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RESEARCH

COVID-19 Alters Inflammatory, Mitochondrial, and Protein Clearance Pathway Genes: Potential Implications for New-onset Parkinsonism in Patients

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Abstract

Several preclinical and clinical studies have shown that SARS-CoV-2 infection is associated with new-onset Parkinson's disease (PD). The overall goal of this study is to uncover how the COVID-19 severity gradient impacts the conventional pathological pathway of PD to inform the identification of at-risk patients and the development of personalized treatment strategies. Transcriptomics analysis of 43 PD pathogenic genes was conducted on nasopharyngeal swabs from 50 COVID-19 patients with varying severity including 17 outpatients, 16 non-ICU, and 17 ICU patients, compared to 13 SARS-CoV-2 negative individuals. The study shows that COVID-19 severity gradient differentially dysregulates PD pathological genes. Dysfunctional lysosomal and mitochondrial processes in outpatients and non-ICU COVID-19 patients was identified as the convergent network of COVID-19—PD interactions. These dysfunctions were later abrogated by the upregulation of the ubiquitin–proteasome system and autophagy-lysosome system in ICU COVID-19 patients. A potential synergistic co-expression and clustering of protein clearance pathway genes with other pathological genes was observed in ICU patients, indicating a possible overlap in biological pathways. Dysregulation of the PD pathopharmacogene, *SLC6A3* was observed in ICU patients, suggesting potential COVID-19-gene-drug interactions. Nasopharyngeal swabs express major PD pathological genes as well as clinically relevant drug processing genes, which could advance studies on PD, including diagnosis, pathogenesis, and the development of disease-modifying treatments. Outpatients and non-ICU COVID-19 patients may face a higher risk of developing new-onset PD, whereas ICU COVID-19 patients may be more susceptible to COVID-19-gene-drug interactions.

Keywords Parkinson's disease · COVID-19 · SARS-CoV-2 · Nasopharyngeal swab · α-synuclein · Mitochondrial dysfunction · Ubiquitin–proteasome system · Autophagy-lysosome system · Pathopharmacogene · Disease–gene-drug interactions · COVID-19–Parkinson's disease interactions

Abbreviations

SNCA	α-synuclein
SNCB	β-synuclein
SNCG	γ-synuclein
EIF4G1	Eukaryotic Translation Initiation Factor 4 Gamma 1
LRRK2	Leucine Rich Repeat Kinase 2
GIGYF2	GRB10 Interacting GYF Protein 2
MAPT	Microtubule Associated Protein Tau
PLA2G6	Phospholipase A2 Group VI
GCH1	GTP Cyclohydrolase 1
GAK	Cyclin G Associated Kinase
SYT11	Synaptotagmin 11
PRKN	Parkin RBR E3 Ubiquitin Protein Ligase
PINK1	PTEN Induced Kinase 1
PARK7	Parkinsonism Associated Deglycase
HTRA2	High Temperature Requirement Protein A2
FBXO7	F-Box Protein 7
TOMM20	Translocase of Outer Mitochondrial Membrane 20
TIMM23	Translocase of Inner Mitochondrial Membrane 23

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TIMM21	Translocase of Inner Mitochondrial Membrane 21
ATP13A2	ATPase Cation Transporting 13A2
UCHL1	Ubiquitin C-Terminal Hydrolase L1
VPS35	Vacuolar Protein Sorting-Associated Protein 35
DNAJC6	Putative Tyrosine-Protein Phosphatase Auxilin
SYNJ1	Synaptojanin 1
DNAJC13	DnaJ Heat Shock Protein Family (Hsp40) Member C13
VPS13C	Vacuolar Protein Sorting-Associated Protein 13 C
GBA	Glucosylceramidase Beta 1
MAP1LC3B2	Microtubule Associated Protein 1 Light Chain 3 Beta 2
LAMP1	Lysosomal Associated Membrane Protein 1
LAMP2	Lysosomal Associated Membrane Protein 2
LAMP3	Lysosomal Associated Membrane Protein 3
LAMP5	Lysosomal Associated Membrane Protein Family Member 5
BST1	Bone Marrow Stromal Cell Antigen 1
HLA-DRB5	Major Histocompatibility Complex, Class II, DR Beta 5
TSPO	Translocator Protein
IL2	Interleukin 2
CCL5	C–C Motif Chemokine Ligand 5
DRD1	Dopamine Receptor D1
DRD2	Dopamine Receptor D2
DRD3	Dopamine Receptor D3
DRD4	Dopamine Receptor D4
SLC6A3	Dopamine Transporter
COMT	Catechol-O-Methyltransferase

Introduction

Coronavirus disease 2019 (COVID-19) is associated with several central and peripheral nervous system symptoms including headache, stroke, ageusia, anosmia, myelitis, myalgia, encephalopathy, and neuropsychiatric manifestations (Farrokhi et al. 2023). This suggests potential interactions with the pathological pathway of neurological diseases (Farrokhi et al. 2023; Iravanpour et al. 2023). Indeed, studies have found a positive association between new-onset neurological diseases such as Parkinson's disease (PD) and infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathological agent that causes COVID-19 (Rahmati et al. 2023a, b). Three different clinical case reports have documented new-onset parkinsonism in COVID-19 patients (Cohen et al. 2020; Faber et al. 2020; Méndez-Guerrero et al. 2020). These patients were less than 60 years old, had no history of PD or exposure to neurotoxicants, and developed PD symptoms less than 2 months after the onset of COVID-19 symptoms. They also exhibited abnormalities in nigrostriatal dopamine transporter (DAT) activity; however, two of the patients responded positively to dopaminergic therapy (Cohen et al. 2020; Faber et al. 2020) but the oldest patient did not respond and had significant symptom improvement without any specific therapeutic intervention(Méndez-Guerrero et al. 2020). One out of the three patients underwent genetic testing for genetic polymorphisms linked to PD and the result was negative (Cohen et al. 2020). This suggests that COVID-19 probably triggered the pathological pathway associated with PD, highlighting the urgent need for preclinical and clinical studies to further validate COVID-19-mediated interactions with PD and its underlying mechanism.

Similar to COVID-19, PD is a multisystem disorder impacting the central and peripheral nervous systems (Leta et al. 2021). The pathophysiological hallmark of PD is the misfolding and aggregation of α-synuclein (AS) into Lewy bodies and Lewy neurites and the consequential death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Benítez-Burraco et al. 2016; Nwabufo and Aigbogun 2022; Iravanpour et al. 2023). PD is clinically characterized by motor and non-motor symptoms with the non-motor symptoms taking the lead in clinical manifestation(Nwabufo and Aigbogun 2022). It is noteworthy that hyposmia, a non-motor symptom of PD overlaps with COVID-19 symptomology and serves as an early indicator of PD during its initial stages(Rey et al. 2018). This is particularly significant as the olfactory system is a key site for the accumulation of AS (Rey et al. 2018). This suggests that the nasal cavity may be a primal target site for investigating COVID-19 – PD interactions for the early detection of at-risk patients.

Previous studies have shown that AS is involved in the innate immune response following viral infection, uncovering a potential pathological network for COVID-19 – PD interactions (Tulisiak et al. 2019). Preclinical studies have started uncovering potential mechanisms by which SARS-CoV-2 infection may interact with the pathological pathway associated with PD (Philippens et al. 2022; Käufer et al. 2022). For example, a preclinical study using male and female SARS-CoV-2-infected Syrian hamsters investigated how SARS-CoV-2 infection impacts brain neuroinflammatory processes and accumulation of neurotoxic proteins including AS, tau, and hyper-phosphorylated tau either at the onset of clinical symptoms and high virus levels in the body (day 3) or after recovery from infection and no detectable virus (day 14) (Käufer et al. 2022). They discovered that viral proteins in the nasal cavity triggered significant microglia activation in the olfactory bulb even after viral clearance. Furthermore, they observed a significant increase in AS, tau, and hyper-phosphorylated tau in

cortical neurons on day 14 post-infection (Käufer et al. 2022). These findings indicate that SARS-CoV-2 infection is associated with elevated expression and accumulation of AS which persist even after the virus is cleared and could potentially contribute to long-term neurological diseases including neurodegenerative diseases such as PD (Iravanpour et al. 2023).

