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Prediction of protein-protein interaction networks and druggable genes associated with parkinson's disease

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Parkinson's disease (PD) affects about 2-3% of the global population over 65 years of age and hence, it is the second most common neurodegenerative disorder in the world. This study explored the key genes and miRNA involved in PD. Microarray dataset (accession number GSE19587) comprising of two regions of medulla: dorsal motor nucleus of vagus (DMNV) and inferior olivary nucleus (ION) was downloaded from Gene Expression Omnibus (GEO) database. A total of 697 DEGs from ION (605 up-regulated genes and 92 down-regulated genes) and 663 DEGs from DMNV (638 up-regulated genes and 25 down-regulated genes) were screened. These DEGs were found to be enriched in 46 (DMNV) and 24 (ION) pathways common in DAVID and Comparative Toxicogenomics Database. In PPI network analysis, *IGF1* and *CD44* were identified as hub genes in DMNV whereas, for ION, the hub genes identified were *CSF2* and *CD44*. In TF-miRNA-target gene networks, an aggregate of 11 transcription factors and 46 miRNA were observed to influence the target genes. In drug-gene interaction studies, *CYP3A5* and *ESR1* had higher connective degrees and hence, they might be novel druggable targets for Parkinson's disease.

Keywords: Differentially expressed genes, Drug-gene interaction, Metabolic pathways, Microarray, Transcription factor

Parkinsonism is an umbrella term that describes any condition that shares similar symptoms associated with movement abnormalities. Parkinsonism was observed in different neurodegenerative conditions such as Parkinson's disease, corticobasal degeneration, dementia with Lewy bodies, Huntington's disease, Wilson's disease, *etc*[1]. Parkinson's disease (PD) is a long term multi-system neurological disease characterized by early motor symptoms including tremor, slowness of movement (bradykinesia), rigidity and instability while walking[2]. As the disease progresses, more non-motor symptoms such as dementia become more common[3]. Even before the onset of motor symptoms, patients may experience difficulties such as anhedonia (inability to feel pleasure in activities that are usually enjoyable), mood associated problems, excessive sweating and constipation. These symptoms are collectively called as pre-motor symptoms[4]. PD affects about 2-3% of the global population over 65 years of age and hence, it is the second most common neurodegenerative disorder in the world[5]. In juvenile Parkinsonism, parkinsonian symptoms and signs can be observed in young individuals aged less than 21 years[6].

Numerous investigations on the pathogenesis of PD indicate two definitive hallmarks such as gradual loss of dopaminergic neurons in pars compacta of substantia nigra and the presence of Lewy body (protein inclusions) in the surviving neurons[7]. Even though the complete etiology of PD remains unknown, it was found that the majority of PD cases arise sporadically and may have associations with both genetic and environmental risk factors[8]. Moreover, the genetic basis of PD progression is well established and the most familial form of the disease have been commonly associated with six genes including *PRKN*, *LRRK2*, *VPS35*, *SNCA*, *PINK1* and *PARK7*[9]. Parkin gene (*PRKN*), encoding E3 ubiquitin ligase parkin is involved in functions associated with quality control and turn over in mitochondria. The major role of parkin is the ligation of ubiquitin to amino acid lysine, an important post-translational modification[10]. *LRRK2* (Leucine-rich repeat kinase 2) encodes 2527 amino acid long protein called dardarin, is linked with various cellular processes like maintenance of cytoskeleton, degradation of autophagic protein and vesicle trafficking. In the case of PD, mutations leading to the up-regulation of *LRRK2* gene were observed and it was widely researched as a target to develop novel therapeutics[11]. *Dardarin* can

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Suppl. Data available on respective page of NOPR

interact with the C-terminal R2 RING-finger domain of parkin and vice versa through the COR domain of *dardarin*. Hence, the co-expression of *dardarin* and *parkin* can cause an elevation in ubiquitination of cytoplasmic protein aggregates of *dardarin.* Also, mutants of *LRRK2* were known to cause neuronal degeneration in primary neurons[12]. *VPS35* (vacuolar protein sorting 35 gene) is a part of the retromer complex that recycles proteins by transporting from endosomes to trans-Golgi network. In multiple cases of PD, a single missense mutation AspD620Asn was widely observed. As the exact mechanism behind the role of mutant *VPS35* in PD is not fully understood, it can only be hypothesized that mutations in *VPS35* could lead to neurodegeneration by disrupting the receptor recycling process[13]. Synuclein (*SNCA*) gene encodes a 140 amino acid long protein called alpha-synuclein and it is mainly responsible for maintaining a sufficient number of neurotransmitter filled synaptic vesicles in presynaptic terminals of neurons. Also, they play a vital role in the movement of microtubules, an important component of the cytoskeleton. Mutations in *SNCA* gene can give rise to abnormal alpha-synuclein in turn which can bring about neuronal death by affecting synaptic functions[14]. *PINK1* encoding PTEN induced kinase 1 is involved in the protection of mitochondria from oxidative stress. Mutations in *PINK1* are associated with mitochondrial dysfunction and progressive reduction of dopamine release over age in the PD population[15]. The gene *PARK7* encodes protein deglycase DJ-1, which is hypothesized to play a crucial role in protecting various types of cells, especially brain cells from oxidative stress. The pathology of PD associated with *PARK7* or DJ-1 remains largely undetermined as PARK7 mutations are less frequent than other types including *PRKN* in PD[16].

Apart from these genes, many other genes and miRNAs are found to have a positive correlation with the progression of PD. Low-density lipoprotein receptor-related protein 10 encoded by gene LRP10 has now emerged as a novel target for PD and it has potential implications towards molecular cascades involved in the pathogenic aggregation of alpha-synuclein[17]. Small non-coding micro RNAs (miRNA) play a crucial role in regulating various metabolic pathways involved in the development and survival of nerve cells. Many genes associated with PD have mi-RNAs as regulatory elements. Studies suggested that two miRNAs (miR-7 and miR-153) act as regulatory elements of synuclein. In the normal population, miR-7 and miR-153 keep alpha-synuclein mRNA under negative control[18]. Aberrant changes in miRNA expression patterns in the PD population have been reported in multiple studies. For instance, miR-133b associated with the development of dopaminergic neurons was reported to be down-regulated in mid-brain samples obtained from the PD population[19]. Another mi-RNA, miR-433 is linked to PD based on its binding activity towards the *FGF20* gene. Alterations in the binding of miR-433 to *FGF20* can elevate synuclein expression levels[20-21].

Identification of key genes and regulatory elements involved in PD progression can accelerate the process of developing new drugs or repurposing existing drugs to alleviate PD. For example, Amantadine, an anti-flu drug, is now widely used to treat PD[22]. In this study, microarray data obtained from NCBI's (National Centre for Biotechnology Information) GEO (Gene Expression Omnibus) database was analyzed to identify differentially expressed genes (DEGs) in the inferior olivary nucleus (ION) and dorsal motor nucleus of the vagus (DMNV) of PD affected and normal individuals. Then DEGs were screened based on Gene Ontology functional (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis. The potential DEGs involved in GO and KEGG pathway analysis were used further to construct protein-protein interaction (PPI) and mi-RNA-transcription factor (TF)-target gene regulatory networks. Finally, the druggable gene targets involved in PD progression were obtained based on the network modules identified in PPI networks. The gene targets identified in this study might offer new insight into the molecular mechanism of PD associated with ION and DMNV. Moreover, this study might provide novel targets for developing therapeutic agents against PD.

