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Elucidation of Gene Signature and Pathway between Gut Microbiome Dysbiosis and Autism Spectrum Syndrome

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Abstract

Due to the increase prevalence in the neurological diseases (ND), it had become a concern where more advancements are required to be discovered in resolving this issue. Despite the current treatment available, the concept of involving the gut-brain axis may provide another possible alternative to treat ND from the site of gut microbiome. To achieve this goal, the association between ND and gut microbiome should be studied. Therefore, this study aimed to explore the possible association between autism spectrum syndrome (ASD) and irritable bowel disease (IBD) from the aspect of differentially expressed genes (DEGs) and their pathways. In this study, a total of four datasets were obtained from Gene Expression Omnibus (GEO), which were GSE111176, GSE13367, GSE36701 and GSE87847. From there, the differentially expressed genes (DEGs) were selected based on the criteria where Log2FC > 1.0 and p-value < 0.05. The functional genomics analysis of shared DEGs were then measured through gene oncology (GO) enrichment on Database for Annotation, Visualization and Integrated Discovery database (DAVID). The biological processes that fulfilled the criteria p-value < 0.05 and false discovery rate (FDR) value < 0.05 were selected for the predictive model development using web-based GeneMANIA. There were 874 shared DEGs between ASD and IBD in total. The common biological processes where the shared DEGs in both ASD and IBD were signal transduction, inflammation and apoptosis process. From the co-expression network, the interaction of DEGs for both ASD and IBD were shown. The protein-protein interaction (PPI) network also demonstrated the DEGs that involved in leukocyte cell-cell adhesion and B cell activation were LYN, JAK3, ITGB1, PTPRC. The interaction between LYN and PTPRC had been examined. In conclusion, the association and the most commonly found biological processes which involved in shared DEGs between ASD and IBD had been examined in this study.

Keywords: Autism spectrum syndrome; differentially expressed genes; Gut-brain axis; Gut microbiome; Gut microbiome dysbiosis

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1. Introduction

Gut microbiome refers to the collection of microorganisms, which include the bacteria, viruses or archaea that inhabit the human body. These gut microbiomes play a crucial role in our daily life to maintain our health. However, when the growth of these gut microbiomes has been altered, it will lead to gut microbiome dysbiosis (GMD) [1]. The main reason which enhances the alteration of the composition of gut microbiome is the combination of stress and external environment [2]. GMD usually increases the patients' risk in developing functional gastrointestinal disorders (FGID). This event will also involve the dysregulation of the gut-brain axis, which refers to the bi-directional interaction between the gut and the brain. As gut microbiome is involved in regulating the gut-brain axis, GMD will lead to the dysregulation of the gut-brain axis [3]. On the other hand, inflammation events will be enhanced when there is alteration of gut microbiome. This will eventually affect the brain signaling and finally lead to the development of neurological diseases such as autism spectrum syndrome (ASD) [4-5]. ASD is a type of neurodegenerative disease, where patients often have difficulties in social interaction with other people.

Since the patients usually have restricted behaviors, they will repeat the same action frequently. Furthermore, ASD patients might also have obstacles in developing language skills and learning. Up to date, there is no medical test or standard treatment available to diagnose and treat

ASD, respectively [6]. Thus, healthcare providers could only provide support to ASD patients to minimize their symptoms so they can live with lesser interference [6]. The discovery of novel treatment for ASD had nowadays becomes a popular topic. Recently, many studies have proved the involvement of GMD among ASD patients [7]. The association between ASD and FGID like irritable bowel disease (IBD) had been discussed frequently as well. IBD patients are usually associated with abdominal pain, bloating and altering bowel habit [8]. Due to the complexity of IBD, it is very difficult to provide treatment for the IBD patients [8-9]. Since there is lack of alternatives in treating both ASD and IBD, this study focused on the discovery of the differentially expressed genes (DEGs) and pathways that associated ASD with IBD. This concept is important to discover alternatives in treating ASD and IBD by targeting those DEGs and pathways.

2. Materials and Methods

Since this research was carried out in dry-lab mode, the materials used in this study are mostly the websites and Microsoft tools such as Gene Expression Omnibus (GEO), Database for Annotation, Visualization and Integrated Discovery database (DAVID) and GeneMANIA.

2.1. Dataset selection and processing

The datasets (GSE111176, GSE13367, GSE36701 and GSE87847) were obtained from Gene Expression Omnibus (GEO) by using the keywords like autism, differentially expressed genes and gut microbiome dysbiosis. The datasets underwent internal and external validation, followed by gene annotation.