To better understand the interplay between COVID-19 and PD, it is imperative to demonstrate how the COVID-19 severity gradient impacts the conventional pathological pathway of PD. This will help identify the stage of the disease that may be likely to interfere with the pathogenesis of PD and allow for individualized treatment of the affected patient population. Furthermore, the involvement of the nasal cavity in the early stage of PD and COVID-19 pathogenesis implies that the nasal specimen may be useful for investigating the interplay between COVID-19 and PD. The overall goal of this current study is to investigate how the COVID-19 severity gradient impacts the expression of genes associated with the pathogenesis of PD in nasopharyngeal swab samples obtained from patients at different COVID-19 severity levels including outpatients, non-intensive care unit (ICU) patients, and ICU patients compared to SARS-CoV-2 negative individuals. This study will help uncover the potential benefits of nasopharyngeal swab samples for PD diagnosis, pathogenesis, and testing of disease-modifying treatments. If this study uncovers the expression of PD pathogenic genes in nasopharyngeal swab samples, it may help identify the impact of COVID-19 on several PD pathological pathways including AS misfolding and aggregation, mitochondrial function, protein clearance pathways, neuroinflammation, and pharmacogenetics following COVID-19 severity gradient. The outcome of this study will help identify COVID-19 patient groups that are at-risk of COVID-19 – PD interactions, allowing for personalized treatment strategies, especially for patients with long COVID.

Methods

The transcriptomics dataset used in this study is based on recently performed clinical studies and RNA-sequencing of nasopharyngeal swab samples obtained from non-COVID-19 and COVID-19 patients, and the datasets can be freely accessed here—https://figshare.com/articles/dataset/Comparative_transcriptomic_analysis_of_nasopharyngeal_swabs_from_individuals_with_and_without_COVID-19/22704403/1. The associated methodology used in the study is described below.

Study design and ethical approval

The clinical study was carried out as previously described (Kotwa et al. 2022; Nwabufo et al. 2024). Population-based infectious disease surveillance is carried out in metropolitan Toronto and Peel, Ontario, Canada (population: 4.2 million in 2021) by the Toronto Invasive Bacterial Diseases Network (TIBDN). Four hospitals (North York—General Division, Scarborough Hospital—General Division, Sunnybrook—Sunnybrook Campus, and Toronto East General Hospital/Michael Garron Hospital) report clinical specimens positive for SARS-CoV-2 to TIBDN's central study office. Those who underwent ambulatory treatment, ICU admission, or non-ICU hospitalization in the collaborating hospitals between October 2020 and October 2021 and tested positive for SARS-CoV-2 by clinical qPCR testing were eligible for the study. People who tested negative for SARS-CoV-2 were used as controls in the study. All the four participating TIBDN hospitals approved the research (REB# 2024–0233-1025, MED-02–011, SUN-5024, and 084–0209-Lab-001), and all patients provided informed consent in accordance with the Declaration of Helsinki.

Collection of clinical data and specimen

As previously described (Kotwa et al. 2022; Nwabufo et al. 2024), data on COVID-19 risk factors, medical conditions, and demographics were collected through participant interviews and chart reviews. Following established practice (Marty et al. 2020), the study staff collected nasopharyngeal swabs from the participants during enrollment at their respective study centers. For further investigation, nasopharyngeal swabs were quickly put into a universal transport medium (Copan Diagnostics, Murrietta, CA).

Extraction of RNA from human nasopharyngeal swabs

On the day of collection, samples were processed at Sunnybrook Research Institute. Nasopharyngeal swabs were aliquoted and kept at −80 °C after undergoing a 20-second vortexing step (Kotwa et al. 2022; Nwabufo et al. 2024). As directed by the manufacturer, 40 µL of nasopharyngeal swab was used for RNA extraction using the QIAmp Viral RNA Mini Kit from QIAGEN (https://www.qiagen.com; Kotwa et al. 2022; Nwabufo et al. 2024).

RNA-sequencing

As previously described (Kotwa et al. 2023; Nwabufo et al. 2024), RNA-sequencing (RNA-seq) was carried out at the University of Toronto's Donnelly Sequencing Centre (http://ccbr.utoronto.ca/donnelly-sequencing-centre). Qubit RNA HS (cat # Q32852, Thermo Fisher Scientific Inc., Waltham, USA) fluorescent chemistry was used to quantify DNase-treated total RNA, and the High Sensitivity RNA ScreenTape (cat # 5067–5579, Agilent Technologies Inc., Santa

Clara, USA) was utilized to calculate the RNA integrity number (RIN) from 5 ng of RNA. The median RIN score was 3, and the lowest RIN recorded was 1.5. The NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB Cat# E7405) was used in conjunction with the NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB Cat# E7765) to build RNA-seq libraries from RNA samples (50 ng). Libraries depleted of ribosomal RNA showed a mean concentration of 15.2 ng/µL. An Agilent Bioanalyzer dsDNA High Sensitivity chip (cat # 5067–4626, Agilent Technologies Inc., Santa Clara, USA) was used to analyze a 1 µL top stock of each purified final library. After size adjustment, equimolar pooling was carried out following library quantification using the Quant-iT dsDNA high-sensitivity kit (cat # Q33120, Thermo Fisher Scientific Inc., Waltham, USA). The final pool was evaluated using an Agilent Bioanalyzer dsDNA High Sensitivity chip, and the NEBNext Library Quant Kit for Illumina (cat # E7630L, New England Biolabs, Ipswich, USA) was used for quantification. Utilizing an S2 flowcell, the hybridized pool was subjected to paired-end sequencing of 150 bp at a final concentration of 320 pM on the Illumina NovaSeq 6000 platform. The sequencing depth was optimized to achieve 65 M per sample and 350 pM loading.

Bioinformatics analysis of human nasopharyngeal swabs

Nasopharyngeal swabs from SARS-CoV-2-positive patients with varying levels of disease severity and SARS-CoV-2-negative individuals were subjected to RNA-seq analysis as previously described (Nwabufo et al. 2024). Seven comparative analyses of genes associated with PD pathological pathways including AS misfolding and aggregation, mitochondrial dysfunction, dysfunctional protein clearance pathways, inflammation, and pharmacogenetics (Kouli et al. 2018; Franco-Iborra et al. 2018; Day and Mullin 2021; Nwabufo and Aigbogun 2022; Coukos and Krainc 2024; Fløtnes et al. 2024) (Table 1) were performed using DESeq2 (Love et al. 2014) as follows: all COVID-19 patients versus negatives, outpatients versus negatives, non-ICU patients versus negatives, ICU patients versus negatives, outpatients versus non-ICU patients, outpatients versus ICU patients, and ICU patients versus non-ICU patients. The paired end fastq files used for sequencing data were evaluated for quality using FASTQC, which can be accessed at https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Fastp v0.21.0 was used for quality filtering with the default parameters (Chen et al. 2018). Salmon v.1.4.0 (Patro et al. 2017)was used to quantify transcripts, and the following parameters were used: –validateMappings, –seqBias, and –gcBias. Salmon's reference index was the human transcriptome from GENCODE v37 (Frankish et al. 2021). Using the txtimport package v.1.22.0, transcript counts (.quant files) were imported into R v4.1.2. The 'regularized log' (rlog) transformation was used to calculate the normalized transcript expression levels for all genes. The result was a data matrix that could be used for additional statistical analysis. DESeq2v.1.34.0 was then used to identify differentially expressed genes with an adjusted p-value of less than 0.05 to account for multiple comparisons in the statistical analysis. With GraphPad Prism® (version 8.0 for Microsoft Windows, Graph Pad Software, San Diego, CA, United States), a box plot of differentially expressed genes was generated. SRplot (https://www.bioinformatics.com.cn/srplot) was used to create heatmaps and volcano plots.

Table 1 Panel of Parkinson's disease pathogenic genes investigated in nasopharyngeal swab samples of COVID-19 and non-COVID-19 individuals

Gene Symbol for α-synuclein Pathology	Gene Symbol for Mitochondrial Dysfunction	Gene Symbol for Dysfunctional Protein Clearance Systems	Gene Symbol for Inflammation	Gene Symbol for Relevant Pharmacogenes
SNCA	*PRKN*	*ATP13A2*	*BST1*	*DRD1*
SNCB	*PINK1*	*UCHL1*	*HLA-DRB5*	*DRD2*
SNCG	*PARK7*	*VPS35*	*TSPO*	*DRD3*
EIF4G1	*HTRA2*	*DNAJC6*	*IL2*	*DRD4*
LRRK2	*FBXO7*	*SYNJ1*	*CCL5*	*SLC6A3*
GIGYF2	*TOMM20*	*DNAJC13*	——	*COMT*
MAPT	*TIMM23*	*VPS13C*	——	——
PLA2G6	*TIMM21*	*GBA*	——	——
GCH1	——	*MAP1LC3B2*	——	——
GAK	——	*LAMP1*	——	——
SYT11	——	*LAMP2*	——	——
——	——	*LAMP3*	——	——
——	——	*LAMP5*	——	——

Results

Patient characteristics

Three samples were ultimately eliminated from the study owing to sample-related problems, out of the 66 participants who were initially enrolled. The remaining 63 participants include 13 SARS-CoV-2 negative individuals and 50 COVID-19 patients comprising 17 outpatients, 16 non-ICU patients, and 17 ICU patients. A comprehensive patient clinical and demographic characteristics have been recently documented (Nwabufo et al. 2024) and detailed in Table 2. In general, age decreased from ICU patients to negative individuals with ICU patients being older and negative individuals being younger (Nwabufo et al. 2024). There were no significant differences in the number of male and female participants across the study groups (Nwabufo et al. 2024). Comorbidities, COVID-19-related complications, and medication usage were more frequent among ICU patients, followed by non-ICU hospitalized patients (Nwabufo et al. 2024). Based on the available medical information, none of the patients had Parkinson's. One of the non-ICU COVID-19 patients had neuromuscular illness, specifically hemiplegia, paraplegia/quadriplegia, and seizure disorder, as well as depression. Two COVID-19 patients in the ICU experienced neuropsychiatric issues: one exhibited a neuropsychiatric syndrome along with delirium, while the other solely had delirium.

