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Exploring Molecular Interactions and Pathways in Major Depressive Disorder (MDD): A Comparative Analysis of Patients With and Without Anxiety

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Abstract

Major Depressive Disorder (MDD) presents as an intricate mental health issue often associated with Anxiety. In this study, we used data from Gene Expression Omnibus (GEO) to investigate how anxiety acts as a comorbidity to MDD at the molecular level. We used NCBI GEO Accession ID: GSE98793 for this study. This microarray dataset examines the gene expression profiles associated with MDD both with and without anxiety. As the outcome of the study, a total of 15 significantly upregulated Differential Expressed Genes (DEGs), 4 significantly down regulated Differential Expressed Genes (DEGs) were found in MDD with anxiety data's and 5 significantly upregulated Differential Expressed Genes (DEGs), 18 significantly down regulated Differential Expressed Genes (DEGs) were found in MDD without anxiety data were obtained from the dataset. After protein-protein interaction networks were generated using STRING DB, the molecular associations for MDD were visualised using Cytohubba, a Cytoscape plugin. We performed a functional enrichment analysis using the DAVID Functional Annotation tool to identify the biological processes and pathways associated with the up-regulated and down-regulated genes in the anxiety and non-anxiety groups. ARG1, CRISP3, DEFA4, LCN2, BPI, and LTF were discovered in the anxiety group, while HLA-DQB1, FAM3B, GATB, and HLA-DQA1 were discovered in the non-anxiety group. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) were used to analyse differentially expressed genes (DEGs). Interestingly, factors like Staphylococcus aureus infection, the intestinal immune network's role in producing IgA, the steps involved in processing and presenting antigens, and the development of Th1 and Th2 cells are all processes that play a role in the illness and its comorbidity.

Keywords: MDD, DEG's, Biomarkers, Pathways, Therapeutic targets

Introduction

Major Depressive Disorder (MDD) is a common mental health conditioncharacterised by an overpowering sensation of sorrow, despair, and a total loss of interest or pleasure in formerly fun things (1). While depression can manifest on its own, it often coexists with anxiety, creating a complex and challenging clinical presentation(2,3).As per the World Health Organization (WHO), depression stands as the foremost cause of disability globally, impacting more than 264 million individuals across all age groups(4).MDD frequently coexists with various other mental health conditions, such as anxiety disorders, obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), and substance-related disorders(5).

Anxiety disorders manifest through symptoms like worrying, fears related to social interactions and performance, sudden or triggered panic attacks, anticipatory anxiety, and avoidance behaviors. Also, it's prevalence includes Generalized anxiety disorder (6.2%), social anxiety disorder (13%), and panic disorder (5.2%). Anxiety

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disorders are associated with physical symptoms, such as palpitations, shortness of breath, and dizziness. Anxiety disorders are among the most common mental health illnesses globally, with an estimated global prevalence of around 3.8%. Anxiety disorders are among the most common mental health illnesses globally, with an estimated global prevalence of around 3.8 %(5).

The combination of MDD with anxiety not only exacerbates the severity of symptoms but also complicates diagnosis and treatment strategies (3,4).The co-occurrence of MDD with anxiety is common, with epidemiological studies demonstrating a high rate of comorbidity between the two disorders. When MDD and anxiety coincide, the severity of symptoms tends to increase, leading to greater impairment in functioning, heightened risk of suicide, and greater resistance to treatment(6).

Due to the intricate nature of Major Depressive Disorder (MDD) and its co-occurrence with anxiety, there is a critical need for research aimed at elucidating the underlying molecular mechanisms and pathways involved in both conditions. Understanding the molecular interactions and pathways associated with MDD with and without anxiety can provide valuable insights into the biological underpinnings of these disorders, potentially leading to the development of more targeted and effective treatment approaches(5,6).

Despite the recognition of MDD with anxiety as a distinct clinical entity, its diagnosis and management present several challenges (1). The overlapping symptomatology between MDD and anxiety, leading to diagnostic confusion and potential underestimation of the severity of either condition. Additionally, treatment approaches for MDD with anxiety often require careful consideration of pharmacological and psychotherapeutic interventions tailored to address both sets of symptoms effectively. The complexity of managing comorbid MDD and anxiety underscores the need for further research to elucidate underlying mechanisms, improve diagnostic accuracy, and develop more targeted treatment strategies to optimize outcomes for individuals affected by these debilitating disorders (4,6).

