Use of Weighted Gene Coexpression Network Analysis To Identify Connectivity Between Gut and Brain Gene Expression.

Tasnin Khan MS[1], Asa Hatami, Ph.D.[2], Chunni Zhu, Ph.D[2], Riki Kawaguchi, Ph.D[2], Swapna Joshi, Ph.D.[1], Han Chen MS[1], Jill M. Hoffman, Ph.D.[1], Ka Man Law[1], Ph.D.[1], Carl Robert Rankin, Ph.D.[1], Varghese John, Ph.D.[2], Daniel H. Geschwind, M.D., Ph.D.[2,3], Charalabos Pothoulakis, M.D.[1], and Elizabeth J. Videlock, M.D., Ph.D[1]

UCLA Health

[1]Vatche and Tamar Manoukian Division of Digestive Diseases, Department of Medicine,
[2]Department of Neurology and [3]Department of Human Genetics, David Geffen School of Medicine at UCLA

Background

- Alterations in the gut brain axis are being recognized as a pathogenic factor for an increasing number of diseases, including neurodegenerative diseases such as Parkinson Disease (PD).
- The extent to which gene expression profiling in the gut and the brain is co-regulated is not well understood.
- Weighted gene coexpression network analysis (WGCNA[1]) uses unbiased hierarchical clustering to reduce gene expression profiling data to modules of highly correlated genes.

Aims/Hypothesis

- To determine and explore the strongest associations between colon and striatum gene expression
- *Hypothesis: Gut-brain connectivity will be strongest for modules related to physiologic mechanisms with a systemic component, such as immune reactivity.*

Methods

RNA sequencing and WGCNA

- WGNCA networks were constructed for distal colon and striatum RNA seq (QuantSeq 3′ FWD) data from mice overexpressing human wild type alpha synuclein (ASO, n=18) and wild type (WT, n=16) at 1 and 3 months.
- Module annotation:
  - Overrepresented gene ontology (GO) terms (GOstats)
  - Cell-types: Hypergeometric test against cell type signatures from the Panglao and Cellmarker databases (single cell data).

Gut-brain associations in matched samples

- Mice with matched colon and striatum data [n=10 ASO (1m/3m: 4/6), n=6 WT (1m/3m 3/3)] were included in this analysis.
- Linear regression, controlling for both age and genotype

Results

- Significant associations between brain and gut modules are shown in Figure 1.
- Module annotations are shown in Tables 1-4.