COVID-19-mediated interactions with α-synuclein pathology

Three out of the 11 genes associated with AS pathology were dysregulated. *SNCG* was upregulated in non-ICU ($p < 0.05$) and ICU ($p < 0.01$) patients (Fig. 1). Similarly, *GAK* was modestly upregulated in both non-ICU and ICU patients ($p < 0.05$; Fig. 1). *LRRK2* was modestly upregulated in ICU patients alone ($p < 0.05$; Fig. 1). All the other investigated genes (*SNCA*, *SNCB*, *EIF4G1*, *GIGYF2*, *PLA2G6*, *GCH1*, *MAPT*, and *SYT11*) associated with AS misfolding and aggregation pathways were not significantly dysregulated (Supplementary Figures S1 and S2).

COVID-19-mediated interactions with mitochondrial function

Three out of the eight genes associated with mitochondrial function were dysregulated by COVID-19. *TOMM20* was strongly downregulated in both outpatients and non-ICU patients in a consistent manner ($p < 0.0001$) compared to negative individuals and was modestly upregulated in ICU patients ($p < 0.05$) relative to outpatients (Fig. 1). *TIMM21* was strongly and modestly downregulated in outpatients ($p < 0.0001$) and non-ICU patients ($p < 0.05$), respectively, compared to negative individuals, and was moderately upregulated in ICU patients ($p < 0.01$) relative to outpatients (Fig. 1). *HTRA2* was modestly downregulated in outpatients ($p < 0.05$) compared to negative individuals and was upregulated in ICU patients ($p < 0.001$) relative to outpatients (Fig. 1). All the other investigated genes (*TIMM23*, *PARK7*, *FBXO7*, *PINK1*, and *PRKN*) associated with mitochondrial function were not significantly dysregulated (Supplementary Figures S2 and S3).

COVID-19-mediated interactions with protein clearance pathways

Six out of the 13 investigated genes associated with protein clearance pathways were impacted by COVID-19. *VPS35* was strongly downregulated in outpatients ($p < 0.0001$) and non-ICU patients ($p < 0.001$) compared to negative individuals, and was upregulated in ICU patients ($p < 0.05$) relative to outpatients (Fig. 2). *MAP1LC3B2* was consistently upregulated in outpatients ($p < 0.05$), non-ICU ($p < 0.05$), and ICU ($p < 0.01$) patients relative to negative individuals with ICU patients having the most upregulation (Fig. 2). Similarly, *SYNJ1* was consistently upregulated in outpatients, non-ICU, and ICU patients ($p < 0.05$; Fig. 2). *LAMP3* was upregulated in outpatients ($p < 0.05$) compared to negative individuals, and modestly downregulated in ICU patients ($p < 0.05$) compared to outpatients (Fig. 2). On the contrary, *LAMP1* was only upregulated in ICU patients relative to outpatients ($p < 0.05$; Fig. 2). *UCHL1* was upregulated in outpatients ($p < 0.05$) and ICU patients ($p < 0.01$) compared to negative individuals with ICU patients taking the lead (Fig. 2). All the other investigated genes (*GBA*, *VPS13C*, *LAMP2*, *DNAJC13*, *ATP13A2, LAMP5,* and *DNAJC6*) associated with protein clearance pathways were not significantly dysregulated (Supplementary Figures S3 and S4).

COVID-19-mediated interactions with inflammatory processes

Two out of the five investigated genes associated with neuroinflammatory processes were impacted by COVID-19. *CCL5* was modestly downregulated in ICU patients ($p < 0.05$) compared to outpatients while *BST1* was modestly upregulated in ICU patients ($p < 0.05$) compared to negative individuals (Fig. 3). All the other investigated genes (*TSPO*, *HLA-DRB5*, and *IL2*) associated with neuroinflammatory processes were not significantly dysregulated (Supplementary Figure S4).

Table 2 Patient clinicopathological and demographic Profiles

Characteristics	Negative, A (n = 13)	Outpatient, B (n = 17)	Non-ICU, C (n = 16)	ICU, D (n = 17)	Significant Differences
Age (years), median (IQR)	30 (44)	47 (25)	58 (24)	65 (14)	A-C**; A-D***; B-D**
Height (cm), median (IQR)	NA	NA	170 (21)	169 (17)	NSD
Weight (kg), median (IQR)	NA	NA	88 (34)	83 (31)	NSD
Symptom onset to specimen collection (days), median (IQR)	NA	2 (3)	5 (5)	4 (4)	NSD
Sex					
Male, *n* (%)	4 (31)	7 (41)	7 (44)	9 (53)	NSD
Female, *n* (%)	8 (62)	10 (59)	9 (56)	8 (47)	NSD
COVID-19 Vaccination Status					
Vaccinated, *n* (%)	8 (62)	11 (65)	7 (44)	1 (6)	NSD
Not vaccinated, *n* (%)	5 (38)	2 (12)	9 (56)	5 (29)	NSD
Complications					
Acute respiratory distress syndrome, *n* (%)	NA	NA	NA	4 (24)	NSD
Pneumonia, *n* (%)	NA	NA	13 (81)	15 (88)	NSD
Respiratory failure, *n* (%)	NA	NA	NA	8 (47)	NSD
Comorbidities					
Cancer condition, *n* (%)	NA	NA	NA	1 (6)	NSD
Cardiac illnesses, *n* (%)	NA	NA	NA	1 (6)	NSD
Diabetes, *n* (%)	NA	NA	NA	5 (29)	NSD
Gastro-intestinal illnesses, *n* (%)	NA	NA	NA	1 (6)	NSD
Neuromuscular illnesses, *n* (%)	NA	NA	1 (6)	NA	NSD
Pulmonary illnesses, *n* (%)	NA	NA	2 (13)	4 (24)	NSD
Rheumatologic illnesses, *n* (%)	NA	NA	1 (6)	NA	NSD
Vascular illnesses, *n* (%)	NA	NA	5 (31)	10 (59)	NSD
Medications					
Amoxicillin + Clavulanic Acid, *n* (%)	NA	NA	NA	1 (6)	NSD
Aspirin, *n* (%)	NA	NA	2 (13)	2 (12)	NSD
Azithromycin, *n* (%)	NA	NA	NA	5 (29)	NSD
Candesartan, *n* (%)	NA	NA	2 (13)	2 (12)	NSD
Capecitabine, *n* (%)	NA	NA	NA	1 (6)	NSD
Cefazolin, *n* (%)	NA	NA	NA	3 (18)	NSD
Ceftazidime, *n* (%)	NA	NA	NA	3 (18)	NSD
Ceftriaxone, *n* (%)	NA	NA	4 (25)	13 (76)	NSD
Celecoxib, *n* (%)	NA	NA	2 (13)	1 (6)	NSD
Ciprofloxacin, *n* (%)	NA	NA	NA	3 (18)	NSD
Clarithromycin, *n* (%)	NA	NA	1 (6)	NA	NSD
Convalescent COVID-19 plasma, *n* (%)	NA	NA	NA	1 (6)	NSD
Dexamethasone, *n* (%)	NA	NA	14 (88)	17 (100)	NSD
Enoxaparin, *n* (%)	NA	NA	NA	1 (6)	NSD
Ertapenem, *n* (%)	NA	NA	NA	3 (18)	NSD
Fluticasone/salmeterol, *n* (%)	NA	NA	NA	1 (6)	NSD
Gentamycin, *n* (%)	NA	NA	NA	1 (6)	NSD
Hydroxychloroquine sulphate, *n* (%)	NA	NA	1 (6)	NA	NSD
Isoflurane, *n* (%)	NA	NA	NA	1 (6)	NSD
Levofloxacin, *n* (%)	NA	NA	NA	1 (6)	NSD
Meropenem, *n* (%)	NA	NA	NA	5 (29)	NSD
Methylprednisolone, *n* (%)	NA	NA	NA	2 (12)	NSD
Moxifloxacin, *n* (%)	NA	NA	1 (6)	NA	NSD
Piperacillin + Tazobactam, *n* (%)	NA	NA	NA	11 (65)	NSD
Prednisolone, *n* (%)	NA	NA	NA	1 (6)	NSD

