Gene expression network analysis of the gut-brain axis supports an association between alpha-synuclein and markers of enteric glial cells

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BACKGROUND

Parkinson's Disease (PD) & the gut-brain axis

- Lewy-type synucleinopathy (LTS) or aggregation of alpha-synuclein (aSyn, encoded by *SNCA*), is a pathological hallmark of PD
- LTS present in colon of PD patients[1-3]
- Constipation common in PD patients and present in animal models of PD
- Molecular mechanisms of the gut-brain axis in PD and effects of colonic aSyn are poorly understood

***In silico* exploration of the gut-brain axis**

- RNA-sequencing data from post-mortem human tissue samples is available (GTEx)[3]
- Weighted gene correlation network analysis (WGCNA)[5]:
  - Uses unsupervised clustering to identify modules of highly correlated genes
  - Each module represented by module eigengene (ME, first principle component)
  - *Hub genes*: high correlation with the ME
  - *Consensus network*: identifies modules present in two related datasets

AIMS

1. To perform WGCNA of sigmoid colon (SC) and cerebral cortex (CC) from the same individuals to identify: a) relationships between gut and brain modules and b) gut-brain consensus modules
2. To compare connectivity of aSyn-containing networks within brain and colon

METHODS

- GTEx data (gene-level counts) obtained from recount2 database,[6] were scaled, prepared for linear modeling using variance stabilization and quantile normalized using the recount, DESeq2 and limma packages in R
- Donors with data from both CC and SC were included (n=29 after removal of 5 outliers)
- WGCNA[5] was used to generate signed networks in CC and SC and consensus networks
- Associated MEs were correlated (Pearson) with an adjusted (Benjamini-Hochberg) p-value <0.05
- Overrepresented gene ontology (GO) terms were determined with the hypergeometric function in the GOstats package (R)

RESULTS

Colon & Brain specific networks

- Clustering of hub genes in CC and SC networks is shown in Figure 1A-B
- Intermodular connectivity is increased in the brain likely due to increased homogeneity of cell type (Figure 1A-B)
- Significant correlations between CC and SC modules (Figure 1C) are notable for a positive correlation of immune-related modules

Colon-Brain consensus network

- Relationships between the 15 consensus modules are shown in Figure 2
- Similar to organ-specific networks (Figure 1C), there is increased connectivity in the brain
- Intermodular connectivity differs in CC and SC, though some relationships are conserved

Connectivity of aSyn-containing module

- The aSyn module was differentially associated in SC (positively) vs CC (negatively) with the "Astrocyte differentiation" module (Figure 3)
- "Astrocyte differentiation" hub genes SRY-box 10 (*SOX10*) and S100 calcium binding protein B (*S100B*) are markers of both enteric glial cells (EGCs) and astrocytes
- Correlations of *SNCA* (gene encoding aSyn) with EGC markers (Table 1) mirrored module relationships
- *Additional EGC markers*: glial fibrillary acidic protein (GFAP) and proteolipid protein 1 (PLP1)
  - GFAP, which was not in the consensus modules (did not cluster similarly in brain and colon) was correlated with aSyn in brain but not colon (Table 1)
  - PLP1 on the X chromosome was excluded from clustering

