BCL2 a Hub Protein in Genes Expressed in Early Parkinsonism Patients

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ABSTRACT

Parkinson's disease is a neurodegenerative disorder affects approximately 10 million people the main pathological characteristics of PD are cell death in the brain's basal ganglia affecting the dopamine-secreting neurons in the substantia nigra. **Method:** We selected GSE6613 dataset from The Gene Expression Omnibus (GEO) it include (50) PD patients in early stages and (22) normal controls were present in the study the study was profiling gene expression from RNA extracted from patient blood .and used microarrays for data collection. Dataset was analyzed using the tool GEO2R then The 1st 300 gene of differentially expressed genes were analyzed in STRING to construct a network of the protein-protein interactions of the differentially expressed genes products with minimum required interaction score is set on high confidence 0,700. **Results:** Nearly(1300) differentially expressed genes out of 21,000 are identified with p value lower than 0.05,then arranged the DEG in ascending manner and the 1st 300 gene were analyze by STRING tool and found that BCL2 and H3C12 hub proteins, The 2nd 300 gene analysis reveal that EGFR, PRKACA and H4C6 are hub proteins, The following 300 gene showed IL10, ESR1 and SNCA are hub proteins and the last 400 gene analysis showed MYC, RPL22, RPS27L, RPL35A and SKP1 are hub proteins. **Conclusion:** BCL2 is a hub protein between the 1st 300 significantly expressed gen in early PD patients ,Gene therapy and The CRISPR/Cas9 gene editing system can be directed to BCL2 that may modulate the path of the disease.

Keywords: Parkinson's disease, neurodegenerative, gene expression, BCL2

INTRODUCTION

Parkinson's disease is a neurodegenerative disorder that affects the movement and balance of a person. It primarily affects older adults, but can occur at any age. While there is no cure for Parkinson's disease, there are treatments available that can manage symptoms and improve quality of life [1].

Risk factors include age, male gender and some environmental factors. The etiology of the disease in most patients is unknown, but different genetic causes have been identified [2].

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there are approximately 10 million people affected with Parkinson's Disease (PD) worldwide and this estimation will increase to 13.5 million by 2030 [3].

PD typically occurs in people over the age of 60, of whom about one percent are affected. In those younger than 50, it is termed early-onset PD, PD results from a complex interaction between genetic and environmental factors [4].

PATHOPHYSIOLOGY OF PARKINSONISM

The main pathological characteristics of PD are cell death in the brain's basal ganglia (affecting up to 70% of the dopamine-secreting neurons in the substantia nigra pars compacta by the end of life) [5].

Alpha-synuclein, a protein encoded by SNCA gene mutations, is the main component of the Lewy bodies that accumulate in the brains of people with PD [6].

In Parkinson's disease, alpha-synuclein becomes misfolded and clumps together with other alpha-synuclein. Cells are unable to remove these clumps, and the alpha-synuclein becomes cytotoxic, damaging the cells [7,8].

in addition, alpha-synuclein activates the non-homologous end joining DNA repair pathway. The aggregation of alpha-synuclein in Lewy bodies appears to be a link between reduced DNA repair and brain-cell death in PD [9].

Other mechanisms include proteasomal and lysosomal systems dysfunction and reduced mitochondrial activity [10].

Parkinson-related genes are involved in the function of lysosomes, organelles that digest cellular waste products. Lysosomal disorders that reduce the ability of cells to break down alpha-synuclein may cause PD [11].

Bandres-Ciga et al., 2020 utilized a high-throughput and hypothesis-free approach to determine biological processes underlying PD using currently available genetic and gene expression data and applied large-scale gene-set specific polygenic risk score (PRS) analyses they nominated specific molecular sub-processes underlying protein misfolding and aggregation, post-translational protein modification, immune response, membrane and intracellular trafficking, lipid and vitamin metabolism, synaptic transmission, endosomal-lysosomal dysfunction, chromatin remodeling and apoptosis mediated by caspases among the main contributors to PD etiology [12].

METHOD

Dataset Selection

The Gene Expression Omnibus (GEO) is an online NCBI repository containing public gene expression data from a variety of studies (Barrett etal.,2013)

GEO was searched for datasets matching "Parkinson's", "early", and "Homosapiens", GSE6613 dataset [13,14] were selected for this study (50) PD patients and (22) normal controls were present in the study, All PD patients were at early stages, according to the Hoehn and Yahr scale (ranges from 1 to 5). the study was profiling gene expression from RNA extracted from patient blood .The study used microarrays for data collection. Microarrays are a chip-based technology that uses oligonucleotide probes to capture strands of complementary DNA (cDNA) [15]. The cDNA is reverse transcribed from the mRNA present in the blood samples

Differential Expression Analysis

The dataset was analyzed using the tool GEO2R which is provided by the Gene Expression Omnibus [16], GEO2R is a browser-based software that processes gene expression values and outputs a table of differentially expressed genes (DEGs) between two groups (PD and control). T tests were used to determine p values.

