ETH zürich

Machine learning to uncover biological interactions

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Antwerp, 27.04.2016

Part I
Testability and correction for multiple hypothesis testing

By Damian Roqueiro

Significant pattern mining

Definition F. Llinares-López et al. KDD 2015

- The goal of *significant pattern mining* is to identify sets of items that occur statistically significantly more often in one class than in the other.

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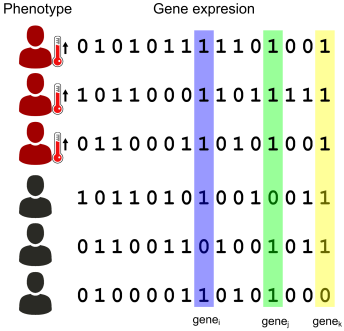
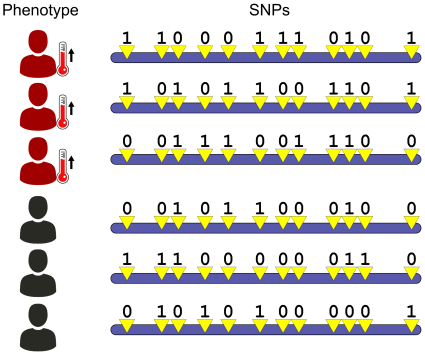


Significant pattern mining

Two other motivating examples

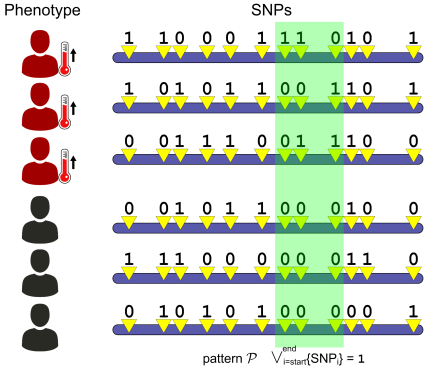


To be discussed in Part II by Laetitia

To be discussed in Part III by Anja

Significant pattern mining

Two other motivating examples



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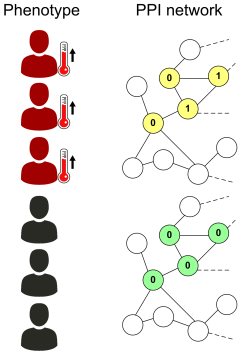
Significant pattern mining

Two other motivating examples



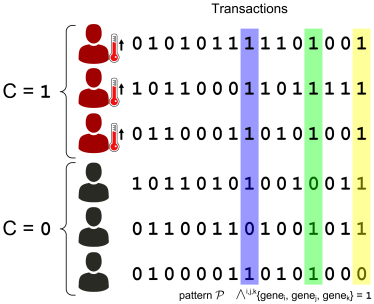
To be discussed in Part III by Anja

To be discussed in Part II by Laetitia

Significant pattern mining

Key aspects

	Pattern $\mathcal{P}$ is present	Pattern $\mathcal{P}$ is not present	
$C = 1$	a	$n_1 - a$	n_1
$C = 0$	$x - a$	$(n - n_1) - (x - a)$	$n - n_1$
	x	$n - x$	n



- Where

n : total number of transactions

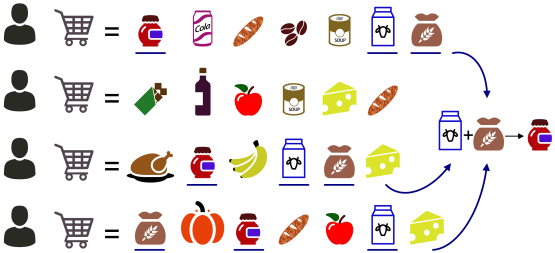
n_1 : number of transactions with class label $C = 1$

x : support of the pattern $\mathcal{P}$, i.e. number of transactions where $\mathcal{P}$ is present

a : support of the pattern $\mathcal{P}$ in transactions of class $C = 1$

What is not significant pattern mining

Frequent itemset mining



Goal: Identify sets of products that are jointly bought by most customers

Significant pattern mining

Statistical association

	Pattern $\mathcal{P}$ is present	Pattern $\mathcal{P}$ is not present	
$C = 1$	a	$n_1 - a$	n_1
$C = 0$	$x - a$	$(n - n_1) - (x - a)$	$n - n_1$
	x	$n - x$	n

