Ontologic Analysis: Challenges for Global Testing

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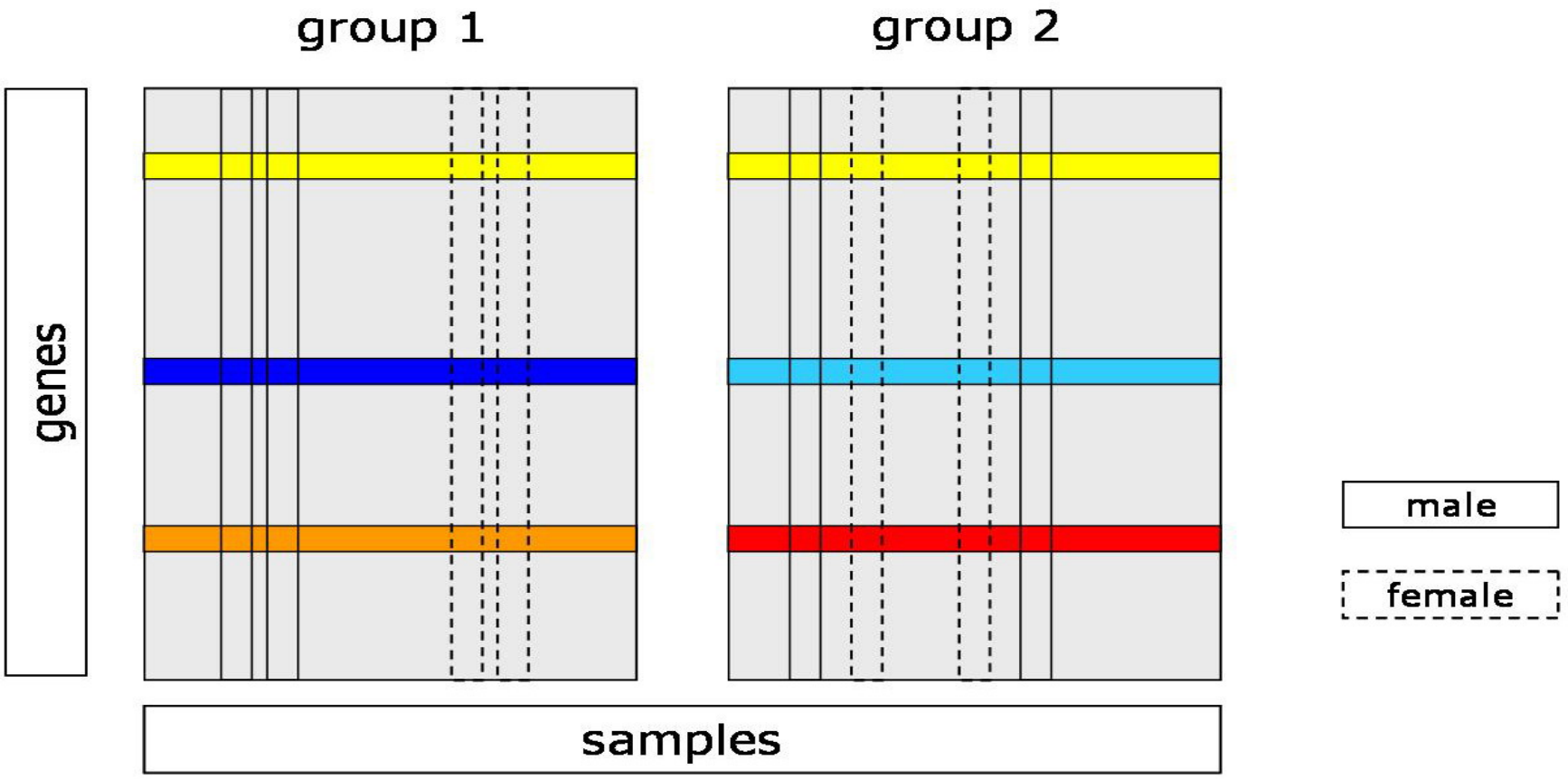
Overview

- Global tests for groups of genes
- Example of flexible linear modeling with `GlobalAncova`
- Gene Ontology analysis
- How to find significant regions in the GO?

