Biomedical Reviews 2024; 35: 115-121
© Bulgarian Society for Cell Biology
ISSN 1314-1929 (online)

BMR Biomedical Reviews

Research article

ELUCIDATION OF HUB GENES AND PATHWAYS ASSOCIATED WITH GUT MICROBIOME DYSBIOSIS AND MILD COGNITIVE IMPAIRMENT

Yao Yang Law[1], Ying Pei Wong[1], Ee Xion Tan[2], and Anna Pick Kiong Ling[1*]

[1]*Division of Applied Biomedical Sciences and Biotechnology, School of Health Sciences, IMU University, Bukit Jalil, Kuala Lumpur, Malaysia,* [2]*Department of Digital Health and Health Informatics, School of Business and Technology, IMU University, Bukit Jalil, Kuala Lumpur, Malaysia*

Bidirectional communication route of brain and gut, gut-brain axis, was suggested to contribute to mild cognitive impairment (MCI) through inflammatory bowel disease (IBD) as gut microbiome dysbiosis occurs. Therefore, this study aimed to predict the gene signatures, and the potential molecular network associated between gut microbiome dysbiosis and MCI. To initiate the study, datasets (GSE13367, GSE140829, GSE36701 and GSE63063) were extracted from Gene Expression Omnibus (GEO) and the differentially expressed genes (DEGs) were selected based on the Log_2 fold change >1.0 with p-value < 0.05 for both MCI and IBD. The shared DEGs between MCI and IBD were identified, and inputted into Database for Annotation, Visualization and Integrated Discovery database (DAVID) to perform gene ontology (GO) for functional enrichment analysis. The functional pathways were selected based on p<0.05 and FDR <0.05. The GeneMANIA was used to construct the protein-protein interaction (PPI) network. This study identified 1061 common shared DEGs between MCI and IBD. Based on the GO enrichment study, majority of the genes were involved in protein binding for molecular function, signal transduction biological process and cytosol for cellular component. The co-expression network showed that leukocyte cell-cell adhesion and Type 1 interferon function might contribute to IBD and MCI. Further elucidation studies on DEGs showed that leukocyte cell-cell adhesion signaling pathway would be the main pathogenesis pathway leading to both diseases, in which 8 genes involved were CD3E, PTPRC, GRAP2, CD86, VAV1, SEMA4D, FYN and LYN. Thus, further studies especially in the perspective of neuroinflammation is worth to be explored.
***Biomed Rev* 2024; 35: 115-121**

Keywords: Gene Ontology Enrichment; Gene signatures; Gut-brain axis; Inflammatory bowel disease; Neurological disorder

Received 1 December 2024, accepted after revision 11 December 2024.

*Correspondence to: Professor Dr. Anna Pick Kiong Ling, PhD, Division of Applied Biomedical Sciences and Biotechnology, School of Health Sciences, IMU University, 126 Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia
Tel: 603-2731 7222 Fax: 603-8656 7229 Email: anna_ling@imu.edu.my
ORCID of corresponding author: 0000-0003-0930-0619 (Anna Pick Kiong Ling)

INTRODUCTION

Mild cognitive impairment (MCI) is the stage between the expected decline in memory and thinking, eventually develop into more serious decline of dementia. This may include problems with memory, language or judgment; thus, they will experience loss in memory, attention and other cognitive tasks over the time. Based on the condition of patients' memory, MCI can be classified into amnestic MCI or non-amnestic MCI [1]. Currently, the exact pathogenesis of MCI is not fully understood, but the damage to brain cell is believed to be the main contributing factor. MCI is also a risk factor for the development of Alzheimer's disease and other forms of dementia [2]. It was reported that gastrointestinal diseases and imbalanced microbiome may contribute to the neurodegeneration and inflammation [3,4]. Bad lifestyle such as poor diet and obesity also may contribute to cognitive decline [3-6]. Nevertheless, these hypotheses are still in infancy research.