- Compute p-value based on a, x, n_1 and n
- Use Fisher's Exact Test R.A. Fisher, 1922
  - 2×2 contingency table
  - Marginals are assumed to be fixed (row and column totals)
- Must guarantee Family-wise Error Rate (FWER) $< \alpha$

Family-wise Error Rate (FWER)

Definition Y. Benjamini and Y. Hochberg, 1995

- Is the probability that at least one false discovery (type I error) occurs in multiple tests

	Number not rejected	Number rejected	
True null hypothesis	U	V	m_0
Non-true null hypothesis	T	S	m_1
	$m - R$	R	m

- V is the number of false positives
- FWER $= Pr(V \geq 1)$
- Increases at most linearly as the number of tests increases
  - Motivates the use of the Bonferroni correction

Multiple hypothesis testing

Adjustement of p-values

- Exponential growth in the number of patterns analyzed

  In our first example, all possible patterns of any size s in N genes, $\sum_{s=1}^{N} \binom{N}{s} = 2^N$
- Therefore, we must correct for multiple hypothesis testing

Bonferroni correction

- For each H_i, with $i = 1 \ldots m$ we obtain a p-value p_i
- Corrected significance level $\delta = \frac{\alpha}{m}$
- Reject H_i if $p_i \leq \delta$
  - If m is large, we incur in loss of statistical power $\rightarrow$ nothing is significant
  - **Question**: Can we correct using $k \ll m$?

Multiple hypothesis testing

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Testability

Deconstructing Fisher's Exact Test

	a	A	Total
Controls	**4**	**6**	10
Cases	**1**	**6**	7
Total	5	12	17

Example: Association test in GWAS

- p-value (two-sided) $= 0.338235$
- Null hypothesis: no association of alleles in cases/controls

- Enumeration of all matrices

$$\begin{bmatrix} 5 & 5 \\ 0 & 7 \end{bmatrix} \leftarrow \begin{bmatrix} \mathbf{4} & \mathbf{6} \\ \mathbf{1} & \mathbf{6} \end{bmatrix} \rightarrow \begin{bmatrix} 3 & 7 \\ 2 & 5 \end{bmatrix} \rightarrow \begin{bmatrix} 2 & 8 \\ 3 & 4 \end{bmatrix} \rightarrow \begin{bmatrix} 1 & 9 \\ 4 & 3 \end{bmatrix} \rightarrow \begin{bmatrix} 0 & 10 \\ 5 & 2 \end{bmatrix}$$

$p = 0.040724$ $\quad p = 0.237557$ $\quad p = 0.407240$ $\quad p = 0.254525$ $\quad p = 0.056561$ $\quad p = 0.003394$

- Where each p is obtained from the hyper-geometric distribution

$$\begin{array}{cc} \begin{bmatrix} x_{11} & x_{12} \\ x_{21} & x_{22} \end{bmatrix} & \begin{array}{c} r_1 \\ r_2 \\ n \end{array} \\ \begin{array}{cc} c_1 & c_2 \end{array} & \end{array} \rightarrow p = \frac{\binom{r_1}{x_{11}}\binom{r_2}{x_{21}}}{\binom{n}{c_1}}$$

- Fisher's p-value

$p = 0.338235 = 0.040724 + 0.237557 + 0.056561 + 0.003394$

Using "biased matrices"

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- Where each p is obtained from the hyper-geometric distribution

$$\begin{array}{cc} \begin{bmatrix}x_{11} & x_{12}\\x_{21} & x_{22}\end{bmatrix} & \begin{array}{c}r_1\\r_2\end{array}\\ \begin{array}{cc}c_1 & c_2\end{array} & n\end{array} \rightarrow p = \frac{\binom{r_1}{x_{11}}\binom{r_2}{x_{21}}}{\binom{n}{c_1}}$$