Materials and Methods

DataSet

We obtained 68 series and 688 samples pertaining to human Major Depressive Disorder by using the search query "Gene Expression AND (("Major Depressive disorder" OR "MDD") AND (Human OR Homosapien))" in the GEO Datasets database (https://www.ncbi.nlm.nih.gov/geo/) (MDD). Based on a comprehensive analysis, we downloaded gene expression profiles using the GSE98793series, which was based on the GPL570 [HG-U133 Plus 2] Affymetrix Human Genome U133 Plus 2.0 Array. There were no human or animal subjects in any of the studies, and the results are publicly accessible online (7,8).

Analysis of DEG's

Using the GEO2R online analytical application (http://www.ncbi.nlm.nih.gov/geo/geo2r), we compared patients with MDD with and without anxiety to healthy controls. Based on Differentially Expressed Genes (DEGs), the investigation was conducted.We used logFC and corrected P-values as part of the investigation. If a gene's absolute logFC was more than or equal to 1.0 and its adjusted P-value was less than 0.05, it was considered a DEG (9).

Interaction Network:

The DEGs were inserted into the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org/)to create a PPI network. The STRING database received multiple gene names from the microarray analysis, both upregulated and downregulated. The database created a PPI network with nodes representing proteins and edges indicating connections between them using these gene names (10).

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Hub Genes Identification

The PPI network topology was then examined using Cytoscape (version 3.10.1; http://www.cytoscape.org/). With the use of the CytoHubba plugin function in Cytoscape, the degree of every protein node was determined. Hub genes were defined as those having ten or more gene degrees in the PPI network. The resulting visualizations provided insight into the key molecular interactions associated with MDD, highlighting potential biomarkers or therapeutic targets for further investigation(7,8).

Gene Ontology

Gene ontology was used to analyse the top ten genes in MDD with and without anxiety that were shown to be up and down-regulated. The DAVID Functional Annotation Tool (https://david.ncifcrf.gov/) was used for this analysis. The DAVID tool was used for analysis once gene symbols that corresponded to the top DEGs were uploaded. Three categories exist for classifying gene functions: molecular function (MF), cellular component (CC), and biological process (BP) (9).

Functional Enrichment Analysis

Using the DAVID Functional Annotation Tool (https://david.ncifcrf.gov/), pathway enrichment analysis was performed on the top 10 genes identified in MDD with and without anxiety. This analysis aimed to reveal molecular pathways and signalling cascades influenced by dysregulated genes, shedding light on the pathophysiological mechanisms in MDD. Statistical significance of enriched pathways was determined by adjusted p-values (< 0.05) through appropriate tests. Post-analysis, dysregulated genes and enriched pathways were visualized using the Science and Research plot (SR plot, https://www.bioinformatics.com.cn/en), illustrating fold change and statistical significance of gene expression data related to both MDD with and without Anxiety. Similarly, the GOpathway enrichment bubbleplot(https://www.bioinformatics.com.cn/plot_basic_gopathway_enrichment_bubbleplot081en) from the KEGG database depicted enriched pathways graphically, with bubble size indicating the number of involved genes and color representing statistical significance(10).

Results & Discussion

Identification of DEG's

The Gene Expression profile gene series dataset (GSE98793) was used in this study. The GSE98793 contained total of 128 samples in that 64 with generalised anxiety disorder, diagnosed by the MINI questionnaire, and 64 without anxiety disorder and 64 healthy controls (Table 1). A total of 45119 DEGs were screened from GSE98793 according to the criterion of $P < 0.05$ and —logFC— ≥ 1.0. In this 18 upregulated DEG's, 5 downregulated DEG's for MDD with Anxiety and 6 upregulated DEG's, 19 downregulated DEG's for MDD without Anxiety were identified (Table 2). We were able to identify all DEGs by comparing samples of MDD with and without anxiety with samples of normal, healthy individuals (2).

Interaction Network and Hub Gene Identification

Analyzed PPI network in MDD with anxiety revealed 14 nodes and 39 edges for upregulated genes and 4 nodes with 1 edge for downregulated genes. In MDD without anxiety, identified 4 nodes and 1 edge for upregulated genes, and 17 nodes with 40

Table 1: Data from GEO Major Depressive Disorder (MDD) With and Without Anxiety

Reference	Sample	GEO	Platform	Normal	MDD with Anxiety	MDD without Anxiety
Leday GGR, 2018	Whole Blood Samples	GSE98793	GPL570	64	64	64

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Table 2: Screening DEG's in MDD with and without Anxiety

