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Exploring Transcriptional Relationships Implicated in Autism and Inflammatory Bowel Diseases Using Systems Biology Approaches

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Abstract

Introduction: Understanding the association among various disorders has remarkably improved their diagnosis and therapies. It has been observed that autism and inflammatory bowel diseases (IBDs) cause a sort of inflammation. Exploring the relationship between them will lead to discovering important involved genes and in turn will eventually help discover any possible common therapeutic protocols. The aim of the present study was to determine the correlation of autism spectrum disorders (ASDs) and IBDs.

Materials and Methods: The common genes associated with autism spectrum disorders and IBDs were retrieved from DisGeNET. SFARI and TRRUST databases were subjected to an in-silico data analysis framework to explore predictive genes and the related pathways.

Results: Eleven genes including *HLA-DRB1*, *MTHFR*, *PON1*, *IL6*, *MTOR*, *SETD2*, *GSTM1*, *APC*, *IFNG*, *SERPINE1* and *MAPK1* regulated by *YY1* and *IRF1* transcription factors were characterized as discriminating molecules which by further screening were enriched in pathways mostly involved in neutrophil apoptosis, neutrophil homeostasis, chemokine biosynthesis and the regulation of immune system response.

Conclusions: According to findings it can be stated that the identified common genes were associated with a wide range of pathogenic mechanisms.

Keywords: Autism Spectrum Disorders, Inflammatory Bowel Diseases, Disease-associated Genes, Network Analysis

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Introduction

The immune system should defend the physiological system against external threats homeostatically. The failure in maintaining the internal stability in facing with the external threats will contribute to controversial autoimmune and inflammatory conditions. Autism spectrum disorders (ASDs) are neurodevelopmental conditions with heterogeneous clinical manifestations which affect different parts of the brain. ASDs are characterized by impairments in social communication such as restricted or repetitive behaviors or interests which are followed by neurological delicacy due to the dysfunctionality of neurons.[1,2] Inflammatory bowel disease (IBD) in two major forms, Crohn's disease (CD) and ulcerative colitis (UC), is an immune-mediated disease which causes the damage of the gastrointestinal tract with an equal prevalence in both sexes.[3] UC is a chronic inflammatory disorder of the colon that causes typical ulcers in the mucosa of the rectum and colon. On the other hand, CD is a chronic inflammatory condition that can influence any part of the gut from the mouth to the anus. From different contributing factors triggering IBD, Vitamin D levels, diet, hormone use, stresses caused by industrialization besides and cigarette smoking have been introduced as risk factors.[4]

There are many possible ways to link between expression data and different genes and pathways being affected in ASDs. Among them, the gene co-expression networks are considered as a way to investigate the etiology of ASDs. Voineagu et al identified co-expressed genes associated with autism.[5] They believe that biological networking is able to detect convergent molecular abnormalities in ASDs and underlying mechanisms. The co-incidence of two or more disorders in an individual is named comfortability and characterizing genes and pathways involved in the related diseases seemingly would be helpful in identifying comorbidity patterns.[6] The relationships

among different autoimmune diseases led to few studies investigating the underlying molecular similarities among immune-mediated disorders.[7,8] Activation of the immune system in ASDs and IBD by alteration in gut microbiota will indeed increase the intraepithelial T lymphocytes and inflammatory cytokines leading to intestinal atrophy followed by malabsorption in these patients.

Although the association between ASDs and IBD has been already presented in a number of research,[9,10] data mining methods can also be considered as a powerful approach towards the understanding of the etiology of these diseases.

Considering the ever growing of omics data, data integration and analysis approaches are great keys for solving the diseases complexities. In this study it was assumed that there is a sort of similarities among ASDs and IBD patients at the gene expression level. We mainly aimed to elucidate potential relationships between ASDs and IBD by in silico data analysis approaches.