Materials and Methods

Data source

In our study, microarray dataset (accession number GSE19587) was downloaded from NCBI's GEO database (https://www.ncbi.nlm.nih.gov/geo/) and it contains expression profile of 22 samples including 6 samples of PD affected inferior olivary nucleus, 6 samples of PD affected dorsal motor nucleus of the vagus, 5 samples of normal inferior olivary nucleus and 5 samples of normal dorsal motor nucleus of the vagus. The samples were derived from individuals aged 74 to 84 years. The dataset was analyzed in the platform GPL571, which is the Affymetrix Human

Genome U133A 2.0 Array [HG-U133A_2]. It consists of more than 22,000 probe sets and 500,000 unique oligonucleotide features.

Screening of DEGs

The preprocessing of the dataset was done using R package oligo (version 1.50.0, https:// www. bioconductor.org/ packages/release/bioc/html/ oligo.html)[23]. The preprocessing of data constitute background correction, log transformation, quantile normalization (to equalize differences between the arrays) and probe normalization (to equalize differences within the probe sets). The RMA (Robust Multichip Average) method of oligo was used to perform data normalization. After data normalization, the differential gene expression analysis was carried out using the classical Bayesian method of limma (version 3.42.0, https://bioconductor.org/packages/ release/bioc/html/limma.html)[24]. For constructing volcano plots, R package Enhanced Volcano (version 1.4.0, https://bioconductor.org/packages/ release/bioc/ html/EnhancedVolcano.html) was used[25]. The DEGs with p-value < 0.05, logFC ≥ 2 (for up-regulated genes) and logFC ≤ 2 (for down-regulated genes) were screened for further analysis. For constructing clustered heat maps, R package pheatmap (version 1.0.12, https://www.rdocumentation.org/packages/ pheatmap/versions/1.0.12) was used[26].

Gene ontology and KEGG pathway analysis

Online tool DAVID (Database for Annotation, Visualization and Integrated Discovery, version 6.8, https://david.ncifcrf.gov/home.jsp) was used to perform GO functional annotation and KEGG pathway enrichment analysis for screened DEGs[27]. The gene ontology functional annotation consists of three categories: Biology Process, Molecular Function and Cellular Component. The significant GO terms and KEGG pathways were selected on the basis of the *P*-value (<0.05) and gene count (≥ 2). In addition, the GOplot package of R (version 1.0.2, https:// wencke. github.io/) was used to visualize the results of functional analysis.

The genes enriched in KEGG pathways were further screened by comparing it with PD associated pathways in the Comparative Toxicogenomics Database (CTD, http://ctdbase.org/). The pathways found in common between DAVID and CTD along with their associated genes were selected for performing further analysis.

Protein-Protein Interaction networks construction

In order to construct PPI networks for genes involved in common pathways, the STRING database (Search Tool for Retrieval of Interacting Genes, version 11.0, https://string-db.org/) was used. STRING is an online tool that comprises of both known and predicted protein-protein interactions[28]. The interactions with PPI score > 0.4 were considered significant and are selected to build PPI networks. For PPI network construction, Cytoscape software (version 3.7.2, https://cytoscape.org/) was used[29]. After network construction, the topological properties associated with the PPI network were determined using cytoNCA plugin of Cytoscape (version 2.1.6, http://apps.cytoscape.org/apps/cytonca)[30]. For centrality measurement, the network parameter was set as without weight. The node degree was used as a measure to screen hub genes from PPI networks. The network modules present within the PPI networks were screened using Cytoscape plugin MCODE (Multi-contrast delayed Enhancement, version 1.5.1, http://apps.cytoscape.org/apps/mcode). MCODE clusters a network as per its topological properties to construct densely connected networks or network modules[31]. Network modules with score value ≥ 5 were screened as significant modules.

TF-miRNA-target gene interaction study

To study the pairwise relationship between transcription factors and target genes in constructed PPI networks, iRegulon plugin of Cytoscape (version 1.3, http://apps.cytoscape.org/apps/iregulon) was used. The iRegulon plugin predicts transcription factor and its target through motif and track discovery method[32]. The parameters set in the analysis include minimum identity between orthologous genes (0.05) and maximum false discovery rate on motif similarity (0.001). Finally, transcription factors with normalized enrichment score (NES) > 4 were selected for further use.

Prediction of miRNAs associated with screened DEGs was done using a web-based gene set analysis toolkit, WebGestalt (http://www.webgestalt.org/ option.php). The parameters selected for prediction include organism of interest: *Homo sapiens*, method of interest: Over-Representation Analysis (ORA), count: ≥ 2 and p-value: <0.05. In addition, miRNAs linked with PD were obtained from Human microRNA Disease Database (HMDD, version 3.2, http://www.cuilab.cn/ hmdd)[33]. Then, TF-miRNA-target gene interaction networks were constructed in Cytoscape software.

Drug-gene interaction prediction

The drug-target gene interaction was predicted using a web-based resource, Drug-gene Interaction database