Table 2 (continued)

Characteristics	Negative, A (n = 13)	Outpatient, B (n = 17)	Non-ICU, C (n = 16)	ICU, D (n = 17)	Significant Differences
Ramipril, *n* (%)	NA	NA	NA	1 (6)	NSD
Remdesivir, *n* (%)	NA	NA	9 (56)	5 (29)	NSD
Telmisartan, *n* (%)	NA	NA	NA	1 (6)	NSD
Tobramycin, *n* (%)	NA	NA	NA	1 (6)	NSD
Tocilizumab, *n* (%)	NA	NA	6 (38)	5 (29)	NSD
Vancomycin, *n* (%)	NA	NA	NA	6 (35)	NSD
Patients with polypharmacy, *n* (%)	NA	NA	2 (13)	6 (35)	NSD
Average polypharmacy use, median (IQR)	NA	NA	5 (0)	10 (12)	NSD
Race					
Arab, *n* (%)	NA	NA	NA	1 (6)	NSD
Chinese, *n* (%)	NA	NA	2 (13)	1 (6)	NSD
Filipino, *n* (%)	NA	NA	NA	1 (6)	NSD
South Asian (East Indian, Pakistani, Sri Lankan, etc.), *n* (%)	NA	NA	1 (6)	2 (12)	NSD
Southeast Asian (Vietnamese, Cambodian, Malaysian, Laotian), *n* (%)	NA	NA	NA	1 (6)	NSD
West Asian (Iranian, Afghan, etc.), *n* (%)	NA	NA	3 (19)	2 (12)	NSD
White/Caucasian, *n* (%)	NA	NA	3 (19)	6 (35)	NSD

Statistical significance was assessed utilizing Kruskal–Wallis test followed by Dunn's multiple comparison test. **, $p < 0.01$, ***, $p < 0.001$. IQR, interquartile range; NA, not available; NSD, no significant difference; ICU, intensive care unit. Polypharmacy was determined by calculating the number of patients taking 5 or more medications. Average polypharmacy use was calculated by dividing the total number of medications for all patients by number of patients. This table was adapted from Nwabufo et al. 2024 (Nwabufo et al. 2024).

COVID-19-mediated interactions with Parkinson's disease-associated pharmacogenes

One out of the six investigated pharmacogenes associated with PD was dysregulated by COVID-19. *SLC6A3* was consistently upregulated in ICU patients relative to negative individuals and outpatients ($p < 0.05$; Fig. 3). All the other investigated genes (*COMT*, *DRD1*, *DRD2*, *DRD3*, and *DRD4*) associated with pharmacogenetic liabilities in PD were not significantly dysregulated (Supplementary Figures S4 and S5).

Molecular interactions between Parkinson's disease pathogenic genes and COVID-19 severity gradient

To identify the primary PD pathological pathways linked to varying COVID-19 severity levels, genes with statistical significance and notable fold changes in expression were examined across seven comparisons of different COVID-19 patient groups (Fig. 4 and Supplementary Figure S6). Then, heatmap was used to evaluate inter- and intra-patient group differences in the expression pattern of individual genes, as well as potential associations among the investigated genes (Fig. 5). Lysosomal and mitochondrial dysfunction appears to be the predominant PD pathological pathway in outpatients and non-ICU patients compared to SARS-CoV-2 negative individuals (Fig. 4). This is demonstrated by the profound statistical downregulation of genes associated with mitochondrial and lysosomal function including *TOMM20*, *TIMM21*, and *VPS35* with outpatients taking the lead (Fig. 4). Interestingly, these three genes are closely clustered together with negative individuals having the highest expression followed by ICU patients while outpatients and non-ICU patients had the lowest expression with outpatients leading the pack (Fig. 5). The lysosomal and mitochondrial dysfunction pathway initially triggered in the outpatient and non-ICU patient groups was abolished in the ICU patient group (Fig. 4). This abolishment appears to have been accomplished by the activation of the protein clearance pathways, specifically, the ubiquitin–proteasome system and autophagy-lysosome system (Fig. 4). This is demonstrated by the upregulation of *UCHL1* and *MAP1LC3B2* genes in ICU patients compared to the negative individuals (Fig. 4). Other genes including *GAK*, *SYNJ1*, *BST1*, *LRRK2*, *SLC6A3*, and *SNCG* were also upregulated in ICU patients (Fig. 4). Intriguingly, *LRRK2*, *BST1*, *SLC6A3*, *MAP1LC3B2*, *SYNJ1*, and *GAK* are closely clustered together and there appears to be some level of uniform expression pattern for these genes across the patient

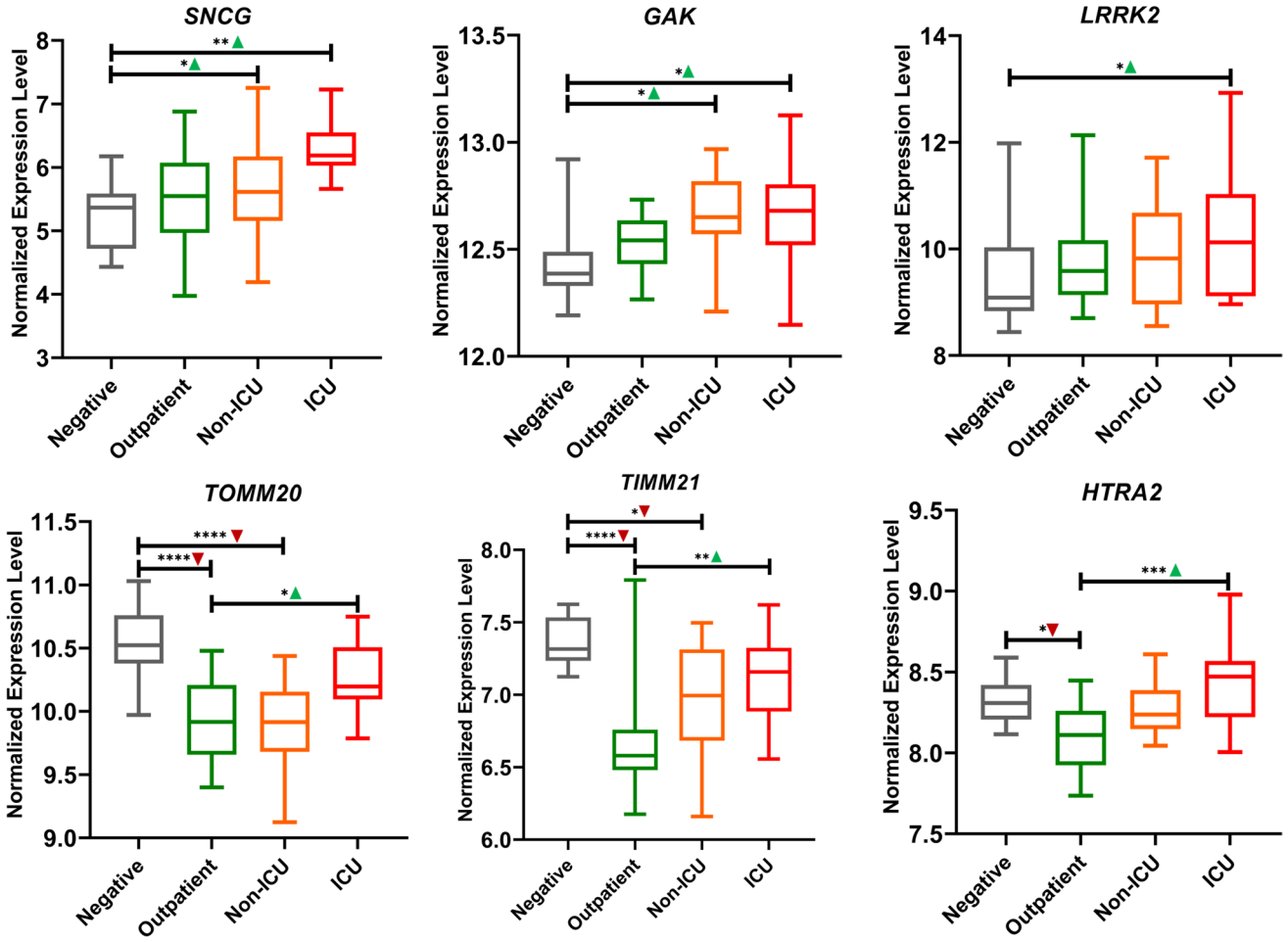

Fig. 1 Transcriptomics analyses comparing gene expression linked to α-synuclein pathology and mitochondrial dysfunction in nasopharyngeal swabs of non-COVID-19 and COVID-19 individuals. The normalized gene expression levels of selected genes associated with α-synuclein pathology and mitochondrial dysfunction were determined by RNA-seq analyses of nasopharyngeal swabs from 50 COVID-19 patients (17 outpatients, 16 non-ICU patients, and 17 ICU patients), along with a comparison to 13 SARS-CoV-2 negative individuals. A box plot is used to illustrate the results. The interquartile range, or range between the first and third quartiles, is shown by the box in the plot, while the whiskers extend to the minimum and maximum values. The median is indicated by the line inside the box. Adjusted p-values less than 0.05 is indicated (*, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$)