Studies have shown that gut microbiome dysbiosis may be associated with MCI [4, 7, 8]. The gut microbiome dysbiosis may be caused by host-specific factors such as genetic inheritance, infections, inflammation, poor diet, xenobiotics and hygiene [9-11], eventually leads to functional gastrointestinal disorders [12-14]. Functional gastrointestinal disorders (FGID) are also called as irritable bowel syndrome (IBS) and functional dyspepsia. It causes chronic symptoms throughout the gut, mainly abdominal pain, dysphagia, dyspepsia, diarrhoea, constipation and bloating [14]. Another condition associated with dramatic changes in gut microbiome is inflammatory bowel disease (IBD) [15-17]. It is a chronic gastrointestinal disease caused by complex interactions between the gut microbiome and host immune response [16, 17]. In normal condition, the enormous taxonomic diversity and functional potency of gut microbiome will be balanced among themselves, providing benefits to host. When gut microbiome dysbiosis occurs, the inflammatory microbial products will trigger host immune response, which leads to mass excretion of cytokines, eventually damage the colonic epithelial cells and manifest chronic inflammatory digestive disease [16]. Based on the study by Venegas *et al*., dysbiosis causes bacteria that ferment fibers and produce short chain fatty acids (SCFAs) to be reduced in patients with IBD, as those are important metabolites in maintaining intestinal homeostasis and strengthening the gut barrier function, reducing SCFs may result in weak intestine [18]. Eventually, the inflammation and ulceration will occur, resulting in abdominal pain, diarrhea, bloody stools, weight loss, and the influx of neutrophils and macrophages [19]. It was suggested that IBD is closely related to the development of MCI by inducing neuroinflammation through gut-brain axis [22-22].

The main pathway that connecting gut and brain is the gut-brain axis, which is the bidirectional pathway involving in the immune system, neuroendocrine system, enteric nervous system, circulatory system and vagus nerve [23, 24]. Its main role is to monitor and integrate gut functions, link emotional and cognitive centres of the brain with peripheral intestinal functions. The secretions of gut microbiome such as microbial-derived metabolites and products, peptides, gut hormones, and neuroactive substances can affect the brain through those pathways. If gut microbiome dysbiosis occurs, the secretive substance presence in the gut will be abnormal, and it will bring negative feedback to the brain, eventually trigger neurological disorders in brain. Thus, targeting the gastrointestinal disease such as IBD can potentially reduce the neurological diseases. The aim of this study was to elucidate and predict the associated gene signatures, and the potential molecular network associated between gut microbiome dysbiosis and MCI.

MATERIALS AND METHODS

Data Selection and Identification

The gene expression datasets were selected from the Gene Expression Omnibus (GEO). The keyword used to search the datasets were 'mild cognitive impairment', 'functional gastrointestinal disorders', 'inflammatory bowel disease', 'gut microbiome' and 'gut brain axis'. Datasets were subjected to both internal and external validation and eventually the selected datasets that meet the criteria were GSE13367, GSE140829, GSE36701 and GSE63063.

Data Mining and Processing

All datasets were sorted into correct sequence before processing. The sorted datasets were then normalised to obtain the $\log_2$fold change value and the p-value were calculated. Then, the gene annotation was performed on each dataset according to the platform used in the array's datasets respectively.

Feature selections and Gene ontology enrichment analysis

The differentially expressed genes (DEGs) were selected based on $\log_2$fold change > 1.0 and p-value < 0.05 for each dataset. A Venn diagram was constructed by using the selected DEGs from MCI and IBD to identify the shared common DEGs. The Database for Annotation, Visualization and Integrated Discovery database (DAVID) was used to perform gene ontology (GO) enrichment for functional genomics analysis based on

the linear regression method. The top ten biological process, cellular component and molecular function were identified based on p-value < 0.05 and Benjamini < 0.05.

Development of Predictive Model

The gene list from biological process was inputted into web-based GeneMANIA to construct a predictive Protein-protein Interaction (PPI) model on the pathogenesis of these diseases.

RESULTS AND DISCUSSION

Identification of shared common DEGs between MCI and IBD

As analysed from the Venn diagram, there were 3124 genes from IBD and 10276 genes from MCI. There were 1061 shared common DEGs identified from MCI and IBD datasets. Noteworthy examples of shared genes are HLA-A, HLA-E, HLA-G, PTPN6, NLRP3 and IRF1. The existence of shared common DEGs indicates that there is a relationship between MCI and IBD. This is the strong evidence that IBD are highly related to neurological disorders, and vice versa, since the gut-brain axis is bidirectional. This indicates that gut microbiome dysbiosis and gastrointestinal disease do contribute to neurological disorders. One of the noteworthy shared genes is NLRP3. Based on the study conducted by He *et al*, the gene NLRP3 is involved in inflammasomes expression that contributes to neuroinflammation. Their study showed that suppression of NLRP3 could decrease the neuroinflammation and alleviate the neuronal loss [20]. For other gastrointestinal disease, preclinical studies showed the relation between altered bowel movement in IBS to decreased inhibitory and increased excitatory neurotransmission [3]. Based on the study conducted by Garofalo *et al*., the use of probiotics could be prominent strategies to improve symptoms severity of IBS, with a reduction of depression and anxiety, as well as pain relief [25]. These changes in gut microbiome have affected the brain condition by gut-brain axis. Contrarily, the study conducted by Kennedy *et al*. proved that daily stressors play a key role in exacerbating symptoms in IBD [26].