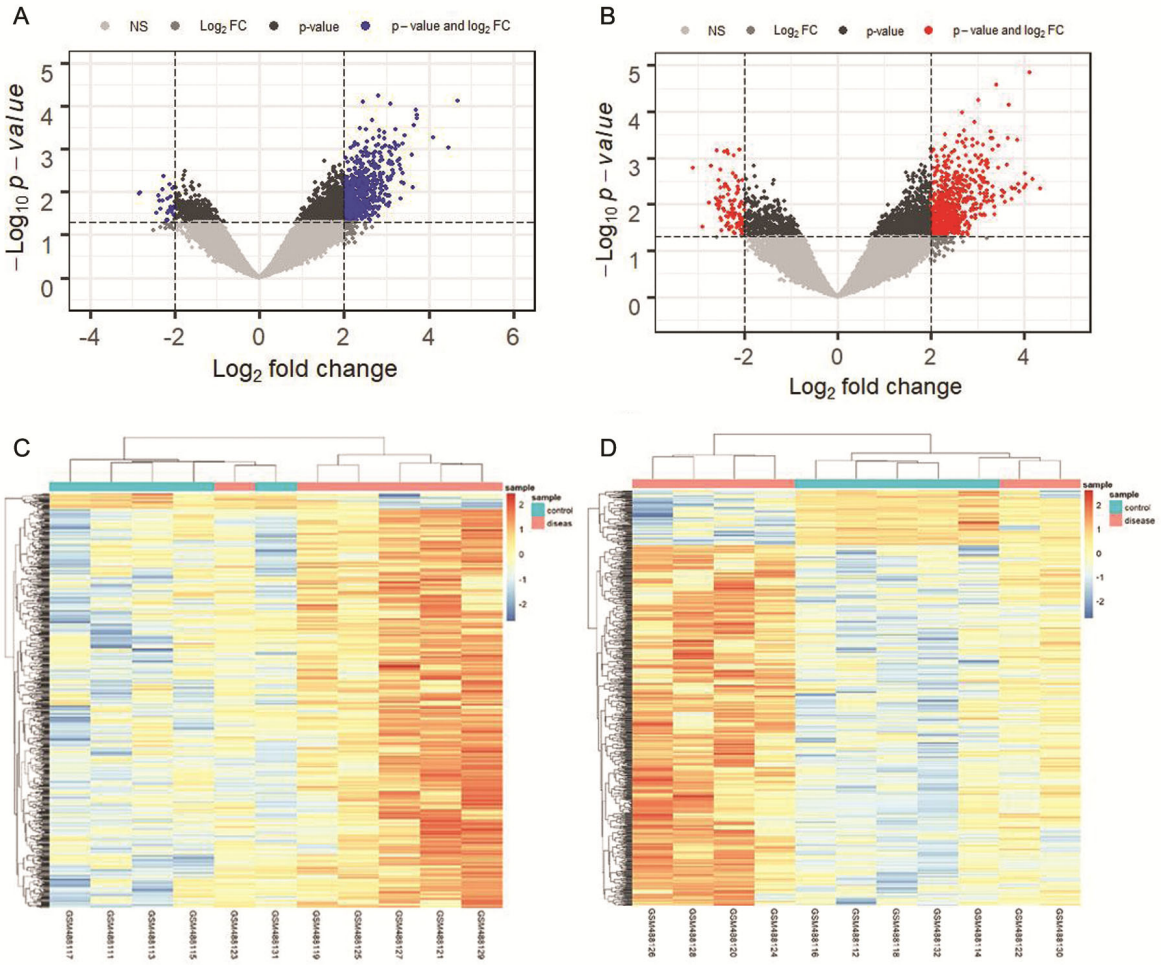

Fig. 1 — The volcano plots (A & B) and clustered heatmaps (C & D) of differentially expressed genes related to Parkinson's disease. (A) and (C) depict the data of DMNV, while (B) and (D) depict the data of ION

(DGIdb, version 3.0.2, http://www.dgidb.org/). DGIdb can predict the potential druggability of selected genes and drug-gene interactions[34]. In our study, genes represented in network modules were selected for the prediction of druggability and drug-gene interactions. The parameters selected are FDA approved drugs and immunotherapeutic agents. Finally, the results were visualized as networks in Cytoscape software.

Results

Screening of DEGs

After normalizing the data, we have screened a total of 697 DEGs from ION and 663 DEGs from DMNV. In ION, 605 genes were up-regulated and 92 genes were down-regulated. In DMNV, 638 genes were up-regulated and 25 genes were down-regulated (Suppl. Tables 1 and 2). Around 62 DEGs (61 up-regulated and 1 down-regulated) were found common between ION and DMNV. The volcano plot and clustered heatmap of screened DEGs from ION and DMNV are displayed in (Fig. 1).

Gene ontology and KEGG pathway analysis

GO analysis indicated that the screened DEGs from ION and DMNV were enriched in 205 and 186 functional terms, respectively, (Suppl. Tables 3 & 4). In ION, up-regulated genes were enriched in 167 functional terms and down-regulated genes were enriched in 38 functional terms. In DMNV, up-regulated genes were enriched in 175 functional terms and down-regulated genes were enriched in 11 functional terms. The majority of the functional terms in ION and DMNV were seen associated with the category biology process. With reference to the p-value, the most significant functional terms were found linked with cellular components. The GO plot and the corresponding Pheatmap for top most GO terms along with their genes

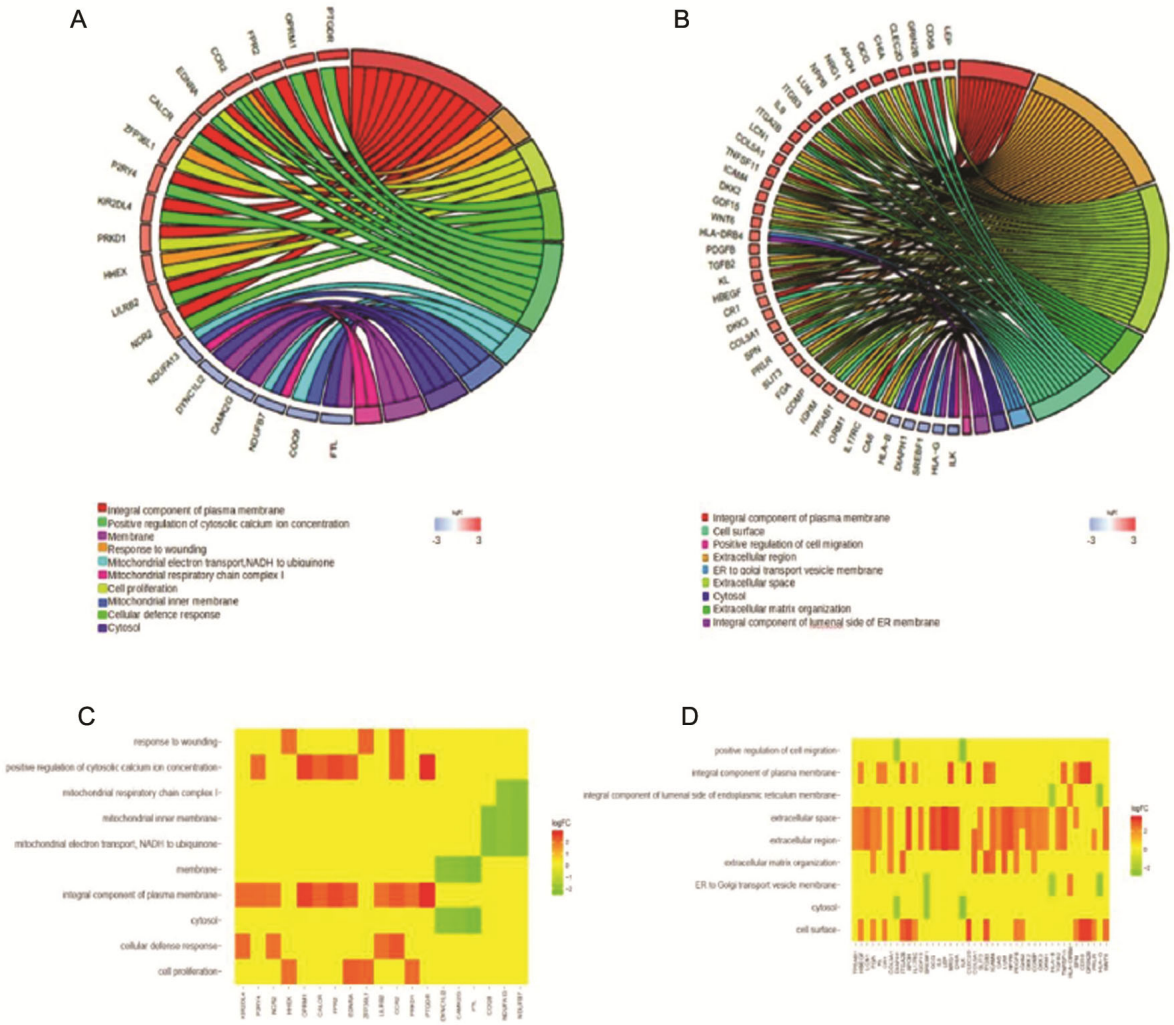

Fig. 2 — Circos plots (A & B) representing links between DEGs and GO annotation terms that are repeatedly enriched along with the heatmaps (C & D) of DEGs shown in circos plot. Red boxes indicate up-regulated DEGs, while blue boxes indicate down-regulated DEGs. (A) and (C) illustrate the data of DMNV, whereas, (B) and (D) illustrate the data of ION