Differential Gene Expression



Differential Gene Expression

Question A:

Which genes differ in expression between biological entities?

→ Single tests for each gene

Question B:

Do functional groups of genes (e.g. pathways, areas in the genome, Gene Ontology terms) contain genes showing differential expression?

→ Global tests for groups of genes

Global Testing

Y : clinical outcome, $X : p \times n$ gene expression matrix
(p genes, n samples)

Approach A: $H_0 : P(Y = 1|X) = P(Y = 2|X)$

Random Coefficient Generalized Linear Model; Score test

Goeman et al. (2004)

R package `globaltest`

Approach B: $H_0 : P(X|Y = 1) = P(X|Y = 2)$

ANCOVA: Comparison of adjusted means; F test

Mansmann, Meister (2005)

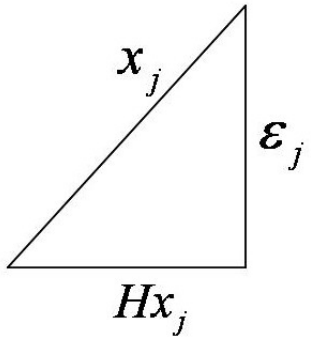
R package `GlobalAncova`

Hypotheses are equivalent by Bayes theorem

GlobalAncova

- Question of interest:
  How is gene expression X influenced by phenotype Y?
- The expectation for gene j follows a linear model
  $E(x_j) = D\beta_j = Hx_j$, with $H = D(D'D)^{-1}D'$
- The design matrix D, e.g. in the two group case and with an additional covariate z, e.g. sex, may look like this

$$\begin{array}{c} \\ \text{sample 1} \\ \text{sample 2} \\ \text{sample 3} \\ \text{sample 4} \\ \dots \end{array} \begin{array}{c} \begin{array}{ccc} \text{Int} & Y & z \end{array} \\ \begin{pmatrix} 1 & 0 & 0 \\ 1 & 0 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 0 \\ & \dots & \end{pmatrix} \end{array}$$



- Residual sum of squares for gene j
  $\varepsilon_j{}'\varepsilon_j$, with $\varepsilon_j = (I - H)x_j$

GlobalAncova

- Question of interest:
  Do we need the variable Y to explain the data X?
  $\rightarrow$ Extra sum of squares principle

- Design matrices:
  $D_{full} = (\mathbf{1}, Y, z)$, $D_{reduced} = (\mathbf{1}, z)$

- Extra residual sum of squares:
  $SSR_{extra} = SSR_{reduced} - SSR_{full}$, with $SSR = \sum_{j=1}^{p} \varepsilon_j{}' \varepsilon_j$

- F statistic:
  $F = MSR_{extra}/MSR_{full}$

GlobalAncova p-values

Theoretical F distribution p-values

Those are not valid in the case of correlations between genes or non-normality

Permutation p-values

Sample labels are permuted and a p-value is estimated as the fraction of corresponding permutation F statistics that are greater than the observed F statistic

Asymptotic distribution of the test statistic

The test statistic has an asymptotic scaled F distribution $\sim b \cdot F(h_1, h_2)$ where b, h_1 and h_2 depend on eigenvalues of the $p \times p$ gene expression covariance matrix and adequate differences of $n \times n$ model hat matrices

Linear Models

The global ANCOVA approach can easily be extended to a general linear model framework with various modeling capabilities

design	full model	reduced model
Various groups	~ `group + cov`	~ `cov`
Continuous variable	~ `dose + cov`	~ `cov`
Time trends in groups	~ `group * time + cov`	~ `group + time + cov`
Gene-gene interaction	~ `gene + cov`	~ `cov`
Co-expression	~ `group + gene + cov`	~ `group + cov`
Differential co-expression	~ `group * gene + cov`	~ `group + gene + cov`
...	...	...

