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Network Gene Analysis of Potential Target for Neurological Disorders through System Biology Approach

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Abstract

Neurological disorders are a major threat to the wellness of individuals. The neurological disorders included in the Global Burden of Disease (GBD) Study—Alzheimer's and other dementias, Parkinson's disease, multiple sclerosis, epilepsy, and headache ailments represent 3 percent of the global burden of illness. Though there are very many diagnostics approaches available for early diagnosis of neurological ailments, systems biology approaches, on the other hand, would attempt to identify the systems which, when altered, change the entire body by a 'Healthy' into a 'disease' state. In the present study, retrieval of selective genes that are related to Neurological Disorders (Epilepsy, Autism, Migraine, Alzheimer's and Parkinson's disease) was done by intense data mining using NCBI (https://www.ncbi.nlm.nih.gov/). The PPI (Protein-Protein Interaction) network was constructed using these genes by the use of STRING 11.0 database. The experimental and co-expression data with 0.400 confidence score were taken as key parameter for PPI network construction in STRING. Further the constructed network was subjected to network analysis and visualization using Cytoscape v 3.8 plug-in Network analyzer. Based on topology parameter betweenness centrality (BC) and node degree INS, AKT1, ALB, IL6 and TP53 genes are identified as the key genes in network. INS gene was obtained as a super hub gene amongst all other genes having the highest betweenness centrality (BC) of 0.0598 and node value of 293. The enrichment analysis of INS, AKT1, ALB, IL6 and TP53 reveals their active role in regulation of pathways and processes which are related to the selected neurological disorder. Thus, the study on these genes along with their pathways and biological mechanism can provide a potential target that may lead to the discovery of potential biomarkers for early detection, diagnosis and monitoring of neurological disorders at different stages.

Keywords: Neurological Diseases, Systems Biology, Network Analysis, Neurodegeneration.

Introduction

Neurology is the branch of science that deals with diseases related to the functioning of the nervous system.[1] Neurological disorders present a large burden on wellness, global disability and life lost.[2] These disorders can be classified as either neurodegenerative or neurodevelopmental disorders. The most recent estimates show that the neurological disorders included in the Global Burden of Disease (GBD)[3,4] Study-Alzheimer's and

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other dementias, Parkinson's disease, multiple sclerosis, epilepsy, and headache ailments [migraine, tension-type headache (TTH), along with medication-overuse aggravation (MOH)] represent 3 percent of the global burden of illness. Although this is a seemingly modest general percent, dementia, epilepsy, migraine, and stroke rank among the highest causes of death and disability.[5]

Neurodegenerative disorders refer to the loss of structure or function of neurons including the death of neurons. These disorders are generally influenced by a combination of genetic, epigenetic and environmental factors, therefore, making them sporadic in nature.[6] Neurodegenerative disorders include diseases like Parkinson's disease, Alzheimer's disease. Parkinson's disease (PD) is an irreversible, progressive and incurable multigenic neurodegenerative disorder that involves progressive loss of mid brain dopaminergic neurons.[7] It affects about 1% of the population above the age of 65 and its prevalence increases with age. It affects 41 people per 100,000 in the age group of 30–40 years old to over 1900 per 100,000 in people over 80 years of age.[8] Alzheimer's disease (AD) is the most common cause of dementia worldwide that accounts for 80% of all dementia diagnoses.[9] It is characterized by progressive decline of two or more cognitive domains, which causes loss of ability to perform basic day-to-day activities.[10] Dementia and Parkinson's disease are among the top 15 ailments having the most significant increase in the last ten years. Epilepsy also resembles neurodegenerative disease due to its association with progressive cognitive decline. [11] It is the most common neurological disorder that affects 50 million people worldwide. The disorder is characterized by occurrence of unpredictable seizures that affect people of all ages.[12] Migraine is another neurological condition that is characterized by recurrent episodes of pulsating headache.[13] It is a common, multifactorial and hereditary neurovascular headache disorder affecting millions of people worldwide, approximately 15% of the population.[14,15] Migraine and epilepsy represent twenty-five and one-fourth of the neurological weight reduction, respectively. With the increasing cases of neurodegenerative disorders, it is now vital to understand the molecular mechanics and behavioral symptoms of these disorders in order to be able to predict the disease.

