Skinner Lab Meeting

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Amyloid bioinformatics project

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Introduction

Questions:

How are amyloid diseases the same, and how are they different?

More specifically:

1) What molecular pathways are common to many or all amyloid diseases?

2) What molecular pathways are unique to certain amyloid diseases (such as prion disease or Alzheimer's disease?)

Rationale:

A bioinformatics approach allowing comparisons of gene expression, gene annotation, and protein-protein and protein-DNA interaction data may answer these questions and provide leads for biomarker discovery

Aims and Methods

1) Comparative microarray analysis:

Create a unified amyloid bioinformatics database using gene transcription data

Outcome: two target lists of genes of interest – by their expression across many amyloid diseases, or by expression unique to individual amyloid diseases

2) Ontology annotation and data integration:

Create a semi-automated text mining and gene annotation tool using known ontologies, as well as with associated gene-disease, protein-DNA and protein-protein interaction data

Outcome: function annotation and clustering of genes in the target lists above

3) Data visualization and network inference:

Create a data visualization and network inference tool, using as input the unified amyloid database and the ontology annotation tool described above

Outcome: concise data visualization and network inference to prioritize target lists of genes and gene products for further analysis as biomarkers

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Comparative microarray analysis

Might be accomplished with simple aggregation of differentially expressed gene (DEG) lists across studies, but:

- this is vulnerable to the varying methods of assessing significance of differential expression across studies
- this does not incorporate data from genes that don't make a given author's cut to be in the DEG list, even though the study may have collected data on those genes
- some DEG lists reported from one study have little or no overlap with another study, making comparisons impossible

- Meta-analysis allows for a more systematic use of all data across studies
    - can obtain data from central repositories such as GEO, in addition to individual studies
    - vulnerable to variation across studies with regard to different microarray platforms and normalization methods
        - but this still allows more data to pass through, and with less bias, than the above approach

Comparative microarray analysis

Can be thought of as a microarray meta-analysis:

- Or, a statistical comparison of gene expression across multiple different studies
- A meta-analysis “pools” data across many studies, increasing sample size, which can:
    - increase statistical power
    - resolve discrepancies between individual studies
    - refine estimates of effect size
    - answer new questions not asked in the original studies
- Each microarray study has multiple samples divided into two groups:
    - with or without disease (Alzheimer's, Parkinson's, prion disease, etc.)
    - for this meta-analysis: +/- disease ~ +/- amyloid formation
        - note that the original studies intended to study a single specific disease, and we're extrapolating to the amyloid formation common to many diseases
            - this may mean wide variation in effect sizes across diseases/models

Basic & Clinical Biostatistics. B Dawson-Saunders & RG Trapp. Lange, 1994

Microarray meta-analysis

Many techniques have been developed to perform microarray meta-analysis:

- Many of them are available as R packages
- The different techniques can be broken down, based on which metric is combined across the multiiple studies:
  - ranks
  - effect sizes
  - p-values - “Fisher's technique”
- Campan and Yang compared these approaches on common datasets and found:
  - rank technique performed poorly
  - effect size techniques had variable performance, depending on the specific statistical model employed
  - p-value technique performed well

Campain and Yang BMC Bioinformatics 2010, 11:408

Microarray meta-analysis

Meta-analysis by combining p-values:

- Combine p-values from individual studies to estimate an overall p-value for each gene across all studies
- p-values come from performing t-tests to compare normalized gene expression values between the disease group and the control group
- Technique originally developed by Fisher in the 1930s
    - one of the more simple techniques
- Not examining effect sizes may make this approach more robust
    - avoids direct data comparison
        - avoids cross-platform and normalization differences
    - good for combining the very disparate data we are after
- Once this technique tells us which genes hold true across multiple studies, we can go back and look at effect direction and size

Ramasamy A et al. PLoS Medicine 2008, 5:e184.

Rhodes DR at el. Cancer Research 2002,62:4427-4433.

Hu P et al. Cancer Informatics 2006: 2 289–300.