After the lists of differentially expressed genes were obtained, they were processed in Google sheets. Non significantly Expressed genes (with p values of greater than 0.05) were removed and the remaining DEGs nearly 1300 gene , they were sorted into an ascending manner from lower to higher

Network Analysis

The 1st 300 gene of differentially expressed genes were analyzed in STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a biological database and web resource of known and predicted protein–protein interactions. The STRING database contains information from numerous sources, including experimental data, computational prediction methods and public text collections. It is freely accessible and it is regularly updated.

STRING construct a network of the protein-protein interactions of the differentially expressed genes products with minimum required interaction score is set on high confidence 0,700 , to create a graph of input genes and how their protein products interact [17] a hub protein is detected for later discussing its possible role in parkinsonism pathogenesis

RESULTS

GSE6613 was analyzed in GEO2R and the lists of nearly 21thousend expressed genes were downloaded. Non significantly Expressed genes (with p values of greater than 0.05) were removed and the remaining differentially expressed genes which were 1300 gene sorted into an ascending manner from lower to higher we took the 1st 300 gene and analyze the by STRING tool to detect the possible

relation between the protein produced from these genes we set the minimum required interaction score on high confidence 0,700, and found the following relation [Figure 1] with gene BCL2 and H3C12 hub proteins.
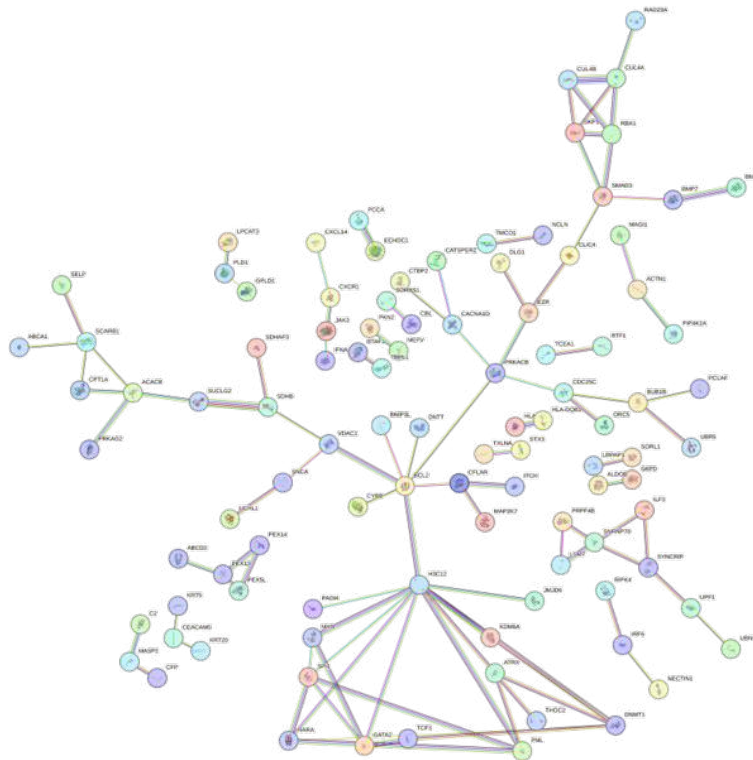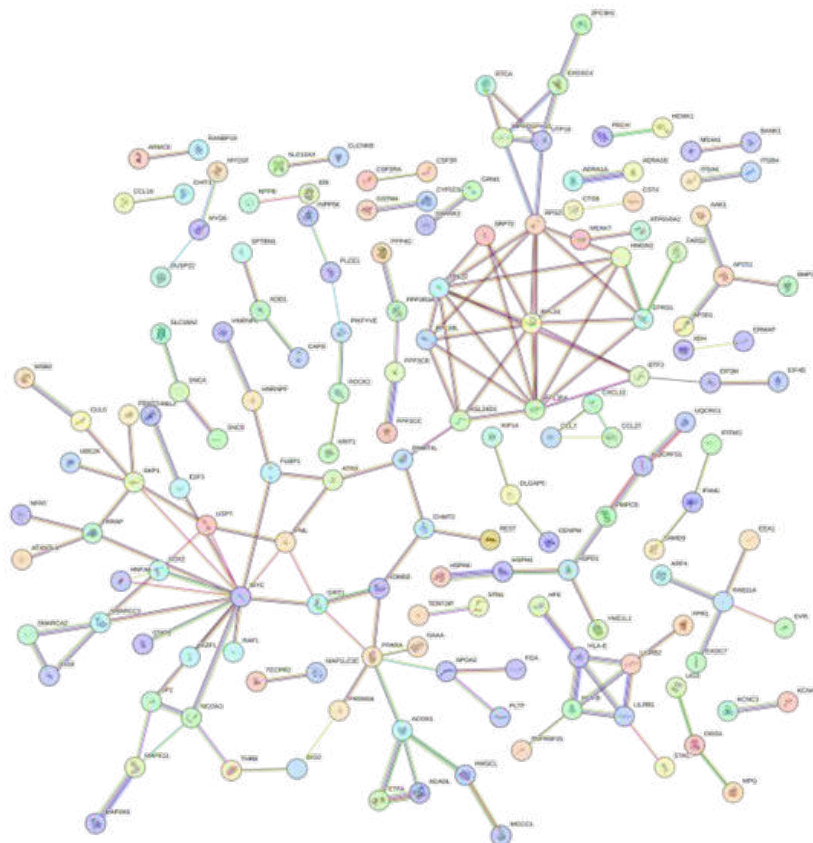