Neurodevelopmental disorders refer to a group of circumstances with onset in the developmental stage.[16,17] These conditions are characterized by impairment of social skills or intelligence.[18] Neurodevelopmental disorders include diseases like Autism. Autism is one of the highest expanding neurodevelopmental disorder. It is developed due to complex interactions between genes and environmental factors and influences many systems in the body like immunological, mitochondrial, gastrointestinal, metabolic and neurological.[19] Diagnosis of autism is generally made through symptoms like social communication, language advancement and repetitive behaviour.[20]

The etiology of neurodegenerative disorders is not clear nonetheless, it also results in varied factors like oxidative stress (ROS), ATP depletion, andreduction of mitochondrial membrane potential.[21,22] The neurological ailments, such as procedures linked to pathological aging are concerned with the increased accumulation of the abnormal protein aggregates in and around neurons that are affected. Oxidative stress and protein misfolding plays a vital role in the pathogenesis and development of neurodegenerative diseases. These diseases are associated with the fibrillar aggregates of misfolded proteins. Neuronal death or apoptosis could be mediated by oxidative stress or ER strain or from both at the cellular level. Consequently, efforts are being made continuously to determine a target protein present in the cell that can protect the cell from oxidative damage and also have the capability to treat neurodegenerative diseases. In order to identify potential drug targets for Autism, Epilepsy, Migraine, Alzheimer's and Parkinson's Disease it is necessary to understand the inherent mechanism of disease pathogenesis. Systems biology approaches can be analyzed as a new area of research that aims to find out biology at the system level, entailing the practical analysis of their dynamics and structure of organisms and cells.[23]

With the inception of network biology, it has become easier to know the entire disease mechanism at the system level by constructing a network based computational model for diseases. Assessing these networks provides valuable information regarding the functional association of genes/proteins at the network that in-turn aids in identifying the maximum potential drug target for disorder among the huge array of genes/proteins from the network. The present in-silico analysis was attempted to research the underlying disease mechanism of five most common neurological disorders in addition to the identification of key genes along with their functional enrichment analysis on the basis of protein-protein interaction network approaches.

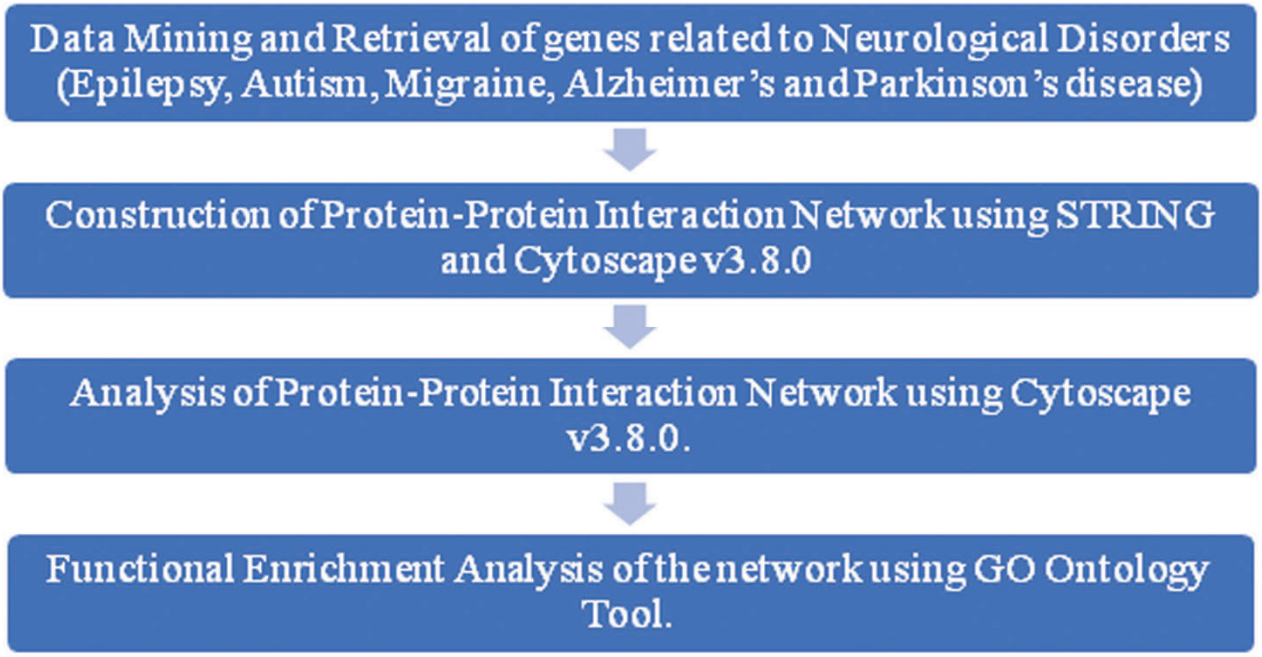

Fig. 1: An overview of methodology.