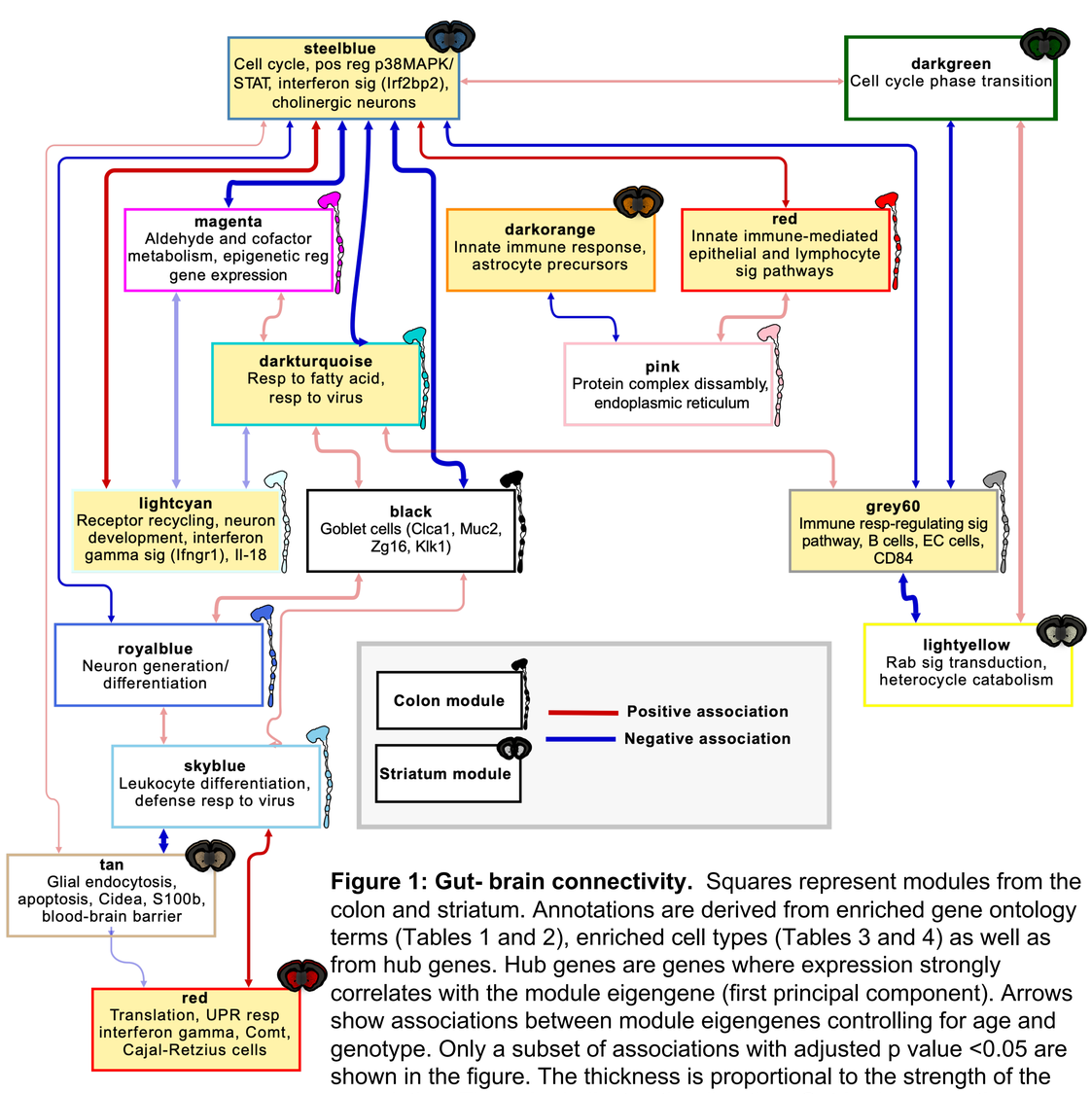

Figure 1: Gut- brain connectivity. Squares represent modules from the colon and striatum. Annotations are derived from enriched gene ontology terms (Tables 1 and 2), enriched cell types (Tables 3 and 4) as well as from hub genes. Hub genes are genes where expression strongly correlates with the module eigengene (first principal component). Arrows show associations between module eigengenes controlling for age and genotype. Only a subset of associations with adjusted p value <0.05 are shown in the figure. The thickness is proportional to the strength of the association. The color indicates the direction. Gut-brain connections are emphasized by stronger intensity of color. Yellow shading is used to highlight modules that may relate to the immune system.