Gene Regulation	MDD with Anxiety	MDD without Anxiety
Upregulated	OLFM4 CEACAM8 MMP8 LTF LCN2 HLA-DQA1 CRISP3 MMP8 CEACAM6 ARG1 DEFA4 CEACAM6 BPI CNTNAP3P2 CNTNAP3 CNTNAP3B TDRD9	HLA-DQB1 FAM3B GATM HLA-DQA1
Downregulated	ERAP2 HLA-DQB1 FAM3B HLA-DQA1	CD177 CEACAM6 PF4V1 TNNT1 GRB10 SDHD LTF FOLR3 MMP8 CEACAM8 TMEM176A ARG1 LCN2 DEFA4 TMEM176B OLFM4 HLA-DRB4 BPI

edges for downregulated genes (Figure 1). The methodological approach chosen for constructing the hub network using Cytoscape, Cytohubba was Degree centrality, as it identifies nodes with the highest number of connections within the biological network, indicating their potential significance in regulating cellular processes and disease mechanisms. For MDD with anxiety, upregulated genes included MMP8, LCN2, CEACAM8, OLFM4, LTF, CRISP3, DEFA4, BPI, CEACAM6, and ARG1. For MDD without anxiety, downregulated genes comprised CEACAM8, MMP8, LCN2, DEFA4, OLFM4, LTF, BPI, CEACAM6, CD177, and FOLR3(10,11) (Figure 2).

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The network includes MDD with anxiety revealed 14 nodes and 39 edges for upregulated genes (a). In MDD without anxiety, identified 17 nodes with 40 edges for downregulated genes (b) (10,11)

Color of node indicates degree of connectedness. The top nine hub genes score from 1 to 9 on the pseudocolor scale, which ranges from red to yellow. On a scale from lowest to greatest, the colours red, orange, and yellow denote the various degrees (10,11).

The matrix metalloproteinase family includes matrix metalloproteinase 8, or collagenase 2, an enzyme that is essential for wound healing, inflammation, and tissue remodelling. Anxiety and Major Depressive Disorder (MDD) patients may have

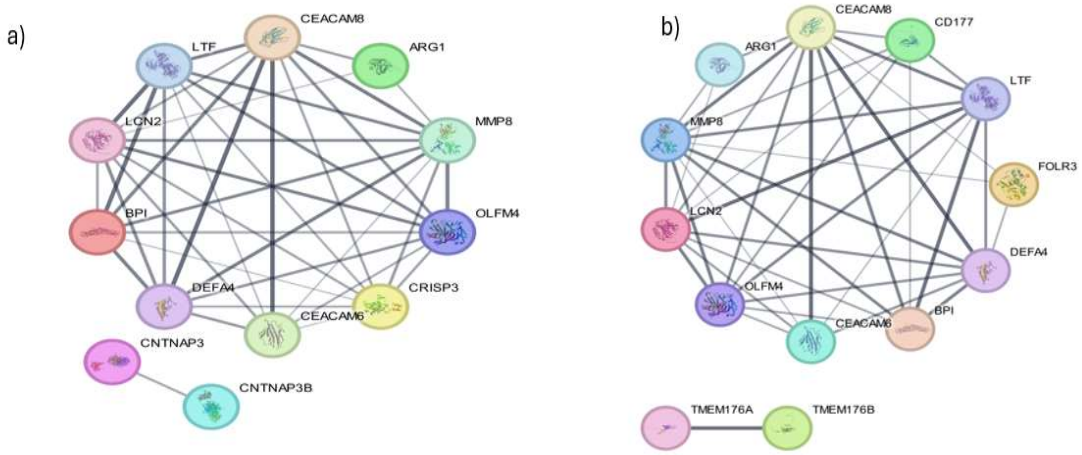

Figure 1: STRING protein-protein interactions network of 20 upregulated and 22 downregulated genes