Example

- *Van t'Veer et al. (2002)* present a gene signature of 70 genes to predict recurrence of breast cancer
- We derived 9 cancer related pathways from a literature research
- Questions:
  Is it possible to relate the signature genes to the pathways?
  Are signature genes co-expressed with pathways?
- Explored clinical outcome: development of distant metastases within 5 years (yes/no)
- For demonstration we pick the cell cycle pathway and signature gene „AL137718“

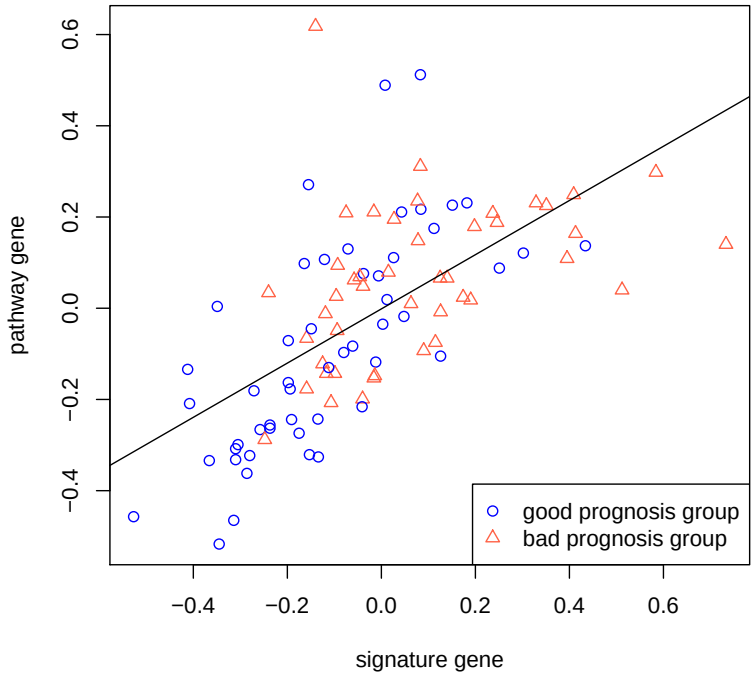
Example

Is there a correlation between the expression of the signature gene and the pathway genes?

Full model: $\sim$ `signature.gene`

Reduced model: $\sim$ `1`

```
$ANOVA
              SSQ    DF         MS
Effect  27.24455    31 0.8788564
Error  117.75240 2914 0.0404092

$test.result

F.value 21.74892
p.value  0.00000
p.perm   0.00000

$terms
[1] "(Intercept)" "signature.gene"
```



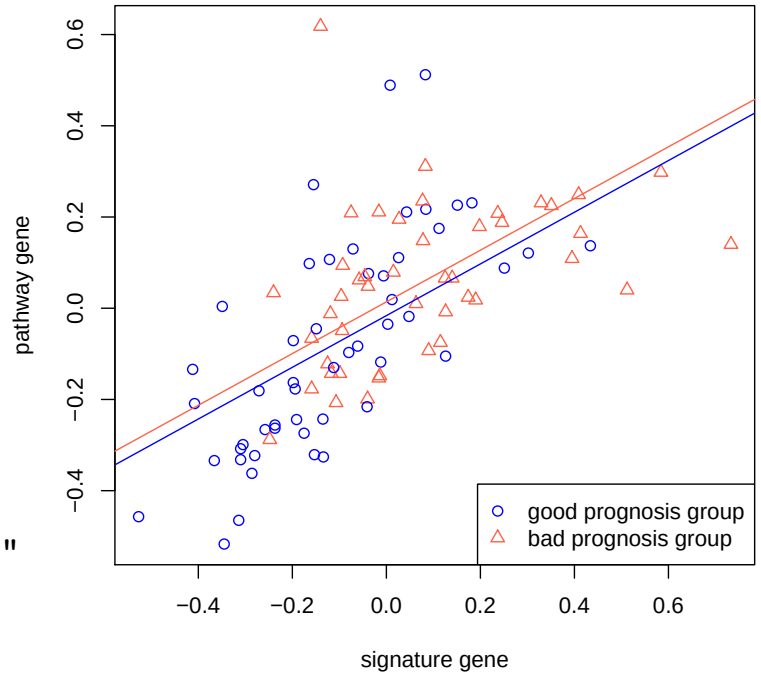
Example

Is there co-expression between signature gene and pathway regarding the clinical outcome?

