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Keywords:	Parkinson's Disease, COVID-19, ANPEP, CD13

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Conflict of Interest:
Nothing to report.

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Main Text

The coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)[1], a new human coronavirus (hCoV) strain. The neuroinvasive potential of SARS-CoV-2[2] and recent research linking advanced age and neurological disease to COVID-19 severity[3] raises concerns for Parkinson's disease patients (PD), who appear particularly susceptible to worse outcomes from COVID-19[4].

Using a combination of publicly available and in-house RNAseq data, we compared the abundance of the SARS-CoV-2 entry factors ACE2 and TMPRSS2 between PD case and control whole blood (WB), frontal cortex (FC) and substantia nigra (SN) tissue. In addition, similar to others[5], we expanded our investigation to include other hCoV entry factors, including ANPEP (HCoV-229E), DPP4 (MERS-CoV) and ENPEP[6], all have been discussed as SARS-CoV-2 co-recpetors[6]. We hypothesised that the expression of hCoV receptor genes in PD patients differs from that of healthy individuals, potentially explaining the links between COVID-19 and worsened outcomes in PD.

*In line with available data (Genotype-Tissue Expression Project[7]), ACE2 was undetectable in WB in all cohorts. No expression was observed in SN tissue in PD cases or controls. Low ACE2 expression was observed in FC tissue, but PD cases and controls the difference was not statistically significant (**Fig 1a**), suggesting that ACE2 expression levels are not likely to be a significant contributor to the reported COVID-19 susceptibility in PD[4]. TMPRSS2 was also undetectable in WB and either brain tissue.*

DPP4 and ENPEP, although undetected in WB, may contribute to hCoV neuroinvasion[2] as both are expressed in FC and SN tissue, however, there was no significant difference in transcript abundance between PD and controls ($p>0.05$, data not shown). Finally, we did not detect any significant correlation between genes and no significant correlation between ACE2, DPP4, ENPEP expression and age in cases or controls in any of the tissues examined ($p>0.05$).

*Intriguingly, we saw significantly elevated ANPEP expression (adjusted $p<0.05$) in PD cases compared to controls in all WB cohorts and FC tissue (**Fig 1b-g**), but not in SN tissue (**Fig 1h**), although we found no correlation between ANPEP expression and age in PD cases or controls in any tissue ($p>0.05$). Interestingly, ANPEP levels were also significantly elevated in prodromal individuals compared to healthy controls ($p=1.8x10^{-8}$, **Fig 1i**).*

Currently, there no evidence that ANPEP, also known as CD13, plays a role in SARS-CoV-2 infection. It is a widely expressed membrane alanyl aminopeptidase. Increased expression is a hallmark of inflammation and has been observed in neurodegenerative disease[8] and reduction of ANPEP activity has been investigated as a possible anti-inflammatory therapy[9]. Thus, elevated ANPEP levels could be associated with the development of PD and conceivably, though its role in inflammation, contribute to the severity of SARS-Cov-2 infection.

We conclude that low ACE2 and a lack of TMPRRS2 expression in vulnerable tissues and WB supports reports that SARS-CoV-2 is rarely seen in the CNS. Further, our data suggest that elevated ANPEP levels may be important to the onset and progression of PD irrespective of COVID-18 and, although requiring further study, could modulate the severity of COVID-19.

Acknowledgments

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Author Roles

1. Research project: A. Conception, B. Organization, C. Execution;
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3. Clinical data: A: Collection, B. Organization;
4. Statistical Analysis: A. Design, B. Execution, C. Review and Critique;
5. Manuscript Preparation: A. Writing of the first draft, B. Review and Critique;

Hannah Lowes – 1B, 2A-B, 3B.
Fiona Robertson – 1C, 2B.
Angela, Pyle - 1B, 2A-B, 3B.
Rafiqul Hussain - 2A, 2B.
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Conflicts of Interest:

The authors declare that they have no competing financial interests.