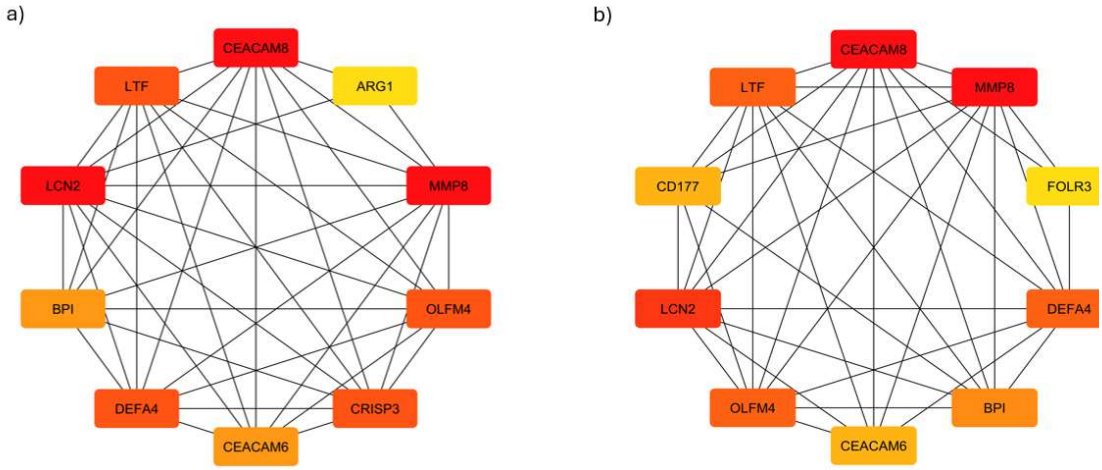

Figure 2: Subnetwork of top nine hub genes from protein-protein interactions network using Cytoscape software

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abnormalities in tissue remodelling processes or increased inflammatory activity, as shown by the overexpression of MMP8 in these patients. Conversely, the downregulation of MMP8 in MDD without anxiety suggests differences in the inflammatory profile or tissue remodeling dynamics specific to this subtype of depression, involving inflammatory and stress response pathways (12).

Tissue inhibitors of metalloproteinases are one of the variables that affect the activity of matrix metalloproteinase 8, which mostly breaks down collagen in the extracellular matrix (TIMPs). Natural inhibitors known as TIMPs bind to matrix metalloproteinases (MMPs) and control their activity. In conditions like MDD with anxiety, the balance between MMPs and their inhibitors, such as TIMPs, influences MMP8 activity. Dysregulation of this balance, characterized by decreased TIMP levels relative to MMPs, could elevate MMP activity, potentially contributing to pathological processes like inflammation, tissue remodeling, and neurodegeneration (12,13). Neutrophil gelatinase-associated lipocalin, also known as lipocalin-2 (LCN2), is one member of the lipocalin family of proteins (NGAL). LCN2 is essential for the movement of iron, the response to inflammation, neurogenesis, and neuroprotection. A specific pathway involving lipocalin-2 (LCN2) in major depressive disorder are Neuroinflammation pathways, Neuroprotection and Neurogenesis pathways, Stress Response pathways, Iron Metabolism pathways(14).

CEACAM8, also known as CD66b or CCG, is a member of the family of carcinoembryonic antigen-related cell adhesion molecules. It is primarily expressed on the surface of neutrophils and plays a role in various immune processes. Some Key roles are Neutrophil activation, Inflammatory Response, Cell Adhesion and Migration, Microbial Defence. If CEACAM8 is indeed dysregulated in MDD with or without anxiety, its functions could potentially impact several pathways implicated in the pathophysiology of depression such as Neuroinflammation, stress response, Blood Brain Barrier integrity. CEACAM8-mediated neutrophil activation might contribute to the inflammatory response and neuroinflammation in MDD, whereas its role in regulating neutrophil adhesion and migration could influence immune cell trafficking and tissue inflammation (15,16).

OLFM4, or olfactomedin 4, is a glycoprotein within the olfactomedin family, engaging in diverse biological processes and implicated in cancer, inflammation, and tissue development. Its role in cell adhesion and migration, inflammation response, neurogenesis, neuroplasticity, and dysregulation of the WNT signalling pathway are among its notable roles. Neuronal function and mood regulation may be affected by OLFM4-mediated modulation of Wnt signalling, which has been associated with depression. (16–18). A member of the transferrin family, Lactotransferrin (LTF), often referred to as lactoferrin, is a multifunctional glycoprotein. With the ability to inhibit inflammation, fight germs, and regulate immunity, it is involved in many different biological processes. It involves in some pathways such as Neuroinflammation, Neuroprotection and iron metabolism. LTF-mediated inhibition of inflammatory cytokines and oxidative stress could have anti-inflammatory and neuroprotective effects, whereas its promotion of immune cell activity could enhance immune responses against pathogens (19).

The male reproductive system, the pancreas, the kidney, the prostate, salivary glands, and other organs all produce cysteine-rich secretory protein 3 (CRISP3), a member of the CRISP family of proteins. CRISP3 has been implicated in several biological processes, such as Regulation of Fertility, Immune modulation, Anti-microbial activity, and Regulation of cell signalling. It involves in some pathways such as Neuroinflammation, Neuroprotection and Neurotransmitter Regulation (20).

DEFA4, also known as defensin alpha 4, is a member of the defensin family of antimicrobial peptides. Small cationic

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peptides called defensins are essential for the innate immune system's protection against microbiological infections. It involves in biological process such as antimicrobial activity, Immune modulation, Inflammatory response, and Epithelial Barrier function. It involves in some pathways such as Neuroinflammation, Stress response and Gut-Brain Axis (20).