Figure 1: The 2nd 300 gene analyze [Figure 2] with EGFR, PRKACA and H4C6 are hub proteins
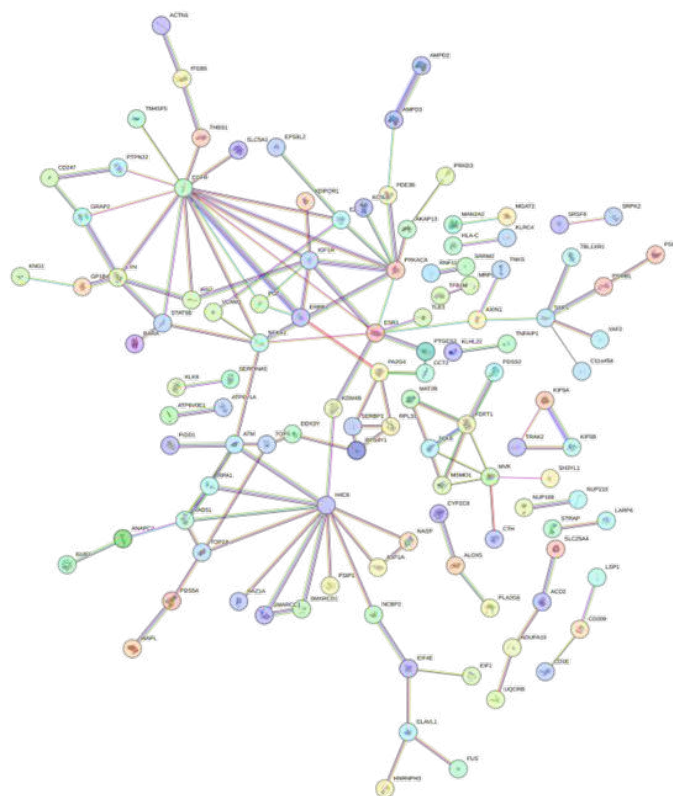
Figure 2: The following 300 gene showed IL10, ESR1 and SNCA are hub proteins.
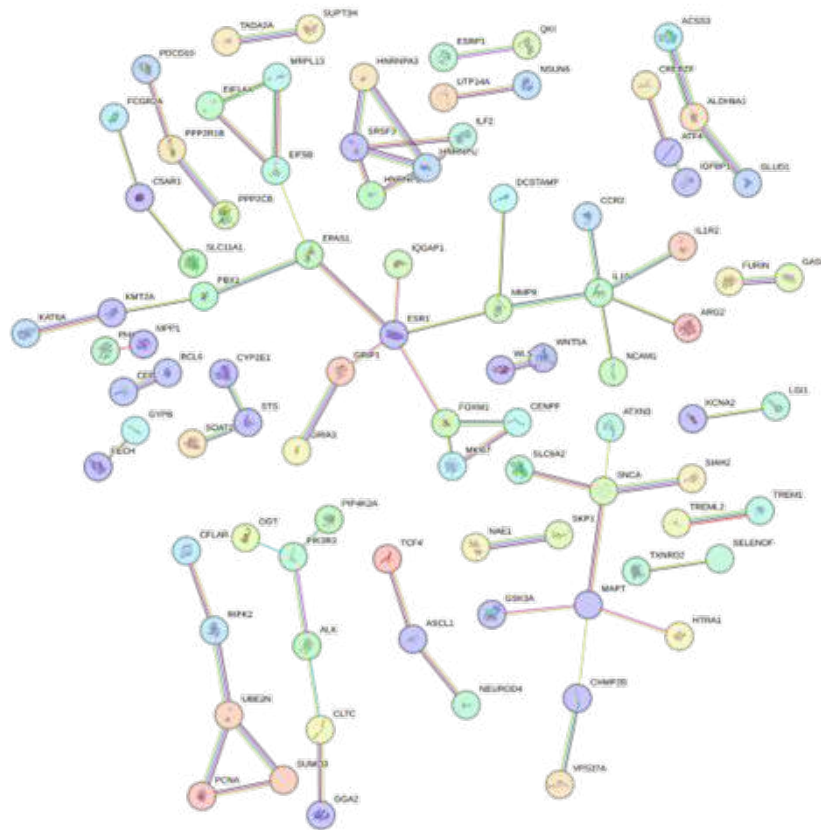
Figure 3: The last 400 gene analysis showed MYC, RPL22, RPS27L, RPL35A and SKP1 are hub proteins.