- Fisher's p-value

$p = 0.338235 = 0.040724 + 0.237557 + 0.056561 + 0.003394$

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- Fisher's p-value

$$p = 0.338235 = 0.040724 + 0.237557 + 0.056561 + 0.003394$$

Using "biased matrices"

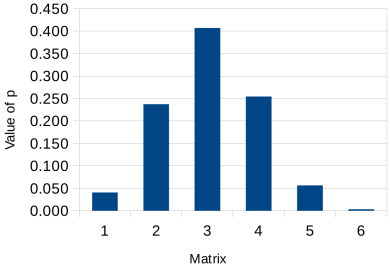
Testability

Minimum attainable p-value

$\begin{bmatrix} 5 & 5 \\ 0 & 7 \end{bmatrix}$	$\begin{bmatrix} 4 & 6 \\ 1 & 6 \end{bmatrix}$	$\begin{bmatrix} 3 & 7 \\ 2 & 5 \end{bmatrix}$	$\begin{bmatrix} 2 & 8 \\ 3 & 4 \end{bmatrix}$	$\begin{bmatrix} 1 & 9 \\ 4 & 3 \end{bmatrix}$	$\begin{bmatrix} 0 & 10 \\ 5 & 2 \end{bmatrix}$
$p = 0.040724$	$p = 0.237557$	$p = 0.407240$	$p = 0.254525$	$p = 0.056561$	$p = 0.003394$
1	2	3	4	5	6

Key elements

- Distribution of p is discrete
- p_{min} in most biased matrix
  - Statistical test on original matrix cannot give a p-value $< p_{min}$



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Minimum attainable p-value

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Most biased matrices (when $r_1 \leq r_2$)

if $r_1 \geq c_1$, then $\begin{array}{ccc} \begin{bmatrix} c_1 & x_{12} - x_{21} \\ 0 & x_{22} + x_{21} \end{bmatrix} & \begin{array}{c} r_1 \\ r_2 \end{array} \\ \begin{array}{cc} c_1 & c_2 \end{array} & n \end{array}$

with $p_{min} = \binom{r_1}{c_1} \Big/ \binom{n}{c_1}$

otherwise $\begin{array}{ccc} \begin{bmatrix} 0 & x_{12} + x_{11} \\ c_1 & x_{22} - x_{11} \end{bmatrix} & \begin{array}{c} r_1 \\ r_2 \end{array} \\ \begin{array}{cc} c_1 & c_2 \end{array} & n \end{array}$

with $p_{min} = \binom{r_2}{c_1} \Big/ \binom{n}{c_1}$

Reducing the Bonferroni correction factor

An illustrative example

- Perform association tests on $m = 5$ SNPs
- Significance level $\alpha = 0.05$
- With Bonferroni correction

  $\rightarrow \delta = \frac{\alpha}{m} = 0.01$

	a	A	Total
Controls	x_{11}	x_{12}	r_1
Cases	x_{21}	x_{22}	r_2
Total	c_1	c_2	n

Id	Observed	Fisher's p-value
SNP_1	$\begin{bmatrix} 2 & 6 \\ 1 & 6 \end{bmatrix}$	0.2
SNP_2	$\begin{bmatrix} 2 & 8 \\ 2 & 7 \end{bmatrix}$	1.0
SNP_3	$\begin{bmatrix} 2 & 8 \\ 7 & 1 \end{bmatrix}$	0.015220
SNP_4	$\begin{bmatrix} 3 & 11 \\ 2 & 7 \end{bmatrix}$	1.0
SNP_5	$\begin{bmatrix} 1 & 9 \\ 3 & 5 \end{bmatrix}$	0.274510

- After correction for multiple hypothesis, there are no statistically significant associations
- How can we improve on these results using the p_{min} of each SNP?