Materials and Methods

Firstly, the curated gene-disease relations of ASDs and IBD was retrieved from DisGeNET v2.0 server (http://www.disgenet.org/web/DisGeNET/). These gene-disease associations have been collected from several databases including UNIPROT, human CTD, PsyGeNET, Orphanet and the HPO. The common genes were further filtered based on SFARI database (https://gene.sfari.org/) which contains genetic variants in ASDs associated genes. Afterwards, a list of 9905 regulatory links between human gene-transcription factors was obtained from TRRUST database (http://www.grnpedia.org/trrust/). These regulatory connections have been collected from PubMed articles and experimentally validated transcriptional regulations consisting of 821 human transcription factors and 2,159 non-TF genes. A network was then built using the co-expressed genes and pathways by a list of common genes related to both ASDs and IBD by utilizing GeneMANIA (https://genemania.org/). Functional classification of common genes was performed by BINGO Cytoscape plugin (http://apps.cytoscape.org/apps/bingo) with hypergeometric test (Benjamini and Hochberg FDR correction) at the significant level 0.001. The significant GO biological terms have been visualized by Enrichment map Cytoscape plugin (http://www.baderlab.org/Software/EnrichmentMap) with Jaccard coefficient 0.001. Transcription factors control the expression of gene within clusters. Therefore, iRegulonCytoscape plugin (http://iregulon.aertslab.org/) was used to discover the possible regulons and co-factors associated with ASDs and IBD. Finally, the Gemma server (http://www.chibi.ubc.ca/Gemma/home.html) was checked for differential expressions among the shared genes. Figure 1A is a schematic representation of the methodologies used here.

Results

DisGeNET is a curated collection of genes carrying genetic variants associated to human diseases from which among the 4753986 potential diseases, 13064 diseases were found to share at least one gene. Among these associations, ASDs was significantly associated (combined score at P value <0.05) with thyroid neoplasms, mesothelioma, and schizophrenia while in IBD the significant associations were seen with stomach neoplasms, osteoporosis, postmenopausal and sepsis. Combined score is computed by taking the log of the P values generated by the Fisher's exact test and multiplying that by the z-score of the deviation from the expected rank. Furthermore, research indicated a higher prevalence of IBD in people with autistic brains.[9,10] Therefore, we were interested in mining ASDs-IBD correlations at transcriptome levels to understand the underlying reasons of potential overlaps. As previously mentioned, ASDs-IBD associated genes were retrieved from the DisGeNET database. Only disease-related genes with more than one evidence from Pfam have been selected for further analysis. Out of 128 ASDs and 880 IBD specific genes, 128 and 279 genes were respectively chosen. Eleven genes including *HLA-DRB1, MTHFR, PON1, IL6, MTOR, SETD2, GSTM1, APC, IFNG, SERPINE1* and *MAPK1* genes were shared among 910 ASDs related genes from the SFARI database (Figure 1), enriched in pathways mostly involved in neutrophil apoptosis, neutrophil homeostasis, chemokine biosynthesis and regulation of immune system response. The GeneMANIA server was used to establish a network of co-expressed genes and pathways in ASDs-IBD

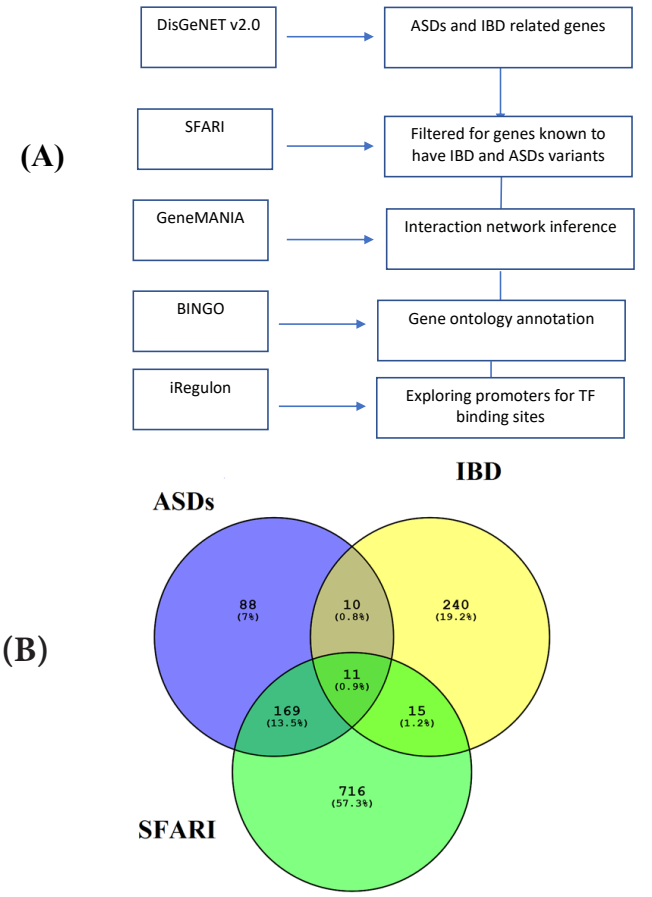

Figure 1. (**A**) Schematic Representation of the Methodology. (**B**) Venn Diagram of Intersection Between ASDs and IBD Genes Retrieved From DisGeNET and SFARI Servers.