Microarray meta-analysis – in practice

Before even getting to the analysis, must contend with difficult issue of massaging data so that the R meta-analysis package will accept them

- Different microarray platforms provide unique links from their probe identifiers to different gene identifiers:
    - Affymetrix links to a combination of GenBank accession number and RefSeq
    - Illumina links to RefSeq only
    - The link between GenBank and Refseq, or any other identifier, such as EntrezID, is not unique:
        - it's many-to-many
        - this requires modifying the expression datasets to incorporate all combinations
        - ideally, would do this in a way to maximize discovery, so that:

            Links with many probes to one gene => select lowest p-value

            Links with one probe to many genes => expand dataset so there's a record for each gene

Microarray meta-analysis – in practice

Data obtained via GEOquery in R, and by ftp from NCBI

Data massaged in both R and PHP/MySQL

Analysis performed using R package metaMA (part of MAMA suite of microarray meta-analysis packages)

So far have worked with 5 studies of human brains with and without Alzheimer's and Parkinson's

Marot G et al. Bioinformatics 2009;25:2692–2699.

Microarray meta-analysis – in practice

Example of how it would work:

- study1: Alzheimer's disease and the normal aged brain – GSE5281

  Affymetrix platform – GPL570

  161 samples/arrays

- study2: Transcriptional analysis of multiple brain regions in Parkinson's disease – GSE20295

  Affymetrix platform – GPL96

  93 samples/arrays

- study3: Genetic control of human brain transcript expression in Alzheimer’s Disease – GSE15222

  Illumina platform – GPL2700

  363 samples/arrays

Microarray meta-analysis – in practice

Example of how it would work:

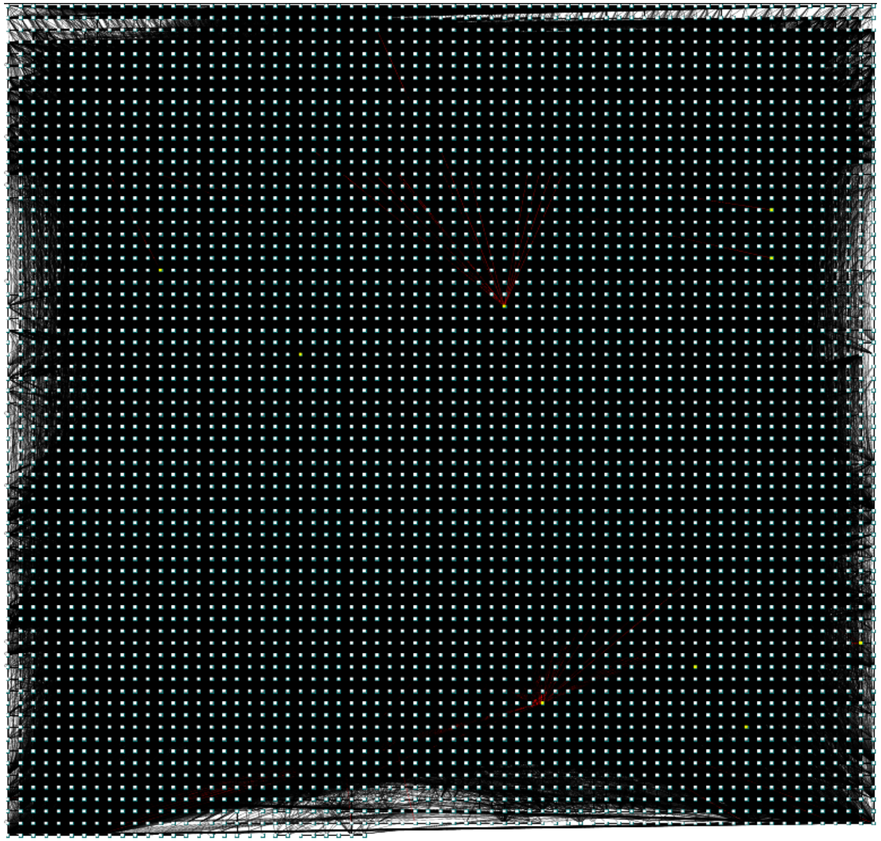
- study1: Alzheimer's disease and the normal aged brain – GSE5281
  Affymetrix platform – GPL570
  161 samples/arrays => for 23 AD, 13 control patients
- study2: Transcriptional analysis of multiple brain regions in Parkinson's disease – GSE20295
  Affymetrix platform – GPL96
  93 samples/arrays => for 15 PD, 15 control patients
- study3: Genetic control of human brain transcript expression in Alzheimer’s Disease – GSE15222
  Illumina platform – GPL2700
  363 samples/arrays => for 176 PD, 187 control patients