Methodology

Intensive data mining and use of various in silico tools was done to form a proper workflow for the computational identification of potential target for neurological disorders.

Data Collection

Retrieval of genes related to Neurological Disorders (Epilepsy, Autism, Migraine, Alzheimer's and Parkinson's disease) was done through immense data mining from NCBI database. Overall, nine hundred and ninety-eight genes regulating neurological disorder in human were retrieved from NCBI database (www.ncbi.nlh.nic.gov). For Autism, Migraine and Parkinson's Disease two hundred genes each and for Alzheimer's Disease, Epilepsy total of one hundred and ninety-nine genes each were reported from NCBI.

Construction of PPI Network

STRING (Search Tool for the Retrieval of Interacting Genes)[24] tool was used for the construction ofprotein-protein interaction network. The network was constructed at the confidence score of 0.400, which means that only interactions above the threshold level of confidence will be used for network construction.

Analysis of PPI Network

The visualization and analysis of the protein-protein interaction (PPI) network was done through Cytoscape 3.8.0 software.[25] Betweenness Centrality (BC) and node degree are the parameters included in Cytoscape Plug-in Network Analyzer. Node is represented by gene and interaction is represented by edges. Edges' number specifies node degree.

Functional Enrichment Analysis

Functional analysis is employed to spot set of an enriched gene with significant function in entire candidate gene list derived from network analysis. Several tools & software are available for analysis among them some widely used are Gene Ontology (GO; http://www.geneontology.org)[26], provides core biological knowledge representation for contemporary biologists, based computationally or experimentally. It represents the gene and its product in term of their biological process, cellular process, and metabolism. The Database for Annotation, Visualization and Integrated Discovery (David; http://david.ncifcrf.gov)[27] provides functional enrichment analysis, functional annotation, clustering, bio-Carta and KEGG pathway mapping, identifying functionally related genes that provide biological significant function derived from the massive dataset. Serval Cytoscape plugins like BiNGO[28], ClueGO[29], Enrichment Analysis and Visualization (ENViz)[30] etc. also are available for analysis supported interaction network and topological parameter.

Table 1: Publicly available functional enrichment analysis tools

Tools	URL
Gene Ontology (GO)	http://www.geneontology.org
The Database for Annotation, Visualization and Integrated Discovery	http://david.ncifcrf.gov
BiNGO	http://apps.cytoscape.org/apps/bingo
ClueGO	http://apps.cytoscape.org/apps/cluego
Enrichment Analysis and Visualization (ENViz)	http://apps.cytoscape.org/apps/enviz

Result

Network based strategy was utilized for the collection of large gene data which can contribute to the prediction of putative candidate genes. The research concentrates on the inter-relationship involving the respective components using the PPI network and aids in the identification of genes linked to the disease. The current study has used the PPI network-based strategy to recognize the most important novel gene in response to the selected neurological disorders.

PPI Network

In the current study, we explored the protein-protein interaction by utilizing the knowledge and the information that is available using STRING 11.0 database. PPI network assembled with 998 genes in STRING contributed to 15,063 interactions