by utilizing the identified 11 common genes (Figure 2). The common genes were additionally investigated to discover the disease-associated motifs and master regulators by iRegulon and TRRUST (Figure 3) where YY1 and IRF1 were found as the most probable regulators. We checked whether gene-transcription factor links from iRegulon should also be presented as experimentally validated links in TRRUST. Finally, as a meta-analysis, the differential expression of common genes was deciphered in the Gemma server. Except the *HLA-DRB1* the other genes have shown differential expressions in ASDs-IBD experiments in cell types and treatments (Figure 4).

Discussion

IBD and the irritable bowel syndrome are the most important immune-mediated intestinal conditions.[11] IBD is a chronic, systemic inflammatory disease with gastrointestinal complaints for which methylated thiopurine metabolites, like 6-methyl mercaptopurine, are commonly administered.[12,13] ASDs share a core set of neurodevelopmental features affecting social and communicational abilities known as complex abnormalities due to both the extreme phenotypic heterogeneity and contributing many genes in these disorders.[14,15] In spite of performing research which has helped to expand our knowledge about IBD and ASDs, questions such as how these are linked and the extent to which the gastrointestinal disorders are common in autistic patients, remained unclear. In this study, we freely employed available data and bioinformatics tools to investigate genes whose interactions are hypothetically challenging in both ASDs and IBD pathogenesis. Furthermore, we tried to elucidate the common underlying molecular pathways in these diseases. Interestingly, the most identified genes were enriched in chemokine biosynthesis. The levels of