Results

Table 1: Enriched Gene Ontology Terms - Striatum

Module	Terms (p-value)
darkgreen	reg of phagocytosis (0.001), peptidyl-AA modif (0.002), cell cycle G2/M phase transition (0.002), N reg of cellular carb MP (0.002), sterol MP (0.003), reg of plasma lipoprot particle levels (0.003), resp to ionizing radiation (0.003), reg of coagulation (0.004), histone deubiquitination (0.007), G1/S transition of mitotic cell cycle (0.008), P reg of prot loc to cell periphery (0.01)
darkorange	N reg of cell adhesion (0.001), resp to external biotic stimulus (0.001), reg of vasculature dev (0.003), Golgi-associated vesicle memb (CC) (0.003), resp to bacterium (0.003), blood vessel morphogenesis (0.004), reg of cell prolif (0.005), defense resp to virus (0.005), reg of imm effector proc (0.018)
lightyellow	Rab protein signal transduction (2.08E-04), cellular nitrogen compound CP (0.001), inorganic anion transp (0.003), small GTPase mediated signal transduction (0.003), G2/M transition of mitotic cell cycle (0.004), peptidyl-serine phosphorylation (0.006)
red	translational initiation (0.002), resp to unfolded prot (0.003), cellular resp to interferon-gamma (0.006), reactive oxygen species MP (0.01), lipid modif (0.018)
steelblue	reg of RNA stability (5.57E-05), ribosomal small subunit biogenesis (9.19E-05), reg of mRNA CP (1.20E-04), endosome to lysosome transp (0.001), peptide biosynth proc (0.001), N reg of G2/M transition of mitotic cell cycle (0.001), N reg of fat cell diff (0.004), vacuolar transp (0.004), neuronal stem cell population maintenance (0.005), internal peptidyl-lysine acetylation (0.006), P reg of p38MAPK cascade (0.007), P reg of STAT cascade (0.008)
tan	reg of cell shape (0.001), fatty acid biosynth proc (0.006), import across plasma memb (0.009), reg of apoptotic proc (0.012), reg of cell shape (0.017), mitochondrion org (0.018), axon ensheathment (0.019)

AA, amino acid; biosynth; biosynthetic; carb, carbohydrate; CP, catabolic process; dev, development; differentiation, diff; endoth, endothelial; imm, immune; loc, localization; memb, membrane; MP, metabolic process; modification, modif; N, negative; P, positive; proc, process; prot, protein; regen, regeneration; reg, regulation; resp, response; sig, signaling; transp, transport;

Table 2: Enriched Gene Ontology Terms - Colon

Module	Terms (p-value)
black	secretory granule (CC) (0.002), digestion (0.005), metal ion transp (0.011), reg of reproductive proc (0.012), reg of ion transp (0.029), carb derivative biosynth proc (0.029), resp to lipid (0.037), proteasome-mediated ubiquitin-dependent prot CP (0.043)
darkturquoise	organelle loc by memb tethering (4.47E-04), resp to virus (0.005), cellular rep to fatty acid (0.006), retrograde vesicle-mediated transp, Golgi to ER (0.007), P regulation of nitric oxide biosynth proc (0.008), vesicle docking involved in exocytosis (0.009), resp to acid chemical (0.011), reg of extracellular matrix org (0.013), neuron maturation (0.016)
grey60	imm response-reg sig pathway (0.003), reg of gene silencing by miRNA (0.003), N reg of gliogenesis (0.003), cytokine MP (0.003), reg of glycolytic proc (0.003), reg of lymphocyte prolif (0.005), prot deacylation (0.006), covalent chromatin modif (0.006), amine MP (0.008), P reg of chemokine production (0.009), reg of purine nucleotide biosynth proc (0.014), reg of oxidative stress-induced cell death (0.019), circadian reg of gene expression (0.02)
magenta	metencephalon dev (0.002), myosin complex (CC) (0.003), terpenoid MP (0.003), reg of gene expression, epigenetic (0.003), prot-DNA complex assembly (0.006), mRNA processing (0.006), cellular resp to extracellular stimulus (0.01), prot targeting to memb (0.01), reg of synaptic vesicle cycle (0.013), epithelial tube morphogenesis (0.016)
pink	prot-containing complex disassembly (0.006), prot-DNA complex assembly (0.013), P reg of cellular prot loc (0.017), mitochondrial gene expression (0.019), P reg of intracellular transp (0.029), ER to Golgi vesicle-mediated transp (0.034)
red	N reg of insulin receptor sig pathway (0.002), maintenance of prot location in cell (0.003), reg of prot modif by small prot conjugation or removal (0.003), reg of wound healing (0.005), reg of cellular resp to insulin stimulus (0.005), autophagy (0.008), transmemb receptor prot tyrosine kinase sig pathway (0.012), imm resp-activating cell surface receptor sig pathway (0.013), prot processing (0.016), N reg of transcription, DNA-templated (0.025), resp to endoplasmic reticulum stress (0.025)
royalblue	forebrain generation of neurons (0.003), N reg of mRNA MP (0.005), central nervous system neuron diff (0.009), P reg of macroautophagy (0.013), neuron projection morphogenesis (0.014), adenylate cyclase-activating G prot-coupled receptor sig pathway (0.015), posttranscriptional gene silencing by RNA (0.015)
skyblue	myeloid leukocyte diff (0.016), defense resp to virus (0.019), embryonic organ morphogenesis (0.031), steroid MP (0.033), reg of translation (0.033), lipid biosynth proc (0.034), N reg of cellular prot MP (0.047)

