organism to any stimulus. Crohn's (CD) and Celiac (CeD) diseases are among chronic inflammatory diseases, and both cause chronic inflammation in the intestines. Both diseases are caused by polygenic, environmental, and lifestyle risk factors. Inflammation can perpetuate disease and cause it to become chronic. For this reason, CD and CeD that choose the intestine as the target organ may trigger each other. Although the relationship between these diseases is widely mentioned in the literature, scanty knowledge and research have been done on the immune mechanisms of these inflammatory diseases. **Aim:** This study aimed to determine hub genes, transcription factors-miRNAs, and protein-chemical interaction networks shared between CD and CeD. **Methods:** The NCBI-GEO datasets were downloaded and analyzed in GEO2R to identify differentially expressed genes (DEGs). STRING tool for Protein-Protein Interaction (PPI) and NetworkAnalyst tool were used for Gene Set Enrichment Analysis (GSEA), Transcription factor (TF) - miRNA Coregulatory Networks, and Protein-Chemical Interactions. **Results and Discussion**: GSE11501 and GSE3365 datasets were utilized to recognize 54 DEGs in CD, and CeD. 13 of these commonly expressed genes were defined as hub genes. GSEA has indicated that these genes are associated with immune system processes, cellular defense response, proteolysis, and apoptosis. KAT6A and SPI1 are transcription factors that direct the continuity of intestinal epithelial cells. Antirheumatic agents and Methotrexate are likely to be used to treat these diseases. **Conclusions:** In conclusion, we think that delayed-type hypersensitivity resulting from epitope propagation is a common immune mechanism of CD and CeD. Given the increasing prevalence of both CD and CeD in the population, it is clear that more studies are needed to understand the shared pathogenesis and overlapping immune mechanisms of these diseases.

Keywords: *Bioinformatics, Celiac disease, Crohn's disease, Differentially Expressed Genes (DEGs), Hub genes, Inflammation*.

ÖZET

Giriş: Kronik inflamatuar hastalıklar, organizmanın herhangi bir uyarana uzun vadeli tepkisidir. Crohn's (CD) ve Çölyak (CeD) hastalıkları kronik inflamatuar hastalıklar arasındadır ve her ikisi de bağırsaklarda kronik inflamasyona neden olur. Her iki hastalığın da poligenik, çevresel ve yaşam tarzı risk faktörlerinden kaynaklandığı düşünülmektedir. Enflamasyon bir hastalığı sürdürebilir ve kronikleşmesine neden olabilir. Bu nedenle bağırsağı hedef organ olarak seçen CD ve CeD birbirini tetikleyebilir. Literatürde bu hastalıklar arasındaki ilişkiden çokça bahsedilmesine rağmen, bu inflamatuar hastalıkların immün mekanizmaları hakkında çok az bilgi ve araştırma yapılmıştır. **Amaç:** Bu çalışma, CD ve CeD arasında paylaşılan hub genlerini, transkripsiyon faktörleri-miRNA'ları, protein-kimyasal etkileşim ağlarını belirlemeyi amaçlamaktadır. **Yöntem:** NCBI-GEO verisetleri indirilip GEO2R'de analiz edilerek ifadesi farklılaşan genler (DEG'ler) belirlendi. Protein-Protein Etkileşimi (PPI) için STRING aracı ve Gen Setleri Zenginleştirme Analizi (GSEA), Transkripsiyon faktörü (TF) - miRNA Ortak Düzenleyici Ağları, Protein-Kimyasal Etkileşimleri için NetworkAnalyst aracı kullanıldı. **Sonuçlar ve Tartışma:** CD ve CeD'deki 54 DEG'leri tanımlamak için GSE11501 ve GSE3365 verisetleri kullanıldı. Bu ortak ifade edilen genlerden 13'ü, hub genleri olarak belirlendi. GSEA, bu genlerin bağışıklık sistemi süreçleri, hücresel savunma tepkisi, proteoliz ve apoptoz ile ilişkili olduğunu vurgulamıştır. KAT6A ve SPI1, bağırsak epitel hücrelerinin devamlılığını yönlendiren transkripsiyon faktörleridir. Bu hastalıkların tedavisinde antiromatizmal ajanlar ve Metotreksat kullanılması mümkündür. **Karar:** Sonuç olarak, epitop yayılımından kaynaklanan gecikmiş tip aşırı duyarlılığın, CD ve CeD'in ortak bir immün mekanizması olduğunu düşünüyoruz. Popülasyonda hem CD hem de CeD'in artan prevalansı göz önüne alındığında, bu hastalıkların ortak patogenezini ve örtüşen bağışıklık mekanizmalarını anlamak için daha fazla çalışmaya ihtiyaç olduğu açıktır.

Keywords: *Biyoinformatik, Çölyak hastalığı, Crohn’s hastalığı, İfadesi farklılaşan genler (DEG'ler), Hub genleri, Enflamasyon*.

