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Beyond dopamine: further evidence of cholinergic dysfunction in Parkinson's disease.

(Commentary on Keo et al. 2021)

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Differences in regional gene expression may partly explain the selective vulnerability of specific brain regions to neurodegeneration in Parkinson's disease. In this article by Keo et al. (2021), the authors examined differential regional gene expression in the anterior and posterior cingulate structural covariate networks (patterns of covariance of grey matter density) of the healthy human brain. They demonstrate a number of highly expressed genes in the cingulate networks which show reduced grey matter integrity in Parkinson's disease, compared to the rest of the cortex. They show that these highly expressed genes are enriched in pathways including synaptic transmission, as well as cholinergic cell markers. Although it is unclear whether this differential pattern of expression is also seen in Parkinson's disease brains, these findings highlight the potential importance of synaptic dysfunction and cholinergic transmission in Parkinson's disease.

Parkinson's disease (PD) affects brain structures unevenly, with some regions and cortical networks affected earlier and more frequently. The reason for this selective vulnerability of some regions over others are not yet clear, but may relate to underlying differences in regional gene expression and cell populations.

In this issue of EJN, Keo et al (Keo *et al.*, 2021) leverage this differential regional and network involvement by examining regional differences in underlying gene expression between two structural covariate networks (patterns of covariance in grey matter morphology) that show disproportionate grey volume atrophy in PD: the anterior and posterior cingulate networks.

The cingulate cortex has an established role in emotion, shifting attention and contextualising and selecting sensory information (Vogt, 2014). A growing body of evidence also suggests that both the anterior and posterior cingulate are preferentially affected in patients with PD and cognitive impairment: grey matter atrophy is seen in Parkinson's dementia within the posterior cingulate (Melzer *et al.*, 2012) and cingulate hypometabolism is correlated with executive function and visual hallucinations in PD (Lewis *et al.*, 2012; Wakamori *et al.*, 2014). We have also recently shown white matter degeneration within the cingulum in PD patients who are at higher risk of subsequent dementia (Zarkali *et al.*, 2021).

To investigate the biological processes and cell types that may be driving selective vulnerability of the cingulate cortex in PD, Keo et al (2021) examined the anterior and posterior cingulate structural covariate networks previously identified by de Schipper et al (de Schipper *et al.*, 2017) as showing reduced integrity in PD. Structural covariance networks are defined by between-region correlations in grey matter characteristics, in this case in grey matter density (de Schipper *et al.*, 2017). These networks are replicable and at least partly explained by patterns of regional gene expression (Alexander-Bloch *et al.*, 2013; Romero-Garcia *et al.*, 2018), although their neurobiological basis is not fully understood.

Using data from the Allen Human Brain Atlas (Hawrylycz *et al.*, 2015), Keo et al. (2021) compared gene expression in the healthy human brain between the two cingulate networks and the remaining seven structural covariance networks that are less affected in PD. They identified 144 genes that were highly expressed in both cingulate networks (200 highly expressed genes in the posterior cingulate and 269 highly expressed genes in the anterior cingulate network), but not in other networks. They then examined the function and cellular composition of these differentially over-expressed genes.

Keo et al. (2021) found that highly expressed genes in cingulate networks were enriched in pathway and gene ontology terms related to synaptic transmission, particularly neurotransmitter-mediated signalling. Alpha-synuclein is important in neurotransmitter release (Cabin *et al.*, 2002) and accumulation of alpha-synuclein oligomers has been associated with synaptic dysfunction (Diógenes *et al.*, 2012; Rockenstein *et al.*, 2014). Regional expression of synaptic transfer genes is correlated with regional grey matter atrophy in PD (Freeze *et al.*, 2018) and synapse-related genes are more highly expressed in regions affected early in Braak staging (Keo *et al.*, 2020). In addition, prior work from our group showed up-weighting of genes related to synaptic signalling and chemical synaptic transmission in regions showing loss of connectivity in Parkinson's patients at risk of dementia (Zarkali *et al.*, 2020) as well as in regions with increased cortical iron deposition in PD (Thomas *et al.*, 2021). In this way, regional variation in expression of genes relating to synaptic transmission is emerging as a common factor from studies, including by Keo et al. (2021), using varying technologies and acquiring imaging data from different PD cohorts, suggesting it is likely to be of significance in neurodegeneration in Parkinson's disease.

Interestingly, Keo et al. (2021) found enrichment of the differentially expressed genes in cholinergic cell markers. Although cell types were derived from mouse and not a purely single-cell dataset (Mancarci et al., 2017), limiting interpretability, this enrichment was consistently seen using two different analyses methods. Parkinson's disease is classically associated with altered dopaminergic transmission, but cholinergic cell involvement, particularly in relation to cognitive impairment is well recognised: the cholinergic neurons in nucleus basalis of Meynert in Parkinson's are involved early in PD (Candy et al., 1983; Hepp et al., 2017), cortical acetylcholine levels are reduced in patients with Parkinson's dementia (Mattila *et al.*, 2001; Hilker *et al.*, 2005; Hall *et al.*, 2014) and cortical nicotinic receptors are reduced in association with dementia severity in patients with PD (Meyer et al., 2009). The healthy cingulate cortex is particularly enriched in nicotinic receptors (Picard et al., 2013). The findings from Keo et al. (2021), suggest that cortical regions normally enriched in nicotinic receptors are particularly vulnerable in Parkinson's disease.

The highly-expressed genes in cingulate networks were identified by Keo et al. (2021) using differential gene expression analysis at the individual gene level. This approach, although appropriately corrected for multiple comparisons by the authors, does not take into account co-expression between genes with similar function (Oldham *et al.*, 2008) or genes with similar spatial patterning of expression (Hawrylycz *et al.*, 2015; Fulcher *et al.*, 2020) which could potentially influence the results. It is also important to note that gene expression data in this study by Keo et al. (2021) were derived from a small number (n=6) of relatively young donors without history of neurological or psychiatric disease (Hawrylycz *et al.*, 2015). Gene expression in PD may differ from the healthy brain. Further transcriptomic datasets integrating larger numbers of age-matched neuroimaging and gene expression profile data from PD patients and controls will help to further elucidate the role of regional gene expression in the selective vulnerability of specific brain regions in PD. Future single-cell transcriptome atlases would further enlighten the diversity of genomic signatures related to cell morphology that is lost when expression levels are aggregated across cell types. Despite these limitations, the study by Keo et al highlights the role of synaptic dysfunction and nicotinic transmission in PD, providing important mechanistic insights into non-dopaminergic PD neurodegeneration.

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