Table 3: Enriched Cell Types - Striatum

Module	Cell Type (Tissue, database)	P value (*FDR <0.05)	Genes
darkorange	Astrocyte precursor cell (Brain, C)	0.0131	S100a6
	Endothelial cells (Vasculature, P)	0.0145	Emcn, Plac8, Clic4, Rgs5
	M1 macrophage (Artery, C)	0.0260	Cdh1
	Endothelial cells (Brain, C)	0.0261	Emcn, Rgs5, Apcdd1
	Pericytes (Vasculature, P)	0.0277	Des, Rgs5
	Mural cell (Brain, C)	0.0360	Rgs5, Tpm2
	Olfactory ensheathing glia (Brain, C)	0.0390	Lhfp, Apcdd1
red	Anterior pituitary gland cells (Brain, P)	0.0173	Pcsk1
	Cajal-Retzius cell (Brain, C)	0.0258	Smad1, Kcnh7, Slc2a13
steelblue	Cholinergic neurons (Brain, P)	0.0280	Chat, Slc5a7
tan	Ependymal cells (Brain, P)	0.0441	S100b, Angptl2

Table 4: Enriched Cell Types - Colon

Module	Cell Type (Tissue, database)	P value (*FDR <0.05)	Genes
black	Goblet cells (Small int., C)	9.96E-08*	Clca1, Zg16, Fcgbp, Klk1, Tff3, Atoh1, Muc2, Rep15, Slc12a8
	Paneth cells (Int. crypt, C)	2.53E-07*	Hepacam2, Reg4, Fcgbp, Klk1, Tff3, Gm1123, Ckmt1, Muc2, Tfcp2l1, St6galnac2, Zfp148, Slc12a8, Peli1, Rnf216
	Goblet cells (GI tract, P)	8.28E-05*	Tff3, Atoh1, Muc4
	Chandelier cell (Brain, C)	5.24E-04*	Gdf10, Pde3a, Peli1
	Goblet cells (Int. crypt, C)	1.28E-03*	Zg16, Reg4, Fcgbp, Klk1, Tff3, Muc2, Rep15, Lpin1, Slc12a8
	EEC precursor (Int. crypt, C)	7.06E-03*	Hepacam2, Reg4, Fcgbp, Tff3, Ckmt1, Dpysl2, Fbxo32, Myt1
	Adipocytes (Connective tissue, P)	0.0308	Gdf10, Cdo1, Lpin1
grey60	EECs (Int. crypt, C)	0.038	Neurod1, Chgb, Cacna1a, Aldh1a1, Tph1
	Microglia (Brain, P)	0.042	Slco2b1, Cd53, Ccr5
magenta	Astrocytes (Brain, P)	0.019	Gja1/Tril/Hmg20a
red	Goblet cells (Small int., C)	0.036	Ostc, Stard3nl, Spdef, Ern2
royalblue	Endothelial cells (Vasculature, P)	0.017	Ptprb, Stab1, Kdr, Pecam1, Madcam1, Rgcc
skyblue	Type II spiral ganglion neuron (Brain, C)	0.019	Ephb2, Dok7, Smim15, Mmp16, Naga, Il3ra, Eef2kmt

Conclusions

- Correlations exist between gene expression in colon and striatum in mice.
- The most highly gut-connected striatum modules are connected to several colon modules which are likely involved in the mucosal immune response.
- “Profiling” the gut-brain axis is feasible and future directions of research include evaluating disease-related changes in a larger sample size.

References: 1. Langfelder P, et al. BMC Bioinformatics. 2008;9:559

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