were shown in (Fig. 2). The top most GO terms in ION were composed of GO:0005887 (integral component of plasma membrane), GO:0005576 (extracellular region), GO:0005615 (extracellular space), *etc*. In DMNV, the top most GO terms were GO:0005887 (integral component of plasma membrane), GO:0009611 (response to wounding), GO:0008283 (cell proliferation), *etc*.

KEGG pathway analysis showed that the screened DEGs from ION and DMNV were enriched in 25 and 31 pathways (Suppl. Table 5). In ION, up-regulated genes were enriched in 13 pathways and down-regulated genes were enriched in 12 pathways. In DMNV, up-regulated genes were enriched in 26 pathways and down-regulated genes were enriched in 5 pathways. The significant pathways in ION comprised of hsa04512 (ECM-receptor interaction), hsa04151 (PI3K-Akt signaling pathway), hsa04080 (Neuroactive ligand-receptor interaction), *etc*. The significant pathways in DMNV contained hsa04080 (Neuroactive ligand-receptor interaction), hsa04151 (PI3K-Akt signaling pathway), hsa04917 (Prolactin signaling pathway), *etc*. The top 10 GO terms and pathways associated with screened DEGs along with their p-value are depicted in (Fig. 3).

In the CTD database, we have screened around 249 pathways associated with Parkinson's disease. Our screened DEGs from ION and DMNV were represented in 24 and 46 pathways in the CTD database. Among these pathways, 14 and 28 pathways belonged to up-regulated genes in ION and DMNV. For down-regulated genes in ION and DMNV, we have found around 10 and 18

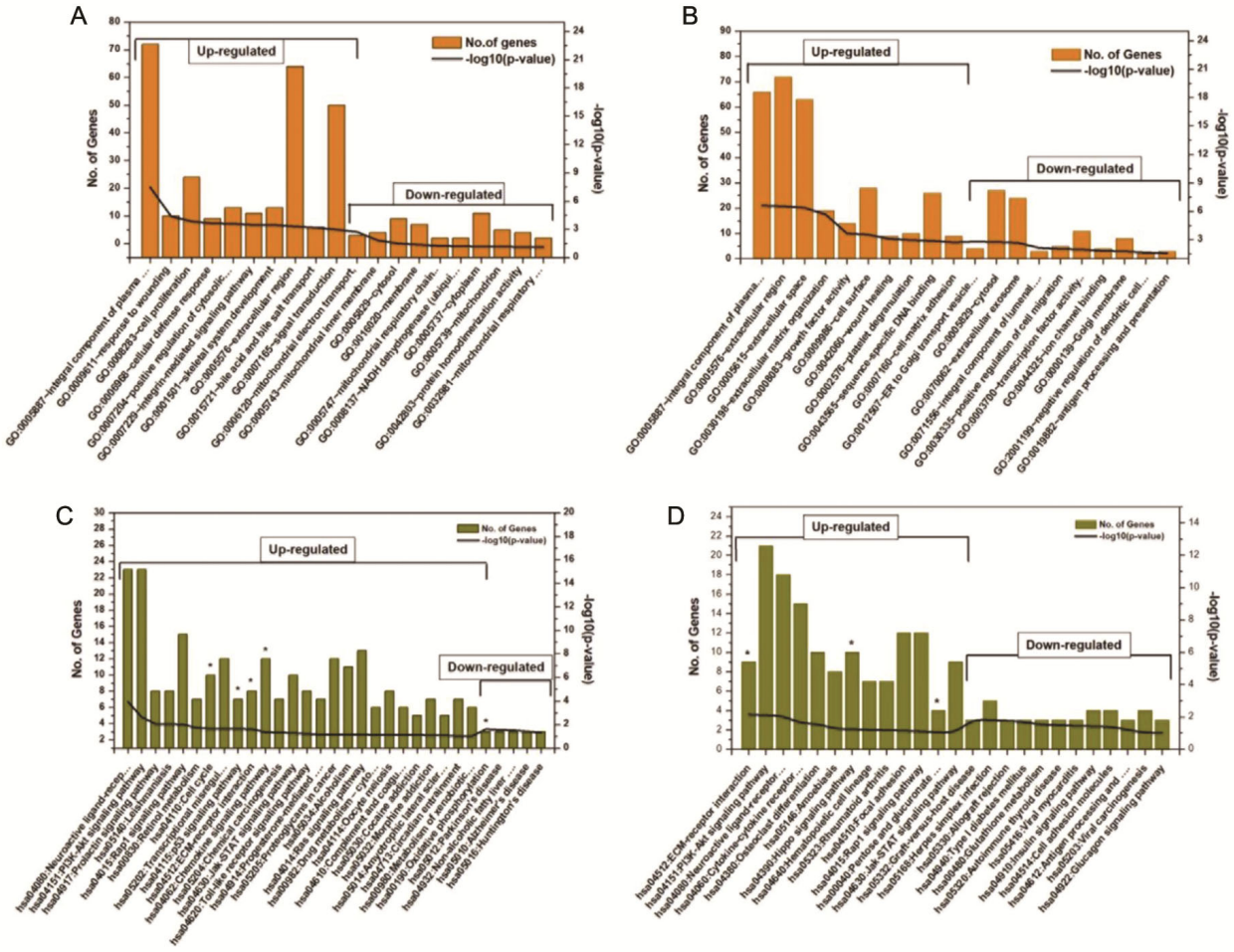

Fig. 3 — Gene ontology function (A & B) and KEGG pathway analysis (C & D) of differentially expressed genes. "*" indicates pathways that are not existed in Comparative Toxicogenomics Database. (A) and (C) represent the data of DMNV, while (B) and (D) represent the data of ION

pathways associated with PD in the CTD database (Suppl. Table 6)

PPI networks and gene modules

For PPI network construction; we have screened the DGEs enriched in common pathways. It contains a total of 84 DEGs in ION (68 up-regulated genes and 16 down-regulated genes) and 114 DEGs in DMNV (111 up-regulated genes and 3 down-regulated genes). For ION, the constructed PPI network consisted of 71 nodes and 225 edges. In the case of DMNV, the PPI network consisted of 101 nodes and 408 edges. After determining the connective degrees of genes in the PPI network, we had found that *CSF2* (Colony stimulating factor 2) and *LEP* (leptin) had higher connective degrees (*CSF2*, degree = 20 and *LEP*, degree = 19) than other genes in ION. Similarly, we had found that *IGF1* (Insulin like growth factor 1) and *CD44* (Cluster of differentiation 44) had higher connective degrees (*IGF1*, degree = 29 and *CD44*, degree = 24) than other genes in DMNV.