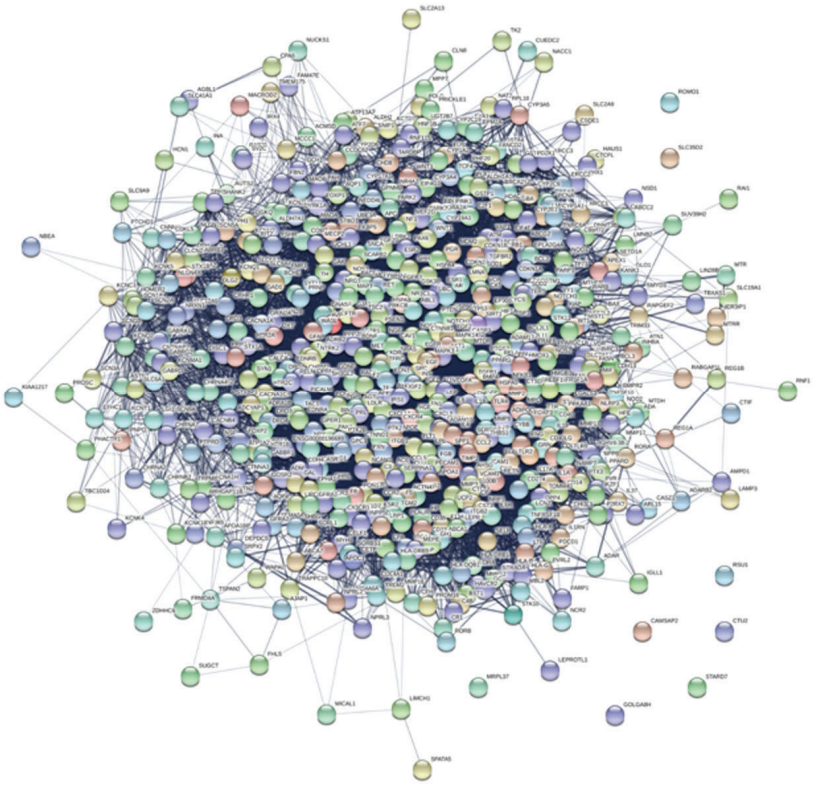

Fig. 2: Overview of PPI Network constructed using STRING 11.0 database. The network includes 15,063 edges (interaction) between 601 nodes respectively based on experiment, co-expression, text mining & co-expression with 0.04 confidence score as the analysis parameter.

Table 2: Obtained Key Genes in Network based on Topological parameter Node Degree and Betweenness Centrality.

Gene name	Node degree	Betweenness centrality
INS	293	0.0598
AKT1	277	0.0375
ALB	271	0.0339
IL6	262	0.0225
TP53	242	0.0301

involving 601 nodes based on parameters such as experimental, database, coexpression, text mining together with all the confidence score (0.04), network density 0.084, average number of neighbors 50.126 and average local clustering coefficient 0.456. (Figure 4.1)

PPI Network Analysis

The PPI networks were constructed inputting total 998 genes, including 200 genes each for Autism, Migraine, and Parkinson's Disease while 199 genes for Alzheimer's Disease and Epilepsy using the STRING database. Obtained networks have been examined and visualized using Cytoscape 3.8.0 plugin Network Analyzer version 2.7. According to a topological parameter Betweenness Centrality and Node Degree: INS, AKT serine/threonine kinase 1 (AKT1), ALB, interleukin 6 (IL6) and tumor protein p53 (TP53) are selected for reflecting a greater tendency to be a hub gene with maximum Betweenness Centrality value and Node Degree. Among these, INS was identified as a super hub gene and had the highest betweenness centrality and node degree in the constructed network. (Figure 4.2)

Various literature finding suggests the active function of INS gene at the central nervous system