1. INTRODUCTION:

Chronic inflammatory diseases are thought to be responsible for more than 50% of deaths today (Furman *et al.*, 2019). These diseases are defined as a prolonged and chronic inflammatory response of the immune system. Inflammation is a response that protects the organism against endogenous or exogenous stimulus such as pathogens or invading cells. This process is complemented by eliminating the stimulus, cleaning damaged cells, and repairing tissue (Netea *et al.*, 2017). Inflammation occurs in three phases: induction, peak, and resolution (Schett

and Neurath, 2018). After the inflammatory stimulus has been removed, control of the resolution phase is necessary to maintain homeostasis. Failure to resolve inflammation leads to chronic inflammatory diseases (McInnes and Schett, 2017; Schett *et al.*, 2013).

Celiac (CeD) and Crohn's diseases (CD) have complex genetic features that manifest with chronic inflammation in the gut, which can be caused by polygenic, environmental, and lifestyle risk factors (Heap and van Heel, 2009). Both diseases can be induced through the innate and adaptive immune systems, and the etiology and immunopathogenesis are still unclear (*Petagna et al.*, 2020; Marafini *et al.*, 2016). These diseases continue throughout their lifetime and adversely affect the quality of life of the individuals suffering from these diseases. CeD causes complaints in the patient depending on the intake of gluten-containing foods such as wheat, rye, and barley and can be inherited with the HLA-DQ2 and HLA-DQ8 gene alleles. Although the certain cause of CD is unknown, it may be affected by changes in the intestinal microbiota. CD and ulcerative colitis (UC) are forming the inflammatory bowel disease group and are often evaluated and confused together.

The mucosal immune system in the gastrointestinal tract consists of intraepithelial lymphocytes (IELs) that act as antigen-presenting cells and the lamina propria containing Peyer's patches and immune cells (Suzuki *et al.*, 2018; Tokuhara *et al.*, 2019). The mucosal immune system is in contact with the peripheral immune system and contributes to the formation of tolerance. The chronic inflammation of the mucosal immune system creates a Th1-like environment and damages the tissues. It has been known that Th1 and Th17 responses are present in CeD and CD diseases (Imperatore *et al.*, 2016; Geremia *et al.*, 2014). Chronic inflammation can perpetuate disease and trigger the other. Previous studies suggest that patients with CD also show a high rate of CeD (Tursi *et al.*, 2005) and that a patient with CeD subsequently develops CD (Lail *et al.*, 2016). Although the inflammatory process and resolution of inflammation are universal, tissue-specific changes are observed. Disturbances in these processes in chronic inflammatory diseases may contain similarities and differences, tissue-specific and disease-specific (Schett and Neurath, 2018; Schett *et al.*, 2013). Although the relationship between these two complex diseases is mentioned in the literature, limited research has been done on the collective immune mechanisms of the diseases (Festen *et al.*, 2011). Therefore, this study determined the similarities between the immune mechanisms in chronic inflammation for CD and CeD that target the same organ.

2. MATERIALS AND METHODS:

2.1. Datasets

Datasets GSE11501 and GSE3365 in NCBI GEO (United States National Center for Biotechnology Information-Gene Expression Omnibus) were downloaded and analyzed in GEO2R for CeD and CD respectively. Due to the complexity of both diseases, special attention was paid to ensure that the expression profiles in datasets were from blood samples. GEO2R is an online tool that facilitates comparing sample groups from a GEO dataset using the GEOquery and limma R packages to identify differentially expressed genes with Bioconductor. The statistical analysis data calculated with GEO2R was transferred to Excel for further processing.

2.2. Differentially Expressed Genes (DEGs)

The downloaded data were imported to excel and the genes with $p < 0.05$ were determined. In each dataset, genes expressing up or down-regulated according to logFC were defined separately. Candidate genes whose expression differentiated according to logFC and $p < 0.05$ were identified. Multiple List Comparator online tools were used to identify differentially expressed genes for both diseases.

2.3. Protein-Protein Interaction (PPI)

Differentiating genes for protein-protein interaction (PPI) network was performed with STRING online tool. STRING is a database that calculates and predicts the physical and functional relationships of protein-protein interactions. This way, interactions between proteins were determined within a confidence score range. The file was imported into Cytoscape 3.9.1, which is a program that can visualize PPI networks and perform advanced statistical analysis with these networks with its plugin.

2.4. Gene Set Enrichment Analysis (GSEA)

Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses were used. Genes whose expressions were changed in mutual for both diseases were associated with GO terms and grouped with their biological process, cellular components, and molecular functions. In the KEGG analysis, the common pathways of these genes were determined. NetworkAnalyst online

tool was used to visualize the GSEA results.

2.5. Transcription factor (TF) - miRNA Coregulatory Networks

The regulatory interaction of hub genes with transcription factors and miRNA was constructed using the RegNetwork repository and visualized with NetworkAnalyst.

2.6. Protein-Chemical Interactions

The interaction network of hub gene proteins with chemicals was created based on the Comparative Toxicogenomics Database (CTD) by NetworkAnalyst.