Gene Ontology and Enrichment Analysis

DAVID was used to perform gene ontology biological process (BP), molecular function (MF) and cellular component (CC) from the shared common DEGs of MCI and IBD. Figure 1 shows the combined bar chart of BP, CC and MF respec-

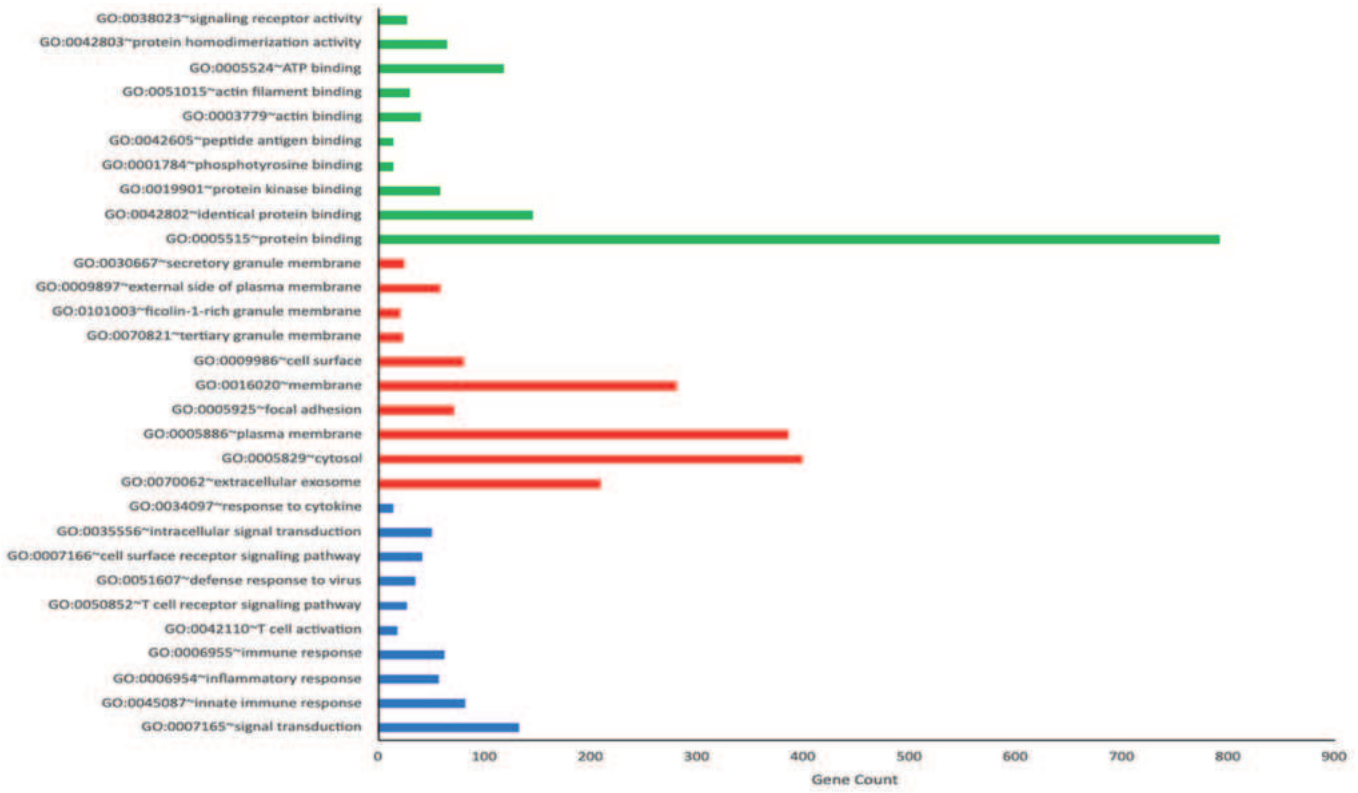


Figure 1. *Top 10 Gene ontology enrichment analysis of the differentially expressed genes among MCI and IBD with biological processes (blue), molecular function (green) and cellular components (red).*

tively. In BP, signal transduction (GO:0007165) is the largest category, which consists of 133 gene counts. In CC, cytosol (GO:0005829) is the largest category with 400 gene counts while in MF, protein binding (GO:0005515) is the largest category with 792 gene counts.