groups (Fig. 5). Similarly, *SNCG* and *UCHL1* are more closely clustered together and also have some level of uniform expression pattern (Fig. 5). When compared to outpatients, ICU patients demonstrate restored mitochondrial and lysosomal functions through the upregulation of *HTRA2*, *TIMM21*, *VPS35*, *TOMM20*, and *LAMP1* (Fig. 4). However, *SLC6A3* was also upregulated and was clustered with genes that appeared to be linked with protein clearance pathways (Figs. 4 and 5). The following genes were clustered together and appeared to have some level of expression pattern across the different patient groups: *HTRA2* and *LAMP1*; *TOMM20*, *TIMM21,* and *VPS35* (Fig. 5). No significant dysregulation was observed for the following comparisons: all COVID-19 positives vs negatives, outpatients vs non-ICUs, and non-ICUs vs ICUs (Fig. 4 and Supplementary Figure S6).

Discussion

In this study, COVID-19-mediated interactions with 43 PD pathogenic genes in nasopharyngeal swab samples from patients with varying disease severity levels were examined. The clinical cohort included 17 outpatients, 16 non-ICU patients, 17 ICU patients, and 13 SARS-CoV-2 negative individuals from the Greater Toronto Area. Given that the nasal cavity is the primal target site for the early stages of both PD and SARS-CoV-2 infection, it was necessary to see whether nasopharyngeal swab expresses clinically relevant pathogenic genes associated with PD to inform its potential utility for early PD diagnosis, disease mechanism studies, investigation of COVID-19 mediated interactions with PD, and testing of potential disease-modifying therapies.

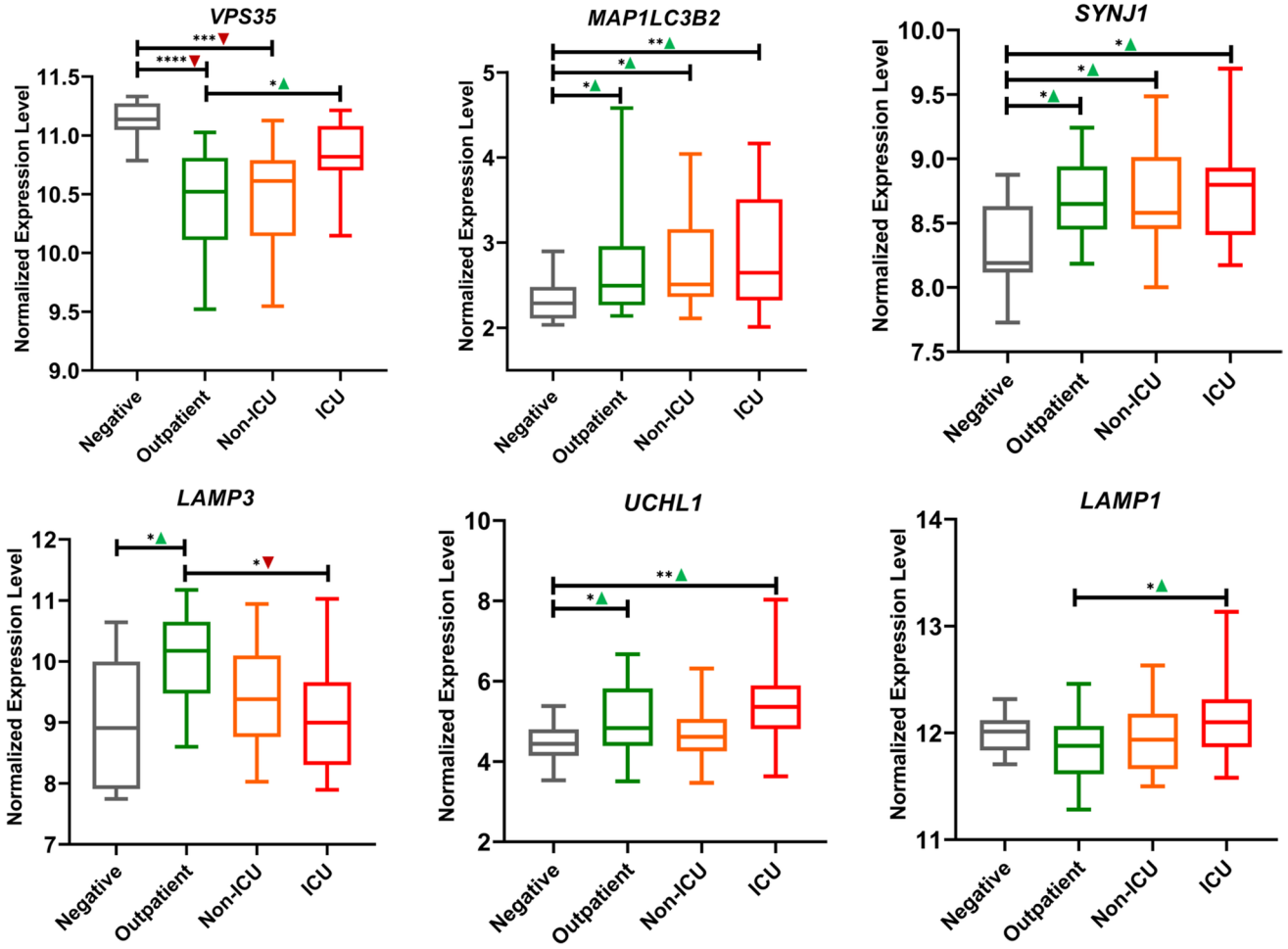

Fig. 2 Transcriptomics analyses comparing gene expression linked to dysfunctional protein clearance systems in nasopharyngeal swabs of non-COVID-19 and COVID-19 individuals. The normalized gene expression levels of selected genes associated with dysfunctional protein clearance systems were determined by RNA-seq analyses of nasopharyngeal swabs from 50 COVID-19 patients (17 outpatients, 16 non-ICU patients, and 17 ICU patients), along with a comparison to 13 SARS-CoV-2 negative individuals. A box plot is used to illustrate the results. The interquartile range, or range between the first and third quartiles, is shown by the box in the plot, while the whiskers extend to the minimum and maximum values. The median is indicated by the line inside the box. Adjusted p-values less than 0.05 is indicated (*, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$)

To the best of current knowledge, this is the first study to investigate the expression of genes associated with the pathological pathway of PD in nasopharyngeal swab samples, as well as the interplay between COVID-19 disease severity levels as demonstrated by hospitalization status and the expression of PD pathogenic genes. This study has uncovered for the first time the expression of major genes linked to PD pathological pathways in nasopharyngeal swab samples. Recently, the nasopharyngeal expression of major drug metabolizing enzymes and membrane transporters involved in processing commonly prescribed drugs, as well as those recommended by the United States Food and Drug Administration for routine assessment of investigational new drugs, was demonstrated by our group (Nwabufo et al. 2024). Of interest is the nasopharyngeal expression of two important membrane efflux transporters, P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) which often play a synergistic role in restricting the penetration of neurotherapeutic agents across the blood–brain barrier (Hou et al. 2012; Liu et al. 2014; Nwabufo 2022; Nwabufo and Aigbogun 2022; Nwabufo et al. 2024). Beyond impacting the disposition of neurotherapeutic agents, P-gp and BCRP are also implicated in the neuropathology of PD (Bartels et al. 2008; Zlokovic 2008), and our group recently showed that P-gp is downregulated in the nasopharyngeal swab of COVID-19 patients (Nwabufo et al. 2024). Moreover, PD pathology initially manifests in the olfactory nucleus and brainstem, progressing in sequential stages to affect the substantia nigra before spreading to other regions of the brain (Braak et al. 2003). Altogether, this indicates that