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1 Datasets

Affymetrix Human Genome U133A Array-GPL96 platform in the GSE3365 dataset and Illumina humanRef-8 v2.0 expression bead chip-GPL6104 platform in the GSE11501 dataset is used. GSE11501 dataset is gene expression of primary leukocytes and includes 110 samples with CeD disease and 22 samples from healthy controls (Table 1). GSE3365 dataset is expression profiles of peripheral blood mononuclear cells and includes 59 samples with CD disease, 26 samples with UC, and 42 samples from healthy controls (Table 1). Each dataset was defined into patient and control groups (except UC), and logarithmic fold change (logFC) and p values were determined in GEO2R (Figure 1).

3.1.2 Differentially Expressed Genes (DEGs)

In order to eliminate the differences in the platforms used in the datasets, the logFC rates are specific to each dataset (|log FC| ≥ 0.05 in GSE3365 and |log FC| ≥ 0.02 in GSE11501), and $p < 0.05$ was determined (Maouche *et al.*, 2008). There were 147 up and 87 downregulated genes in CeD disease, while 1506 up to 1343 downregulated genes in CD disease (Table 2). Multiple List Comparator online tools detected 54 DEGs in both diseases (Figure 1E).

3.1.3 Protein-Protein Interaction (PPI)

PPI networks were performed with STRING online tool among 54 determined DEGs (Table 3). Possible functional pathways between proteins emerged via PPI networks. 28 edges were detected between 54 nodes with STRING default settings (Figure 2A). Analysis results for generated PPI network were PPI enrichment p-value: 2.98e-05 and average local clustering coefficient: 0.407. Then, the file was imported to Cytoscape 3.9.1 and analyzed with its plugin CytoHubba. It predicts key nodes in the PPI network using various algorithms using shortest paths and centrality. Hub genes were calculated and visualized according to a degree (Figure 2B). The analysis showed that 13 of these genes were hub genes (Table 3). ANPEP, CXCR1/2, and GZMB genes are those with ≥ 4 connections from the hub genes. The expression graphs of these genes in CeD and CD diseases were generated separately (Figure 3).

3.1.4 Gene Set Enrichment Analysis (GSEA)

GSEA was evaluated by NetworkAnalyst online tool according to hub genes (Figure 4). GO terms and KEGG pathway analysis results FDR < 0.5 are significant. Considering KEGG pathway analysis, hub genes are mostly associated with the Phospholipase D signaling pathway (CXCR2, CXCR1, GAB2), epithelial cell signaling in Helicobacter pylori infection (CXCR2, CXCR1). GO Biological process results highlight the relationships with immune system process (CTSL, GZMA, CXCR1, CXCR2, NCF4, KAT6A, GAB2, CD160), cellular defense response (CXCR2, CD160), proteolysis (CTSL, GZMA, ANPEP, GZMH, GZMB), cellular component disassembly involved in the execution phase of apoptosis (GZMA, GZMB). GO Cellular component information shows the familiarities with an immunological synapse (GZMA, GZMB), lytic vacuole (CTSL, CXCR2, NCF4), lysosome (CTSL, CXCR2, NCF4), plasma membrane part (GZMA, GZMB, CXCR2, NCF4, ANPEP, CD160), vacuole (CTSL, CXCR2, NCF4), Plasma membrane (GZMA, GZMB, CXCR2, NCF4, ANPEP, CD160, FPR2, CXCR1, GAB2), intrinsic to the plasma membrane (CXCR2, NCF4, ANPEP, CD160). GO Molecular function data indicated links with endopeptidase activity (ANPEP, CTSL, GZMH, GZMA, GZMB), peptide receptor activity (FPR2, CXCR1, CXCR2), serine-type endopeptidase activity (GZMH, GZMA, GZMB), peptidase activity (ANPEP, CTSL, GZMH, GZMA, GZMB), serine-type peptidase activity (GZMH, GZMA, GZMB), serine hydrolase activity (GZMH, GZMA, GZMB), cytokine binding (CXCR1, CXCR2), cytokine receptor activity (CXCR1, CXCR2), phosphatidylinositol binding (NCF4, GAB2).

3.1.5 Transcription factor (TF) - miRNA Coregulatory Networks

A comprehensive network analysis involving TF and miRNA was performed to

understand the regulation mechanism of hub genes. Eleven of the hub genes (CD160, GZMH, KAT6A, GAB2, CTSL, ANPEP, GZMB, PI3, NCF4, CXCR1, CXCR2) were connected in the TF - miRNA network (Figure 5A). KAT6A is a transcription factor linked with SPI1, USF1, MXI1, and 23601 TFs. SPI1 is the transcription factor most associated with hub genes in the regulatory network. Since miRNAs are connected with at most two hub genes, it is unclear whether they are involved in the regulation mechanism of both diseases.

3.1.6 Protein-Chemical Interactions

According to CTD, chemical substances or drug molecules associated with at least three of the hub genes have been identified (Figure 5B). The ones with the most connections are Nickel (ANPEP, CXCR1, GZMA, FPR2, NCF4, CTSL, PI3, CD160, GZMH, GZMB, CXCR2), Antirheumatic Agents (ANPEP, GZMA, FPR2, NCF4, CTSL, GZMH, GZMB, CXCR2), (+)-JQ1 compound (CXCR1, GAB2, GZMA, NCF4, KAT6A, CD160, GZMB), Aflatoxin B1 (ANPEP, CXCR1, GAB2, FPR2, KAT6A, CD160, CXCR2), Methotrexate (ANPEP, GZMA, FPR2, NCF4, PI3, CXCR2), Lipopolysaccharides (CXCR1, GZMA, PI3, CD160, GZMH, CXCR2).