Full model: $\sim$ `metastases + signature.gene`

Reduced model: $\sim$ `metastases`

```
$ANOVA
              SSQ    DF          MS
Effect  21.27201    31 0.68619383
Error  116.90453 2883 0.04054961

$test.result

F.value 16.92233
p.value  0.00000
p.perm   0.00000

$terms
[1] "(Intercept)" "metastases"  "signature.gene"
```



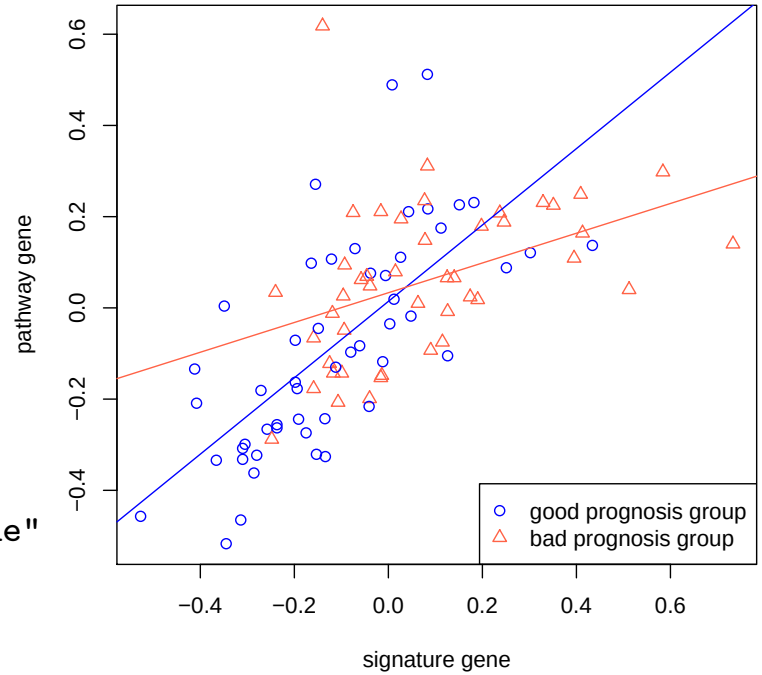
Example

Is there differential co-expression between a signature gene and a pathway regarding the clinical outcome?

Full model: $\sim$ `metastases * signature.gene`

Reduced model: $\sim$ `metastases + signature.gene`

```
$ANOVA
                SSQ   DF          MS
Effect    3.694421   31 0.11917486
Error   113.210105 2852 0.03969499

$test.result

F.value 3.002265e+00
p.value 5.741837e-08
p.perm  5.000000e-04

$terms
[1] "(Intercept)"  "metastases"  "signature.gene"
[4] "metastases:signature.gene"
```