Reducing the Bonferroni correction factor

An illustrative example

- Perform association tests on $m = 5$ SNPs
- Significance level $\alpha = 0.05$
- With Bonferroni correction

  $\rightarrow \delta = \frac{\alpha}{m} = 0.01$

	a	A	Total
Controls	x_{11}	x_{12}	r_1
Cases	x_{21}	x_{22}	r_2
Total	c_1	c_2	n

Id	Observed	Fisher's p-value
SNP_1	$\begin{bmatrix} 2 & 6 \\ 1 & 6 \end{bmatrix}$	0.2
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- After correction for multiple hypothesis, there are no statistically significant associations
- How can we improve on these results using the p_{min} of each SNP?

Reducing the Bonferroni correction factor

Eliminate tests where $p_{min} < \alpha$ N. Manthel, 1980

Id	Observed	Fisher's p-value	Most biased	p_{min}
SNP_1	$\begin{bmatrix} 2 & 6 \\ 1 & 6 \end{bmatrix}$	0.2	$\begin{bmatrix} 0 & 8 \\ 3 & 4 \end{bmatrix}$	0.076923
SNP_2	$\begin{bmatrix} 2 & 8 \\ 2 & 7 \end{bmatrix}$	1.0	$\begin{bmatrix} 0 & 10 \\ 4 & 5 \end{bmatrix}$	0.032508
SNP_3	$\begin{bmatrix} 2 & 8 \\ 7 & 1 \end{bmatrix}$	0.015220	$\begin{bmatrix} 1 & 9 \\ 8 & 0 \end{bmatrix}$	0.000206
SNP_4	$\begin{bmatrix} 3 & 11 \\ 2 & 7 \end{bmatrix}$	1.0	$\begin{bmatrix} 0 & 14 \\ 5 & 4 \end{bmatrix}$	0.003745
SNP_5	$\begin{bmatrix} 1 & 9 \\ 3 & 5 \end{bmatrix}$	0.274510	$\begin{bmatrix} 0 & 10 \\ 4 & 4 \end{bmatrix}$	0.022876

- SNP_1 is eliminated from the analysis, its $p_{min} > \alpha$. It is untestable
- Then, $k = 4$ and $\delta = \frac{\alpha}{k} = 0.0125$. Yet, no statistically association after correction

Reducing the Bonferroni correction factor

Eliminate tests where $p_{min} < \alpha$ N. Manthel, 1980

Id	Observed	Fisher's p-value	Most biased	p_{min}
SNP_1	$\begin{bmatrix} 2 & 6 \\ 1 & 6 \end{bmatrix}$	0.2	$\begin{bmatrix} 0 & 8 \\ 3 & 4 \end{bmatrix}$	0.076923
SNP_2	$\begin{bmatrix} 2 & 8 \\ 2 & 7 \end{bmatrix}$	1.0	$\begin{bmatrix} 0 & 10 \\ 4 & 5 \end{bmatrix}$	0.032508
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- SNP_1 is eliminated from the analysis, its $p_{min} > \alpha$. It is untestable
- Then, $k = 4$ and $\delta = \frac{\alpha}{k} = 0.0125$. Yet, no statistically association after correction

Reducing the Bonferroni correction factor

Tarone's method R.E. Tarone, 1990

procedure main($\mathcal{H}$, α)
▷ $\mathcal{H}$: Set of all hypotheses
▷ α: Nominal significance level
  $k \leftarrow 0$
  repeat
    $k \leftarrow k + 1$
    $\mathcal{T} \leftarrow$ `get_testable_set`($\mathcal{H}$, $\frac{\alpha}{k}$)
  until $k \geq |\mathcal{T}|$
▷ Ready to perform Fisher's Exact Tests
  $\delta \leftarrow \frac{\alpha}{k}$
  `perform_fisher_exact_tests`($\mathcal{H}_{\mathcal{T}}, \delta$)

function `get_testable_set`($\mathcal{H}$, δ)
▷ Determine all testable hypotheses
  $m \leftarrow |\mathcal{H}|$
  $\mathcal{T} \leftarrow \emptyset$
  for $i \leftarrow 1, m$ **do**
    if `is_testable`($\mathcal{H}_i$, δ) **then**
      $\mathcal{T} \leftarrow \{\mathcal{T}\} \cup i$
  return $\mathcal{T}$

function `is_testable`(h, δ)
▷ Check if hypothesis h is testable
  $p_{min} \leftarrow$ `compute_min_pvalue`(h)
  if $p_{min} > \delta$ **then**
    return False
  return True