3.2. Discussion

This study used bioinformatics tools to evaluate the common immune mechanisms in CD and CeD diseases. Comparative analyses revealed 13 hub genes (GZMB, GZMA, GZMH, CD160, CXCR1, CXCR2, ANPEP, FPR2, GAB2, PI3, NCF4, CTSL, and KAT6A) in these diseases with complex genetics. GZMB has a central role in the pathway between identified hub genes. ANPEP, CXCR1/2, GZMB from hub genes, degree ≥ 4. However, considering the complex mechanism of these diseases, all of the hub genes found are important. GSEA results of hub genes show that they are associated with biological processes of the immune system, cellular defense response, proteolysis, and apoptosis. According to KEGG, the Helicobacter pylori pathway highlights the similarity of the gut microbiome between diseases, while the phospholipase D signaling pathway indicates that tissue damage is repaired by inducing cell proliferation. Finally, TFs causing differential gene expression and possible drug candidates are discussed.

Granzymes are serine protease granules secreted by CTL (Cytotoxic T lymphocytes) and NK (Naturel Killer) to induce cell death. There are different types, such as GZMA, GZMB, GZMH, GZMK, and GZMM. The most well-known type is GZMB, which induces caspase-dependent and independent apoptosis and pyroptosis. GZMA is not different from GZMB and it also has a role in DNAdegredation (Beresford *et al.*, 2001). Unlike other granzymes in the formation of the immune response, GZMH contributes to the antiviral response by acting on the cytolysis of the target cell. Expressions of all granzyme types are up-regulated in CD and down-regulated in CeD. GZMB, GZMA, and GZMH expressions were studied separately in CeD and CD diseases, and it was mentioned that they might have a biomarker role (Kim *et al.*, 2018; Marafini *et al.*, 2016; James *et al*., 2021; Jaeger *et al*., 2021; Trzupek *et al.*, 2020; Aguilar *et al.*, 2021).

CXCR1/2 chemokine receptors belong to the G-coupled-protein-receptor family and are expressed by endothelial cells. IL-8 binds to receptors and slows down, followed by phagocytosis. It also provides the formation of proteolytic enzymes and ROS (Reactive oxygen species) components required for oxidative damage. CXCR1/2 expressions are down-regulated in CD but up-regulated in CeD. It has been mentioned that the expression of chemokine receptors alters in CeD and CD diseases (Cheluvappa *et al.*, 2018; Lauxmann *et al.*, 2021).

Aminopeptidase N (ANPEP) terminates the digestion of peptides in the small intestine and takes part in processing peptide hormones and regulatory peptide molecules secreted by various cells. It can also increase presentation to T cells by cleavage of peptides bound to MHC II molecules in antigen-presenting cells. Therefore, it contributes to inflammation (Dong *et al.*, 2000). ANPEP expression is down-regulated in CD but up-regulated in CeD. While CeD disease is associated with genes encoding HLA DQ2 and DQ8, MHC II class genes have also been linked with CD disease (Yang *et al.*, 1999).

CD160 antigen is expressed by NK, CTL, and IELs, leading to cell activation and differentiation. It can bind broadly to classical/non-classical MHC class I molecules (Agrawal *et al.*, 1999). Thus, it can play an immune-regulatory role between antigen-presenting cells and lymphocytes (Le Bouteiller *et al.*, 2002). CD160 expression was up-regulated in CD and down-regulated in CeD. Proteins with N-Formylmethionine residue synthesized by bacteria stimulate FPR2 and provide chemotaxis of neutrophils and granulocytes. In this way, it activates the phosphatidylinositol-calcium secondary messenger via the G-protein and directs phagocytosis. FPR2 expression is down-

regulated in CD but up-regulated in CeD. GAB2 acts as a gatekeeper in the intracellular activation of SHP2, PI3K, Grb2, ERK, and AKT. It plays a role in mast cell-related inflammation with PI3K activation (Simister and Feller, 2012). GAB2 expression is down-regulated in CD but up-regulated in CeD. Peptidase inhibitor-3 (PI3), or Elafin, is a specific inhibitor of elastase secreted from neutrophils in epithelial cells (Paczesny *et al.*, 2009). In this way, it prevents the proteolysis of the epithelial tissue and ensures its persistence. PI3 expression is down-regulated in CD but up-regulated in CeD. NCF4 is involved in regulating the NADPH-oxidase enzyme system, which creates ROS products in cell defense. It also has a role in PI3K signaling. NCF4 expression is down-regulated in CD but up-regulated in CeD. It has been shown that SNPs in NCF4 increase the risk of developing CD disease (Muise *et al.*, 2012). Cathepsin L1 (CTSL) is a lysosomal cysteine protease, although it can be secreted in inflammation and result in apoptosis (Gomes *et al.*, 2020). It also assists in the elimination of bacteria with its ability to control neutrophil elastase (Belaaouaj *et al.*, 2000). CTSL expression was downregulated in both CD and CeD.