After network analysis, we have found functional modules in constructed PPI networks using the MCODE plugin (Suppl. Table 7). For ION, one gene module (module 1) consisted of 7 nodes and 21 edges with a score value of 7.000 were obtained. In that module, *DRD2* (Dopamine receptor D2) was a hub gene with a connective degree of 11. In the case of DMNV, a total of three network modules were observed. The first module (module 2a) consisted of 16 nodes and 81 edges (score = 10.800), in which *GNGT1* (Guanine nucleotide-binding protein G(T) subunit gamma-T1) was a hub gene (degree = 23). The second module (module 2b) consisted of 19 nodes and 45 edges (score = 5.000) with *CD44* (Cluster of differentiation 44) as a hub gene (degree = 24). The third network module (module 2c) consisted of

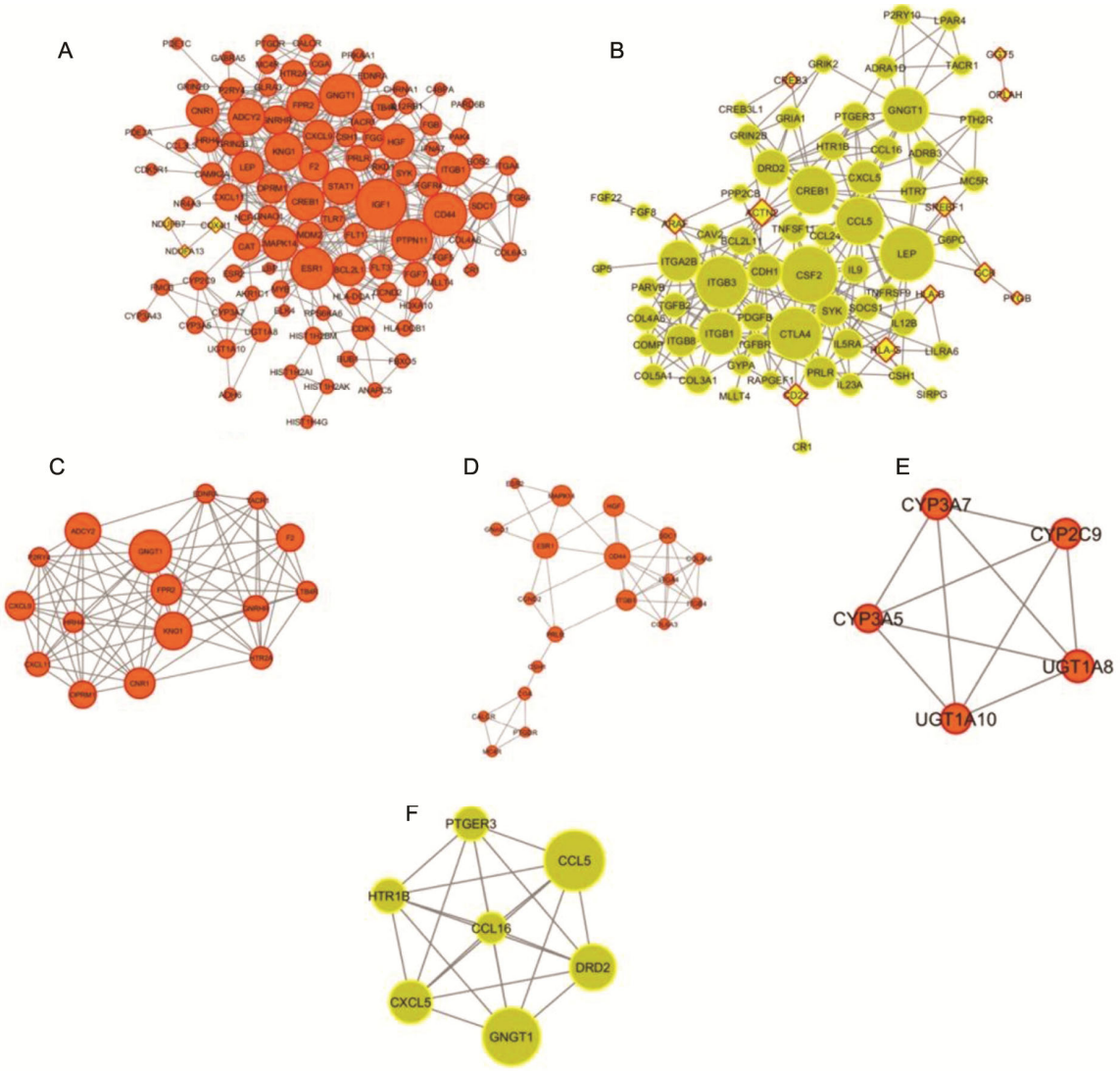

Fig. 4 — Protein-Protein Interaction networks (A & B) and their corresponding gene modules (C-F). Circle indicates up-regulated genes and rhombus indicates down-regulated genes. (A) depict the data of DMNV, while (B) depict the data of ION. (C-E) represents gene modules 2a, 2b and 2c, whereas (F) represents module 1. Node size is proportional to the degree. A large node indicates higher degree

5 nodes and 10 edges (score = 5.000) with *UGT1A8* (UDP glucuronosyltransferase family 1 member A8) as a hub gene (degree = 7). The PPI network of ION and DMNV with their corresponding gene modules are illustrated in (Fig. 4).

TF-miRNA-gene target networks

TF-miRNA-gene target networks constructed for ION and DMNV (Suppl. Table 8) are shown in (Fig. 5). The TF-miRNA-gene target network of ION consisted of 82 nodes (6 transcription factors, 20 miRNA and 56 target genes) and 207 edges. The transcription factor comprised of *DDX20* (DEAD-box helicase 20), *IKZF1* (IKAROS Family Zinc Finger 1), *SPIB* (Spi-B Transcription Factor), *NKX2-5* (NK2 Homeobox 5), *YY1* (Yin Yang 1) and *ESRRA* (Estrogen Related Receptor Alpha). Some of the important miRNAs provided by WebGestalt included *miR-29a* (degree = 7), *miR-29b* (degree = 7), *miR-29c* (degree = 7), *etc*. In addition, 2 micro RNA precursors such as *let-7e* (degree = 6) and *let-7g* (degree = 6) were existed in Human microRNA Disease Database. The hub genes in TF-miRNA-gene target network of ION were *DDX20* (degree = 25), *IKZF1* (degree = 24) and *SPIB* (degree = 23).

