Integrated multi-omics approach identified molecular mechanism and pathogenetic processes of COVID-19 that affect patient with Parkinson’s disorder

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ABSTRACT
The novel coronavirus named SARS-CoV-2 has emerged at the end of 2019, which causes coronavirus disease (COVID-19). Recent case reports of COVID-19 patients have revealed the onset of Parkinson’s disease (PD) symptoms in patients who do not have a family history of the PD. However, till recently, no genetic impact or mechanisms that may induce Parkinsonism in COVID-19 patients or after COVID-19 have been found. This study aimed to detect the commonly dysregulated genes, transcriptional regulators, and pathways between PD and COVID-19. We integrated genome-wide transcriptomic datasets from peripheral blood mononuclear cells (PBMC) samples from COVID-19 and PD and associated pathways. Our study revealed 81 upregulated and 48 downregulated differentially expressed genes (DEGs) shared between PD and COVID-19. These dysregulated genes were involved in key pathways “mitochondrion structure organization”, “cell activation in immune response”, and “signalling by interleukins”. Our analysis showed RELA, TP53 and SP1 TFs that may regulate the upregulated DEGs. We have discovered key dysregulated genes and characterized the biological processes of commonly dysregulated in COVID-19 and PD, which could be used for the design of personalized treatment of PD following COVID-19.

1. Introduction
At the end of 2019, a novel virus termed SARS-CoV-2 emerged in Wuhan, China. This novel virus is responsible for a respiratory illness called coronavirus disease (COVID-19). The SARS-CoV-2 is a member of coronaviruses, which are single-stranded RNA viruses that usually infect a wide range of vertebrate hosts (Cui et al., 2019). The patients infected with coronaviruses present mild symptoms of cold (Weiss and Navas-Martin, 2005). In the last decades, three types of coronaviruses (SARS-CoV-1, MERS-CoV, SARS-CoV-2) have emerged and caused severe respiratory tract infection. These three viruses have caused the loss of lives of many people. SARS-CoV-1 infected about 8000 people worldwide in 2002–2003 while MERS-CoV infected ~2,500 people. Recent SARS-CoV-2, the causative agent of COVID-19, which has become a massive threat for people worldwide (Li et al., 2019). Several factors including age, comorbid diseases impact the outcome of the disease (Guan et al., 2020).

Parkinson’s disorder (PD) is a chronic neurodegenerative disease that shows neurodegeneration of neurons that secrete dopamine in the substantia nigra part of the midbrain. The typical symptoms of PD are shaking, stiffness, tremor, walking imbalance. Various factors including genetics and environment are considered for the development and progression of PD. The influence of SARS-CoV-2 infection and its implications on PD, on the other hand, remains unclear (Schirinzi et al., 2020). Many patients are concerned about the possibility of COVID-19 infection, since a few cases...
studies have shown exacerbation of parkinsonian symptoms during infection, as well as poor treatment outcomes in PD patients (Antonini et al., 2020; Cilia et al., 2020; Hainque and Grabli, 2020). Within 2–5 weeks of infection, hospitalized patients with COVID-19 developed acute clinical parkinsonism, according to at least three published case reports (by Méndez-Guerrero et al., Cohen et al., and Faber et al.) (Méndez-Guerrero et al., 2020; Cohen et al., 2020; Faber et al., 2020). Among the three patients, two responded to the treatment of dopaminergic medication (Hainque and Grabli, 2020; Pantell et al., 2013), and the rest of one patient recovered spontaneously (Cilia et al., 2020). These three patients did not have any family history of PD, or prodromal PD (Hainque and Grabli, 2020). Brain imaging of these patients showed the decreased function of nigrostriatal dopamine systems, which is close to PD (Cohen et al., 2020). Brundin et al. has proposed three potential mechanisms for the repaid development of parkinsonism following SARS-CoV-2 as discussed herein (Brundin et al., 2020). Firstly, vascular insults due to COVID-19 in various organs including the brain that may damage the nigrostriatal systems as seen close to vascular parkinsonism (Fabbrì et al., 2021). Secondly, inflammation has been considered as risk factors for PD which suggests the acute systemic infection as seen in COVID-19 may trigger neuroinflammation and death of nigral dopamine secreting neurons. Midbrain dopamine secreting neurons are vulnerable to systemic inflammation. A prior research found higher levels of IL-6 in COVID-19, while a study found a disruption in the kynurenine pathway in COVID-19 (Thomas et al., 2020). Both processes have been related to Parkinson’s disease (Johnson et al., 2019; Heilman et al., 2020). This evidence supports the idea that COVID-19 infection could trigger Parkinson’s disease. Numerous studies have been published to characterize the COVID-19 transcriptomic dysregulations (Blanco-Melo et al., 2020; Islam et al., 2020; Lieberman et al., 2020; Fagone et al., 2020). However, there are no molecular processes or pathways that could explain the connections between PD and COVID-19.

