alzheimer's association AAIC21 POSTER

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IDENTIFYING NEUROINFLAMMATION IN OMICS DATASETS WITH A COMPUTATIONAL BIOLOGICAL NETWORK MODEL OF REACTIVE ASTROGLIOSIS

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Introduction and Objectives

Introduction

- Reactive astrogliosis is a change in the molecular phenotype of astrocytes in CNS injuries and diseases, including neurodegenerative diseases such as Alzheimer's disease[1].
- Signaling changes in reactive astrogliosis are heterogeneous and correspond to specific injuries[1].
- Neuroinflammation (and neurodegenerative diseases) induces a neurotoxic "A1" phenotype, and ischemic stroke induces a protective "A2" phenotype[1].
- There is a core set of pan-reactive genes that are regulated by both inflammation and ischemia[1].
- Transcriptomics allows us to capture large-scale changes in gene expression in different disease states; but gaining mechanistic understanding is challenging[2].
- Here, we present a new tool for interpreting transcriptomic data within the context of reactive astrogliosis.

Objectives:

To construct causal biological network (CBN) models for interpreting astrogliosis transcriptomic data, which can:

- Facilitate comparison of the extent of reactive astrogliosis between different conditions in a dataset[2].
- Identify key molecular drivers of reactive astrogliosis in the dataset to aid mechanistic interpretation and help identify disease-specific reactive astrogliosis states[2].

Methods

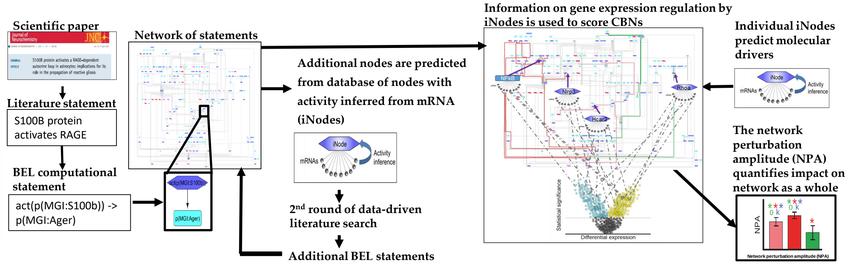

Figure 1. An overview of the causal biological network (CBN) approach[2].

CBN construction:

- Mechanistic statements from the literature were captured in a structured computational format with the Biological Expression Language (BEL)[2].
- BEL statements captured the relationships (edges) between molecular entities (nodes)[2].
- The statements were assembled into a CBN backbone[2].
- A database of scorable nodes was used for scoring the network and predicting additional nodes for data-driven expansion.
- The database contains gene expression regulation information on the nodes, which is used to infer their activity (inferred iNodes).
- Transcriptomic data from GSE35338[1] was used to predict additional iNodes for data-driven expansion of the network. These nodes were added through a 2nd round of focused publication search for mechanistic papers on astrogliosis

The criteria for including publications in the CBN were:

- Primary scientific experimental data — no reviews.
- Mechanistic signaling information derived from astrocytes.
- Inclusion of human, mouse, or rat *in vivo* or *in vitro* astrocyte studies.
- *In vitro:* primary cells, iPSC-derived cells, or organotypic cultures.
- Exclusion of signaling data from immortalized cell lines, cancer models, or other cell types.

CBN scoring:

- The iNodes present in the backbone nodes that were iNodes formed a second, scorable layer[2].
- The CBN model was scored with GSE75246, a publicly available transcriptome dataset of astrocytes, neurons, and microglia isolated from mice 24 hours after a systemic lipopolysaccaride (LPS) injection that induced neuroinflammation.[3]
- The impact on the network as a whole was scored with the network perturbation amplitude (NPA) algorithm, and the top dysregulated iNodes identified the key individual molecular drivers[2].

Results

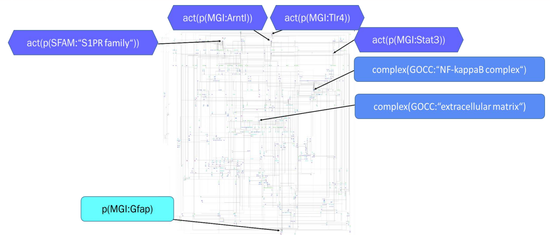

Figure 2. An overview of the pan-reactive astrogliosis CBN. The inserts show the highly connected NF-κB complex, extracellular matrix, GFAP, STAT3, S1PR family, TLR4, and ARNTL nodes.