In the case of DMNV, the TF-miRNA-gene target network consisted of 85 nodes (5 transcription factors, 26 miRNA and 54 target genes) and 188 edges. The transcription factors in the network included *DDX4*

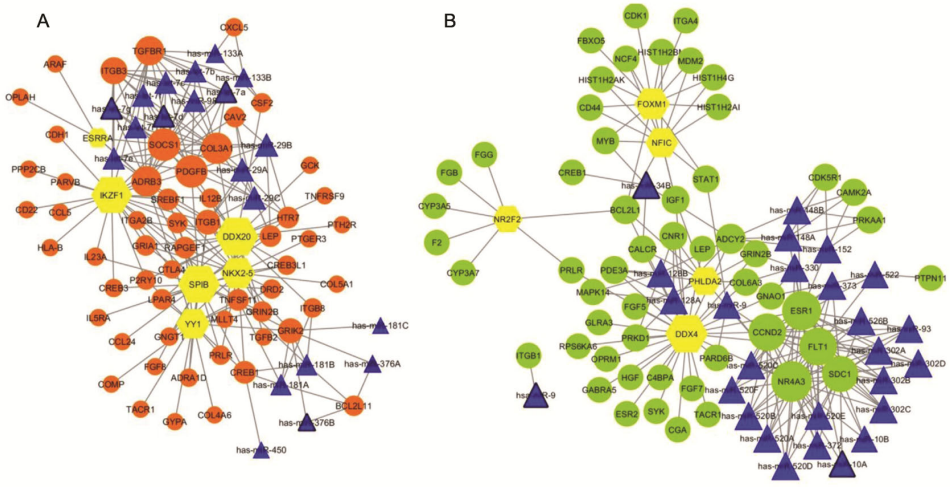

Fig. 5 — TF-miRNA-target gene networks of (A) DMNV; and (B) ION. Circles represent target genes, hexagons represent transcription factors and triangles represent miRNA. Triangles with black border indicate the existence of miRNA in Human microRNA Disease Database

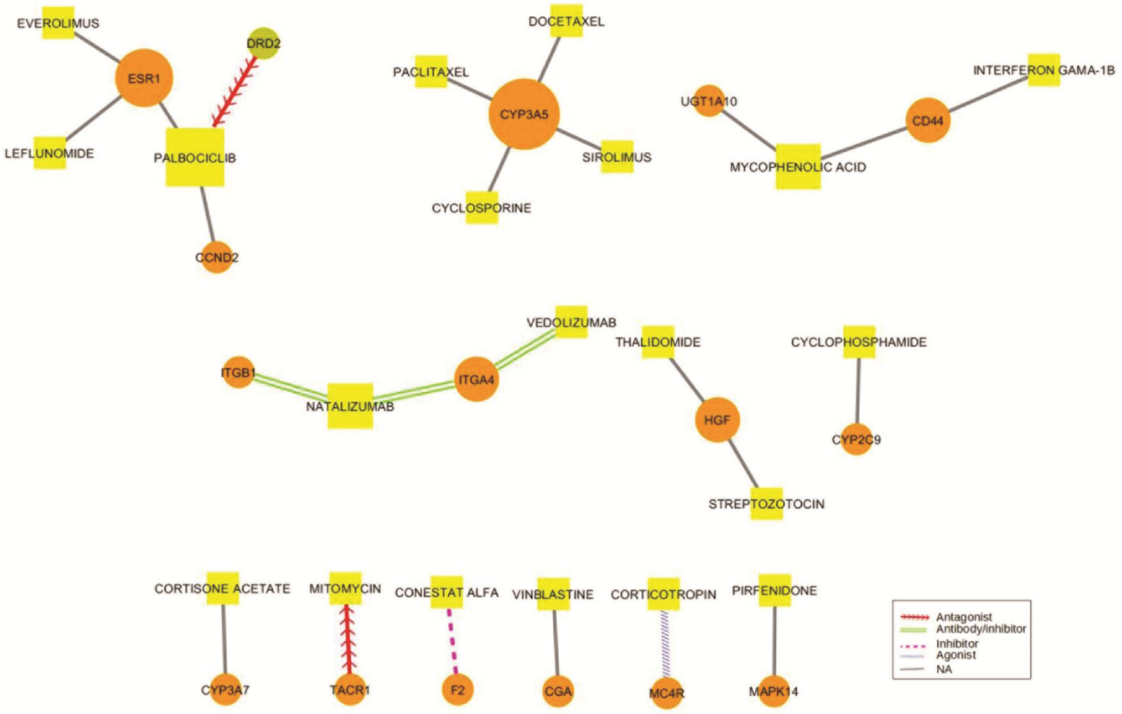

Fig. 6 — Drug gene interaction networks. Orange circles represent DEGs of DMNV, green circles represent DEGs of ION and yellow squares represent the drug

(DEAD-box helicase 4), *FOXM1* (Forkhead Box M1), *NFIC* (Nuclear Factor I C), *PHLDA2* (Pleckstrin Homology Like Domain Family A Member 2) and *NR2F2* (Nuclear Receptor Subfamily 2 Group F Member 2). Among the miRNAs, the miRNA with higher connective degrees included *miR-148a* (degree = 5), *miR-148b* (degree = 5), *miR-152* (degree = 5), *etc*. Moreover, *miR-34b* (degree = 4), *miR-9* (degree = 4) and *miR-10a* (degree = 3) had also existed in Human microRNA Disease Database. Among the nodes, transcription factor, *DDX4* was observed to be a hub gene with highest connective degree (degree = 29).

Drug-gene interactions

For ION and DMNV, an aggregate of 24 drug-gene interactions was predicted in DGIdb database and it includes 16 genes and 20 drugs (Fig. 6). The major type of interactions observed between the selected genes and identified drugs were antagonist, agonist, inhibitor and

anti-body/inhibitor. Based on their connective degrees, *CYP3A5* (Cytochrome P450 Family 3 Subfamily A Member 5, degree = 4), *ESR1* (Estrogen Receptor 1, degree = 3) of DMNV might be potentially druggable genes for PD. In the case of ION, no significant drug-gene interactions were found.

Discussion

PD is a clinical illness that manifests itself as a neurodegenerative disorder. The perception of PD as just the movement disorder is void as it accompanies a plethora of symptoms that are not associated with movements such as depression, sleep disorders, cognitive abnormalities, *etc*. Even with effective therapeutic interventions such as L-DOPA (dopamine) treatment, deep brain stimulation, *etc*., PD is still incurable[5]. Hence, the search for novel therapeutic targets and therapies for PD remains persistent. Recently, *MCL1* (apoptotic regulator of *BCL2* family) was suggested as a new therapeutic target for PD[35]. This suggestion was made based on the fact that a reduction in dopaminergic neurons because of the depletion of *MCL1* was observed in the parkin knockout mouse. Hence, enhancing *MCL1* could be beneficial as it can reduce the mortality of dopaminergic neurons by inhibiting proapoptotic *BCL2* factors[36]. Motor symptoms associated with PD are largely due to the loss of dopaminergic neurons in the substantia nigra pars compacta. While regions of basal ganglia are widely explored for their role in PD progression, only a little focus in this concern is offered towards other regions of the brain, especially medullar regions. MRI studies demonstrate that the brain stem region DMNV was differentially affected in PD. So, in our study, we analyzed the microarray expression profile of DMNV and ION in the nearby medullar region to extract possible biomarkers and druggable genes for PD, as affected DMNV was linked with pre-motor symptoms of PD[37].