BPI, or bactericidal/permeability-increasing protein, is a protein primarily known for its antimicrobial properties. As a component of the innate immune system, it starts to break down the cell membranes of a variety of Gram-negative bacteria to function as a strong antibacterial agent against them. It involves in biological process such as antimicrobial activity, Inflammatory Response and Endotoxin Neutralization. It involves in some pathways such as Neuroinflammation, Stress Response and Gut-Brain Axis (21).

CEACAM6, short for carcinoembryonic antigen-related cell adhesion molecule 6, is one of the many members of the CEACAM family. It is a glycoprotein that is primarily involved in cell adhesion and signalling processes. It involves in biological process cell Adhesion, Immune Regulation and Signal Transduction. It involves in some pathways such as Neuroinflammation, Stress response, Cell Adhesion and Neuroplasticity (22).

One enzyme that may convert arginine to ornithine and urea is ARG1, which stands for Arginase1. Important to the effort to eliminate excess ammonia from the body, it is part of the urea cycle. Additionally, ARG1 is involved in regulating arginine availability for various cellular processes, including protein synthesis, nitric oxide (NO) production, and immune function. It involves in some pathways such as Neuroinflammation, Nitric oxide signalling and Immune Dysregulation (23).

One of the members of the family of receptors that facilitate the absorption of folates into cells is FOLR3, which is another name for folate receptor gamma (FR-gamma). While the placenta is the most common site of expression, other tissues, including the brain, have also shown signs of its presence. The transport of folates across cell membranes is facilitated by folate receptors, which are essential in folate metabolism. It involves in biological process such as Folate transport, Neurotransmitter synthesis and one carbon metabolism. It involves in some pathways such as Neurotransmitter dysfunction and Epigenetic Regulation (24).

CD177, also known as neutrophil specific antigen NB1, is a glycoprotein receptor primarily expressed on the surface of neutrophils. It plays a role in neutrophil biology and immune responses, Endothelial Adhesion, and Inflammatory Responses. It involves in some pathways such as Neuroinflammation, Blood-Brain Barrier dysfunction and Stress Response (25).

Functional Enrichment Analysis of DEG's

GO function analyses and the KEGG pathway enrichment analysis DEGs were carried out by DAVID. The enriched GO terms were categorized into biological process (BP), cellular component (CC), and molecular function (MF) ontologies.

The majority of DEGs associated with MDD with anxiety in BP are Anti-bacterial humoral response, Innate Immune Response and Cellular response to polysacchrides. These process posses the intricate interplay between the immune system and mental health, particularly in mood disorders like MDD. Anxiety disorders frequently co-occur with MDD, indicating shared underlying mechanisms. Dysregulation of innate immune responses, characterized by the activation of immune cells and the release of pro-inflammatory cytokines, may contribute to the development and maintenance of depressive and anxious symptoms. Moreover, heightened cellular responses to bacterial components such as LPS and increased antibacterial humoral responses suggest a state of chronic inflammation, which has been implicated in the etiology of both mood and anxiety disorders(26).

CC indicated significant enrichment in Extracellular space, Extracellular region,

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specific granule lumen involved in intercellular communication and signaling pathways in the pathophysiology of this condition.The importance of the extracellular environment, including the space between cells and within specific cellular compartments, in regulating various physiological processes, including immune responses and neuroinflammation. The dysregulation of genes associated with these cellular components may reflect alterations in communication between immune cells, glial cells, and neurons, contributing to the development and maintenance of depressive and anxious symptoms. Furthermore, the specific enrichment of genes in granule lumens suggests a potential role for secretory pathways and vesicle trafficking mechanisms in mediating cellular responses to stress and inflammatory stimuli in MDD with anxiety(27).

MF analysis showed enrichment in Lipopolysaccharide Binding, Iron ion binding, and Serine type endo peptidase activity.This suggests potential link between immune dysregulation, oxidative stress, and neuroinflammation in the pathophysiology of this condition. Lipopolysaccharide (LPS) binding proteins are integral components of the innate immune response, recognizing bacterial cell wall components and initiating inflammatory signaling pathways. The upregulation of genes involved in LPS binding may reflect heightened immune activation and chronic low-grade inflammation observed in individuals with MDD and anxiety. Additionally, iron ion binding proteins are critical for various physiological processes, including neurotransmitter synthesis and oxidative stress regulation. Dysregulation of iron metabolism and increased iron accumulation have been implicated in both mood disorders and anxiety disorders, contributing to oxidative stress and neuronal damage. Furthermore, the enrichment of serine-type endopeptidase activity suggests potential alterations in proteolytic processing and regulation of neuroinflammatory mediators, further implicating immune-inflammatory pathways in MDD with anxiety(27).