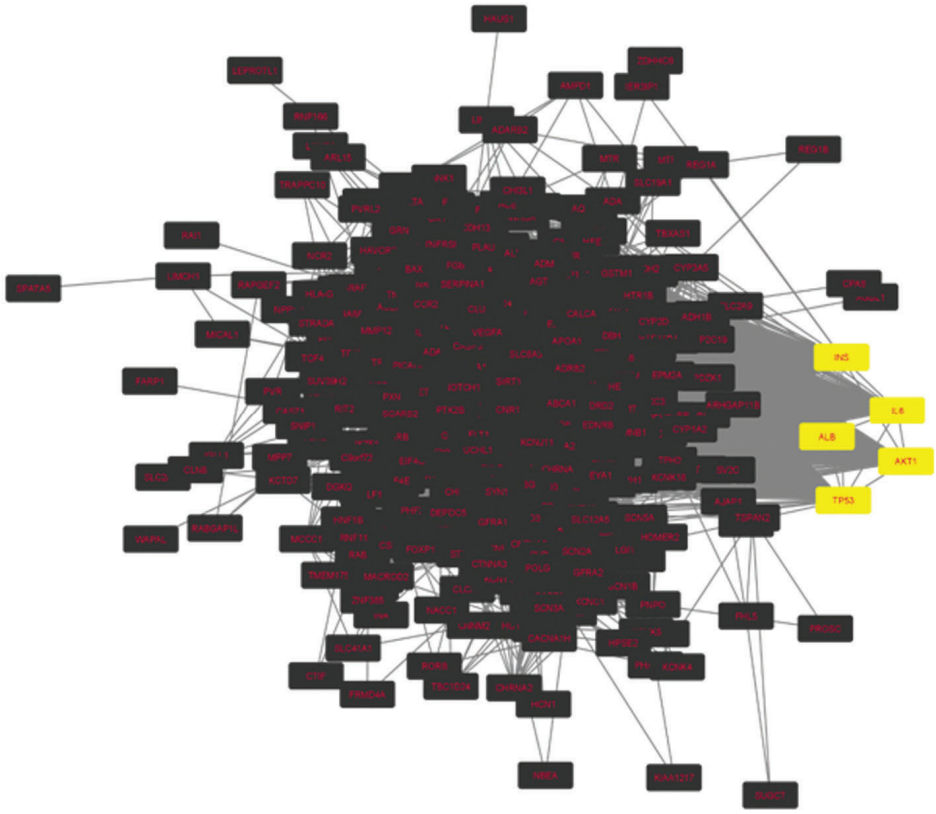

Fig. 3: PPI Network constructed using Cytoscape 3.8.0. Network. The node with yellow colors represents the key genes in the network having the highest betweenness centrality and node degree.

Table 3: Functional Enrichment analysis of the key genes in PPI Network.

ID/Pathway	Term	P-value	Associated Genes
Go_0033554	Cellular response to stress	1.7259E-10	(ALB, AKT1, TP53, CASP3, ABL1)
Go_0006950	Response to stress	2.9094E-5	(INS, AKT1, TP53, ALB, IL6)
Go_0080134	Regulation of response to stress	2.8276E-21	(INS, ALB, AKT1, IL6, TP53)
Go_0010941	Response to stimulus	1.05E-03	(IL6, ALB, AKT1, TP53, INS)
Go_0035466	Regulation of signaling pathway	1.13E-04	(IL6, AKT1, TP53, INS)

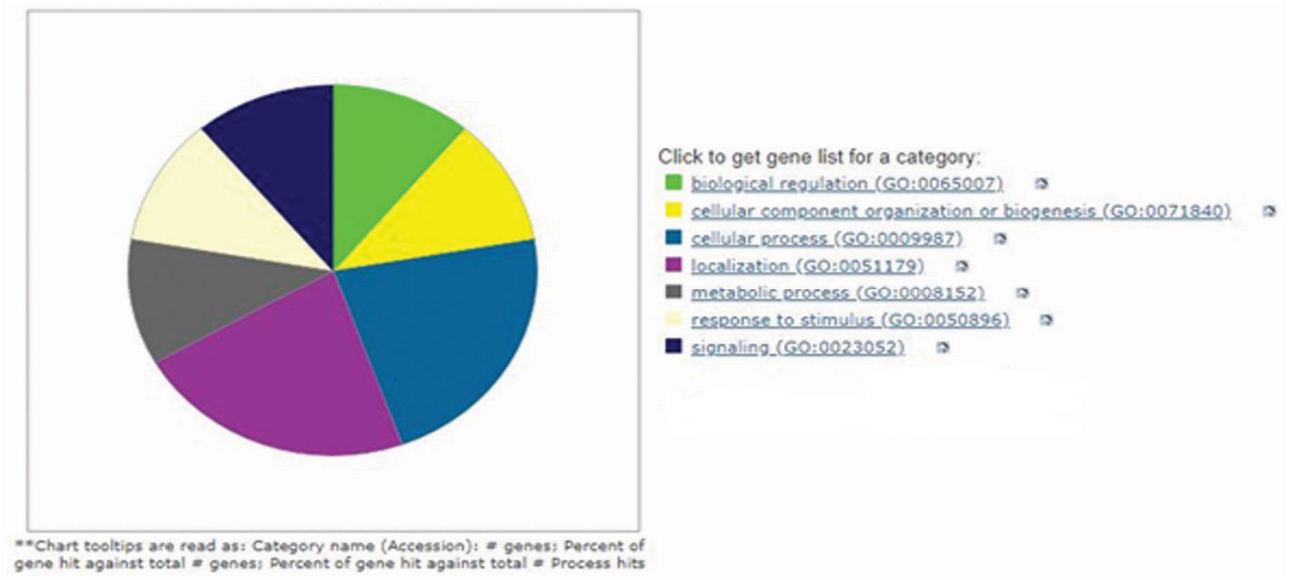

Fig. 4: Biological Process ontology pie chart obtained by functional enrichment analysis via PANTHER GO tool.
Source: https://www.mdpi.com/2079-7737/11/4/555?type=check_update&version=1