In this study, by examining genome-wide gene expression data from COVID-19 and PD, we have proposed a multi-omics integration approach to uncover dysregulated transcriptomic signatures and pathogenetic mechanisms that may explain the effect of COVID-19 on the development of PD. Furthermore, dysregulated gene signature, molecular pathways, gene ontologies, and transcriptional regulators that may underlie PD in COVID-19 were investigated in this study (Fig. 1).

2. Materials and methods

2.1. Transcriptomic data acquisitions

We have queried NCBI Gene Expression Omnibus (GEO) database to detect the suitable datasets generated from PBMCs from COVID-19 and PD patients. We retrieved two RNA-Seq datasets (GSE152418) that contained gene expression profiling of 16 PBMC samples of COVID-19 cases and 17 healthy controls (Arunachalam et al., 2020). We also obtained a PBMC COVID-19 dataset (Accession number: CRA002390) from the Genome Sequence Archive. This dataset contained gene expression profiles of 3 COVID-19 cases and 3 controls (Xiong et al., 2020).

We then queried the GEO database with the keyword Parkinson’s disorder peripheral blood mononuclear cells (PBMCs) of COVID-19 and Parkinson’s disease (PD) that result in three microarray transcription datasets with criteria 1) mRNA expression data ii) should contain cases and controls iii) human peripheral blood mononuclear cells (PBMCs) samples (Table 1). GSE49126 dataset contained 50 samples, where 30 PD cases and 20 healthy controls from PBMC. GSE100054 contained 19 PBMC samples, which 10 PD cases and 9 healthy controls. GSE22491 contained gene expression of 18 PBMC samples, which 10 PD cases and 8 healthy controls. The detailed characteristics of the datasets are presented in Table 1.

2.2. Data processing and differential expression analysis

We have preprocessed the COVID-19 PBMC two datasets in the DESeq2 R package. The individual datasets were analyzed and identified differentially expressed genes (DEGs) using a statistical threshold an absolute log-fold change (logFC) >=1 and Benjamini-Hochberg corrected adjusted p-value FDR < 0.05. We then performed a meta-analysis of these two datasets using Fisher’s meta-analysis method implemented in the metaRNASeq R package.
The three microarray datasets of PD PBMC were processed and meta-analyzed using Network Analyst (NA) web utility tool (Zhou et al., 2019). First of all, we used two normalization procedures of datasets, namely variance stabilizing normalization (VSN) algorithm (Konishi, 1985), followed by quantile normalization (Hansen et al., 2012). Combat procedure embedded into NA was also employed to adjust study batch effect in meta-analysis. The normalized datasets were used for differential gene expression analysis using R package LIMMA (linear models for microarray data) (Ritchie et al., 2015). Finally, we performed a meta-analysis of three PBMCs datasets using Fisher’s meta-analysis method as used for COVID-19 datasets.

2.3. Functional enrichment analysis into the DEGs
We used a web utility “Metascape” for enrichment analysis (Zhou et al., 2019). The Metascape uses various sources of data including Gene Ontology, KEGG, Reactome, MSigDB for functional enrichment analysis. Metascape provides non-redundant terminology (Zhou et al., 2019). To detect significant ontologies, Metascape uses the hypergeometric test and Benjamini-Hochberg p-value correction algorithm. A Bonferroni corrected p-value < 0.05 was considered for significant terms selection.

2.4. Transcription factor analysis
We also used Metascape to identify the transcriptional regulatory transcription factors (TFs) for the identified DEGs (Zhou et al., 2019). Metascape uses the TRRUST database, which is a manually curated database of human and mouse transcriptional regulatory networks (Han et al., 2015; Han et al., 2018).

2.5. Protein-protein interaction analysis
We have performed protein–protein interaction analysis (PPI) of the proteins, by using protein interactome data via MetaScape (Zhou et al., 2019). The modules of the PPI network was explored using MCODE plugin embedded in MetaScape (Zhou et al., 2019).