According to GO analysis, BP was more abundant in Major Depressive Disorder (MDD) without Anxiety. This covers functions such as the MHC class-II protein complex, the adaptive immune response, and the processing and presentation of polysaccharide antigen by MHC class-II. The MHC class-II protein complex plays a pivotal role in antigen presentation to CD4+ T cells, crucial for coordinating the adaptive immune response. Dysregulation of MHC-II expression or function may disrupt antigen presentation, potentially contributing to neuroinflammation. Aberrant adaptive immune responses, characterized by T cell dysfunction, cytokine signaling alterations, and antibody production irregularities, have been implicated in the chronic low-grade inflammation. Disturbances in the processing and presentation of polysaccharide antigens by MHC class-II molecules could disrupt immune homeostasis observed in MDD without the comorbidity Anxiety(28).

The CC analysis revealed an enrichment in the lumenal side of the endoplasmic reticulum membrane, the MHC class II protein complex, and the integral component of the lumenal side of the endoplasmic reticulum membrane. Dysregulation within the endoplasmic reticulum (ER) can instigate cellular stress responses like the unfolded protein response (UPR), pivotal for maintaining protein homeostasis. In MDD pathogenesis, ER dysfunction may ensue, fostering the accumulation of misfolded proteins, consequently activating the UPR. Prolonged ER stress can lead to neuronal apoptosis, fuel neuroinflammation, disrupt synaptic plasticity and neurotransmitter signaling, thereby undermining neural circuits involved in mood regulation. Additionally, ER stress perturbs neurotrophic factor levels, including brain-derived neurotrophic factor (BDNF), exacerbating neuronal vulnerability, and further compromising cognitive function. Furthermore, ER-mitochondria crosstalk disruption, attributable to ER stress, fosters mitochondrial dysfunction and oxidative stress, amplifying neuronal damage and depressive symptomatology.

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The MHC class II protein complex implies perturbations in immune regulation. The MHC class II molecules are instrumental in antigen presentation to CD4+ T cells, a process pivotal for immune response coordination. Dysfunctions in MHC class II expression or function could contribute to aberrant immune responses, inflammation and these mechanisms implies in MDD without the comorbidity Anxiety(29).

The MFanalysis revealed an enrichment for peptide antigen binding, MHC class II protein complex binding, and MHC class II receptor activation (8,9).The enrichment in peptide antigen binding indicates alterations in the recognition and binding of specific antigens, potentially implicating dysregulation in immune response pathways. In MHC class II protein complex binding signifies disturbances in the interaction between antigens and MHC class II molecules, which are essential for antigen presentation to immune cells. Dysfunctions in this process may lead to impaired immune recognition and response, contributing to chronic inflammation observed in MDD. In MHC class II receptor activation suggests alterations in downstream signaling pathways following interaction with MHC class II molecules. This activation may modulate immune cell function and cytokine production, further influencing neuroinflammatory processes implicated in MDD without the comorbidity Anxiety(30).

Ten improved biological mechanisms, cellular constituents, and molecular operations. The X-axis shows the molecular functions, while the vertical axis shows the number of genes.

KEGG pathway analysis revealed that the differentially expressed genes (DEGs) associated MDD with Anxiety patients were primarily concentrated in pathways related to Inflammatory Bowel Disease (IBD), Rheumatoid Arthritis (RA), Th1 and Th2 Cell differentiation, Phagosome, Systemic Lupus erythematosus (SLS). Simultaneously, there was enrichment in similar pathways for autoimmune thyroid disease, inflammatory bowel disease, viral myocarditis, and major depressive disorder without anxiety (Figures 3 and 4)(7,8).

These are the top ten DEG-enriched KEGG pathways. The pathways are shown on the vertical axis, while the number of genes is shown on the X-axis.

The association between Inflammatory Bowel Disease (IBD) and the co-occurrence of MDD with Anxiety underscores the roles of the DEFA4 and LCN2 genes. DEFA4 encodes human defensin 4, which has antimicrobial functions in the gut, while LCN2 encodes lipocalin-2, which is involved in regulating inflammation and iron homeostasis. Changes in the expression of these genes may influence intestinal inflammation and gut microbiota composition, which could play a role in the pathogenesis of IBD. Furthermore, their roles in the neuroimmune axis might be relevant to understanding MDD with Anxiety, a condition that is frequently observed alongside IBD(31).