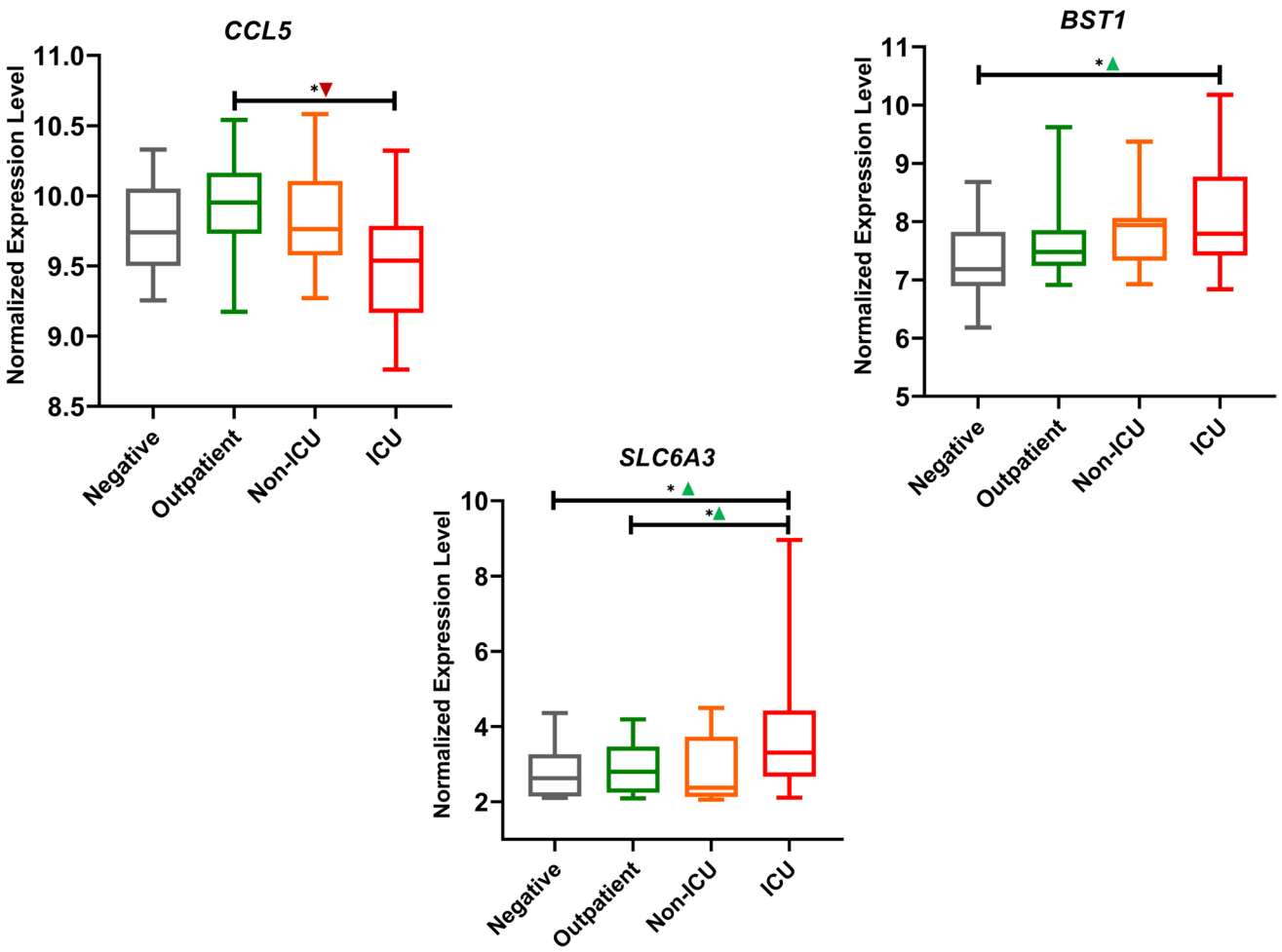

Fig. 3 Transcriptomics analyses comparing gene expression linked to inflammatory reactions and Parkinson's disease-associated pharmacogene in nasopharyngeal swabs of non-COVID-19 and COVID-19 individuals. The normalized gene expression levels of selected genes associated with inflammatory reactions and Parkinson's disease-associated pharmacogene were determined by RNA-seq analyses of nasopharyngeal swabs from 50 COVID-19 patients (17 outpatients, 16 non-ICU patients, and 17 ICU patients), along with a comparison to 13 SARS-CoV-2 negative individuals. A box plot is used to illustrate the results. The interquartile range, or range between the first and third quartiles, is shown by the box in the plot, while the whiskers extend to the minimum and maximum values. The median is indicated by the line inside the box. Adjusted p-values less than 0.05 is indicated (*, $p < 0.05$)

nasopharyngeal swab samples may be used for studying PD pathology, early diagnosis, and evaluation of neurotherapeutic agents, potentially addressing the longstanding issue of inadequate models for studying PD (Nwabufo and Aigbogun 2022). Nonetheless, further studies are required to validate the correlation between PD pathology in nasopharyngeal swabs compared to the central nervous system.

From a pathological standpoint, there appears to be a convergent network of interactions amongst multiple pathways including neuroinflammation, AS misfolding and aggregation, mitochondrial dysfunction, and dysfunctional protein clearance processes in PD (Coukos and Krainc 2024). In PD, mutations and environmental factors trigger AS aggregation, disrupting cellular processes like protein trafficking and lysosomal function (Outeiro and Lindquist 2003; Cooper et al. 2006; Xilouri et al. 2009; Mazzulli et al. 2011; Bellucci et al. 2011), making AS a putative target for the development of disease-modifying treatments (Kakish et al. 2016; Nwabufo et al. 2019, 2021; Aigbogun et al. 2022). Dysfunctional lysosomes and mitochondria impair the removal of damaged organelles and cytosolic components, leading to AS accumulation in Lewy bodies (Chen et al. 2015; Shahmoradian et al. 2019). In this current study, it is evident that dysfunctional lysosomes and mitochondria are the major convergent pathways implicated in COVID-19-mediated interactions with PD pathological pathways in both outpatients and non-ICU COVID-19 patients, with the effect being more statistically pronounced in outpatients (Fig. 4). On the contrary, the dysfunctional lysosomes and mitochondria observed in both outpatients and non-ICU patients appear to be abrogated by