3. Results

3.1. Identification of common transcriptional signatures between COVID-19 and PD PBMCs
We retrieved three peripheral blood mononuclear cells (PBMCs) PD data (accession no: GSE49126, GSE100054, GSE22491), for a total of 87 samples that included 50 PD cases and 37 healthy controls. The three datasets were processed and we performed a meta-analysis of three PBMCs datasets using Fisher’s method. Our meta-analysis detected 2186 differentially expressed genes (DEGs), where 933 upregulated and 1253 downregulated DEGs (FDR < 0.05) (Table 2 and Table S1).

Table 2
Top 20 differentially expressed genes identified via meta-analysis in Parkinson’s disease PBMCs compared to controls.

| Entrez ID | Name       | Combined T stat | FDR    |
|----------|------------|-----------------|--------|
| 64403    | CDH24      | 74.32           | 8.60E-10 |
| 84552    | PARD6G     | 68.27           | 6.95E-09 |
| 10313    | RTN3       | 67.63           | 6.95E-09 |
| 50674    | NEUROG3    | 65.20           | 1.50E-08 |
| 3975     | LHX1       | 64.53           | 1.50E-08 |
| 388630   | TRABD2B    | 64.49           | 1.50E-08 |
| 29101    | SSU72      | 63.84           | 1.75E-08 |
| 6656     | SOX1       | 62.76           | 2.28E-08 |
| 23120    | ATPI10B    | 62.26           | 2.28E-08 |
| 125950   | RAVE1      | 62.22           | 2.28E-08 |
| 83855    | KLF16      | 62.14           | 2.28E-08 |
| 64839    | FBXL17     | 62.04           | 2.28E-08 |
| 8464     | KDSR1      | 61.95           | 2.28E-08 |
| 7070     | THY1       | 60.78           | 3.05E-08 |
| 285601   | GPI150     | 60.39           | 4.09E-08 |
| 284346   | ZNF755     | 59.94           | 4.74E-08 |
| 27158    | NDOR1      | 59.64           | 5.14E-08 |
| 2020     | EN2        | 59.40           | 5.43E-08 |
| 3196     | TLX2       | 59.09           | 5.94E-08 |
| 57863    | CADM3      | 58.08           | 6.84E-08 |

analysis of three PBMCs datasets using Fisher’s method. Our meta-analysis detected 2186 differentially expressed genes (DEGs), where 933 upregulated and 1253 downregulated DEGs (FDR < 0.05) (Table 2 and Table S1).

Two PBMCs COVID-19 RNA-Seq transcriptomic datasets (accession no: GSE152418 and CRA002390) contained a total of 39 samples, 19 cases, and 20 healthy control. These two datasets were processed and we performed a meta-analysis to identify DEGs using the same Fisher’s methods. Our meta-analysis of PBMC COVID-19 showed 3272 significant (FDR < 0.05) genes (Table 3 and Table S2).

In order to identify concordant overlapped DEGs between two pathologies (Table S3) namely, PD and COVID-19, we compared the DEGs. Our analysis identified a total 129 DEGs, 81 upregulated DEGs (COX15, NOL8, SSU72, B3GNT7, RCSD1, RNF167, G6PD, ABHD8, EMC6, TRIM6, KMT2C, STARD7, TRIM62, PGBD4, UAP11, APOL4, LETM1, ARPC5, HELZ2, TMEM106A, TMEM63B, KRIT1, RAB11B, CUL1, PRDX3, CIC, ZDHHC5, MRPS23, SPATA2L, ACVR1B, CIZ1, SHARPIN, CAMKK2, KCTD12, CTS2, PNKD, RAB7A, SPPL2B, GPR153, OGDH, USP7, BCAM, PTKs, ZNF467, NKX1, HAGH, BARD1, CREB3L4, NRROS, PRDX3, CIC, ZDHHC5, MRPS23, SPATA2L, ACVR1B, CIZ1, SHARPIN, CAMKK2, KCTD12, CTS2, PNKD, RAB7A, SPPL2B, GPR153, OGDH, USP7, BCAM, PTKs, ZNF467, NKX1, HAGH, BARD1, CREB3L4, NRROS, and Table 3).