Output (Note that I did not group by patient – this is just to show workflow):

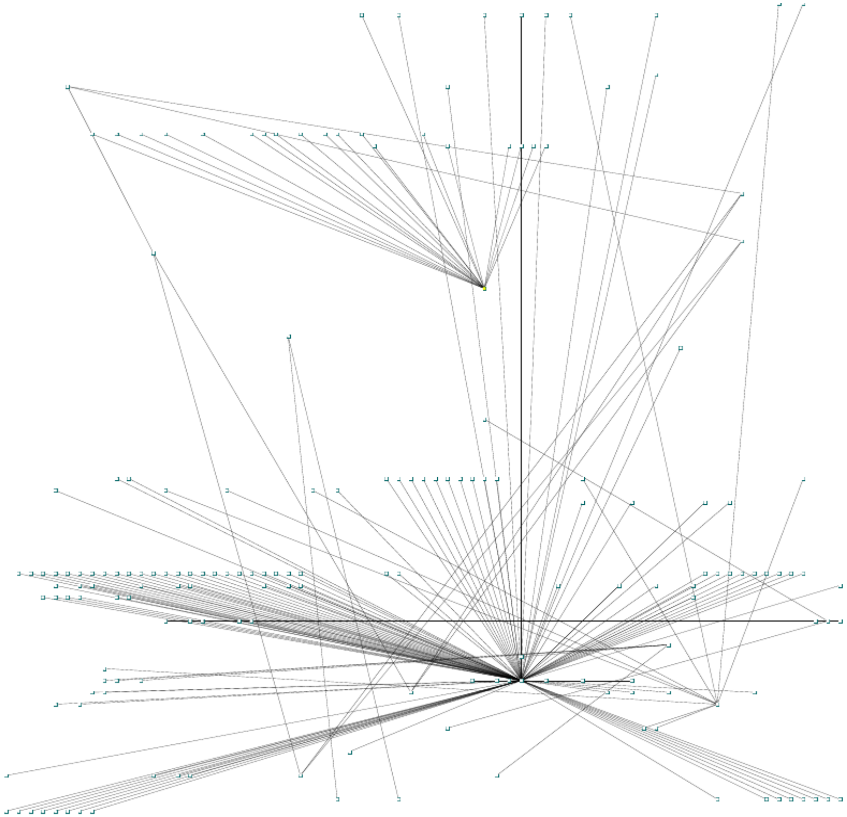
	#DEGs	
study1	1565	
study2	0	=========> (because not grouping by patient...the paper did find DEGs*)
study3	1405	
AllIndStudies	2562	
Meta-analysis	945	=========> DEGs not seen in individual studies: 28 “discoveries”

*Zhang Y et al. American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 2005; 137B:5 –16.

Pulling the “discovery” genes of interest, and their nearest interacting proteins, from a protein-protein interaction network in Cytoscape:



>>



Next steps

Assimilate more microarray data into analyses

- human, mouse and yeast for now
- from both GEO and manually individual studies

Resolve patient grouping and many-to-many issues, likely in PHP

- consider dividing data into brain regions as an option in analyses

Stats review with Rendahl

- consider other meta-analysis techniques

Develop/refine meta-analysis output displays in Cytoscape