The majority of DEGs in both ION and DMNV were up-regulated and are enriched in common pathways such as Neuroactive ligand-receptor interaction, PI3K-Akt signaling pathway, Rap1 signaling pathway, *etc*. These pathways were also represented in the CTD database. The genes associated with neuroactive ligand-receptor interaction pathways include *DRD1* and *DRD2*. Dopamine receptor D1, encoded by *DRD1* is responsible for neuronal growth and development while, Dopamine receptor D2, encoded by *DRD2* plays a crucial role in the regulation of synthesis and utilization of dopamine[38]. Dysregulation in *DRD1* and *DRD2* expression might lead to an increase in neuronal mortality. Although most of the parasympathetic preganglionic motor neurons are cholinergic, some of the neurons of the dorsal motor nucleus of the vagus are dopaminergic[39]. Hence, dopaminergic neurons could be affected by dysregulation of *DRD1* and *DRD2* in PD. In south indian population, the genetic variants of *DRD2* along with COMT (Catechol O-methyl transferase) and MAOB (Monoamine oxidase B) loci increases the suscepability to PD[40]. Down-regulated DEGs in DMNV were enriched in pathways such as Parkinson's disease (hsa05012) and Alzheimer's disease (hsa05010). NADH: ubiquinone oxidoreductase subunit B7 (*NDUFB7*), NADH:ubiquinone oxidoreductase subunit A13 (*NDUFA13*) and cytochrome c oxidase subunit 4I1 (*COX4I1*) are the down-regulated DEGs in DMNV associated with PD. Malfunctions in mitochondrial complexes are well documented in PD cases especially in complex I. Also, studies indicate the presence of oxidatively damaged mitochondrial complex I and are functionally impaired[41]. Hence, variants in mitochondrial complex I genes or their altered expression could increase the risk of developing PD.

In PPI network analysis, *IGF1* and *CD44* were identified as hub genes in DMNV. Insulin-like growth factor 1 (*IGF1*) is known to have neuroprotective effects and many studies indicated the presence of *IGF1* overexpressing neural progenitor cells in PD to protect dopaminergic neurons[42]. In TF-miRNA-target gene networks, we have identified 11 transcription factors that could be associated with PD progression in DMNV and ION. Among these TFs, *DDX4* of DMNV had the highest score (>5). *YY1* (Yin Yang 1) of DMNV plays an important role in nerve cell proliferation and has neuroprotective effects. Apart from transcription factors, miRNA also have important functions related to the regulation of gene expression in PD. In our study, we have found a total of 26 miRNA in DMNV and 20 miRNA in ION were associated with the screened DEGs. Among these miRNA, *miR-34b* was found to be down-regulated in the early stage of PD and it is linked to demodulation of mitochondrial function[21]. In addition, we have discovered that *CYP3A5* and *ESR1* might be potential therapeutic targets for PD based on drug-gene interaction studies. *CYP3A5* is one of the important genes involved in the metabolism of L-DOPA. The presence of *CYP3A5* mRNA in extrahepatic tissues (including various regions of the brain such as mid brain, basal ganglia, *etc*.) was reported and it might have a role in clearing toxins from the brain[43]. Studies

indicated that *ESR1* and *ESR2* polymorphisms have a significant association with altered risk of PD[44]. So, we believe that our findings could provide insight into the molecular pathology of PD pertaining to DMNV and ION. Also, the gene predicted from this study can be subjected to further *in silico* evaluations[45].

Conclusion

In our study, we have identified a total of 697 genes in ION and 663 genes in DMNV were differentially expressed in PD when compared to normal individuals. In the PPI networks constructed, *IGF1* and *CD44* for DMNV similarly, *CSF2* and *LEP* for ION were found to be the hub genes and could play an important role in PD progression. In TF-miRNA-target gene networks, an aggregate of 11 transcription factors and 46 miRNA were observed to influence the target genes. In addition, we have discovered that *CYP3A5* and *ESR1* might be potential therapeutic targets for PD. Furthermore, experimental studies are required to be made to get a deeper insight into the role of these genes in PD.

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Conflict of interest

All authors declare no conflict of interest.