Reducing the Bonferroni correction factor

Tarone's method R.E. Tarone, 1990

- Intuition

  At the end of the loop we have $k \geq |\mathcal{T}|$

  This implies:

$$|\mathcal{T}| \leq k$$
$$\alpha|\mathcal{T}| \leq \alpha k$$
$$\frac{\alpha}{k}|\mathcal{T}| \leq \alpha$$

  Therefore FWER $\leq \delta|\mathcal{T}| \leq \alpha$

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- With $k = 1$, $\delta = 0.05$, $\mathcal{T} = \{2, 3, 4, 5\}$

  Condition $k \geq |\mathcal{T}|$ is False $\rightarrow$ next iteration

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- With $k = 2$, $\delta = 0.025$, $\mathcal{T} = \{3, 4, 5\}$

  Condition $k \geq |\mathcal{T}|$ is False $\rightarrow$ next iteration

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- With $k = 2$, $\delta = 0.025$, $\mathcal{T} = \{3, 4, 5\}$

  Condition $k \geq |\mathcal{T}|$ is False $\rightarrow$ next iteration

- With $k = 3$, $\delta = 0.0167$, $\mathcal{T} = \{3, 4\}$

  $k \geq |\mathcal{T}|$ evaluates to True $\rightarrow$ Stop

Id	Observed	Most biased	Min. p-value
SNP_1	$\begin{bmatrix} 2 & 6 \\ 1 & 6 \end{bmatrix}$	$\begin{bmatrix} 0 & 8 \\ 3 & 4 \end{bmatrix}$	0.076923
SNP_2	$\begin{bmatrix} 2 & 8 \\ 2 & 7 \end{bmatrix}$	$\begin{bmatrix} 0 & 10 \\ 4 & 5 \end{bmatrix}$	0.032508
SNP_3	$\begin{bmatrix} 2 & 8 \\ 7 & 1 \end{bmatrix}$	$\begin{bmatrix} 1 & 9 \\ 8 & 0 \end{bmatrix}$	0.000206
SNP_4	$\begin{bmatrix} 3 & 11 \\ 2 & 7 \end{bmatrix}$	$\begin{bmatrix} 0 & 14 \\ 5 & 4 \end{bmatrix}$	0.003745
SNP_5	$\begin{bmatrix} 1 & 9 \\ 3 & 5 \end{bmatrix}$	$\begin{bmatrix} 0 & 10 \\ 4 & 4 \end{bmatrix}$	0.022876

Reducing the Bonferroni correction factor

Tarone's method R.E. Tarone, 1990

repeat
  $k \leftarrow k + 1$
  $\mathcal{T} \leftarrow \texttt{get\_testable\_set}(\mathcal{H}, \frac{\alpha}{k})$
until $k \geq |\mathcal{T}|$

▷ $k = 3$
▷ $\delta = \frac{\alpha}{k} = 0.0167$
▷ $\mathcal{T} = \{3, 4\}$

▷ Perform Fisher's Exact Test on SNP_3 and SNP_4

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$SNP_3 \rightarrow p\text{-value} = 0.015220$

$SNP_4 \rightarrow p\text{-value} = 1.0$

- SNP_3 is statistically significant at level δ

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Reducing the Bonferroni correction factor

Tarone's method R.E. Tarone, 1990

- Contrast to Bonferroni correction with $m = 5$
  - $\delta = \frac{\alpha}{5} = 0.01$
  - No significant association would have been found

