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Short communication

Human coronaviruses in idiopathic Parkinson's disease: Implications of SARS-CoV-2's modulation of the host's transcriptome

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A B S T R A C T

Objective: A recent study on the effects of SARS-CoV-2 infection on the host's transcriptome indicated the perturbation of several pathways associated with neurodegeneration, including but not limited to Parkinson's and Huntington's diseases. The purpose of this study was to determine overlapping pathways between iPD vs. Controls and those associated with SARS-CoV-2 infection.
Methods: Gene set enrichment analyses (GSEA) were performed on gene expression data from tissues donated by idiopathic Parkinson's disease patients (iPD). These included dorsal motor nucleus of the vagus (DMNV), substantia nigra (SN), whole blood (WB) and peripheral blood mononuclear cell samples (PBMC). Enriched pathways detected by GSEA results were subsequently compared to (a) those retrieved by two independently constructed SARS-CoV-2 – host interactomes, as well as (b) previously published pathway data. For all analyses, a false discovery rate (FDR) <0.05 was considered statistically significant.
Results: Analysis of iPD data revealed multiple immune response and viral parasitism -related pathways (FDR < 0.05). Head-to-head comparisons as well as confirmatory analyses revealed several pathways and gene ontology (GO) terms overlapping between iPD tissues and SARS-CoV-2 induced transcriptomic changes: "Parkinson's Disease" and "Huntington's Disease" (overlapping in DMNV, ION, SN, and WB; FDR < 0.05), "NAFLD" (overlapping in DMNV, SN, PBMC and WB; FDR < 0.05), mRNA surveillance and proteostasis pathways (All datasets; FDR < 0.5), among others.
Conclusion: The overlap noted in this comparative transcriptomic study outlines the potential contribution of human coronaviruses in the pathogenesis of iPD. Furthermore, given SARS-CoV-2's neuroinvasive potential, closer scrutiny is warranted towards its contribution in the long-term development of neurodegenerative disease.

1. Introduction

Since its emergence as a human pathogen, SARS-CoV-2's neurotropism has been the subject of several clinical and translational studies. (Lu et al., 2020; Ramani et al., 2020) A recent study by Bojkova and colleagues(Bojkova et al., 2020) was the first to provide a comprehensive atlas of SARS-CoV-2 acute infection transcriptome and translatome alterations induced on the host cell. Their results included perturbed pathways associated with neurodegenerative disease (Parkinson's and Huntington's disease), non-CNS disease associated with neurodegeneration (Non-alcoholic fatty liver disease; NAFLD) as well as neurodegenerative disease-associated pathways involved in mRNA surveillance, ribosome and proteostasis and proteostasis, mitochondrial stress, and endoplasmic reticulum (ER) trafficking.

On the premises of (a) the clinical and molecular correlates between idiopathic Parkinson's disease (iPD) and these pathways(Chi et al., 2018) (b) SARS-CoV-2 neurotropism and potential CNS entry via the olfactory nerve(Vavougios, 2020a) (c) known associations between human coronaviruses (HCoVs) and neurodegeneration,(Dube et al., 2018) this study aimed to perform a head to head comparison between iPD and SARS-CoV-2 transcriptomics in search for overlapping, perturbed pathways.

2. Materials and methods

2.1. Curated idiopathic Parkinson's disease datasets from gene expression omnibus

For this study, gene set enrichment analyses (GSEA) previously performed on curated, gene expression omnibus (GEO) datasets (presented as iPD vs. control samples) GSE19587 (Dorsal motor nucleus of the vagus; DMNV - 6 vs. 5; Inferior olivary nucleus; ION – 6 vs. 5), GSE7261 (Substantia Nigra; SN - 16 vs. 9), GSE6613 (Whole Blood; WB – 50 vs. 22), GSE54536 (Peripheral Blood Mononuclear Cells; PBMC – 4 vs. 4); data available from DOI:10.17632/tg37fc2s3y.2).

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2.2. Head-to-head comparisons and comparative transcriptomics between SARS-CoV-2 – host pathways and iPD

For SARS-CoV-2 transcriptomics, significantly enriched pathways in iPD datasets were compared head to head with previously identified pathways from an in vitro experiment of SARS-CoV-2 infection.(Bojkova et al., 2020) Genes comprising these pathways (henceforth named Interactome A; I_A), as well as a gene signature of predicted SARS-CoV-2 interactors (henceforth named Interactome B; I_B)(Guzzi et al., 2020) were subsequently used in confirmatory over-representation analysis (ORA). Both ORA and GSEA results were subsequently compared for identical and related (members of the same sub/super family) pathways.