References

1 Bradley WG, *Neurology in clinical practice: principles of diagnosis and management* (Elsevier, Philadelphia) 2004, 293.
2 Dickson DW, Neuropathology of Parkinson disease. *Parkinsonism Relat Disord*, 46 (2018) S30.
3 Schrag A, Horsfall L, Walters K, Noyce A & Petersen I, Prediagnostic presentations of Parkinson's disease in primary care: a case-control study. *Lancet Neurol*, 14 (2015) 57.
4 Sveinbjornsdottir S, The clinical symptoms of Parkinson's disease. *J Neurochem*, 139 (2016) 318.
5 Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag A & Lang AE, Parkinson disease, *Nat Rev Dis Primers*, 3 (2017) 17013.
6 Niemann N & Jankovic J, Juvenile parkinsonism: Differential diagnosis, genetics, and treatment. *Parkinsonism Relat Disord*, 67 (2019) 74.
7 Tolosa E, Wenning G & Poewe W, The diagnosis of Parkinson's disease. *Lancet Neurol*, 5 (2006) 75.
8 Kalinderi K, Bostantjopoulou S & Fidani L, The genetic background of Parkinson's disease: current progress and future prospects. *Acta Neurol Scand*, 134 (2016) 314.
9 Klein C & Westenberger A, Genetics of Parkinson's disease. *CSH Perspect Med*, 2 (2012) 1.
10 Arkinson C & Walden H, Parkin function in Parkinson's disease. *Science*, 360 (2018) 267.
11 Alessi DR & Sammler E, LRRK2 kinase in Parkinson's disease. *Science*, 360 (2018) 36.
12 Smith WW, Pei Z, Jiang H, Moore DJ & Liang Y, Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proc Natl Acad Sci U S A*, 102 (2005) 18676.
13 Vilariño-Güell C, Wider C, Ross OA, Dachsel JC, Kachergus JM, Lincoln SA, Soto-Ortolaza AI, Cobb SA, Wilhoite GJ & Bacon JA, VPS35 mutations in Parkinson disease. *Amer J Hum Genet*, 89 (2011) 162.
14 Stefanis L, α-*Synuclein* in Parkinson's disease. *Cold Spring Harb Perspect Med*, 2 (2012) 1.
15 Zhi L, Qin Q, Muqeem T, Seifert E L, Liu W, Zheng S, Li C & Zhang H*,* Loss of PINK1 causes age-dependent decrease of dopamine release and mitochondrial dysfunction. *Neurobiol Aging*, 75 (2019) 1.
16 Nuytemans K, Theuns J, Cruts M & Van BC, Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. *Hum Mutat*, 31 (2010) 763.
17 Quadri M, Mandemakers W, Grochowska MM, Masius R, Geut H, Fabrizio E, Breedveld GJ, Kuipers D, Minneboo M, Vergouw LJM, Mascaro AC, Yonova-Doing E, Simons E, Zhao T, Fonzo ABD, Chang HC, Parchi P, Melis M, Guedes LC, Criscuolo C, Thomas A, Brouwer RWW, Heijsman D, Ingrassia AMT, Buonaura GC, Rood JP, Capellari S, Rozemuller AJ, Sarchioto M, Chien HF, Vanacore N, Olgiati S, Yah-Huei Wu-Chou YH, Yeh TH, Boon AJW, Hoogers SE, Ghazvini M, IJpma AS, van IJcken WFJ, Onofrj M, Barone P, Nicholl DJ, Puschmann A, Mari MD, Kievit AJ, Barbosa E, Michele GD, Majoor-Krakauer D, van Swieten JC, de Jong FJ, Ferreira JJ, Cossu G, Lu CS, Meco G, Cortelli P, van de Berg WDJ & Bonifati V,LRP10 genetic variants in familial Parkinson's disease and dementia with Lewy bodies: a genome-wide linkage and sequencing study, *Lancet Neurol*, 17 (2018) 597.
18 Mouradian MM, MicroRNAs in Parkinson's disease. *Neurobiol Dis*, 46 (2012) 279.
19 Kim J, Inoue K, Ishii J, Vanti WB, Voronov SV, Park C, Chang K, Lee S H, Cho D & Suh H, A MicroRNA feedback circuit in midbrain dopamine neurons. *Science*, 317 (2007) 1220.
20 Wang G, van der Walt JM, Mayhew G, Li YJ, Züchner, S, Scott WK, Martin ER & Vance JM*,* Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of α-*synuclein. Amer J Hum Genet*, 82 (2008) 283.
21 Miñones-Moyano E, Porta S, Escaramís G, Rabionet R, Iraola S, Kagerbauer B, Espinosa-Perilla Y, Ferrer I, Estivil X & Marti E, MicroRNA profiling of Parkinson's disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. *Hum Mol Gent*, 20 (2011) 3067.
22 Hubsher G, Haider M & Okun MS, Amantadine: the journey from fighting flu to treating Parkinson disease. *Neurology*, 78 (2012) 1096.
23 Carvalho BS & Irizarry RA, A framework for oligonucleotide microarray preprocessing. *Bioinformatics*, 26 (2010) 2363.
24 Smyth GK, *Limma: linear models for microarray data* (Springer, New York) 2005, 397.

25 Blighe K, EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling, *R package version 1.2. 0*, (2019).

26 Kolde R, Pheatmap-package: Pretty heatmaps. *R package*, (2015).

27 Sherman BT & Lempicki RA, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*, 4 (2009) 44.

28 Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva N, Morris JH & Bork P, STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acid Res*, 47 (2018) D607.

29 Smoot ME, Ono K, Ruscheinski J, Wang PL & Ideker T, Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics*, 27 (2010) 431.

30 Tang Y, Li M, Wang J, Pan Y & Wu FX, CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. *Biosystems*, 127 (2015) 67.

31 Bandettini WP, Kellman P, Mancini C, Booker OJ, Vasu S Leung SW, Wilson JR, Shanbhag SM, Chen MY & Arai AE, Multi Contrast Delayed Enhancement (MCODE) improves detection of subendocardial myocardial infarction by late gadolinium enhancement cardiovascular magnetic resonance: a clinical validation study. *J Cardiovasc Magn Reson*, 83 (2012) 1.

32 Verfaillie A, Imrichová H, Van de Sande B, Standaert L, Christiaens V, Hulselmans G, Herten K, Sanchez M, Potier D & Svetlichnyy D, iRegulon: from a gene list to a gene regulatory network using large motif and track collections. *PLoS Comput Biol*, 10 (2014) 1.

33 Huang Z, Shi J, Gao Y, Cui C & Zhang S, HMDD v3. 0: a database for experimentally supported human microRNA–disease associations. *Nucleic Acids Res*, 47 (2018) D1013.

34 Collins FS & Varmus H, A new initiative on precision medicine. *N Engl J Med*, 372 (2015) 793.

35 Robinson EJ, Aguiar S, Smidt MP & Van der Heide LP, MCL1 as a Therapeutic Target in Parkinson's Disease?. *Trends Mol Med*, 25 (2019) 1056.

36 Ekholm-Reed S, Baker R, Campos AR, Stouffer D, Henze M, Wolf DA, Loring JF, Thomas EA & Reed SI, Reducing Mcl-1 gene dosage induces dopaminergic neuronal loss and motor impairments in Park2 knockout mice. *Commun Biol*, 125 (2019) 1.

37 Braak H, Del TK, Rüb U, Devos RA, Steur ENJ & Braak E, Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*, 24 (2003) 197.

38 Paul ML, Graybiel AM, David JC & Robertson HA, D1-like and D2-like dopamine receptors synergistically activate rotation and c-fos expression in the dopamine-depleted striatum in a rat model of Parkinson's disease. *J Neurosci*, 12 (1992) 3729.

39 Hayakawa T, Takanaga A, Tanaka K, Maeda S & Seki M, Distribution and ultrastructure of dopaminergic neurons in the dorsal motor nucleus of the vagus projecting to the stomach of the rat. *Brain Res*, 1006 (2004) 66.

40 Kumudhini N, Uma A, Devi YP, Naushad SM, Mridula R, Borgohain R & Kutula VK, Impact of COMT H108L, MAOB int 13 A>G and DRD2 haplotypeon the susceptability to Parkinson's disease in south Indian subjects. *Indian J Biochem Biophys*, 50 (2013) 436.

41 Keeney PM, Xie J, Capaldi RA & Bennett JP, Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. *J Neurosci*, 26 (2006) 5256.

42 Ebert AD, Beres AJ, Barber AE & Svendsen CN, Human neural progenitor cells over-expressing IGF-1 protect dopamine neurons and restore function in a rat model of Parkinson's disease. *Exp Neurol*, 209 (2008) 213.

43 McFadyen MC, Melvin WT & Murray GI, Regional distribution of individual forms of cytochrome P450 mRNA in normal adult human brain. *Biochem pharmacol*, 55 (1998) 825.

44 Palacios N, Weisskopf M, Simon K, Gao X, Schwarzschild M & Ascherio A, Polymorphisms of caffeine metabolism and estrogen receptor genes and risk of Parkinson's disease in men and women. *Parkinsonism Relat Disord*, 16 (2010) 370.

45 Jennifer DR, Malathy BR & Ariya SS, *In vitro* and *In silico* studies on the biochemistry and anti-cancer activity of phytochemicals from *Plumbago zeylanica. Indian J Biochem Biophys*, 58 (2021) 272.