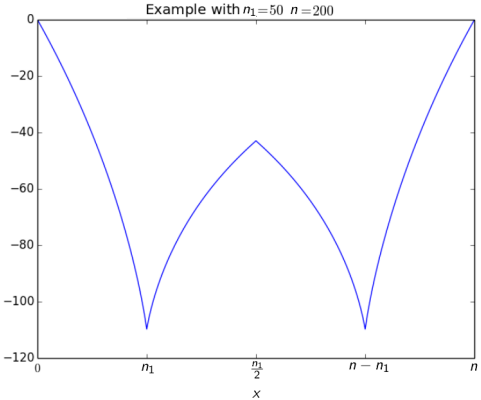
Id	Observed	Fisher's p-value
SNP_1	$\begin{bmatrix} 2 & 6 \\ 1 & 6 \end{bmatrix}$	0.2
SNP_2	$\begin{bmatrix} 2 & 8 \\ 2 & 7 \end{bmatrix}$	1.0
SNP_3	$\begin{bmatrix} 2 & 8 \\ 7 & 1 \end{bmatrix}$	0.015220
SNP_4	$\begin{bmatrix} 3 & 11 \\ 2 & 7 \end{bmatrix}$	1.0
SNP_5	$\begin{bmatrix} 1 & 9 \\ 3 & 5 \end{bmatrix}$	0.274510

Final thoughts

Pre-computing minimum attainable p-values

	Pattern $\mathcal{P}$ is present	Pattern $\mathcal{P}$ is not present	
$C = 1$	a	$n_1 - a$	n_1
$C = 0$	$x - a$	$(n - n_1) - (x - a)$	$n - n_1$
	x	$n - x$	n

- Margins are assumed to be equal for all H_i, e.g. imputed data in GWAS association test
- Therefore, p_{min} can be computed as a function of x



Conclusions of Part I

Key points

- Introduced key aspects of significant pattern mining
- Discussed the concept of minimum attainable p-value
- Applied the Tarone method to obtain a corrected significance level δ_k
- Found $k \ll m$ to correct for multiple hypothesis

Conclusions of Part I

Key points

- Introduced key aspects of significant pattern mining
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In Parts II and III

- How are the patterns defined?
- What test statistic is used?
- How is the search space pruned?
- Are the final results correlated in any way? Post-processing?

References

- R.A. Fisher. On the interpretation of χ^2 from contingency tables, and the calculation of P. Journal of the Royal Statistical Society 85 (1): 87–94. (1922)
- N. Mantel. Assessing laboratory evidence for neoplastic activity. Biometrics 36, 381-399. Correction, Biometrics 37, 875. (1980)
- R.E. Tarone. A modified Bonferroni method for discrete data. Biometrics 46, 515 (1990)
- F. Llinares-López, D.G. Grimm, D.A. Bodenham, U. Gieraths, M. Sugiyama, B. Rowan, K. Borgwardt. Genome-wide detection of intervals of genetic heterogeneity associated with complex traits. Bioinformatics. 31(12):i240-9 (2015)

Part II
Genome-wide genetic heterogeneity detection with categorical covariates

By Laetitia Papaxanthos

Outline

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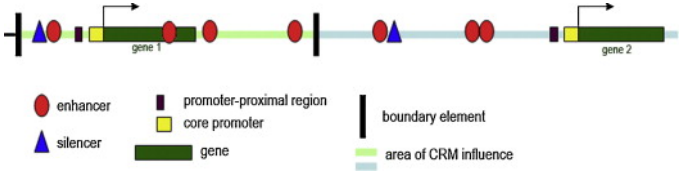
Motivation

- Genetic heterogeneity: the phenomenon under which several variants have a common effect on a phenotype.
- High-order interactions discovery methods for complex traits, an attempt to explain the missing heritability.
- Detection of contiguous interactions between SNPs can reveal local Gene-Gene, cis-regulatory elements (CRE)-Gene or CRE-CRE interactions, ≈10bp to 100kb away.

Source: A systems biology approach to understanding cis-regulatory module function Cell and Developmental Biology, Jeziorska 2009



Propositions: Fast Automatic Interval Search (FAIS) and FastCMH

Baseline

10^5 SNPs lead to $\approx 10^9$ pairs of SNPs, $\approx 10^{14}$ triplets...

- Test high-order interactions: all genomic contiguous intervals, without prior discrimination of region function or length.
- Correct the multiple hypothesis testing problem by controlling FWER using Tarone.
- Scalable to > 500000 SNPs and > 5000 samples.