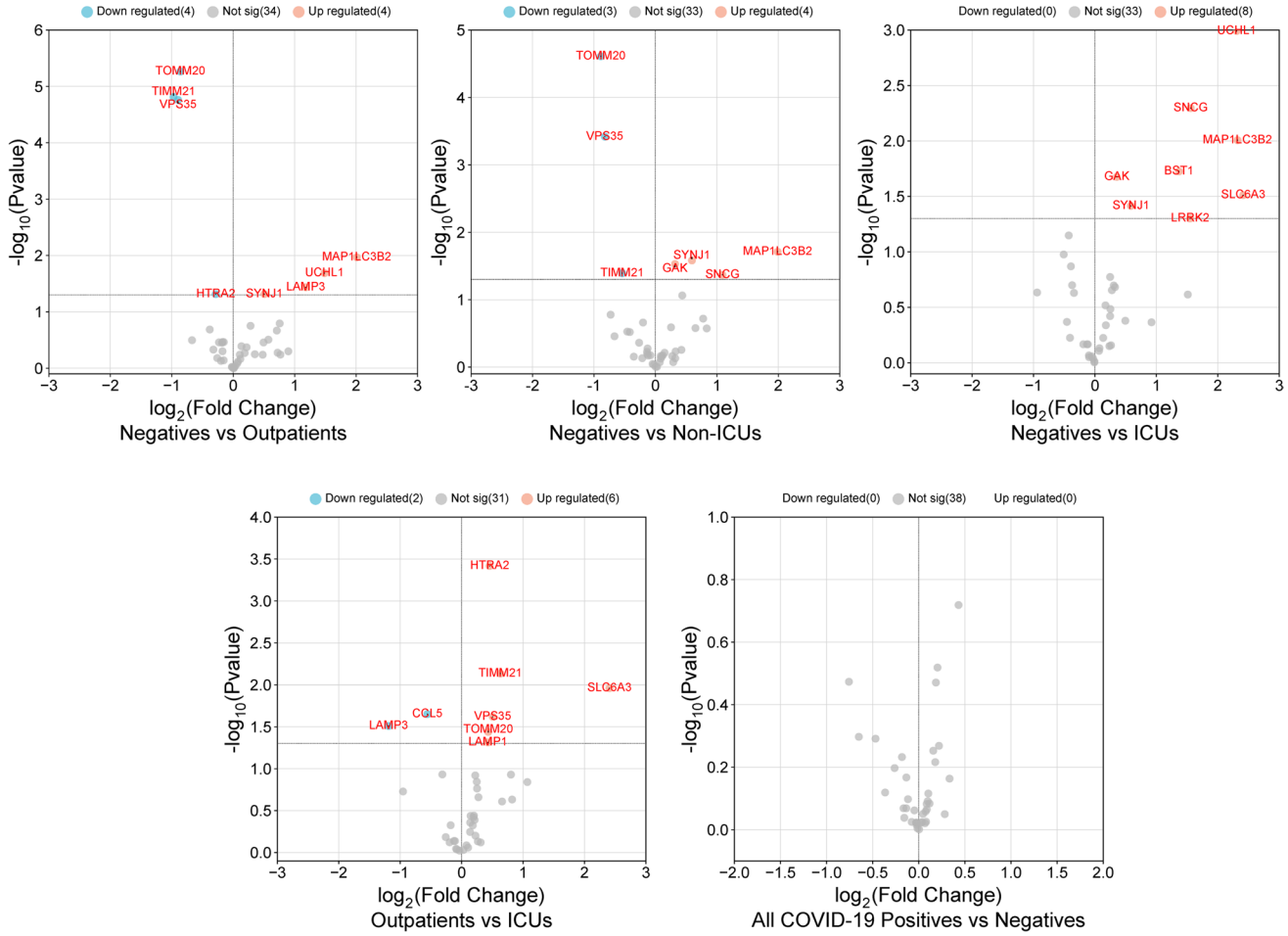

Fig. 4 Volcano plot representing differential gene expression for the investigated Parkinson's disease-associated pathogenic genes in nasopharyngeal swabs of non-COVID-19 and COVID-19 individuals. The differential gene expression results of the investigated Parkinson's disease-associated pathogenic genes were determined by RNA-seq analyses of nasopharyngeal swabs from 50 COVID-19 patients (17 outpatients, 16 non-ICU patients, and 17 ICU patients), along with a comparison to 13 SARS-CoV-2 negative individuals. Five comparative analyses of 43 gene expressions associated with Parkinson's disease pathological pathways including AS misfolding and aggregation, mitochondrial dysfunction, dysfunctional protein clearance pathways, inflammation, and pharmacogenetics were performed using DESeq2 as follows: negatives versus outpatients, negatives versus non-ICUs, negatives versus ICUs, outpatients versus ICUs, and all COVID-19 positives versus negatives. SRplot was used to plot the volcano, and a foldchange threshold of 1 was used. A gene is represented by each point on the figure, with the y-axis denoting statistical significance (such as a -log10 adjusted p-value) and the x-axis showing the log2 fold change. Red indicates upregulated genes, blue indicates downregulated genes, and gray indicates non-significant genes

the upregulation of ubiquitin–proteasome and autophagy-lysosome protein clearance pathways (Fig. 4).

Mitochondrial dysfunction is characterized by altered intrinsic homeostasis which plays a critical role in the pathophysiology of PD (Franco-Iborra et al. 2018). The mitochondrial function relies on importing nuclear-encoded proteins through two key complexes: the translocase of the outer membrane (TOM20) complex and the translocase of the inner membrane (TIM23) complex (Shiota et al. 2015; Wiedemann and Pfanner 2017). TIM21, a component of the TIM23 complex, bridges TOM20 and TIM23 by binding to TOM22 in the intermembrane space (Albrecht et al. 2006). Previous in vitro and in vivo studies showed that complex I inhibition impaired mitochondrial protein import by downregulating TOM20 and TIM23 protein expression, with the consequential in vitro accumulation of aggregated proteins inside mitochondria and the downregulation of mitochondrial chaperones (Franco-Iborra et al. 2018). However, in vitro overexpression of TOM20 or TIM23 abolished most of the pathological changes such as mitochondrial dysfunction and dopaminergic cell death (Franco-Iborra et al. 2018). Similarly, an *ex-vivo* study confirmed the decreased expression of TIM23 and TOM20 protein levels in substantia nigra protein homogenates from PD patients compared to age-matched controls (Franco-Iborra et al. 2018). Interestingly, downregulation of *TOMM20* and *TIMM21* was observed

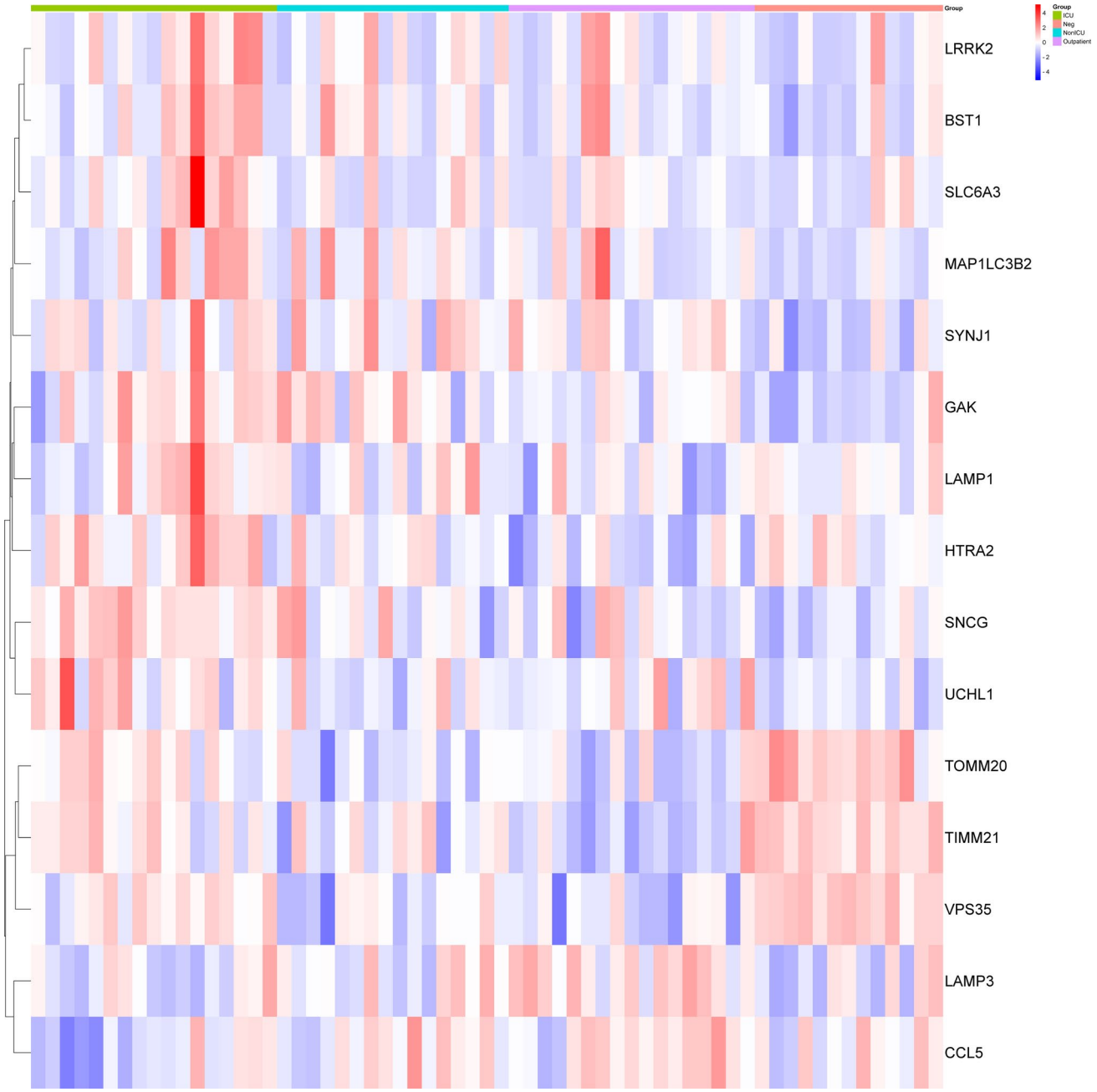

Fig. 5 Heatmap illustrating differentially expressed Parkinson's disease pathogenic genes in nasopharyngeal swabs of non-COVID-19 and COVID-19 individuals. The normalized gene expression levels of the investigated Parkinson's disease-associated pathogenic genes were determined by RNA-seq analyses of nasopharyngeal swabs from 50 COVID-19 patients (17 outpatients, 16 non-ICU patients, and 17 ICU patients), along with a comparison to 13 SARS-CoV-2 negative individuals. Individual study participants are represented by the columns, and each row corresponds to a gene. The color intensity reflects the normalized expression level, with warmer colors (red) denoting upregulated expression and cooler colors (blue) denoting downregulated expression. There is also a color scale bar with red standing for high expression levels, white for intermediate expression, and blue for low expression levels. This demonstrates the unique gene expression patterns found in the various study groups

in both outpatients and non-ICU patients in a synergistic manner although *TOMM20* downregulation was more pronounced in non-ICU patients compared to *TIMM21* (Figs. 1 and 4). However, the compromised *TOMM20 -TIMM21* import machinery appears to be fairly rescued in the ICU patients, in a manner consistent with the strong upregulation of the mitochondrial protease chaperone, *HTRA2* in ICU patients (Figs. 1 and 4). In corroboration with the findings

of this present study, a recent study found that SARS-CoV-2 infection leads to reduced expression of nuclear-encoded and mitochondrial-encoded mitochondrial genes, resulting in mitochondrial dysfunction (Guarnieri et al. 2023). This was observed in nasopharyngeal samples and autopsy tissues from COVID-19 patients, as well as in tissues from SARS-CoV-2-infected hamsters and mice (Guarnieri et al. 2023).