Based on the analysis, the most genes in BP belong to signal transduction (GO:0007165), innate immune response (GO:0045087), immune response (GO:0006955) and inflammatory response (GO:0006954) with 133 counts, 82 counts, 58 counts and 63 counts, respectively. In CC, cytosol (GO:0005829) is the largest category with 400 gene counts while in MF, protein binding (GO:0005515) is the largest category with 792 gene counts. The gene, PIK3CD is found in these four BP. Abnormal PIK3CD signaling is involved in neurological disorders, whereby studies have proven that increased PIK3CD expression is found in patients with neurological disorder. Its expression is found in the Central Nervous System (CNS) and responsible in axonal growth, dendritic branching, and microglial activation. PIK3CD is an important signaling molecule in the context of brain development, maturation, and disease. The study also suggests that the altered PIK3CD transcription will result in dysregulation of proinflammatory cytokine signaling [27].

Co-expression and Pathway Network Analysis

The PPI network of co-expression network of IBD and MCI were produced through GeneMANIA. Further analysis in Figure 2 showed that the biological processes that contributed significantly in IBD and MCI are leukocyte cell-cell adhesion (red nodes) and Type 1 interferon (blue nodes) function. Furthermore, based on the research, HLA-A, HLA-E, HLA-G, PTPN6 and IRF1 genes contributed to both functional characteristics. Following the analysis in this study, the leukocyte cell-cell adhesion signaling pathway is largely involved in IBD and MCI pathogenesis (Figure 3). Based on the analysis on leukocyte cell-cell adhesion signaling pathway, there are 5 pairs of genes that have significant interaction that causes IBD and MCI (Table 1).

Based on the protein-protein interaction (PPI) network of co-expression network of IBD and MCI constructed, the top 10 genes involved are HLA-A, HLA-B, HLA-F, HLA-G, BTN3A1, BTN3A2, LCN2, PI3, RSAD2 and IFI44L. There are 5 genes involved in both leukocyte cell-cell adhesion and Type 1 interferon function, which are HLA-A, HLA-E, HLA-G, PTPN6 and IRF1. Leukocyte cell-cell adhesion contributes to defense system against pathogenic and infectious diseases, thus resulting in inflammation [28]. As evidence, neutrophil activation in the presence of pro-inflammatory mediator tumor

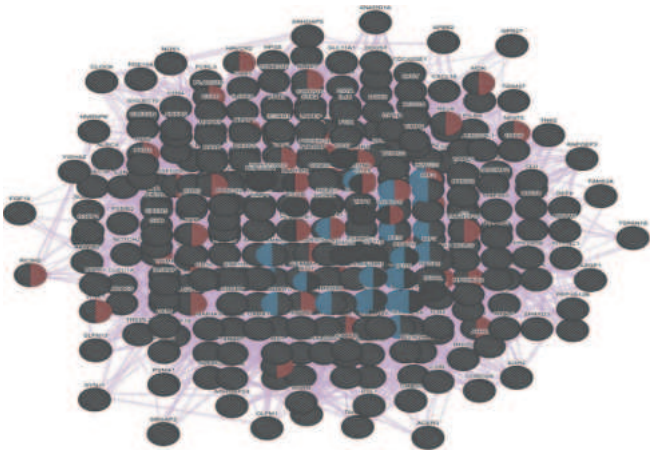


***Figure 2**. Co-expression network analysis of IBD and MCI with significant leukocyte cell-cell adhesion (red nodes) and Type 1 interferon function (blue nodes).*