Propositions: Fast Automatic Interval Search (FAIS) and FastCMH

Categorical confounder correction with FastCMH

- Corrects for multiple categorical confounders such as phenotypical traits (age, height...) and population structure.
  - Enables to increase the number of samples by combining world-wide GWASs.

FAIS: Genome-wide detection of intervals of genetic heterogeneity associated with complex traits, Bioinformatics (2015), F. Llinares-Lopez, D. Grimm, D. Bodenham, U. Gieraths, M. Sugiyama, B. Rowan, K. Borgwardt
FastCMH: Genome-wide genetic-heterogeneity discovery with categorical covariates, submitted to Bioinformatics (2016), F. Llinares-Lopez*, L. Papaxanthos*, D. Bodenham, D. Roqueiro, COPDGene, K. Borgwardt

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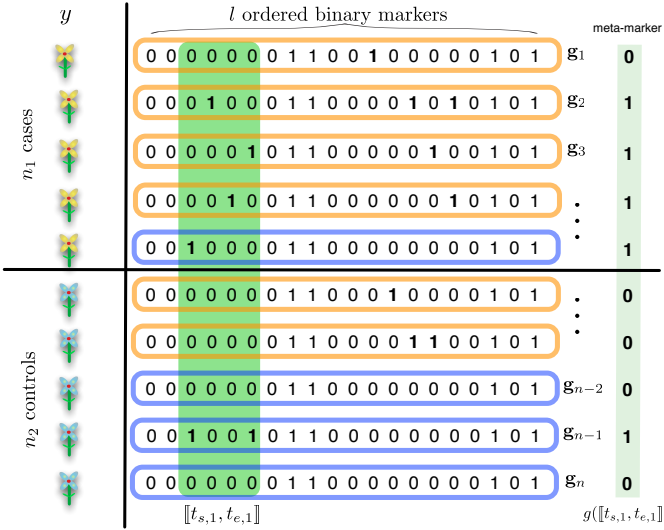
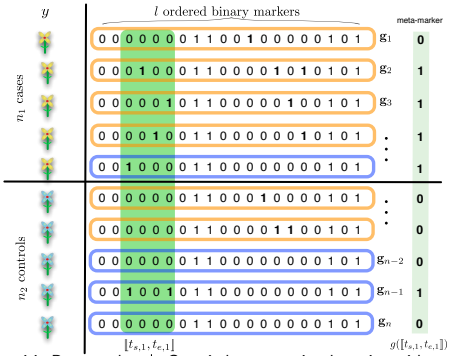
Genomic intervals coded as meta-markers in GWAS datasets



Association testing between meta-markers and phenotype

Variables	*Meta-marker* $= 1$	*Meta-marker* $= 0$	Row totals
$y = \text{case}$	a	$n_1 - a$	n_1 cases
$y = \text{control}$	$x - a$	$n_2 - (x - a)$	n_2 controls
Col totals	x	$n - x$	n



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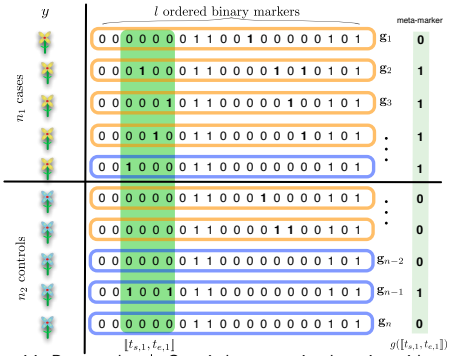

- Notation:
  - Genomic interval: $[\![t_e, t_s]\!]$
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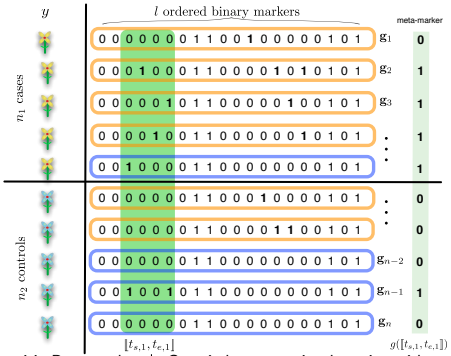