Differentially expressed genes in both diseases are indicated by a pathway divided into three branches. These loops are included processes; the induction of various cell death pathways (apoptosis, pyroptosis, lysis) after the presentation of antigen-presenting cells (APC) with MHC class I and II molecules, IL-8-directed neutrophils being attracted to the region to provide phagocytosis and ROS, on the other hand, stimulating cell proliferation to prevent tissue damage. These processes can suggest us a delayed-type hypersensitivity response resulting from epitope propagation. Delayed-type hypersensitivity responses occur when susceptible individuals are exposed to the same antigen (Warrington *et al.*, 2011). Epitope propagation refers to developing an immune response against epitopes different from the disease-causing epitope (Powell and Black, 2001). Thus, initial responses to the infectious agent can lead to a series of independent responses to different self-epitopes, resulting in autoimmunity. While CeD disease is known to emerge result of re-exposure to gluten, it is predicted that CD disease may also occur as a result of delayed-type hypersensitivity (Fujimoto *et al.*, 2019). Furthermore, it has been shown that CD can develop through microbiome interactions and alter gene expressions in the gut (Sudhakar *et al.*, 2022). The results of our bioinformatic analyses suggest that delayed-type hypersensitivity response resulting from epitope propagation may be a common immune mechanism in CD and CeD.

Apart from these genes associated with inflammation, KAT6A is an acetyltransferase that acetylates histones (H3, H4) and is involved in chromatin remodeling. It also acts as a co-activator for several transcription factors. For example, it acetylates p53 and affects its transcriptional activities (Rokudai *et al.*, 2013). In this way, it plays an important role in developing hematopoietic stem cells (Yang and Ullah, 2007). KAT6A expression was up-regulated in both CD and CeD. As a transcription factor, SPI1 plays a role in differentiating macrophages or B cells or in gene expression activation (Oikawa *et al.*, 1999). It also has the function of regulating alternative splicing of target genes. KAT6A and SPI1 drive the inflammatory mechanism for deciding between survival and death of intestinal epithelial cells.

Protein-chemical network analysis results, Nickel, which has the most connection, can make an organism more susceptible to autoimmune diseases (Drenovska *et al.*, 2020). Antirheumatic agents and Methotrexate are grouped in the disease-modifying antirheumatic drugs (DMARDs) category and used to slow disease progression in rheumatoid arthritis and other autoimmune diseases (Benjamin *et al.*, 2022). Methotrexate has been used in the treatment of CD for over 25 years (Feagan *et al.*, 1995) and may be used to treat CeD, given the drug's immune-modulating role (Caio *et al.*, 2019). Since CD and CeD are chronic inflammatory diseases, these drugs can be used in the treatment to reduce inflammation and pain. JQ1 compound has got the potential to be used as an anti-cancer agent by inhibiting cell proliferation and suppressing BRD4 target genes (Jiang *et al.*, 2020). Lipopolysaccharides originate from gram-negative bacteria and have an evolutionary role in triggering the inflammatory response (Tucureanu *et al.*, 2017).

4. CONCLUSIONS:

In this study, it was detected DEGs between CD and CeD via bioinformatics tools. It was identified 13 DEGs as hub genes, and the authors believe that they may play a role in the immune mechanism of diseases. Hub genes are associated with the induction of various cell death pathways, triggering ROS and stimulating proliferation. The similarity of immune mechanisms between CD and CeD is related to epitope propagation and delayed-type hypersensitivity. As a result of TF-miRNA network analysis, it was revealed that KAT6A, one of the

hub genes, and SPI1 are TFs that define the direction of tissue damage or repair in the gut. It has been suggested that antirheumatic agents and Methotrexate can be used in treating CD and CeD by protein-chemical network analysis. Since this study is limited throughput in certain datasets, there is a need for experimental and clinical studies to reveal the similarities and differences between CD and CeD.

5. DECLARATIONS

5.1. Study Limitations

Only GSE11501 and GSE3365 datasets were used in this study because the only blood sample datasets belong to CD and CeD diseases in NCBI-GEO.

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5.4. Competing Interests

Authors have no financial interests that are directly or indirectly related to the work submitted for publication.