2.2. Feature selections and Functional genomics analysis

The differentially expressed genes (DEGs) were determined through log2 fold change > 1.0 and p-value < 0.05 in selecting the commonly shared DEGs. The functional genomics analysis of commonly shared DEGs was studied through gene oncology enrichment analysis using Database for Annotation, Visualization and Integrated Discovery database (DAVID). From there, the biological processes (BP), molecular functions (MF) and cellular components (CC) of commonly shared DEGs were obtained through the criteria p-value < 0.05 and FDR value < 0.05.

2.3. Predictive model development

Commonly shared DEGs from the Top 10 biological processes selected to study protein-protein interaction. The predictive models produced using web-based GeneMANIA.

3. Results and discussion

3.1. Differentially expressed genes within ASD and IBD

From the Venn diagram shown in Figure 1, there are a total of 874 commonly shared DEGs between both ASD and IBD. This result suggested the association between ASD and IBD. It was suggested that dysregulation of gut-brain axis had significantly increased the cases of ASD and IBD [10]. It also proved that ASD children usually had higher chance to be presented with GI symptoms like diarrhoea and constipation compared to normal children or children with other developmental delays [11-12]. Severity of GI symptoms is dependent on the severity of the ASD symptoms [10-13]. Besides, the alteration of gut microbiome could also be found in both ASD and IBD. There are approximately 90% of ASD children, who has complications of GMD [14]. Thus, it was suggested that gut microbiome is one of crucial components to determine causes of ASD and IBD [11-12]. On the other hand, the chronic inflammatory event had been found in ASD children with GI symptoms although its clinical significant remains unclear. In both ASD and IBD patients, presence of abnormalities like neuroinflammation and altered number of immune cells such as cytokines are indicated [15-16]. Therefore, development and pathogenesis of ASD and IBD can be related to the immune responses. This may suggest the involvement of shared DEGs between ASD and IBD in neuroinflammatory event. Recently, the effects of MIA were studied when discussing about the pathology for both ASD and IBD. It was suggested that MIA can cause autism-like behaviour among children. Immune cells such as cytokines and chemokines usually are elevated in MIA. The alterations in these immune cells will eventually lead to enhancement of inflammatory phenotype, where the inflammation will be developed in foetal brain [15-16]. Besides, increase of immune cells will also cause intestinal disruption and promote immune responses in GI tract [16]. Subsequently, lead to development of several GI problems like IBD.

3.2. Gene oncology enrichment analysis

Figure 2 shows the result obtained from gene oncology enrichment analysis of 874 commonly shared DEGs between ASD and IBD for BP, MF and CC. In BP, the gene count that participate in signal transduction is the highest. Moreover, the CC with most of the gene count is at cytosol. Furthermore, the mostly involved MF is the protein binding. Although the factors remain unclear, ASD was said to be involved in inflammation, transcription, and translation as well as epigenetics. There are studies supported that the interaction of different signaling pathways in ASD play the roles in pathophysiology of ASD. These pathways can be categorized into 4 categories as shown in Table 1. In category 1, the mutations on transcription factors will lead to the dysregulation in important transcription and translational pathways. Some studies suggested that the alterations in these pathways can bring bad impact on brain function and development [12-17-18]]. In category 2, the modification of synapse structure contributed to the signal transduction process due to the abnormalities in synaptic proteins.

The alteration of synaptic proteins will influence the neuronal networks in the brain [12-19]. Moreover, the over-translation of transcripts, which is category 3, can also lead to neuronal complications such as ASD [20] while category 4 is related to the immunoinflammatory responses. The increase and alteration in microglia and astrocyte activity in immune dysfunction had been indicated among ASD patients. This significant finding had also been proven by the detection of high gene expression of both microglial and astrocyte markers [21]. In addition, the neuroinflammation can also contribute to ASD. It was suggested that most of the expressed genes like TREM2, IL2RB and TH1TH2 in ASD patients are involve in neuroinflammation event [22-23]. Neuroinflammation event can lead to the alterations of brain and synaptic functions. Therefore, the immune mediators like cytokines and microglia will be affected, which will eventually lead to synaptic dysfunction in brain tissue [20]. Recently, many studies had identified the abnormal cytokines level, such as high level of proinflammatory cytokines and low level of anti-inflammatory cytokines in ASD.