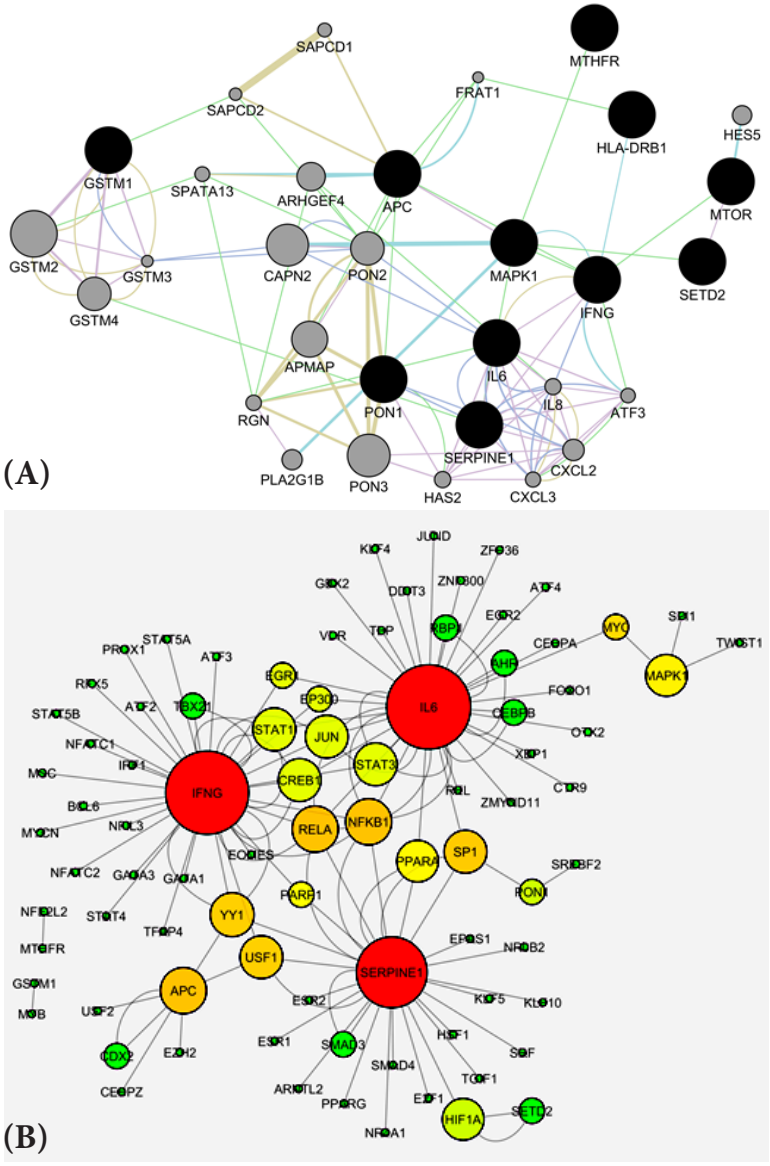

Figure 2. (**A**) Schematic Representation of Co-Expressed Genes Established by Common Genes Underlying Autism and Inflammatory Bowel Diseases by GeneMANIA server. (**B**) Interaction Network Established by Highly Interconnected Genes. Network Units with More Connectivity have been Illustrated Bigger and Darker.

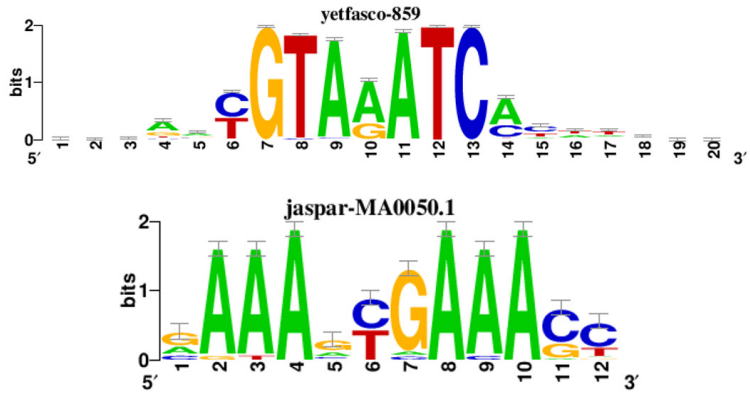

Figure 3. YY1 and IRF1 Transcription Factors Were Enriched as the Most Potential Master Regulators Governing the Expression of a Network of Gene Sets. iRegulon software was used to explore master regulator (right) and motifs in upstream of shared genes between ADSs and IBD.

Figure 4: Expression Profile of Differentially Expressed Genes in IBD and ASDs From Gemma Server.

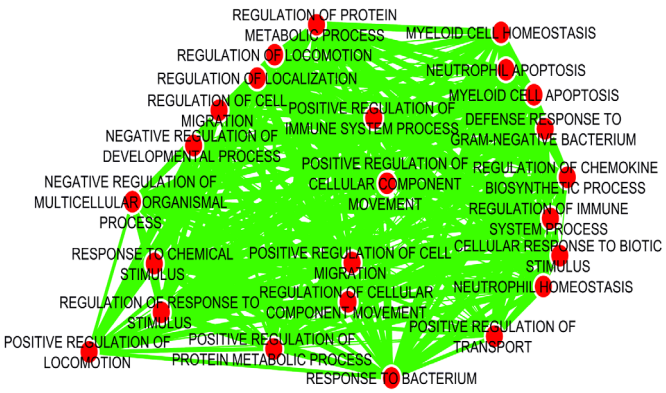

Figure 5. Functional Classification of Biological Processes in Which Differential Expressed Common Genes in ASDs and IBD Supposed to be Involved. The GO terms considered significant based on hypergeometric test with Benjamini and Hochberg FDR correction and significance level 0.001 by BINGO app. The bars have been arranged top to down illustrating the number of genes and significance level assigned to each GO term.