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Table 1. *Quantities of data sets used in the research.*

Dataset	Platform	Healthy	CD	CeD	Total
GSE11501	GPL6104	22	0	110	132
GSE3365	GPL96	42	59	0	101

Table 2. *The list of top 20 genes whose expression is up or down regulated in CeD and CD diseases.*

CeD		CD	
Up Regulated	***Down Regulated***	***Up Regulated***	***Down Regulated***
HLA-DRB1	DHRS9	NPRL2	CXCL8
HLA-DRB5	ATP5O	MYCBP2	EPB42
CSF3R	RBM12B	CLIC3	CXCL1
MBOAT7	MS4A7	MARK2	SLC6A8
IL17RA	FAM26F	MIR4800	CYP1B1
MYO1F	VAMP5	SNRNP70	PF4V1
PGGHG	SLC25A28	XCL2	CXCL3
ANPEP	CLC	CYLD	TMEM158
SORL1	RPL24	KLRF1	IL1R1
FAM129A	CTSL	RUFY3	THBS1
ARID3A	MYL12A	ZFAND6	CXCL2
PI3	PDZK1IP1	DDX3Y	ITGA2B
FFAR2	PYHIN1	KRI1	ALAS2
NRGN	ATP5C1	RBBP6	THBS1
BRD4	CD160	MYOM2	SDC2
ADAM8	GZMB	MIR664B	SDC2
PPP3R1	GBP5	ILF3	ABLIM3
KLF6	LILRA3	CAPN3	CCL2
NAMPT	GZMA	TNPO3	MYL9
ATG16L2	GZMH	KLF6	SERPINB2

Table 3. *Differentially expressed genes in CeD and CD diseases and hub genes marked an asterisk.*

ADAM8	BIRC3	FFAR2	KLF6	PDZK1IP1	RNF24
ADGRG3	BRD3	FPR2*	LRRN3	PGGHG	SLC25A37
AKR1C3	BUB3	GAB2*	LTBP3	PI3*	SORL1
ALPL	CD160*	GZMA*	MBOAT7	PPP3R1	SZRD1
ANPEP*	CTSL*	GZMB*	MGAM	PTAFR	TIMP2
APBB1IP	CXCR1*	GZMH*	MME	RASGRP2	TLE3
ARHGAP26	CXCR2*	HIPK2	NAMPT	RBX1	TNFAIP2
ATP5C1	DYSF	KAT6A*	NCF4*	RERE	TNFRSF10C
BASP1	FAM129A	KDM2A	NRGN	RNF19B	ZMYM6

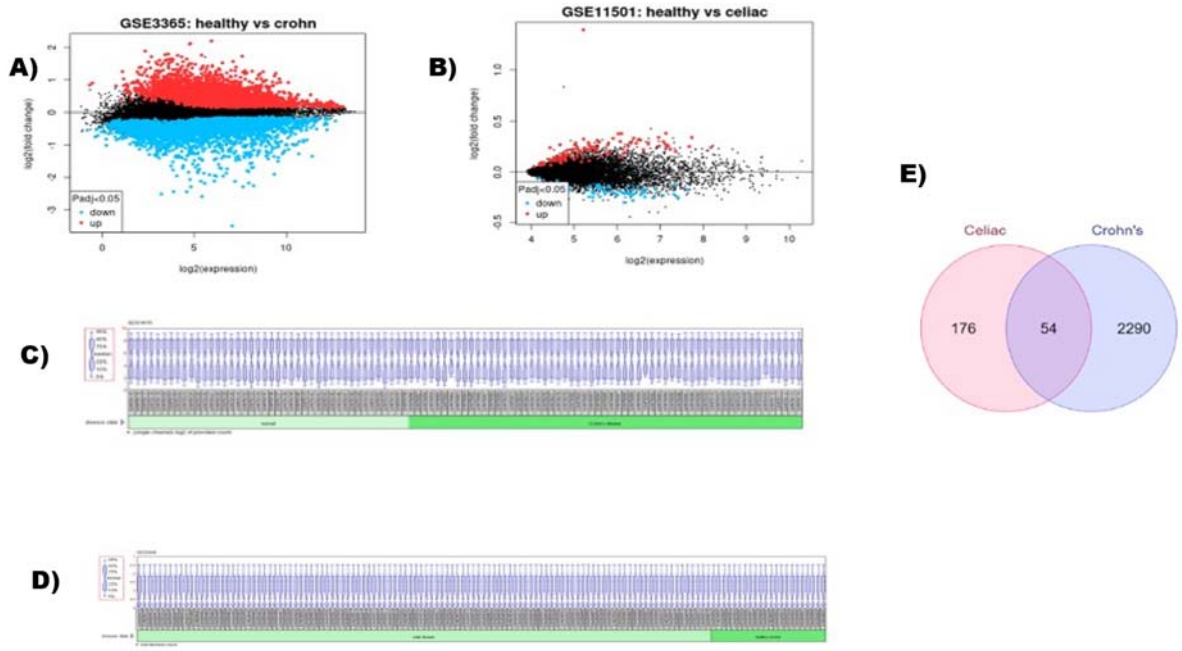

Figure 1. A) *The volcano plot with logFC values,* ***B)*** *The volcano plot with logFC values,* ***C)*** *The box plot with value distribution of CD disease dataset.* ***D)*** *The box plot with value distribution of CeD disease dataset.* ***E)*** *Differentially expressed genes in CD and CeD diseases.*