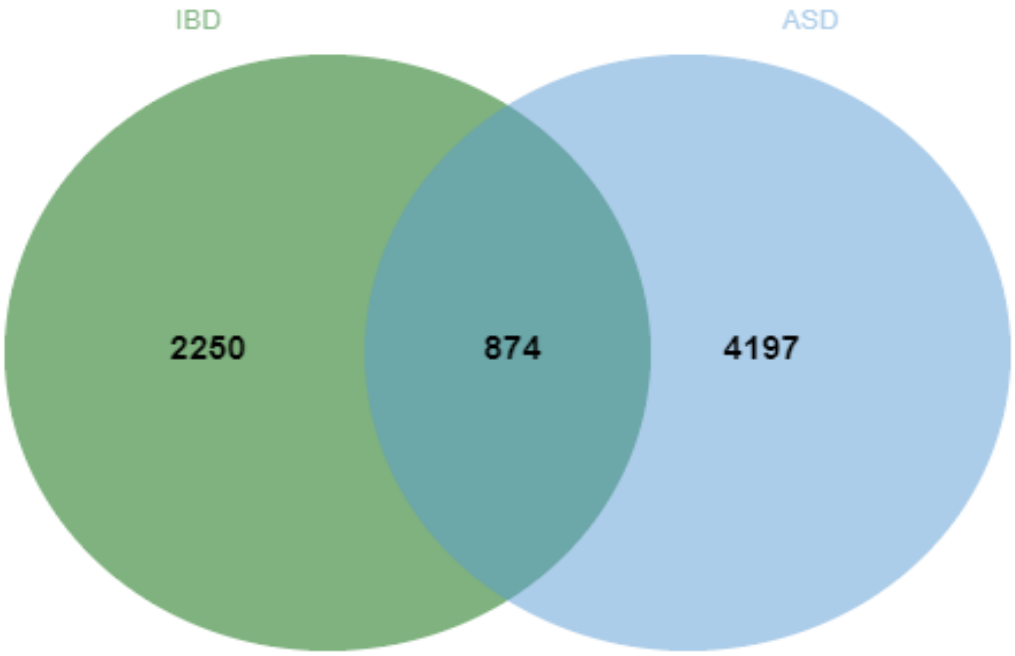

Figure 1: A Venn diagram showing the shared DEGs between ASD and IBD

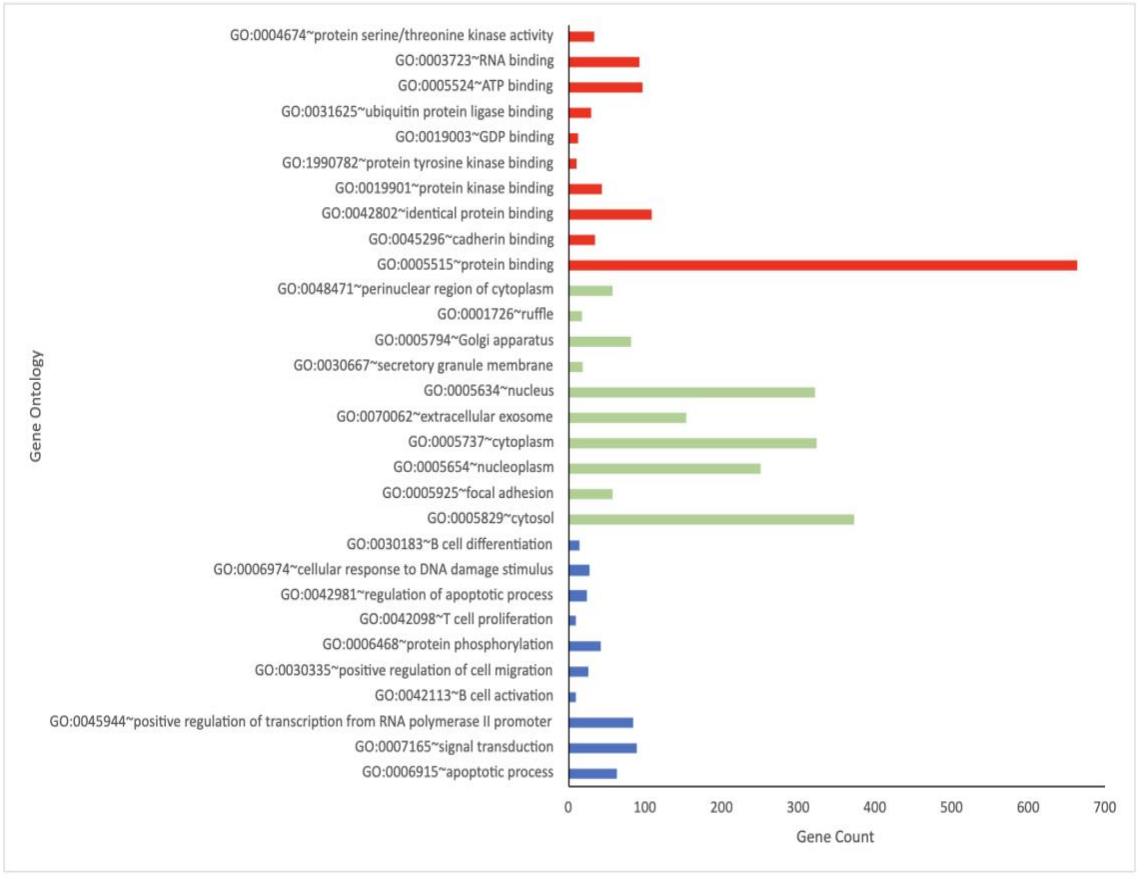

Figure 2: Top 10 Gene ontology enrichment analysis of the differentially expressed genes among ASD and IBD. Blue coloured bar refers to BP, green coloured bar refers to CC, while red coloured bar refers to MF.