RESULTS

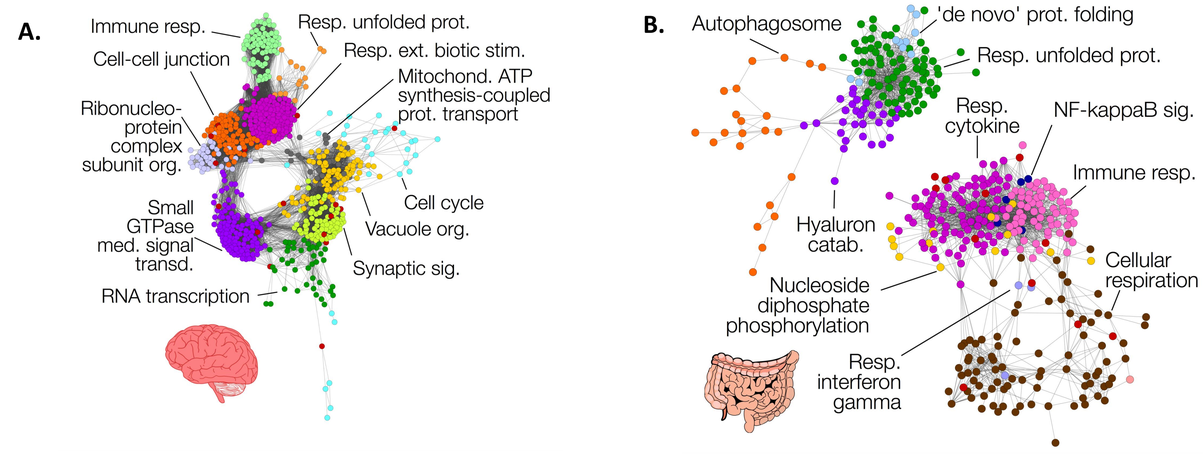

Figure 1: Brain (A) and colon (B) specific gene networks and Interconnectivity of modules (C). Networks of hub genes from brain (A) and colon (B) WGCNA were organized in Cytoscape by edge weight derived from Topological Overlap Matrices. Significantly correlated module eigengenes (MEs) are shown in C. Brain and colon MEs are grey and brown. Node size is proportional to module size (range 54-1196). Red and blue edges indicate positive and negative correlations. Correlations between brain and colon are highlighted by dashed lines. Annotations are from top overrepresented GO terms.

Prot., protein; Resp., response; sig., signaling; catab., catabolism; org., organization; ext. external; stim., stimulus; transd., transduction; proc., process; phos,phosphorylation; reg., regulation

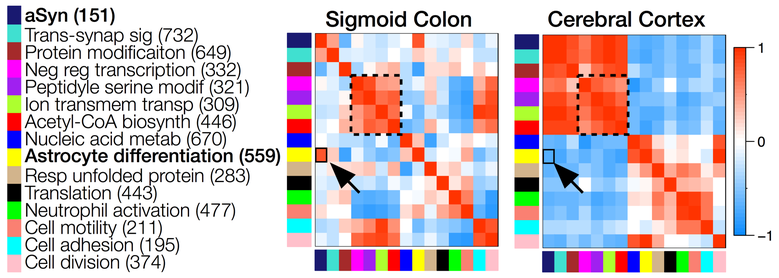

Figure 2: Intermodular connectivity of consensus networks in sigmoid colon and cerebral cortex. Correlations between consensus modules show that many intermodular relationships are preserved (e.g. area outlined in black dotted line). The correlation of the module containing aSyn (SNCA) and the astrocyte module is highlighted by an outline and arrow. Annotations are top overrepresented GO terms. Numbers are module sizes.

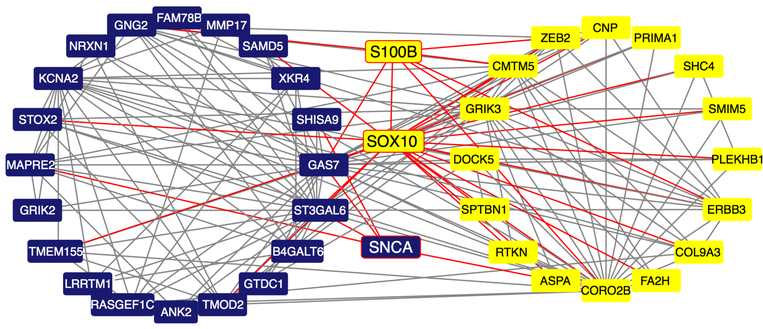

Figure 3: aSyn/Glial network in the colon. Network of hub genes from the aSyn (blue) and "Astrocyte differentiation" (yellow) modules. Edge weights are based on the colon Topological Overlay Matrix. Edges connected to S100B, SOX10 and SNCA are red.

Table 1: Correlations between aSyn (SNCA) and enteric glial cell/astrocyte markers in colon and brain

	S100B	*SOX10*	*GFAP*
Colon *SNCA*	0.79 (3.45e-07)	0.704 (2.01e-05)	-0.149 (0.441)
Brain *SNCA*	-0.621 (0.000324)	-0.0773 (0.69)	-0.395 (0.0342)

Values are Pearson correlation coefficients (p value).

CONCLUSIONS

- The GTEx data set is a valuable resource to explore gene networks relevant to the gut-brain axis
- EGCs may function differently than astrocytes in response to aSyn and/or the tissue response to aSyn may differ in CC vs SC
- The interaction of aSyn and EGCs in the colon merits further investigation in disease and animal models

REFERENCES: **1.** Beach TG, et al. Acta Neuropathologica. 2008;116:277-88. **2.** Lebouvier T, et al. Gut. 2008;57(12):1741-3. **3.** Lionnet A, et al. Acta Neuropathol. 2017. **4.** Carithers LJ, et al. Biopreserv Biobank. 2015;13(5):311-9. **5.** Langfelder P, et al. BMC Bioinformatics. 2008;9:559. **6.** Collado-Torres L, et al. Nat Biotechnol. 2017;35(4):319-21.

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