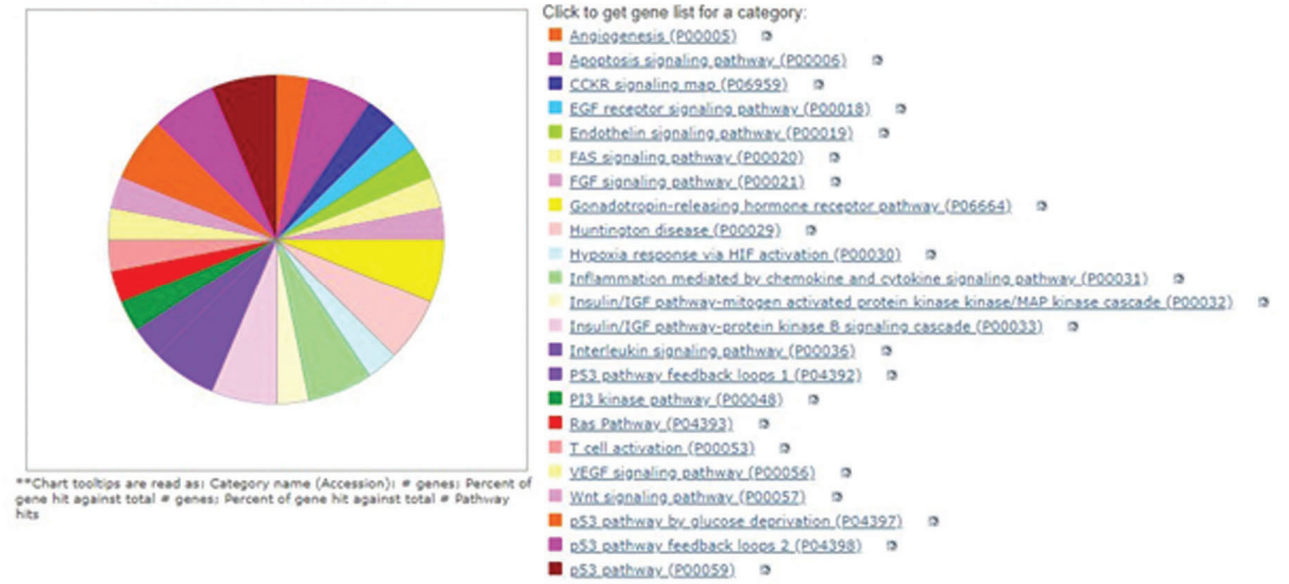

Fig. 5: Pathway ontology pie chart obtained by functional enrichment analysis via PANTHER GO tool.
Source: https://www.mdpi.com/2079-7737/11/4/555?type=check_update&version=1