Figure 4:

DISCUSSION

PD is neurodegenerative disease with symptoms include, Motor Symptoms of PD include:Tremor often described as a "pill-rolling" tremor,Bradykinesia: Slowness of movement, making simple tasks difficult and time-consuming. Muscle stiffness, Impaired posture and balance , Non-Motor Symptoms include constipation and disruption of normal sense of smell, are present in PD, often at early stages Speech changes ,Sleep problems , Cognitive changes like memory problems, difficulties with planning and organizing, and dementia in later stages, Mood and behavior changes: Depression and anxiety [18,19].

In order to promote effective treatments, it is essential to diagnose PD in early stages but, this is relatively difficult as, early stage symptoms of PD usually not specific enough to PD like constipation Some PD cases are due to genetics and could potentially be diagnosed via genetic screening, but the vast majority of PD cases arise spontaneously (idiopathic Parkinson's disease) and genetic mutations may not be as apparent [20].

the understand of genetic expression in early stages of disease is a step to understanding its impact on PD pathogenesis. How the upregulation and the down regulation of these genes expression and the relation between them impact disease mechanism so in light our road as reared pathological changes in early stages, early diagnosis through proposing candidate gene and protein as marker that can be also target for treatment ,Answering these questions is complicated by the large number of potential candidate genes. we prioritize candidate genes for further study, Which its protein represent a hub protein for multiple interconnection with other significantly expressed genes, we will focus on BCL2 which appear to be a hub protein in the 1st 300 gene significantly expressed in parkinsonism patients group in compare to healthy normal volunteers BCL2: This gene encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells such as lymphocytes [21] these BCL2 proteins, required for proper development of the dopaminergic system, may also cause vulnerability of this system in the adult, as these BCL2 proteins have recently been linked to Parkinson's disease [22].

Maintaining a balance between apoptosis (removal damaged or aged cells) and autophagy(selective degradation of damaged cellular organelles and protein aggregates) is critical for cell fate, especially for neurons. neurodegenerative diseases such as Parkinson's disease (PD), is associated with imbalance between autophagy and apoptosis so restoring the balance is a promising way for the treatment of PD,BCL2 family members engage in cross talk between apoptosis and autophagy [23]. It is proposed that excessive oxidant stress induced by metabolism of dopamine (DA), plays a major role in the pathogenesis of Parkinson's disease through initiating apoptosis in cultured, post-mitotic sympathetic neurons. Bcl-2 is a proto-oncogene that blocks apoptosis. and a powerful inhibitor of DA toxicity [24] Bcl-2 binds to and inhibits TMEM175 (a lysosomal potassium channel), activity. Increased TMEM175 function inhibits mitophagy, disrupts mitochondrial homeostasis, and increases production of reactive oxygen species (ROS). TMEM175 is an important regulatory site in the apoptotic signaling pathway and a potential therapeutic target for Parkinson's disease (PD) [25].

Our result showed BCL2 as a hub protein in a net include the following genes CFLAR, PRKACB, H3C12, CYBB, VDAC1, BNIP3L and DNTT.