All analyses were performed by the GeneTrail 2.0 web service, (Stockel et al., 2016) with False Discovery Rates (FDRs) <0.05 considered statistically significant.

3. Results

Comparative transcriptomics revealed several identical and related GSEA-derived pathways overlapping across iPD blood, CNS and the SARS-CoV-2 human interactome ORA analyses (Tables 1 and 2).

3.1. Significantly enriched pathways related to immunity, response to infection and dysproteostasis in the iPD datasets

GSEA of iPD tissues revealed multiple significantly enriched

Table 1
Convergent, significantly enriched pathways between peripheral blood and the CNS (i.e. DMNV) across the SARS-CoV-2 perturbed pathways (Bojkova et al., 2020) and iPD datasets.

Gene Ontology Term	Database	GSE19587		GSE7621	GSE6613	GSE54536
SARS-CoV-2 Human Translatomics		**DMNV**	**ION**	**SN**	**WB**	**PBMC**
Golgi-ER-trafficking	GOBP	R	R	R	R	R
Base excision repair	GOBP	0	0	R	R	0
rRNA processing	GOBP	I	I	I	I	I
Nonsense mediated decay	GOBP	I	R	R	R	R
Translational elongation	GOBP	I	I	I	I	I
Translational initiation	GOBP	R	R	I	R	R
Mitotic metaphase and anaphase	GOBP	0	0	R	R	0
mRNA processing	GOBP	R	R	R	R	R
Regulation of mRNA stability	GOBP	I	I	I	I	I
nucleobase-containing compound biosynthetic process	GOBP	R	R	R	R	R
SRP-dependent cotranslational protein targeting to membrane	R / GOBP	I	I	0	I	I
Huntington's disease	KEGG	I	I	I	I	0
Parkinson's disease	KEGG	I	I	I	I	0
Non-alcoholic fatty liver disease (NAFLD)	KEGG	I	0	I	I	I
Eukaryotic translation termination	RC	I	0	0	I	0
Selenoamino acid metabolism	RC	R	0	0	R	R
Signaling by ROBO receptors	RC	0	0	0	0	0
Ribosome	RC	0	0	0	0	0
MHC class II antigen presentation	RC	0	I	I		0
Asparagine N-linked glycosylation	RC	0	0	0	0	0
COPI-mediated anterograde transport	RC	I	0	I	0	0
Mitotic prometaphase	RC	0	I	0	R	0
HSP90 chaperone cycle	RC	0	0	0	R	0
Spliceosome	RC	R	R	R	R	0

R represents related pathways (either members of the same sub / superfamily), whereas I denotes identical terms; 0 corresponds to null overlap. Black and white density for the heatmap have been allocated accordingly, with white to darker shade corresponding to the procession $I > R > 0$. GOBP: Gene Ontology Biological process; KEGG: Kyoto Encyclopedia of Genes and Genomes; RC: Reactome; RC: Reactome. DMNV: Dorsal motor nucleus of the vagues; ION: inferior olivary nucleus; PBMC: Peripheral blood mononuclear cells; WB: whole blood; iPD: Idiopathic Parkinson's Disease.

Table 2
Convergent, significantly enriched pathways between peripheral blood and the CNS across the SARS-CoV-2 perturbed pathways (Guzzi et al., 2020) and iPD datasets.

Gene Ontology Term	Database	GSE19587		GSE7621	GSE6613	GSE54536
SARS-CoV-2 Human Interactome-based pathways		**DMNV**	**ION**	**SN**	**WB**	**PBMC**
Ribosomal scanning and start codon recognition	RC	I	I	I	I	I
Translation initiation complex formation	RC	I	I	I	I	I
GTP hydrolysis and joining of the 60S ribosomal subunit	RC	I	I	I	I	I
L13a-mediated translational silencing of Ceruloplasmin expression	RC	I	I	I	1	I
Huntington's disease	KEGG	I	I	I	I	0
Influenza A	KEGG	0	0	I	0	I
Transcriptional misregulation in cancer	KEGG	0	0	I	0	0
Apoptosis	KEGG	0	0	I	0	0
Small cell lung cancer	KEGG	0	0	I	0	0
Oxidative phosphorylation	KEGG	I	I	I	I	0
MicroRNAs in cancer	KEGG	0	0	I	0	0
NF-kappa B signaling pathway	KEGG	0	0	I	0	I
Phagosome	KEGG	I	I	I	0	I
Epstein-Barr virus infection	KEGG	I	0	0	0	I
RNA transport	KEGG	I	0	0	I	0