The CRISP3, LCN2, and DEFA4 genes are important for elucidating the complex relationship between Rheumatoid Arthritis (RA) and MDD with Anxiety. Changes in CRISP3, LCN2, and DEFA4 genes are linked to Rheumatoid Arthritis (RA) through their involvement in inflammatory pathways and immune system dysfunction. Evidence also suggests their potential connection to mood and anxiety disorders, likely due to their roles in neuroinflammation and immune responses relevant to psychiatric conditions. This underscores the bidirectional relationship between inflammation and mood disorders, indicating that genetic variations affecting immune regulation may impact susceptibility to mood and anxiety disorders as well(32).

The roles of the CRISP3, LCN2, and DEFA4 genes in the differentiation of Th1 and Th2 immune cells may be informative regarding the immune aspects of MDD with Anxiety. Dysregulation of Th1/Th2 balance can perpetuate chronic inflammation and neuroinflammation observed in major depressive disorder (MDD) and anxiety by disrupting the immune system's ability to

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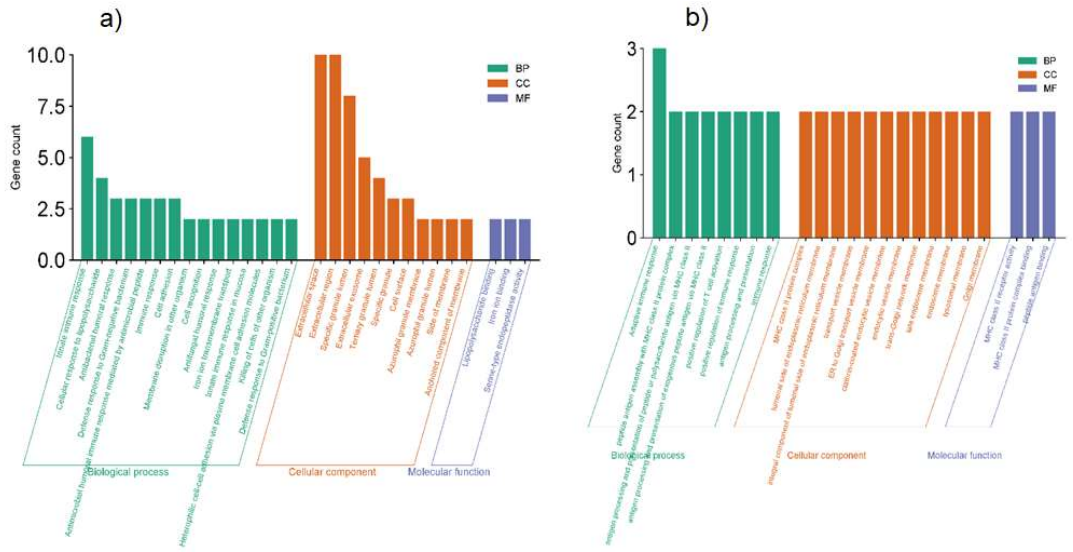

Figure 3: Gene ontology analysis performed using DAVID on DEG's identified from MDD with and without anxiety samples