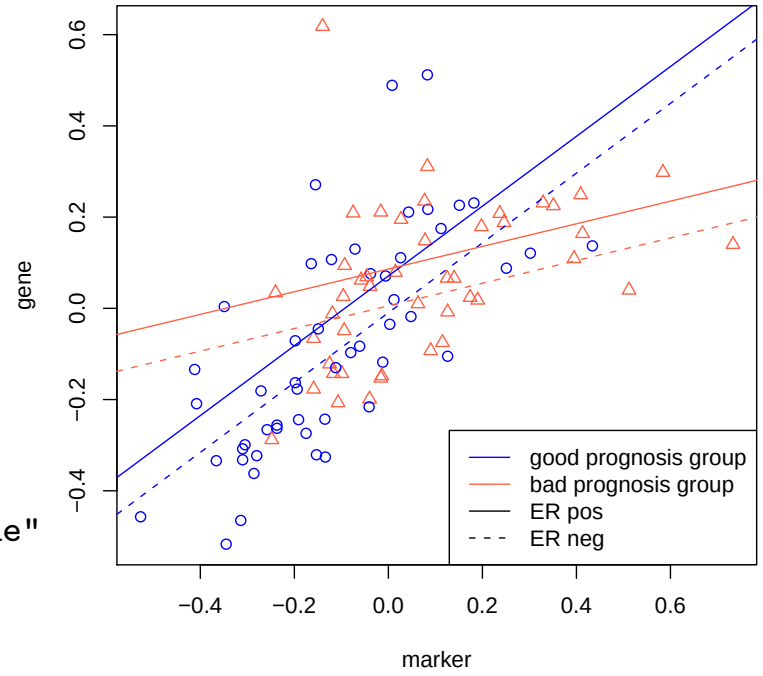
Example

With covariate adjustment

Full model: ∼ `metastases * signature.gene + ER`

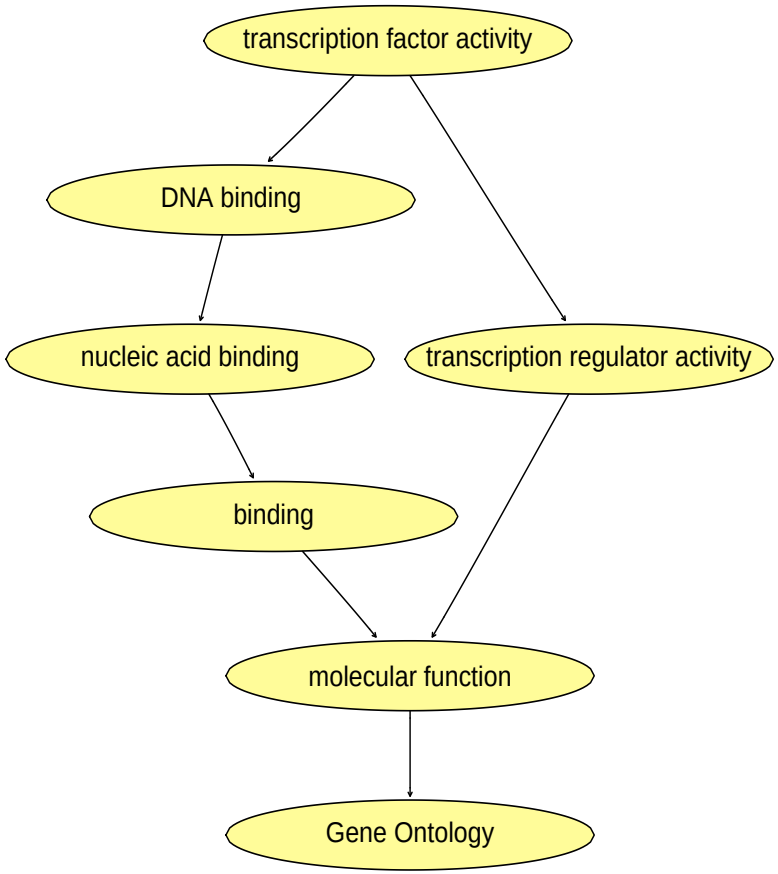
Reduced model: ∼ `metastases + signature.gene + ER`

```
$ANOVA
                SSQ DF         MS
Effect    3.665263 31 0.1182343
Error   107.548645 31 0.0381243

$test.result

F.value 3.101284e+00
p.value 2.031171e-08
p.perm  5.000000e-04

$terms
[1] "(Intercept)"  "metastases"  "signature.gene"
[4] "ER"  "metastases:signature.gene"
```



Gene Ontology

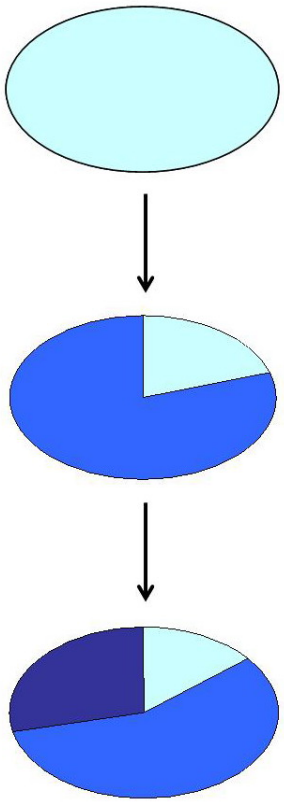
- The Gene Ontology (GO) is a controlled vocabulary to describe gene and gene product attributes (*http://www.geneontology.org/*)
- Three Ontologies: Molecular Function, Biological Process, Cellular Component
- Relations between GO terms are displayed in directed acyclic graphs – direction from specific to general terms