"I" denotes identical terms; 0 corresponds to null overlap. Black and white for the heatmap have been allocated accordingly, with white to darker shade corresponding to the procession $I > 0$. GOBP: Gene Ontology Biological process; KEGG: Kyoto Encyclopedia of Genes and Genomes; RC: Reactome. DMNV: Dorsal motor nucleus of the vagues; ION: inferior olivary nucleus; PBMC: Peripheral blood mononuclear cells; WB: whole blood; iPD: Idiopathic Parkinson's Disease.

pathways associated with the immune response, viral and bacterial infection, and mRNA surveillance and dysproteostasis (See DOI:10.17632/tg37fc2s3y.2 for a complete list; FDR < 0.05). Viral infection pathways were salient in both CNS and peripheral blood pathways. Notably, known and predicted SARS-CoV-2 host interactors in the human transcriptome such as FYN and CTSB(Gkogkou et al., 2020; Iadecola et al., 2020) were also part of dysregulated gene networks in both CNS and peripheral blood datasets (FDR < 0.05).

3.2. Head to head comparisons with previously identified SARS-CoV-2 – induced pathways

Head to head comparisons between these aforementioned pathways and those extracted by in vitro SARS-CoV-2 host proteomics(Bojkova et al., 2020) revealed overlap between selenoaminoacid metabolism, translation surveillance and ER trafficking pathways. Significant overlap was identified in 4 out of 5 iPD tissues was noted for "NAFLD", "Parkinson's Disease" and "Huntington's disease" pathways, and variable overlap (up to 5 out of 5 iPD tissues) for translation surveillance pathways (Table 1).

3.3. Confirmatory ORA on SARS-CoV-2 in vitro and in silico interactomes reveals significantly enriched pathways associated with neurodegenerative disease

Confirmatory ORA of Interactome A confirmed these results and furthermore uncovered a novel 13 gene "Parkinson's Disease" signature; This signature was detected in peripheral blood, DMNV, ION, datasets (Supplementary Data 1 and Supplementary Table 1), comprised of genes associated with the pathogenesis of Parkinson's Disease.

Overlapping identical pathways between iPD and ORA analysis of Interactome B included ribosomal function, L13a-mediated translational silencing of Ceruloplasmin expression (5 out of 5 iPD tissues), oxidative phosphorylation and Huntington's disease (4 out of 5 iPD tissues; Table 2).

Furthermore, gene ontology – biological process derived, viral transcription related pathways were significantly enriched and overlapping across GSEA and ORA analyses (FDR < 0.05; Available from DOI:10.17632/tg37fc2s3y.2).

4. Discussion

Head to head comparisons between SARS-CoV-2 induced host transcriptomic modulations and those detected in iPD tissues revealed significant overlap in pathways associated with Parkinson's disease, Huntington's Disease, NAFLD, as well as pathways associated with translational surveillance and proteostasis.

Previous research has determined that the neurotropism of HCoVs such as the OC43 strain, extends from outside-in neuroinvasion to neuron-to-neuron dissemination via axonal transport.(Dube et al., 2018) As a case in point, OC43 may simultaneously induce apoptotic cascades and dysproteostasis (culminating in ER / misfolded protein stress), while.

paradoxically preserving neurons from ER/misfolded protein stress – related apoptotic cascades.(Desforges et al., 2014) In the setting of iPD, this particular latency mechanism would contribute to aSyn

accumulation in tandem with indolent neuroinflammation resulting from the delayed onset of apoptosis. At the time of writing, there were no reports of SARS-CoV-2 induced parkinsonism; three such cases have been reported to date,(Merello et al., 2020) confirming a phenotypic relationship between iPD and SARS-CoV-2 infection.