Table 1: Different categories of signaling pathways which can interact and involve in ASD pathophysiology.

Category	Pathways
1	Dysregulation in transcriptional and translational pathways
2	Alterations of synaptic proteins
3	Over-translation of transcripts
4	Immunoinflammatory responses

Hence, these abnormalities can be considered as the biomarkers of neuroinflammation among ASD patients [12-21]. The involvement of inflammation event could possibly explain the processes such as T cell and B cell activation, positive regulation of cell migration and regulation of apoptotic process in BP in Figure 2. In addition to ASD, the alteration in immune cells like microglia can also influence the gut microbiome. It was suggested that neuroinflammation can cause the changes in the composition of the *Proteobacteria* like *Escherichia* spp., *Shigella* spp. and *Succinivibrio* spp. in the gut, which then contribute to GMD. Therefore, this indicates the influence of neuroinflammation event to ASD and GMD [24-25].

3.3. Co-expression network analysis

A model that explain the co-expression network analysis of ASD and IBD with significant cell-cell adhesion and B cell activation was generated from GeneMANIA. There are six DEGs that presented with significant cell-cell adhesion and B cell activation, which are JAK3, CTLA4, SYK, LAPTM5, PTPRC and LYN. All these six DEGs are all involve in inflammatory event. For instance, SYK involves in signal transduction to activate or regulate B cells, which promote inflammation event that associated with the pathogenesis of ASD and GMD [26]. On the other hand, LAPTM5 not only suggested to be involved in pathogenesis of IBD, but this DEG also involves in neuronal inflammation and apoptosis processes, which will eventually contribute to ASD [27]. Not only that, the alteration in CTLA4 also found in ASD patients. CTLA4 will involve in neuroinflammation event, which result from the interaction between microglia cells and T cells. Hence, this significantly indicate the participation of CTLA4 in the pathogenesis of ASD and GMD [28]. In short, majority of the DEGs for ASD and IBD are involve in inflammation process, which is common characteristic in both ASD and IBD. Some DEGs also promote apoptotic situation and altering signal transduction.

3.4. Protein-Protein Interaction (PPI) Network

Another model, which presents the lleukocyte cell-cell adhesion and B cell activation signaling pathways involves in IBD and ASD pathogenesis was also produced from GeneMANIA. Only a total of four DEGs are involved in both signalling pathways. They are LYN, JAK3, ITGB1, PTPRC. There is a limited study where only a pair of interacted DEGs could be found, which is the interaction between LYN and PTPRC. Both LYN and PTPRC had been confirmed to be involved in inflammatory and immunity pathways for ASD and GMD [29-31]. LYN acts in regulating various immune cells. Thus, the dysregulation of LYN will badly influence the regulation of gut microbiome and inflammatory response. The dysregulation of LYN is often found among IBD patients [31-33]. There are studies also suggested the involvement of PTPRC in leukocytes apoptosis process [34]. The dysregulation of PTPRC mainly lead to the pathogenesis of chronic GI symptoms and ND like ASD. The importance of PTPRC can also be discussed in cellular activities such as cell growth, cell proliferation and the apoptosis of the cells, where the shared DEGs for ASD and GMD are commonly involved [29-31-35]. Moreover, JAK3 had been suggested to participate in the pathogenesis of ASD and GMD. JAK3 plays important role in mediating the cytokines and hormone transmission. When there are abnormalities in JAK3, the amount of cytokines will be affected and contribute to serious complications like ASD and GMD. The alterations in cytokines will contribute to neuroinflammation that can lead to ASD. Studies also suggested that deficiency in JAK3 will lead to alterations of gut microbiome composition and lead to GMD [36-38]. Besides, the role of ITGB1 in maintaining the composition of several types of microorganisms had also been studied. Thus, the composition of gut microbiome will be altered when there are abnormalities in ITGB1. This had clearly demonstrated the association of ITGB1 in the pathogenesis of GMD. When there is dysregulation of gut microbiome, it can also give rise to other ND like ASD [39-40].

4. Conclusions

In a nutshell, the association between GMD and ASD had been successfully identified in this study. The shared DEGs and pathways associated between GMD and ND have also been discussed. With the current knowledge of the DEGs, further studies on both ASD and GMD should be continued, particularly in discovering more alternatives. For instance, with their shared DEGs, it is hoped that some possible treatments through inhibition of certain significant pathways for both ASD and GMD could be discovered.

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Statement on the conflict of interest

The authors declare no conflict of interest.

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