Finding consistent disease subnetworks in data with extremely small sample sizes

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Affymetrix GeneChip Array

Source: Affymetrix
Typical Analysis Workflow

- Gene expression data collection
- DE gene selection by, e.g., t-statistic
- Classifier training based on selected DE genes
- Apply the classifier for diagnosis of future cases

Terminology: DE gene = differentially expressed gene

Image credit: Golub et al., Science, 286:531–537, 1999
Hierarchical Clustering

Percentage of Overlapping Genes

- Low % of overlapping genes from diff expt in general
  - Prostate cancer
    - Lapointe et al, 2004
    - Singh et al, 2002
  - Lung cancer
    - Garber et al, 2001
    - Bhattacharjee et al, 2001
  - DMD
    - Haslett et al, 2002
    - Pescatori et al, 2007

<table>
<thead>
<tr>
<th>Datasets</th>
<th>DEG</th>
<th>POG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate Cancer</td>
<td></td>
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<tr>
<td>Top 10</td>
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<tr>
<td>Top100</td>
<td>0.54</td>
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</tr>
</tbody>
</table>

“Most random gene expression signatures are significantly associated with breast cancer outcome”

Individual Genes

• Suppose
  – Each gene has 50% chance to be high
  – You have 3 disease and 3 normal samples

• How many genes on a microarray are expected to perfectly correlate to these samples?

• Prob(a gene is correlated) = 1/2^6
• # of genes on array = 30,000
⇒ E(# of correlated genes) = 469

⇒ Many false positives
• These cannot be eliminated based on pure statistics!
Gene Regulatory Circuits

- Each disease phenotype has some underlying cause
- There is some unifying biological theme for genes that are truly associated with a disease subtype
- Uncertainty in selected genes can be reduced by considering biological processes of the genes
- The unifying biological theme is basis for inferring the underlying cause of disease subtype
Overlap Analysis: ORA

ORA tests whether a pathway is significant by intersecting the genes in the pathway with a pre-determined list of DE genes (we use all genes whose t-statistic meets the 5% significance threshold), and checking the significance of the size of the intersection using the hypergeometric test.

Disappointing Performance

DMD gene expression data
- Pescatori et al., 2007
- Haslett et al., 2002

Pathway data
- PathwayAPI, Soh et al., 2010

Graph: subnetwork agreement vs. sample size (N) for upregulated in DMD.
Issue #1 with ORA

- Its null hypothesis basically says “Genes in the given pathway behaves no differently from randomly chosen gene sets of the same size”

- This may lead to lots of false positives

- A biological pathway is a series of actions among molecules in a cell that leads to a certain product or a change in a cell. Thus necessarily the behaviour of genes in a pathway is more coordinated than random ones
Issue #2 with ORA

- It relies on a pre-determined list of DE genes
- This list is sensitive to the test statistic used and to the significance threshold used
- This list is unstable regardless of the threshold used when sample size is small
Issue #3 with ORA

- It tests whether the entire pathway is significantly differentially expressed.

- If only a branch of the pathway is relevant to the phenotypes, the noise from the large irrelevant part of the pathways can dilute the signal from that branch.
**GSEA in Gene Permutation Mode**

- **Issue #2 is mostly solved**
  - Does not need pre-determined list of DE genes
  - But gene ranking (based on t-test p-value) is still unstable when sample size is small

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Note: Class label permutation mode cannot be used when sample size is small
Better Performance

upregulated in DMD

subnetwork agreement

sample size (N)

GSEA
ORA
ORA-Paired: Paired Test and New Null Hypothesis

- Let $g_i$ be genes in a given pathway $P$
- Let $p_j$ be patients
- Let $q_k$ be normals

- Let $\Delta_{i,j,k} = \text{Expr}(g_i, p_j) - \text{Expr}(g_i, q_k)$

- Test whether $\Delta_{i,j,k}$ is a distribution with mean 0

- **Issue #1 is solved**
  - The null hypothesis is now “If a pathway $P$ is irrelevant to the difference between patients and normals, then the genes in $P$ are expected to behave similarly in patients and normals”

- **Issue #2 is solved**
  - No longer need a pre-determined list of DE genes
  - Sample size is now much larger
    - $\# \text{patients} + \# \text{normals}$
    - $\# \text{patients} \times \# \text{normals} \times \# \text{genes in } P$
Much Better Performance

upregulated in DMD

subnetwork agreement

sample size (N)

ORA-Paired
PFSNet
GSEA
ORA
NEA-Paired: Paired Test on Subnetworks

- Given a pathway $P$
- Let each node and its immediate neighbourhood in $P$ be a subnetwork
- Apply ORA-Paired on each subnetwork individually

- Issues #1 & #2 are solved as per ORA-Paired

- Issue #3 is partly solved
  - Testing subnetworks instead of whole pathways
  - But subnetworks derived in fragmented way
Even Better Performance

upregulated in DMD

subnetwork agreement

sample size (N)
ESSNet: Larger Subnetworks

- Compute the average rank of a gene based on its expression level in patients
- Use the top $\alpha\%$ to extract large connected components in pathways
- Test each component using ORA-Paired

- Gene rank is very stable
- Issues #1 - #3 solved
Fantastic Performance

upregulated in DMD

subnetwork agreement

sample size (N)

ESSNet
NEA-Paired
ORA-Paired
PFSNet
GSEA
ORA
More Datasets Tested

- DMD
- NOR
- ALL
- AML
- BCR-ABL
- E2A-PSX1

Sample size (N) vs. treatment agreement.
Do ESSNet results agree on small datasets vs big datasets?

<table>
<thead>
<tr>
<th>sample size (N)</th>
<th>Precision</th>
<th>Recall</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>10</td>
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<td>0.93</td>
</tr>
</tbody>
</table>

- The table above uses ESSNet’s results on entire datasets as the benchmark to evaluate ESSNet’s results on small subsets of the datasets.
- The precision (i.e., agreement) is superb, though some subnetworks are missed when smaller datasets are analysed.
Leukemias: IL-4 Signaling in ALL

For the Leukemia dataset (in which patients are either classified to have acute lymphoblastic leukemia or acute myeloid leukemia), one of the significant subnetworks that is biologically relevant is part of the Interleukin-4 signaling pathway, see figure 6b (supplementary material). The binding of Interleukin-4 to its receptor (Cardoso et al., 2008) causes a cascade of protein activation involving JAK1 and STAT6 phosphorylation. STAT6 dimerizes upon activation and is transported to the nucleus and interacts with the RELA/NFkB1 transcription factors, known to promote the proliferation of T-cells (Rayet and Gelines, 1999). In contrast, acute myeloid leukemia does not have genes in this subnetwork up-regulated and are known to be unrelated to lymphocytes.
Concluding Remarks

• Consistent successful gene expression profile analysis needs deep integration of background knowledge

• Most gene expression profile analysis methods fail to give reproducible results when sample size is small (and some even fail when sample size is quite large)

• Logical analysis to identify key issues and simple logical solution to the issues can give fantastic results
Acknowledgements

• My students
  – Donny Soh
  – Dong Difeng
  – Kevin Lim
  – Li Zhenhua

• & collaborator
  – Choi Kwok Pui

• MOE


• Kevin Lim, Zhenhua Li, Kwok Pui Choi, Limsoon Wong. ESSNet: Finding consistent disease subnetworks in data with extremely small sample sizes. In preparation