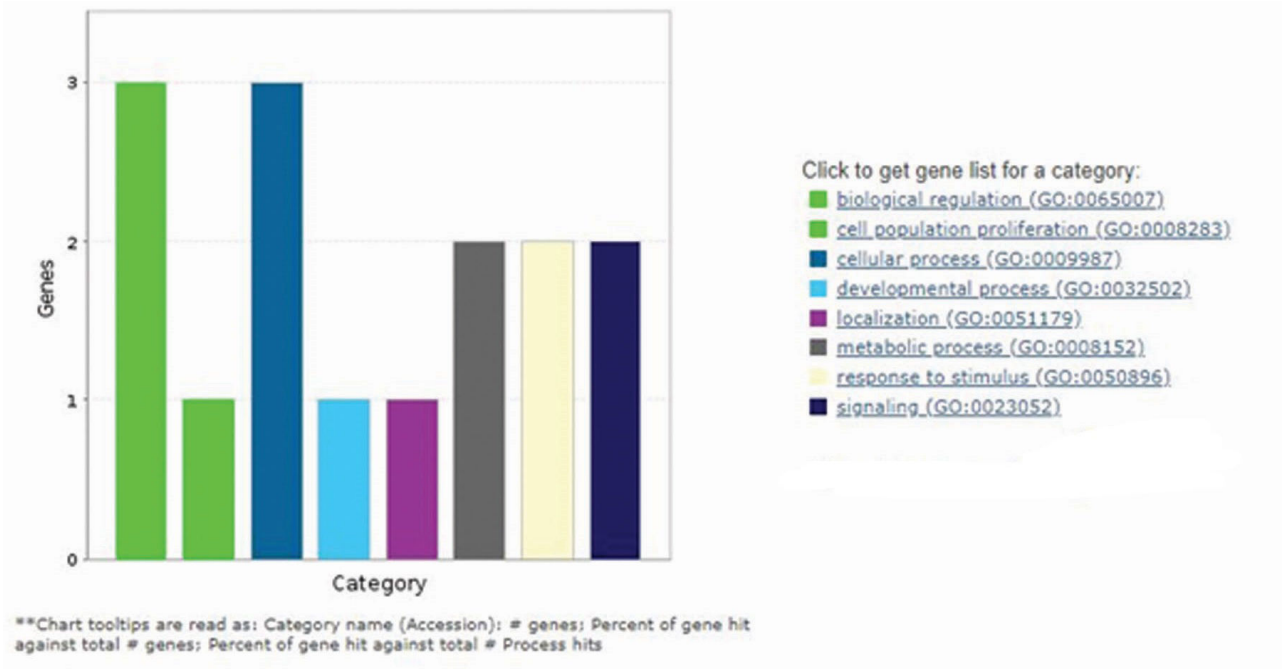

Fig. 6: Biological Process ontology bar chart obtained by functional enrichment analysis via PANTHER GO tool.
Source: https://www.mdpi.com/2079-7737/11/4/555?type=check_update&version=1

(CNS) influences feeding behavior and body energy stores, the metabolism of fats and glucose from the liver and adipose, and various facets of memory and cognition. INS2 production in the thymus is widely accepted to play a role in immune tolerance (Fan et al., 2010; Pugliese et al., 1997). INS might even influence the development or progression of Alzheimer's disease. AKT1 gene are a key modulator of the AKT-mTOR signaling pathway controlling the speed of the procedure for newborn neurons integration during adult neurogenesis, including proper neuron positioning, dendritic growth, and synapse formation.

Functional Enrichment Analysis

To carry out the functional annotation of the important genes in the network, enrichment analysis was performed by using David and panther gene ontology software. The GO biological process that were observed to be enriched included processes like the regulation of response to stress, cellular response to stress and more (Table 2).

Conclusion

The prediction and diagnosis of most of the neurological disorders are still difficult to examine. They only process through serval neurological test or examination. With emerging significance of system biology and network based computational model approaches, based on interpreting large expression data from omics study and constructing on protein-protein interaction network specific to disease or disorder, has supplied far most possibility for obtaining a potential target through system biology approaches. PPI networks offer a simplified summary of this web of interactions which happen in a cell. The huge amounts of sequence data which were created are leveraged to make better predictions of interactions and functional relationships between proteins, in addition to individual protein functions. By incorporating experimental procedures for discovering PPIs and computational procedures for prediction, a great deal of useful information on PPIs are created, such as numerous high-quality databases.

The main aim of the present study was the identification of key genes and pathways involved in stress-mediated neurodegeneration by carrying out the analysis of protein-protein interaction network. The PPI network constructedincludes approximately 15,063 interactions and 601 nodes. Based on network topology parameter, that is, betweenness centrality and node degree INS, AKT1, ALB, IL6 and TP53 were identified as the key genes in the constructed network. The INS gene was obtained as a super hub gene among all other genes. This gene had the highest betweenness centrality (BC) and node degree. The enrichment analysis of these genes alsoreveal their active role in regulation of the pathways and biological processes which are related to the selected neurological disorder.

PPI network evaluation is a vital mechanism to comprehend all of the biological processes out of system biology perspective in addition to additionally utilized in evaluation and prediction of corresponding therapies, giving a theoretical foundation for the research of novel drug targets.

Thus, the system biology approaches may play a significant role in understanding the underlying biological and functional mechanism of complex neurobiology of disease at the system level that may lead to the discovery of potential biomarkers for early detection, diagnosis & monitoring of neurological disorders at different stages.

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