The literature backbone of the pan-reactive astrogliosis CBN was:

- Constructed from 70 publications and contained 458 nodes connected by 816 edges

After data-driven expansion, the CBN was:

- Constructed from 123 publications and contained 879 nodes and 1587 edges.
- Most connected nodes:
  - Upstream
    - S1PR family of sphingosine 1-phosphate receptors (2 indegree edges, 34 outdegree edges)
    - Extracellular matrix proteins (1 indegree edges, 33 outdegree edges)
    - ARNTL1 (1 indegree, 24 outdegree)
    - TLR4 (3 indegree, 21 outdegree)
  - Downstream
    - NF-κB complex (39 indegree edges, 20 outdegree edges)
    - GFAP (34 indegree edges, 2 outdegree edges)

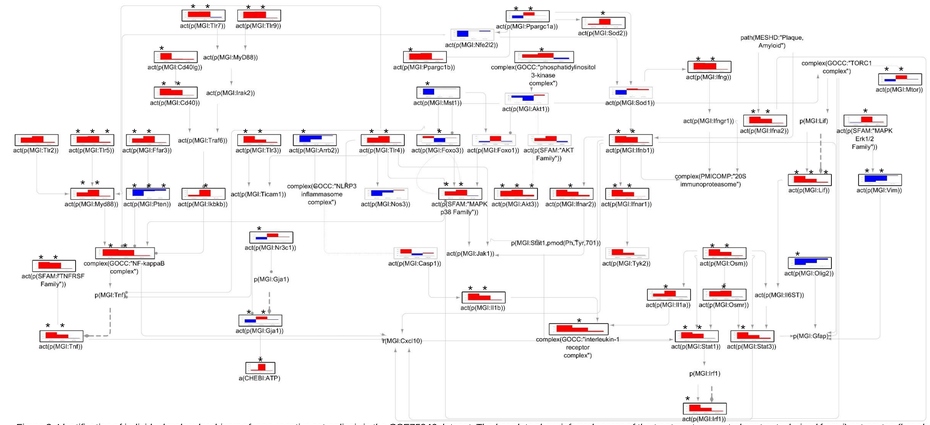

*Figure 3. Identification of individual molecular drivers of pan-reactive astrogliosis in the GSE75246 dataset. The bar plots show inferred scores of the treatment vs. control contrasts derived from i) astrocytes (bar plots on the left side), ii) microglia (bar plots in the middle) ,and iii) neurons (bar plots on the right side) isolated from LPS-treated mice in comparison to the corresponding cells isolated from vehicle-treated mice. Red and blue indicate inferred up- and downregulation, respectively. Inferred adjusted p values <0.01 are indicated by *. Arrow legends: →:causes an increase in; -● causes a decrease in; --► self-relationship.*

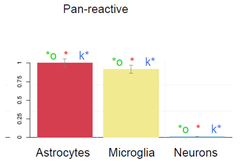

*Figure 8: Bar plots representing the NPA scores obtained following the analysis of the datasets (GSE75246) derived from astrocytes, microglia or neurons isolated from LPS-treated mice in comparison to the corresponding cell types isolated from vehicle-treated mice using the pan-reactive astrogliosis CBN model. Abbreviations: *: confidence interval and significance of the inferred perturbation, "o: the "O"-statistic for specificity of downstream mRNA nodes , k": the "K"-statistic for backbone topology specificity in the NPA calculation.*

- The NPA scores indicated that the pan-reactive astrogliosis network was perturbed in astrocyte and microglia samples to a much larger extent than from isolated neurons in LPS-treated mice.
- The analysis of individual molecular drivers implicated several inflammatory receptors in LPS-induced neuroinflammation including the:
  - Toll-like receptors (TLRs)
  - Interferons (IFNs)
  - Interleukin IL1 and IL6 families signalling
  - Tumor necrosis factor alpha
- These signalling pathways converged downstream on the master inflammatory regulator nuclear-factor NF-KB
- Other key downstream pathways included:
  - Signal Transducer And Activator Of Transcription (STAT) 1 and 3
  - AKT signaling pathways

Discussion

- The CBN was constructed with a literature-driven approach and expanded with an unbiased data-driven approach with nodes inferred to be differentially regulated in the GSE35338 dataset[1].
- The most connected nodes in the CBN model had neuroimmune (i.e., TLR4 and NF-KB) or structural functions (GFAP and extracellular matrix proteins).
- For proof of concept, we analyzed a public neuroinflammation dataset[3] with the CBN.
- The NPA analysis confirmed that the CBN captures neuroimmune signaling in astrocytes and microglia.
- The molecular drivers identified are in line with those in previous reports of LPS-induced signaling, where LPS binds to TLR4 to induce a neuroinflammatory cascade that converges on NF-KB[4].

Conclusions

- The CBNs can be used as a substrate for scoring high-throughput data to provide a disease-specific mechanistic understanding of the differences between diseased and healthy astrocytes in transcriptomic datasets.
- These models can be used to identify disease-specific profiles of reactive astrogliosis, assess gene expression profiles in individuals with CNS diseases for subject classification, generate hypotheses on the mode of action of novel drug candidates, and predict the drivers of treatment outcomes.
- The pan-reactive astrogliosis CBN as well as CBNs focused on A1-like neuroinflammatory and A2-like ischemic stroke phenotype will be made publicly available as research tools on www.causalbionet.com

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