Figure Legend

Figure 1| Whole blood and brain tissue gene expression in PD cases and controls. ***a)*** *ACE2 expression in FC tissue from 29 PD cases and 44 controls using RNAseq obtained from the European Nucleotide Archive (ENA-FC, p>0.05).* ***b-e)*** *ANPEP expression in WB using RNAseq obtained from the PPMI (where b=404 PD patients diagnosed <2 years versus 166 healthy controls (HC), $p=7.2x10^{-3}$, c= 171 patients harboring a mutation LRRK2, GBA, or SNCA (GENPD) versus 223 unaffected individuals who harbor a mutation in LRRK2, GBA (GENUN), $p=1.4x10^{-3}$, d= 172 patients who have or a first-degree relative with a mutation in LRRK2, GBA, or SNCA (REGPD) versus 147 unaffected individuals who have or a first-degree relative with a mutation in LRRK2, GBA, or SNCA (REGUN), $p=1.2x10^{-3}$, and e= all PPMI PD combined versus all controls combined (747 versus 536 , $p=1.7x10^{-8}$).* ***f)*** *ANPEP expression in WB using RNAseq data obtained from ICICLE-PD (24 PD versus 24 matched controls, $p=2.9x10^{-3}$).* ***g)*** *ANPEP expression in ENA-FC tissue (29 PD versus 44 controls, $p=2.1x10^{-7}$).* ***h)*** *ANPEP expression in SN tissue (7 PD cases versus 5 controls, p>0.05).* ***i)*** *ANPEP expression in WB from 56 prodromal individuals compared to PPMI-WB healthy controls (166 HC, $p=1.7x10^{-8}$). In all analyses, age at sampling, sex, postmortem interval (PMI) and RNA integrity number (RIN, where available) were evaluated for differences between groups and assessed as potential confounders in the analyses. Boxplots show median, 25th and 75th percentile (boxes) and 95th percentile (whiskers). *'s denote significance threshold, where ***=<10^{-3}, ****=<10^{-4} and *****=<10^{-5}. Red triangles indicate mean.*

References

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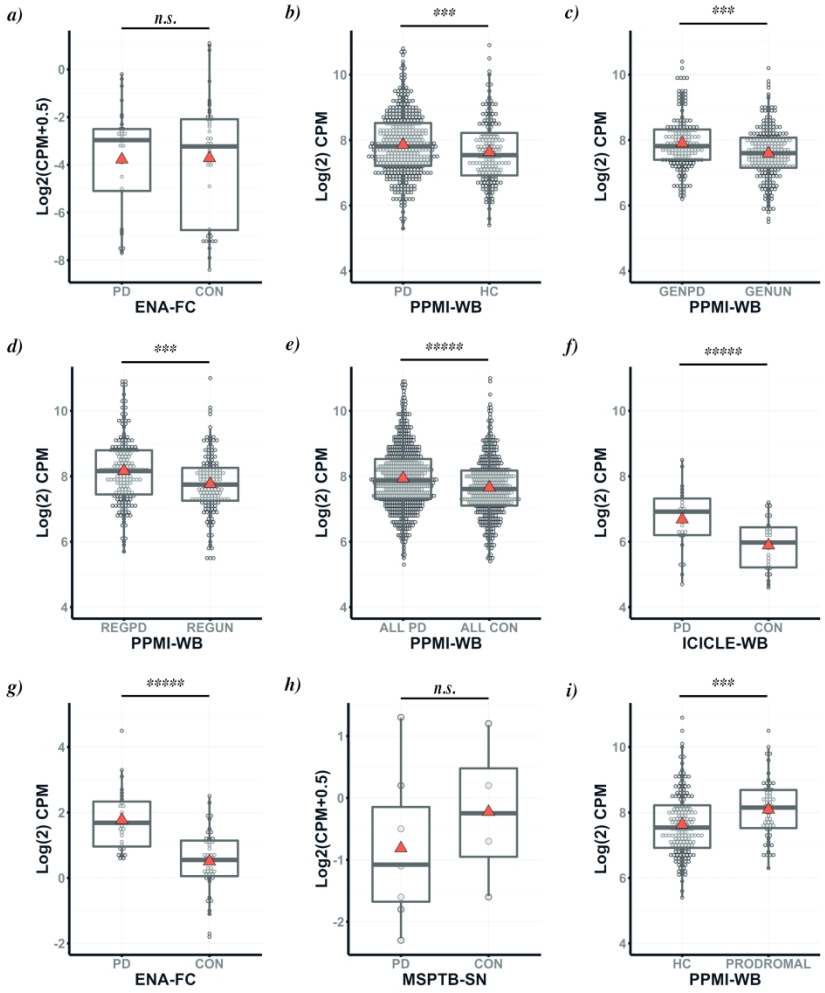

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