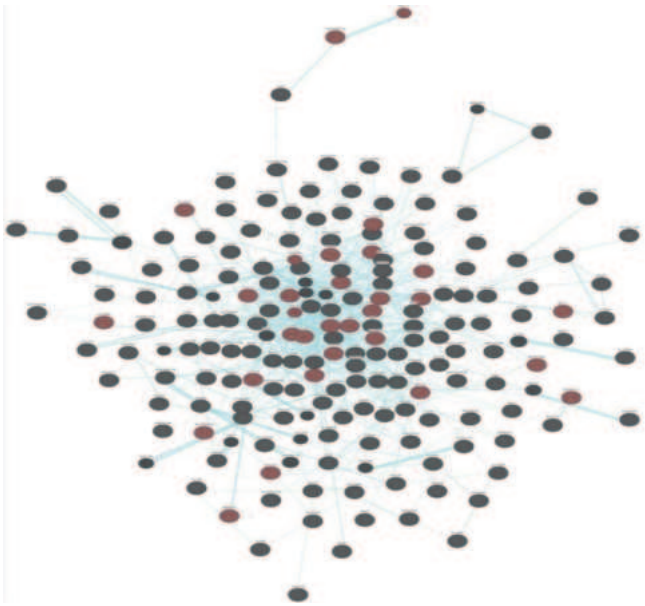


***Figure 3.** Leukocyte cell-cell adhesion signaling pathway involves in IBD and MCI pathogenesis.*

***Table 1.** Genes pairs that contributed to the IBD and MCI.*

Gene 1	Gene 2	Weight
CD3E	PTPRC	0.389806
GRAP2	CD86	0.217637
VAV1	CD86	0.198658
PTPRC	SEMA4D	0.137304
FYN	LYN	0.077532

necrosis factor-α (TNFα) requires adhesion to underlying substrate for maximal responses [28]. They also contribute to inflammatory diseases and autoimmunity [28]. The type-I interferon is responsible in inflammation, immunoregulation, tumor cells recognition, and T-cell responses [29].

They activate macrophages and NK cells to elicit an antiviral response [29]. The upregulation or mutation of type 1 interferon can cause auto-inflammatory and autoimmune diseases [30]. HLA-A gene is critical to the cytotoxic t-cell controlled immune response to viruses and other intracellular pathogens. The study conducted by Wang *et al*. shows that HLA-A contribute to neurodegeneration due to alteration of hippocampal volume [31]. This is further proven by the study conducted by Tang *et al*. that gut microbiome dysbiosis do affect structure and function of the hippocampus, through the vagus nerve pathway, systemic pathway and immune pathway by producing microbial metabolites, resulting in elevation of the permeability of the mucosa-intestinal barrier and blood-brain barrier. This will cause hippocampal inflammation, and result in neurodegeneration [32]. HLA-G act as a shield against inflammatory aggression by inhibiting NK cell activity and tissue infiltration by T cells. The over-expression of HLA-G could reduce clearing of intestinal microorganisms, resulting in secondary chronic inflammation [33]. The study conducted by Wißfeld *et al*. stated that reduction of PTPN6 will trigger inflammatory gene transcription, phagocytosis and phagocytosis-associated oxidative burst in macrophages and microglia [34]. The gene IRF1 responsible in regulating pro-inflammatory activating stimuli, suppressing the inflammation process, thus dysregulation of IRF1 will contribute to inflammation of neural cell and gut [35].

The leukocyte cell-cell adhesion signaling pathway is largely involved in IBD and MCI pathogenesis, in which the gene pairs involved are CD3E-PTPRC, GRAP2-CD86, VAV1-CD86, PTPRC-SEMA4D and FYN-LYN. The CD3E-PTPRC pathway contributes to immune response, whereby the CD3E plays an important role in coupling antigen recognition to several intracellular signal-transduction pathways, whereas PTPRC regulates B- and T-cell receptor signaling [36, 37]. They contribute to defense of pathogen invasion, and may stimulate inflammation in both neuron or gastrointestinal tract. GRAP2 mediates the activation and signal transduction of T cells by producing signaling complexes, while provides costimulatory signals necessary for T cell activation and survival [38, 39]. Therefore, GRAP2-CD86 pathway contributes to the activation and maintenance of T cells in adaptive immune system, and potentially result in inflammation. VAV1 regulates cell surface receptor signaling pathway that involved in phagocytosis, which is macrophages that cause inflammation through the production of interleukin-1, interleukin-6, and TNF-alpha [40]. LYN involved in the regulation of mast cell degranulation, and erythroid differentiation [41]. The degranulation of mast cell will release histamine, tryptase, serotonin, cytokines, chemokines, and lipid mediators, which are all pro-inflammatory mediators [42]. The research conducted by Mkaddem *et al*, proved that LYN and FYN are responsible in activating immunoreceptors to direct homeostasis or inflammation by regulating FcR-ITAM signals [43]. These pathways may be the key of neuroinflammation as they are involved in immune response and inflammation process.

CONCLUSION

The study has proven that gut microbiome dysbiosis and MCI shared the similar gene signature and molecular pathway on their pathogenesis. However, due to its unclear pathogenesis and difficulty in diagnosis, the number of studies on MCI is still very much lacking as compared to other neurological disorders, which also contributed to the major limitation of this study. Hence, more advanced studies are required for better understanding on MCI. The future study based on elucidation of gene to identify more shared DEGs in higher accuracy, and eventually lead to a more reliable findings can be used to screen the MCI among the population.

CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have any conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the IMU University for funding this project under the project number [BMSc I-2023(08)].

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