specific cytokines and chemokines have been found to be elevated in IBD patients.[16-18] In accordance, the presence of chemokines *CXCL* 2 and 3 in GeneMANIA network reveals the potential of inflammation in these patients (Figure 1A). In the generated regulatory network, *NFKB1, STAT3, STAT1, RELA, MYC* and *YY1* TFs, interleukin 6 (IL6), *SERPINE1*, and interferon gamma (*IFNG*) showed the most connectivity. Functional classification of genes involved in regulatory network was performed using FDR correction at significant level 0.001 (Figure 5). The GeneMANIA constructed co-expression network demonstrated a distribution of interleukins that implies an indispensable role of interleukins in immune-mediated disorders. Noteworthy, interleukins especially inflammatory cytokines like *IL6* exhibited more than a two folds changes up-regulation in both ASDs and IBD patients when compared to healthy donors (Figure 4). Further, by inspecting 1KB from the upstream of ASDs-IBD associated genes to characterize the disease-specific *cis*-regulatory motifs, *YY1* and *IRF1* were identified as the most significant regulators of the common genes. The *IRF1* transcription factor with the highest connectivity (Figure 1B), has documented roles in the regulation of interleukin

genes.[19] IL6, as one of the most important neuroimmune interleukins, has been demonstrated to be implicated in several neurological disorders by neuronal circuitry imbalances.[20,21] More interleukins were exhibited to induce proliferation of innate intraepithelial lymphocytes. The IL6 is mostly generated from innate immune cells like, neutrophils where in turn IL6 induced signaling can be primarily seen in a relatively small number of IL6 responsive transcription factors such as STAT3.[22,23] IL6 showed a mild up-regulation in both ASDs and IBD patients (Figure 4). In network of genes, both IL6 and STAT3 showed high connection seemingly as a part of chronic intestinal inflammation cascade. Among the ASDs and IBD common genes, interferon genes like *IFNG* were identified which were seemingly regulated by *NFKB1*, *IFR1*, *STAT1*, *YY1* and *EFGR1*. These interferons participate in inflammatory and apoptotic events (Figure 1B, Figure 2) among which *IFNG* showed a mild to high up-regulation in ASDs and IBD except in the cell types-based experiments. *IFNG* variants have been showed to be related to IBD as an indicative of a more aggressive disease.[24] *IFNG* was shown as a competent of IFNG/JAK-STAT-dependent signature complex and a molecular link between immunity and neural circuits implicated in behavior and inflammation.[25,26] Plasminogen activator inhibitor-1 (*SERPINE1*) with the highest connectivity in network was also shown to have a significant association with autism.[27] *SERPINE1* and gene signatures in its downstream as a putative regulator, have also been reported to be directly related to IBD as a functional regulator.[28,29] In Gemma expression profiles of common genes in IBD, except the experiments regarding infliximab treatment in which *APC* and *GSTM1* showed a >2 fold down-regulation, the other genes were up-regulated. However, the expression of genes in ASDs experiments was more sporadic and experiment dependent. Adenomatous polyposis coli's (APC's) was displayed as a pivotal regulator of synaptic adhesion and signal transduction pathways crucial for normal cognition.[30] ASDs are highly polygenic moreover multiple genes were reported to be associated to them.[31,32] However, inflammation mediated by chemokine, interferon, neutrophil apoptosis, and biosynthesis covered most of common genes between IBD and ASDs, which is in agreement with previous researched showing the proven roles of immune and inflammatory events in autoimmune diseases like IBD and likewise neuroinflammations.[33-39]

However, this analysis included some limitations; IBD and ASDs related genes were retrieved from autosomal recessive and formed 1-27 IBD. Additionally, x-linked, macrocephaly- autism syndrome and high functioning autism related genes were included in the study. One therefore should be more specific to drive the exact types of disease-disease links using strict criterion.

Conclusions

According to findings, several genes and transcription factors were highlighted in this study which is thought to interact with each other which is resulted to the development of ASDs-IBD majorly enriched in chemokine biosynthesis as well as neutrophil apoptosis and homeostasis.

A similar expression level of common genes in ASDs and IBD fairly could show a tight transcriptional relationship of these diseases in promoting each other. Furthermore, grouping the genes in chemokine biosynthesis, might be biologically relevant tonecessity in declining the expression of them to inhibit inflammatory responses in these patients.

To conclude, the conserved genes obtained by bioinformatics data analysis like interferons, IRF1 and interleukin genes could be proposed for future research for investigating their roles.

Authors' Contributions

FI designed the experiment, analyzed the data and wrote the manuscript. The rest of the authors have equally contributed to the edition and writing of the manuscript.

Conflict of Interest Disclosures

The authors declared no conflicts of interest.

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