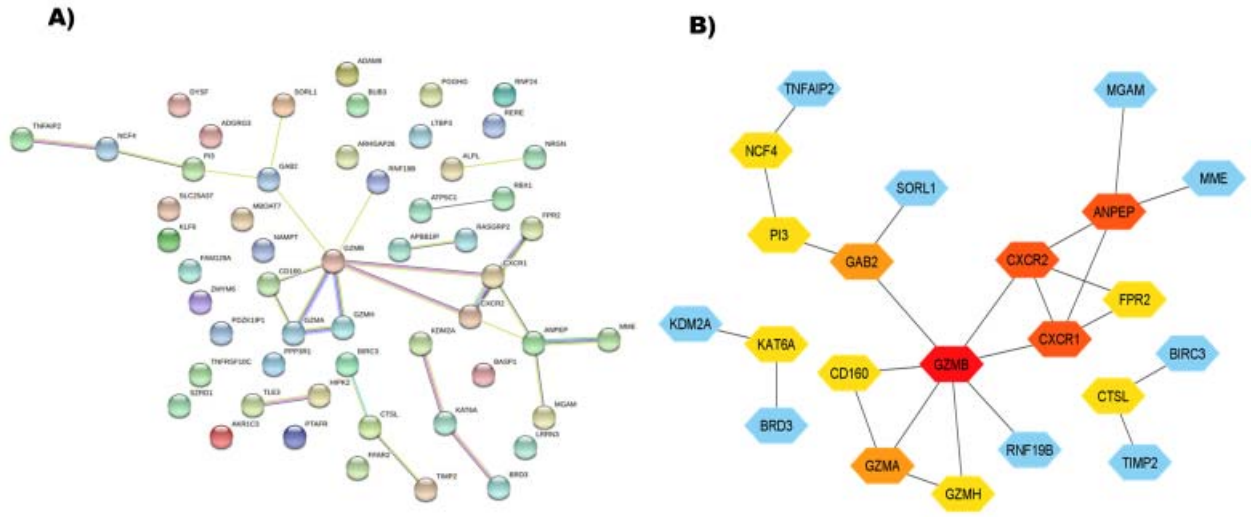

Figure 2. A) *PPI interactions with STRING.* ***B)*** *13 hub genes whose degree reduces from red to blue ones.*

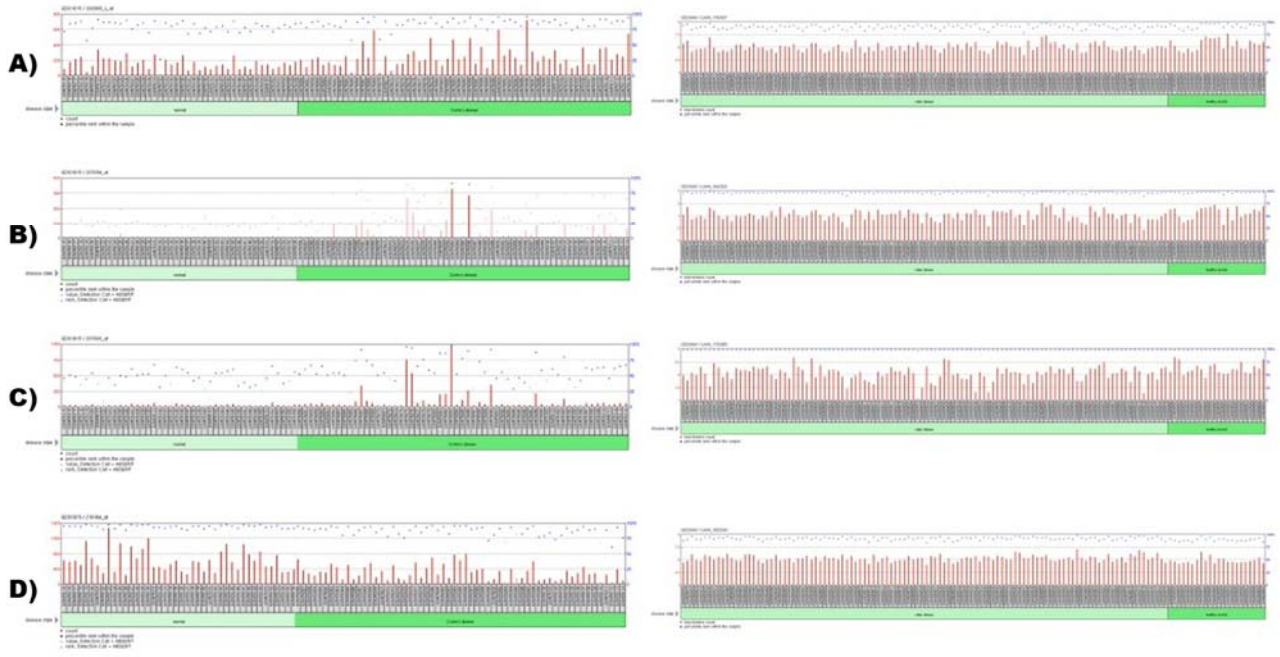

Figure 3. A) *ANPEP expression charts.* ***B)*** *CXCR1 expression charts.* ***C)*** *CXCR2 expression charts.* ***D)*** *GZMB expression charts.*