Table 3
Top 20 differentially expressed genes identified via meta-analysis of COVID-19 PBMCs compared to controls.

| Ensembl ID | Gene symbol | FDR     | Fold change |
|------------|-------------|---------|-------------|
| ENSCG0000021196 | IGH1 | 0.00   | 5.46         |
| ENSCG0000211972 | IGHV3-66 | 0.00   | 5.09         |
| ENSCG0000211972 | IGLV2-28 | 0.00   | 5.04         |
| ENSCG0000165948 | IFI2L1 | 0.00   | 4.79         |
| ENSCG0000211967 | IGL2 | 0.00   | 4.69         |
| ENSCG0000211967 | IGL3 | 0.00   | 4.63         |
| ENSCG0000211974 | IGHV2-70D | 0.00 | 4.62         |
| ENSCG0000211949 | IGHV3-23 | 0.00   | 4.42         |
| ENSCG0000159189 | C1QC | 0.00   | 4.35         |
| ENSCG0000211946 | IGHV3-20 | 0.00   | 4.34         |
| ENSCG0000235381 | IGLV1-41 | 0.00 | 4.13         |
| ENSCG0000253998 | IGLV2-29 | 0.00   | 4.11         |
| ENSCG0000276566 | IGHV1D-13 | 0.00 | 4.00         |
| ENSCG0000136315 | AL355922.1 | 0.00 | 3.89         |
| ENSCG0000282651 | IGHV5-10-1 | 0.00 | 3.88         |
| ENSCG0000211964 | IGHV3-48 | 0.00   | 3.75         |
| ENSCG0000211943 | IGHV3-11 | 0.00   | 3.68         |
| ENSCG0000211958 | IGHV4-1 | 0.00   | 3.66         |
| ENSCG0000211968 | IGHV1-58 | 0.00   | 3.64         |
| ENSCG0000243063 | IGHV3-7 | 0.00   | 3.59         |
SBF1, ARHGAP1, SLC37A2, UBXN11, ARPC1B, RAB34, CYB5R3, N4BP1, PRKACA, SHISA7, VSIG2, BMP8B, NFE2, DUSP7, SLC27A1, CCR2, TFG, SLC37A4, DVL3, MBD6, DOK3, ITMA, MKD, HECTD3, OGFR, RUSC2, PORCN, ALG3, CPTP, PEX10, HEMGN, SMCR8) were commonly modulated between COVID-19 and PD.

Our analysis identified 48 downregulated DEGs (PDCD5, CDCA4, ZNF227, CCR3, RBM6, FAM8A1, RPRD1A, ZNF121, DDX21, RNF139, ACP1, OTULIN, PHF11, CHRN, PSMA1, BORA, LSG1, RNF103, CAMP, ZBTB1, CCDC82, ID1, DCP2, TMEM201, CD36, TRMT12, SCCE, FAM76B, CHI3L1, MITD1, RCHY1, CLEC7A, METTL2A, CEP70, TAF5L, CENPH, HSDL1, HNRNPH3, HSP90AA1, MCM9, AP4S1, SCRn3, SLC45A4, WTAP, THUMP1, NMI, MTM7F3, DBR1) were commonly modulated between COVID-19 and PD.

3.2. Identification of common functional gene ontology terms in COVID-19 and Parkinson’s disorder PBMCs

To illustrate the biology of the overlapping DEGs found, we conducted an enrichment analysis of genes. In this way, we identified several ontologies and pathways that overlapped between PD and COVID-19 (Figs. 3B–4). Our analysis showed the common gene ontologies of the upregulated DEGs between PD and COVID-19 “mitochondrion organization”, “cellular protein catabolism”, “co-factor metabolic process”, “cellular component disassembly”, “apoptotic signalling pathways”, “positive regulation of organelle organization”, “nervous system development”, “negative regulation of cellular component organization”, “organelle localization” (Figs. 3–4). Among the significant ontologies enriched by downregulated DEGs were “Cell activation in immune systems”, “Signalling by interleukins”, “Metabolism of RNA”, “Positive regulation of cell death” (Figs. 3–4).

3.3. Prediction of transcription factor overlapping between COVID-19 and Parkinson’s disorder PBMCs

To provide putative transcriptional regulators of the common modulated DEGs between PD and COVID-19, we identified that RELA, TP53, and SP1 were involved in the expression of commonly upregulated genes in COVID-19 and PD. However, we did not obtain any significant TFs that may be involved in the regulation of DEGs which were downregulated DEGs (Fig. 5).

3.4. Functional module analysis of proteins encoded by the DEGs

To dismantle the molecular interactions of the proteins encoded by the DEGs which were commonly modulated in COVID-19 and PD, we investigated protein–protein interaction (PPI) analysis. Our analysis showed the presence of four modules in the PPI networks (Fig. 6). The modules were found involved in various infection and metabolism associated pathways (Fig. 6B,C).