Gene Ontology

- Genes known to be associated with some attributes are mapped to corresponding GO terms

- Inheritance: each gene associated with some term is also mapped to all its ancestors



Biological Questions

- Provide biological meaning to a list of genes found differentially expressed by means of an over-representation analysis
  → Gene set enrichment approaches

- Find biological coherences regarding differential gene expression
  → Holistic approaches

- Find essentially enriched terms given the relationship structure of the GO
  → GO inheritance approaches

Some Methods

Gene set enrichment approaches

- Define a list of differentially expressed genes and score GO terms using the hypergeometric distribution
- Define a Kolmogorov-Smirnov like running sum test statistic for ranked genes *(Subramanian et al. (2005))*

Holistic approaches

- Score GO terms directly using `GlobalAncova` *(Mansmann and Meister (2005))* or `globaltest` *(Goeman et al. (2005))*
- Category approach *(Gentleman (2005))*

GO inheritance approaches

- Decorrelating the GO (*Alexa et al. (2006)*)
- Parent-child approach (*Grossmann et al. (2006)*)

Drawbacks of Gene Set Enrichment

- Loss of information because of two separated steps
- Small but consistent differential expression is not detected
- Dividing genes into differentially and non-differentially expressed genes is artificial
- p-value correction is crucial (correlations between genes, power of detecting genes, ...)

How to Find Significant Regions in the GO?

- Since many tests are performed some correction for multiple testing is required
- There are already various adjustment methods but it would be desirable to incorporate the structure of the GO
- GO inheritance approaches make use of parent – child relationships in order to find truly enriched nodes
- Those are modifications of classical gene set enrichment
- Alternative: Find significant regions in the graph based on the family of global null hypotheses

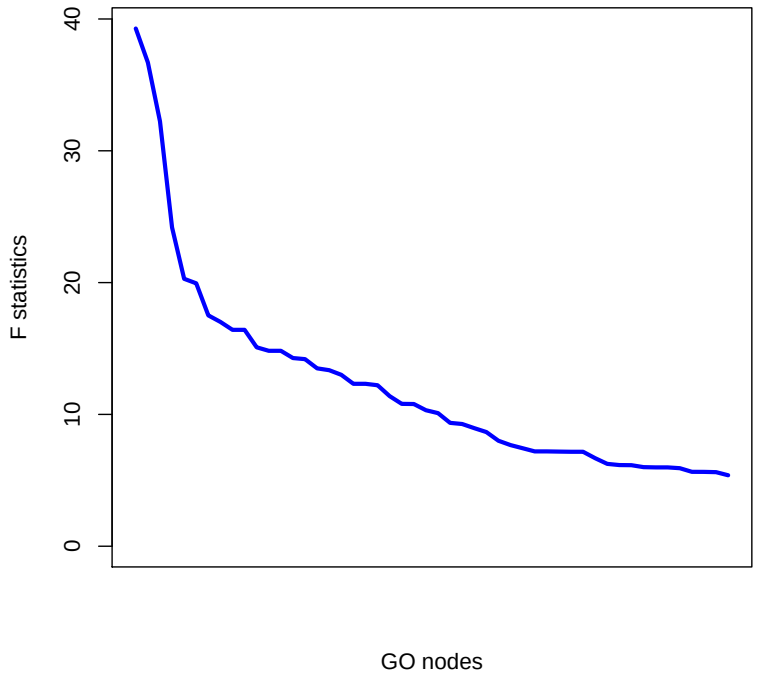
How to Find Significant Regions in the GO?

- For each of the N nodes of the GO graph consider the null hypothesis of no differential expression and corresponding global test statistic F_n
- P_{H_0} denotes the distribution of vector $(F_n)_{n=1,\ldots,N}$ under the family of null hypotheses
- Applying global tests to all nodes yields observed values of the test statistics F_n^{obs}
- Goal: Find a set of nodes $W \subset \{1, \ldots, N\}$ for which

$$P_{H_0}\left\{F_w > F_w^{obs}, \text{ for all } w \in W\right\} < \alpha$$

How to Find Significant Regions in the GO?