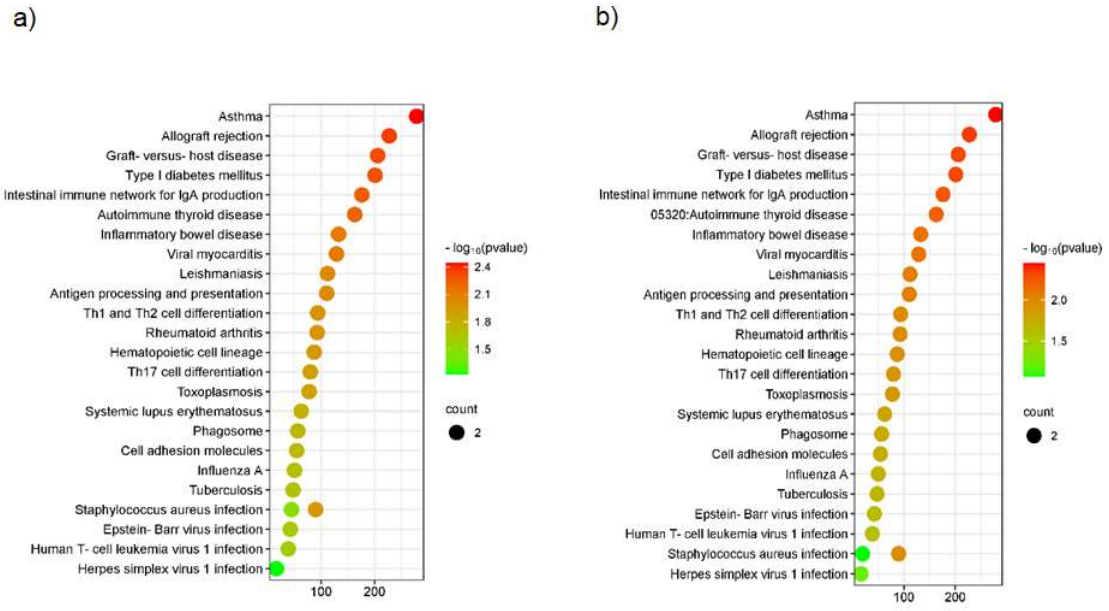

Figure 4: KEGG pathway enrichment analysis performed using DAVID and KEGG Bubble on DEG's identified from MDD with and without anxiety samples

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regulate inflammation. When this balance is skewed, it can lead to persistent activation of inflammatory responses throughout the body, exacerbating symptoms of depression and anxiety. Additionally, dysregulated Th1/Th2 balance can result in neuroinflammation, contributing to changes in brain chemistry and function associated with MDD and anxiety. Ultimately, these immune dysregulations worsen symptoms, impair neurotransmitter function, and perpetuate the underlying conditions(33).

LCN2 and BPI are key regulators in the phagosome pathway, which is essential for pathogen clearance and cellular debris removal. Variations in the expression of these genes could potentially influence this pathway, leading to altered immune responses. Such changes may be implicated in the development of MDD with Anxiety, although the exact mechanisms linking immune function to psychiatric conditions remain to be clarified(34).

In Systemic Lupus Erythematosus (SLE), the genes LCN2 and DEFA4 are involved in immune regulation and inflammation, which are prominent features of SLE. While dysregulation of these genes is associated with SLE, their role in MDD with Anxiety is complex and potentially involves multiple pathways. It is hypothesized that immune and inflammatory processes influenced by these genes might also play a role in psychiatric symptoms observed in SLE; however, this relationship is not fully understood and warrants further investigation(35).

DEFA4 encodes alpha-defensin, integral to innate immunity and inflammatory responses, while LCN2 encodes lipocalin-2, a mediator of both innate immunity and neuroinflammation. In conditions such as Inflammatory Bowel Disease (IBD), characterized by heightened immune activation and gut inflammation, DEFA4 and LCN2 may exacerbate systemic inflammation and influence the neuroimmune axis, potentially impacting mood, and anxiety regulation. This association is significant in Major Depressive Disorder (MDD), where neuroinflammation is implicated, suggesting a link between immune dysregulation and psychiatric symptoms. Understanding DEFA4 and LCN2's roles in the neuroimmune axis could shed light on the co-occurrence of MDD with Anxiety, particularly in individuals with conditions like IBD, where shared inflammatory pathways may contribute to gastrointestinal and psychiatric symptoms. This underscores the bidirectional relationship between immune activation, neuroinflammation, and mood disorders, highlighting the need to consider the interconnectedness of physical and mental health in these comorbid conditions(36).

Conclusion

Our study explores the complex relationship between Major Depressive Disorder (MDD) with and without Anxiety, uncovering the underlying molecular mechanisms and pathways associated with these comorbid conditions. Through analyzing differentially expressed genes (DEGs) and conducting functional enrichment studies, we identified distinct molecular signatures and biological pathways characteristic of each condition. The identification of DEGs associated with MDD with Anxiety and MDD without Anxiety offers valuable insights into the unique molecular profiles of these disorders. Notably, we observed dysregulation of genes involved in immune response, inflammation, neurogenesis, and stress regulation, highlighting the intricate interplay between the immune system and mental health.

Functional enrichment analysis revealed pathways enriched in immune dysregulation, inflammatory processes, and neuroinflammation, emphasizing the crucial role of the immune system in the pathophysiology of MDD with Anxiety. Furthermore, the identification of specific pathways such as Inflammatory Bowel Disease, Rheumatoid Arthritis, and Th1/Th2 Cell Differentiation further clarifies the complex connections between psychiatric disorders and immune-mediated conditions.

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Our findings contribute to a deeper understanding of the molecular underpinnings of MDD with and without Anxiety, laying a foundation for the development of more targeted and effective treatment approaches. By elucidating the molecular mechanisms driving these disorders, our study paves the way for personalized interventions addressing the underlying pathophysiology and optimizing outcomes for individuals affected by these debilitating conditions. However, further validation through in vitro and in vivo studies is necessary for full comprehension.

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