Information about each gene and its protein is extracted from Gen database of NICB

Identifier: ENSP00000312455, CFLAR the protein encoded by this gene is a regulator of apoptosis and is structurally similar to caspase-8. However, the encoded protein lacks caspase activity and appears to be itself cleaved into two peptides by caspase-8. Several transcript variants encoding different isoforms have been found for this gene, and partial evidence for several more variants exists [26].

Identifier: ENSP00000359719, PRKACB

The protein encoded by this gene is a member of the serine/threonine protein kinase family. The encoded protein is a catalytic subunit of cAMP (cyclic AMP)-dependent protein kinase, which mediates signalling though cAMP. cAMP signaling is important to a number of processes, including cell proliferaton and differentiation. Multiple alternatively spliced transcript variants encoding distinct isoforms have been observed.it show Broad expression in brain [27].

Identifier: ENSP00000352252, H3C12

Histones are basic nuclear proteins that are responsible for the nucleosome structure of the chromosomal fiber in eukaryotes. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form an octamer, around which approximately 146 bp of DNA is wrapped in repeating units, called nucleosomes. The linker histone, H1, interacts with linker DNA between nucleosomes and functions in the compaction of chromatin into higher order structures. This gene is intronless and encodes a replication-dependent histone that is a member of the histone H3 family. Transcripts from this gene lack polyA tails but instead contain a palindromic termination element. This gene is found in the small histone gene cluster on chromosome 6p22-p21.3 [28].

Identifier: ENSP00000367851, CYBB

Cytochrome b (-245) is composed of cytochrome b alpha (CYBA) and beta (CYBB) chain. It has been proposed as a primary component of the microbicidal oxidase system of phagocytes. CYBB deficiency is one of five described biochemical defects associated with chronic granulomatous disease (CGD). In this disorder, there is decreased activity of phagocyte NADPH oxidase; neutrophils are able to phagocytize bacteria but cannot kill them in the phagocytic vacuoles. The cause of the killing defect is an inability to increase the cell's respiration and consequent failure to deliver activated oxygen into the phagocytic vacuole. [29].

Identifier: ENSP00000378487, VDAC1

This gene encodes a voltage-dependent anion channel protein that is a major component of the outer mitochondrial membrane. The encoded protein facilitates the exchange of metabolites and ions across the outer mitochondrial membrane and may regulate mitochondrial functions. This protein also forms channels in the plasma membrane and may be involved in transmembrane electron transport. Alternate splicing results in multiple transcript variants. Multiple pseudogenes of this gene are found on chromosomes 1, 2 3, 6, 9, 12, X and Y [30]

Identifier: ENSP00000370003, BNIP3L

This gene encodes a protein that belongs to the pro-apoptotic subfamily within the Bcl-2 family of proteins. The encoded protein binds to Bcl-2 and possesses the BH3 domain. The protein directly targets mitochondria and causes apoptotic changes, including loss of membrane potential and the release of cytochrome c [31].

Identifier: ENSP00000360216, DNTT

This gene is a member of the DNA polymerase type-X family and encodes a template-independent DNA polymerase that catalyzes the addition of deoxynucleotides to the 3'-hydroxyl terminus of oligonucleotide primers. In vivo, the encoded protein is expressed in a restricted population of normal and malignant pre-B and pre-T lymphocytes during early differentiation, where it generates antigen receptor diversity by synthesizing non-germ line elements (N-regions) at the junctions of rearranged Ig heavy chain and T cell receptor gene segments. Alternatively spliced transcript variants encoding different isoforms of this gene have been described [32].

All these proteins are related to apoptosis, mitochondrial membrane transport, DNA replication and DNA supercoiling.

Gene therapy is a clinical tool that may eventually provide therapeutic benefit to patients suffering from movement disorders through a few potential mechanisms: direct correction of the pathogenic mechanism, neuroprotection, neurorestoration or symptom control. The therapeutic mechanism is therefore dependent on knowledge of disease pathogenesis and the required temporal and spatial specificities of gene expression [33].

The CRISPR/Cas9 system is a gene editing and engineering tool for the regulation of gene expression or repair that has developed in medicine and displays a wide perspective of the human genome [34] so directing these tools to BCL2 may modulate the path of the disease.

CONCLUSION

BCL2 is a hub protein between the 1st 300 significantly expressed gen in early PD patients ,Gene therapy and The CRISPR/Cas9 gene editing system can be directed to BCL2 that may modulate the path of the disease.

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