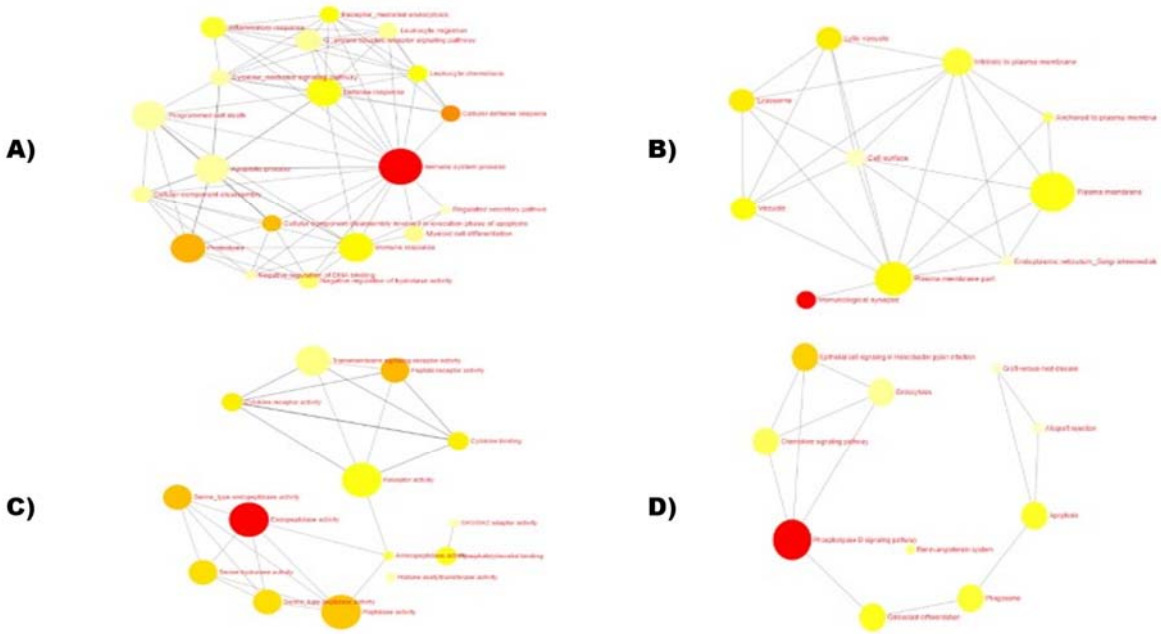

Figure 4. *GSEA results visualized in NetworkAnalyst. The darkness and size of the circles indicate significance.* ***A)*** *GO Biological Process.* ***B)*** *GO Cellular Component.* ***C)*** *GO Molecular Functions.* ***D)*** *KEGG Pathway.*

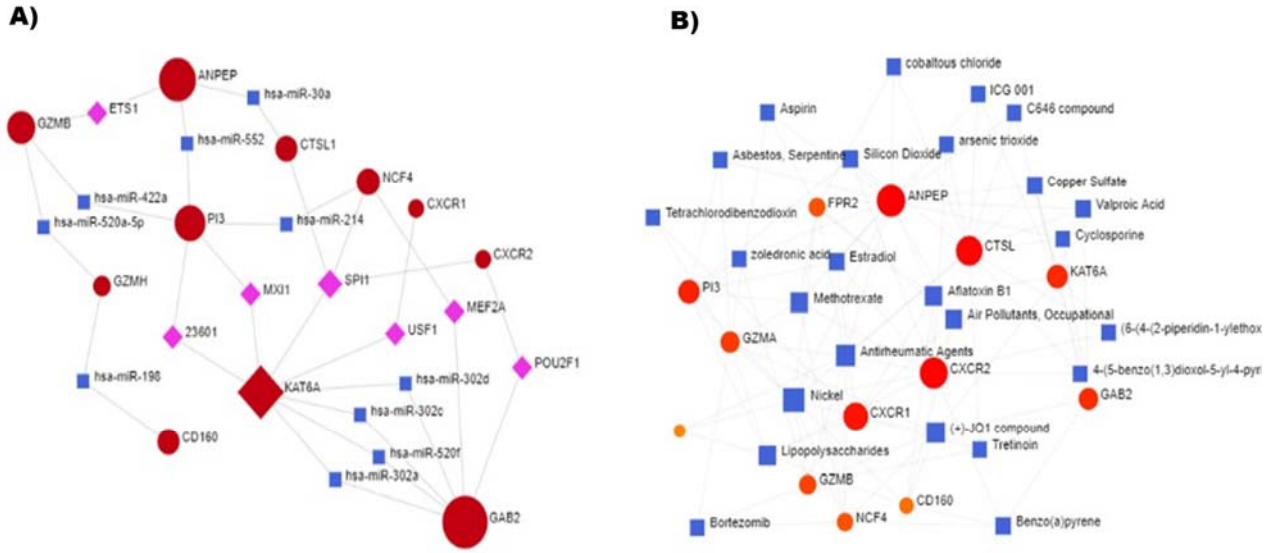

Figure 5. A) *TF - miRNA Coregulatory Networks.* ***B)*** *Protein-Chemical Interactions.*