- First idea to define subset W: Sort nodes by corresponding observed statistics and find suitable cutoff
- The approach is carried out permutation based



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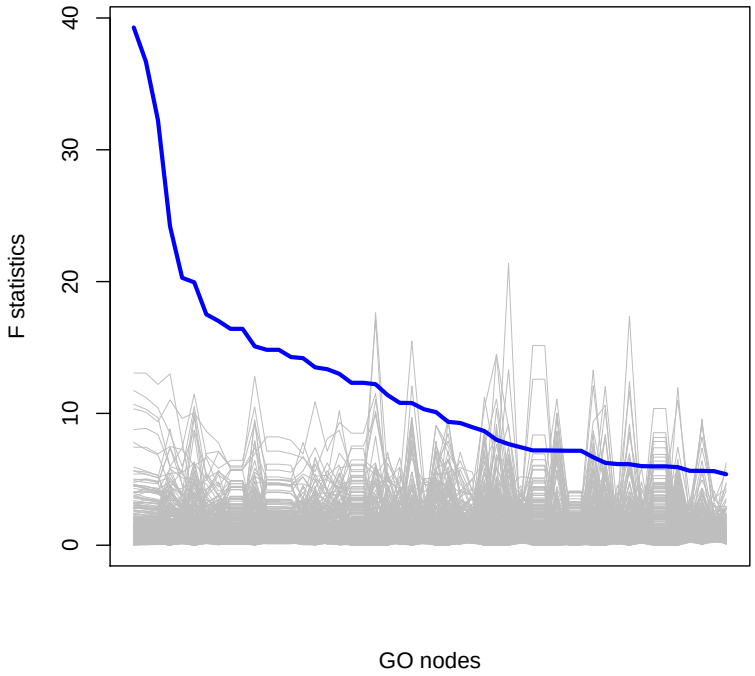

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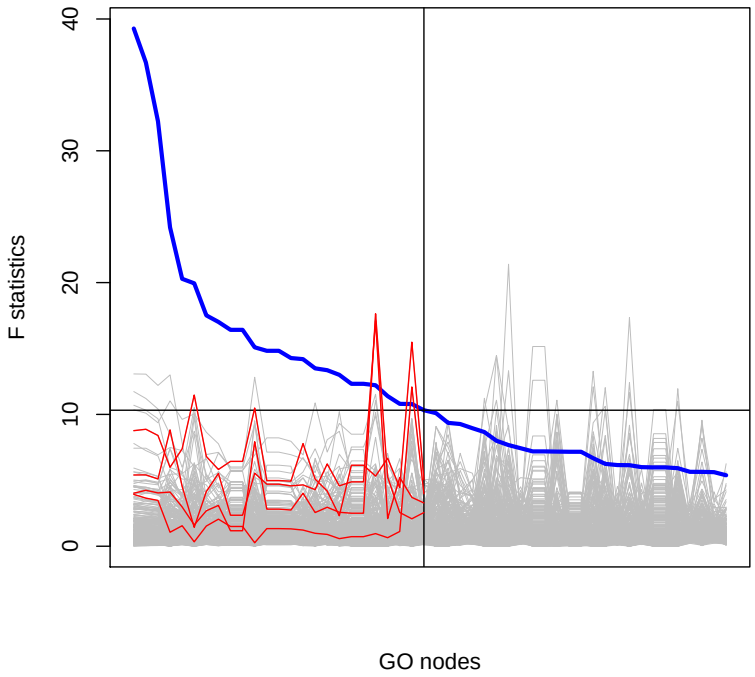

Outlook

- Define subset W of interesting terms by ordering nodes according to the graph structure
- Use full annotation of GO terms or only the 'node-specific' genes (without genes of respective descendant nodes)
- Use complete annotation but shrink expression values of offspring genes before calculating global statistics
- MANOVA approach with additional variable indicating whether a gene is specific at a node

Literature

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