4. Discussion

Comorbidities are considered as immense risk factors for increased vulnerability to COVID-19 and poor therapeutic outcome. Among the comorbidities, neurological disorders came into...
a greater risk for PD since some case reports showed the development of PD following COVID-19. The neurological symptoms in COVID-19 patients appear to be severe to mild. Despite the three case reports described the development of PD after COVID-19 presenting a great threat to the massive development of PD in response to COVID-19 (Méndez-Guerrero et al., 2020; Cohen et al., 2020; Faber et al., 2020).

The cellular and mechanism that may lead to COVID-19 induced PD are not understood yet. Therefore, characterizing critical genes and pathogenetic pathways in COVID-19 and PD is very important to understand the possible links of development of PD following COVID-19. In this study, for the first time, we identified commonly modulated common genes and molecular processes altered between PD and COVID-19 in PBMC. To this aim, we utilized whole-genome PBMC transcriptomic datasets of COVID-19 and PD via systems biology analysis. Our analysis detected 81 upregulated and 48 downregulated significant DEGs were common between two pathologies suggesting a possible molecular link between COVID-19 and PD. The upregulated genes were involved in several biological processes including “mitochondrion structure organization” among others. There is substantial evidence has been presented that suggests the mitochondrial dysfunction is involved in the aetiology and pathogenesis of PD since the inhibitor of electron transport chain complex I cause PD (Winklhofer and Haass, 2010; Perier and Vila, 2012; Keane et al., 2011). The mitochondria are involved in crucial cellular processes including energy metabolism, calcium homeostasis, and program cell death. Disruption of mitochondria is involved in the neuronal cell death of substantia nigra of PD, substantia nigra is the part of the brain mainly involved in PD development. In line with this evidence, our study suggests impairment of mitochondrial structure organization pathways might be involved in PD and COVID-19.

Our study demonstrated that the downregulated common genes are predominantly involved in immune systems related processes namely, “cell activation in immune response”, “Signalling by interleukins”. There is much compelling evidence that suggests the increased expression of genes in central nervous systems and peripheral nervous systems, infiltration of immune cells into CNS, the altered composition of immune cells in peripheral nervous systems, etc are implicated in the development of PD as discussed herein (Kannarkat et al., 2013; Tansey and Romero-Ramos, 2019). Moreover, various attempts have been taken to decipher inflammatory molecules, cells involved in the development of PD. Investigation of post-mortem brain and cerebrospinal fluids of PD showed the presence of inflammatory cytokines suggesting neuroinflammation in affected brain regions of PD (Tiwari and Pal, 2017). Alterations of various interleukins including IL-6 have been suggested as a marker of increased mortality in PD (Dufek et al., 2015). In line with this evidence, we hypothesized that alteration of signalling by interleukins pathways is involved in COVID-19 induced PD. Increasing evidence also has been suggested the impaired activation of immune systems, specifically the “cytokine storm” found in COVID-19 results from uncontrolled host immune response that is considered to be associated with COVID-19 complications, which supports that systemic immune systems alter-
The dysregulated common transcriptional signature and molecular pathways between PD and COVID-19 is involved with COVID-19 (Geerlings and Hoepelman, 1999; Catanzaro et al., 2020).

Finally, we identified TFs (RELA, TP53, and SP1) that may regulate the common DEGs between COVID-19 and PD. Our results identified RELA TFs, a major component of NF-kB, which are considered as the master regulators of inflammation and apoptosis (Bellucci et al., 2020; Lanzillotta et al., 2015). The NF-kB/RELA is involved in neurodegenerative diseases including PD (Lanzillotta et al., 2015). Many lines of evidence showed the crucial role of RELA/NF-kB in PD (Lanzillotta et al., 2015). Previous studies showed the dysregulated increased expression of RELA in nigral dopaminergic neurons and glial cells in PD patients (Lanzillotta, 2015; Ghosh et al., 2007). Inhibition of RELA prevents loss of dopaminergic neurons and downregulated expression of RELA provides neuroprotection in PD (Ghosh et al., 2007). We also identified key TFs namely RELA, TP53 which were involved in the expression of common DEGs. The detected pathways and genes in this study could be therapeutic targets in PD patients with COVID-19. Considering crucial importance of presented hub genes and pathways shared between COVID-19 and PD, experimental assessment of hub genes and pathways in clinical samples in COVID-19 and PD are proposed to further clarify the shared mechanism.