Conversely, the epigenetic control of intraneuronal copper may be another such pathway targeted by intracellular pathogens following neuronal egress. In the setting of iPD, loss of intraneuronal copper homeostasis and transcript surveillance are two interdependent mechanisms by which aSyn misfolding and aggregation can occur.(Vavougios, 2020b) A final interesting point is NAFLD, recently identified within the patterns of liver injury among COVID-19 patients(Ji et al., 2020) and identified as a pathway in the study by Bojkova et al.(Lu et al., 2020). NAFLD stands at the crossroads between diabetes, iPD and cardiovascular disease, with known yet not fully elucidated correlates with neurodegenerative disease.(Ghareeb et al., 2011) In the setting of iPD and HCoV's, the modulation of both macrophage phenotypes and fatty acid metabolism, with the latter being another finding by Bojkova et al.'s study.(Bojkova et al., 2020)

SARS-CoV-2's tropism for olfactory epithelium and the gut fulfills two of the original Braak's hypothesis(Braak et al., 2003) conditions for the pathogen – human interface initiating the presymptomatic phase of iPD. While its recent emergence precludes its identification as the elusive Braak's pathogen, Bojkova et al.'s study has nevertheless pointed towards the modus operandi of HCoVs for the candidates.

The mechanism by which HCoVs and SARS-CoV-2 specifically contribute to parkinsonism and iPD will likely involve either introducing or unmasking perturbations in genes associated with dysproteostasis, and specifically autophagy – mechanisms subverted by betacoronaviruses as a strategy of immunoevasion and latency. (Vavougios, 2020c) This concept would explain how host factors such as FYN and CTSB, physiologically mediating aSyn degradation,(Bellomo et al., 2020) would be subverted in viral processes. Notably, single nucleotide variations of both FYN and CTSB have been shown to confer increased risk of iPD.(Panicker et al., 2019) Other candidates, such as the 14-3-3 may also fit within this concept.

Currently, three lines of evidence on SARS-CoV-2's neurotropism: (a) an infection experiment on brain organoids indicating the priming of tau pathology,(Ramani et al., 2020) (b) the emergence of COVID-19 associated parkinsonism(Merello et al., 2020) and (c) olfaction and memory impairments with neuroanatomical correlates.(Lu et al., 2020) These studies suggest that SARS-CoV-2 may either introduce diffuse disorders of proteostasis not limited to aSyn (i.e. Aβ and Tau), or conversely, that it unmasks and accelerates pre-existing disorders on presymptomatic individuals. Overall, these points along with the results of this study suggest that closer scrutiny on the transcriptomic and genetic level is warranted in NeuroCOVID-19.

The results of this study should be interpreted within the context of its limitations. The data-driven hypothesis presented here is based on head to head comparisons of studies with different protocols (i.e. including inclusion criteria, sample procedures, array technology among others). In order to minimize the effect of their heterogeneity, data were reanalyzed and comparisons were performed at the last step, i.e. GSEA; furthermore, the use of public datasets and web-service analytical tools aimed to make the workflow of the study entirely reproducible. Another important consideration is the conceptual difference between Guzzi et al.'s SARS-CoV-2 signature, which was extracted by master regulator analysis vs. Bojkova et al.'s acute phase transcriptomics. The overlap between perturbed pathways from each study and the iPD datasets should thus be considered bearing in mind that iPD pathogenesis is an indolent process progressing over years or even decades. Thus, Guzzi et al.'s signature would cover the extent of perturbations, whereas Bojkova et al.'s assortment of pathways would represent the extent perturbations associated with the underlying process.

Summarizing the results of this study, perturbed pathways in Bojkova et al.'s SARS-CoV-2 effect on the human translatome indicated that the novel coronavirus may affect mechanisms involved in neurodegeneration in the setting iPD. Performing a confirmatory GSEA across iPD datasets and head-to-head comparisons with a different SARS-CoV-2 human interactome revealed several overlapping perturbed pathways.

The current study outlines a modus operandi, exemplified by SARS-CoV-2, that may produce a disease phenotype (i.e. parkinsonism, respiratory syndrome, gastroenteritis) by a "fixed" set of inflicting epigenetic modulations (i.e. epigenetic silencing of ceruloplasmin, elicitation of PD genes associated with the phagosome in the periphery and neuroprotection in the CNS) in different cell types (i.e. neurons, respiratory tract epithelia). Further targeted experiments are required to confirm these in silico findings, and examine the potential of an occult HCoV as the putative Braak's pathogen.

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Credit author statement

George D. Vavougios was the sole author of this study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable/Single Author.

Availability of data and materials

Not applicable.

Competing interests

None declared.

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Authors' contributions

Single Author.

Data availability

All datasets and gene signatures are publicly available. Analysis files not included here are available upon request. The entirety of the analyses is replicable.

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