VPS35 was downregulated in both outpatients and non-ICU patients (Figs. 2 and 4) indicating dysfunctional protein clearance pathways, particularly, the autophagy-lysosome pathway. VPS35, a component of the retromer complex, plays a key role in endosomal protein sorting and trafficking, an essential component of the lysosomal protein clearance pathway (Zavodszky et al. 2014; McGough et al. 2014). Complete deletion of VPS35 results in abnormalities in lysosomal structure, function, and protein composition due to the buildup of incorrectly sorted proteins from the endomembrane system (Daly et al. 2023). Furthermore, the D620N mutation in *VPS35* causes late on-set PD (Vilariño-Güell et al. 2011; Zimprich et al. 2011). A recent study showed that SARS-CoV-2's ORF3a protein regulates and alters late endosomes, and lysosomal positioning and function (Walia et al. 2024).

On the contrary, the protein clearance pathway was restored in ICU patients with the upregulation of *UCHL1*, a marker of the ubiquitin–proteasome pathway (Figs. 2 and 4). Dysregulation of *UCHL1* is implicated in the pathophysiology of PD. The mRNA and protein expression levels of UCHL1 were reduced in the brains of idiopathic PD patients (Choi et al. 2004; Barrachina et al. 2006). *UCHL1* knockout mice exhibited complete degeneration of presynaptic terminals at neuromuscular junctions, compromised synaptic transmission, as well as gradual paralysis, and premature mortality (Chen et al. 2010). Furthermore, increased *MAP1LC3B2* (which encodes LC3-II) expression levels in ICU patients (Figs. 2 and 4) reflect an increase in the number of autophagosomes, supporting the activation of the autophagy-lysosome system (Barth et al. 2010).

Intriguingly, *SNCA* and *SNCB* expression were not significantly affected across the COVID-19 patient groups compared to SARS-CoV-2 negative individuals (Supplementary Figure S1). However, *SNCG* expression was upregulated in both non-ICU and ICU patients with ICU patients taking the lead (Figs. 1 and 4). Compared to *SNCA* and *SNCB*, *SNCG* has the highest relative expression in the substantia nigra across brain regions despite having limited pathological involvement with PD (Brás et al. 2021). Pathological accumulation of *SNCG* has been found in the brains of PD patients but not in the brains of controls and other neurodegenerative disorder patient groups (Galvin et al. 1999). Furthermore, overexpression of mouse SNCG resulted in SNCG aggregation, neuronal loss, motor deficits, and premature death, consistent with the pathophysiology and symptomology of PD (Ninkina et al. 2009; Peters et al. 2012). Although the physiological function of SNCG is unknown (Patel and Bordoni 2024), the findings from this study suggest a potential role in driving protein clearance pathways, particularly the ubiquitin–proteasome system and autophagy-lysosome system. This speculation arises from observations of its co-expression pattern with genes associated with protein clearance in ICU patients (Figs. 4 and 5). The same applies to *GAK*, *SYNJ1*, *BST1*, *LRRK2*, and *SLC6A3* (Figs. 4 and 5). In PD, robust connections have been established through numerous GWAS and subsequent post-GWAS analyses for genes such as *SNCA*, *RAB29*, *MAPT*, *BST1*, *GAK*, *LRRK2*, and *HLA-DRB5*, among others (Escott-Price et al. 2015; Nalls et al. 2019). *GAK*, for instance, plays a role in synaptic endocytosis, bridging this pathway in sporadic PD to the rare forms associated with *DNAJC6* and *SYNJ1* monogenic PD (Day and Mullin 2021). It is fascinating how certain genes have both common variants linked to higher sporadic disease risk and rare variants causing monogenic PD, hinting at shared biological pathways (Day and Mullin 2021; Coukos and Krainc 2024).

Evidently, the dysregulation of PD pathogenic genes by COVID-19 coupled with validated conventional genetic abnormalities associated with PD pathogenesis highlights the importance of personalized treatment for PD patients with COVID-19 comorbidities, as well as COVID-19 patients at risk of new-onset PD. Various genetic variations linked to dopamine action and metabolism impact medication responses and side effects in PD (Day and Mullin 2021). These variations involve genes such as dopamine receptors (*DRD*), dopamine transporter (*SLC6A3*), and catechol-O-methyltransferase (*COMT*) (Contin et al. 2005; Corvol et al. 2011; Kraemmer et al. 2016). Interestingly, *SLC6A3* was upregulated in ICU patients (Figs. 3, 4, and 5), suggesting potential COVID-19-gene-drug interactions for ICU patients with PD comorbidity characterized by *SLC6A3* pharmacogenetic liability. Our group have provided guidance on how to address disease-mediated interactions with drugs (Nwabufo and Bendayan 2022; Nwabufo et al. 2023; Nwabufo 2023) and recommend that healthcare team members consider this at the point of care when treating COVID-19 patients with PD comorbidity or new on-set PD.

Study limitations

The interpretation of the outcome of this study should be considered alongside its limitations. The sample size is relatively small, and full clinical information on all the subjects were not available. Although the available clinical information for these patients indicates that none of them have PD, other comorbidities these patients may have, as well as any potential underlying PD genetic liabilities and ongoing

medications may impact the expression of the investigated genes. However, the absence of abnormal expression patterns in the investigated genes, as depicted in the heatmap (Fig. 5), suggests that dysregulation patterns for these genes are not solely driven by the expression level of a single patient. Nonetheless, future studies should investigate the potential role of age, sex, and comorbidities in driving the interaction between COVID-19 and PD. As shown in Table 2, ICU patients exhibited a higher burden of comorbidities and were receiving multiple medications, both of which may influence gene expression patterns. Therefore, future studies should further investigate the potential impact of ongoing medication on the observed gene expression changes in this patient population.

Conclusions

This study shows that COVID-19 mediates interactions with PD pathological genes differentially for patients with varying levels of disease severity. The study showed that the convergent network of COVID-19-PD interactions was dysfunctional lysosomal and mitochondrial processes in outpatients and non-ICU COVID-19 patients. These dysfunctions were later abrogated by the upregulation of the protein clearance pathways, particularly, the ubiquitin–proteasome system and autophagy-lysosome system in ICU COVID-19 patients. A potential synergistic co-expression and clustering of protein clearance pathway genes with other pathological genes, specifically, *GAK*, *SYNJ1*, *BST1*, *LRRK2*, *SLC6A3*, and *SNCG* was observed in ICU COVID-19 patients, suggesting a potential overlap in biological pathways. Given the underlying genetic liabilities, as well as COVID-19-mediated dysregulation of some PD pathological genes such as *SLC6A3*, personalized treatment strategies for COVID-19 patients with PD comorbidities or new on-set PD is recommended to mitigate potential adverse drug effects or limited drug response that may be caused by COVID-19-gene-drug interactions. The outcome of this study have shown that nasopharyngeal swabs express major PD pathological genes as well as clinically relevant drug processing genes including drug metabolizing enzymes and membrane transporters, making it a potential specimen for investigating PD diagnosis, understanding its pathogenesis, and assessing the effectiveness of potential treatments aimed at modifying the course of the disease. Further research with a large sample size and age/sex-matched patient groups will help validate the identified interactions between COVID-19 and PD pathological pathways and clarify the cellular network in the nasopharyngeal swab responsible for the observed expression of PD-related pathogenic genes. Given that PD is a slowly progressive neurodegenerative disorder and COVID-19 is a relatively acute illness, a longitudinal study is essential to determine whether the observed changes from this present study may contribute to the eventual development of new-onset PD.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11481-025-10215-4.

Author contributions CKN: Conceptualization, methodology, data curation, formal analysis, visualization, software, writing – original draft, writing – review and editing, funding acquisition, and resources.

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Data availability All relevant data are presented in the manuscript and the associated transcriptomics data is freely available at https://figshare.com/articles/dataset/Comparative_transcriptomic_analysis_of_nasopharyngeal_swabs_from_individuals_with_and_without_COVID-19/22704403/1.

Declarations

Ethics approval and consent to participate All the four participating TIBDN hospitals approved the research (REB# 2024–0233-1025, MED-02–011, SUN-5024, and 084–0209-Lab-001), and all patients provided informed consent.

Conflict of interests CKN was a former employee of Gilead Sciences and was involved in the development of remdesivir. CKN is employed by OneDrug Inc. The author does not have any conflicting interests associated with this research.

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