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- Corresponding p-value based on entries a, x, n_1 and n_2
  - Fisher's Exact Test or Pearson's χ^2 Test

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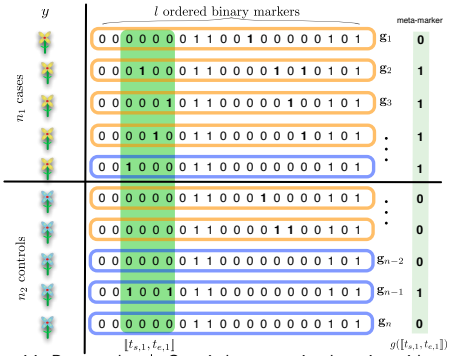

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- Corresponding p-value based on entries a, x, n_1 and n_2
  - Fisher's Exact Test or Pearson's χ^2 Test
  - How to correct for confounders?

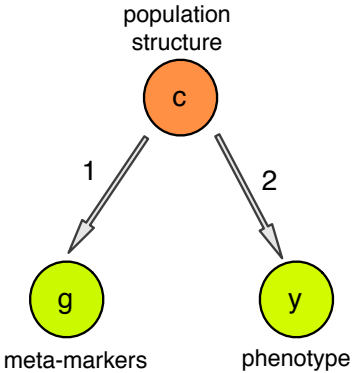
How to correct for confounders ?

Definition

- In statistical genetics, a confounder c is an extraneous variable that influences two conditionally independent variables, for example a phenotypic trait y and a marker g.

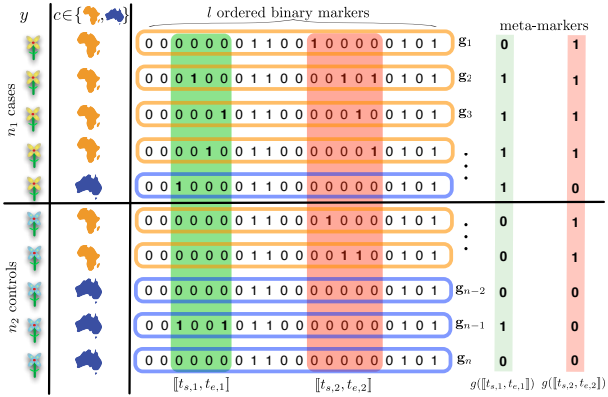
  $y \not\perp\!\!\!\perp g$ but $y \perp\!\!\!\perp g | c$

- It leads to **spurious associations** between the phenotypic trait y and the meta-marker g.



Illustration

Examples of non-confounded and confounded genomic intervals



Correcting for confounders with the Cochran-Mantel-Haenszel (CMH) Test

$\mathbf{g}([\![t_e, t_s]\!]) \in \mathcal{R}^n$ is a meta-marker and k the number of classes of the confounder.
For each class h we define:

- the contingency tables entries: $n_{1,h}$, $n_{2,h}$, x_h and a_h.

CMH Test

The CMH-test is based on the k-vectors $\mathbf{a}$, $\mathbf{x}$, $\mathbf{n}_1$ and $\mathbf{n}_2$.

$$T(\mathbf{a}, \mathbf{x}, \mathbf{n_1}, \mathbf{n_2}) = \frac{\left(\sum_{h=1}^{k} a_h - E(a_h)\right)^2}{\sum_{h=1}^{k} Var(a_h)}$$

$$= \frac{\left(\sum_{h=1}^{k} a_h - x_h \frac{n_{1,h}}{n_h}\right)^2}{\sum_{h=1}^{k} \frac{n_{1,h}}{n_h}\left(1 - \frac{n_{1,h}}{n_h}\right) x_h \left(1 - \frac{x_h}{n_h}\right)}$$

Correcting for confounders with the CMH Test

Corresponding p-value $\Psi(\mathbf{a}, \mathbf{x}, \mathbf{n_1}, \mathbf{n_2})$

$$\Psi(\mathbf{a}, \mathbf{x}, \mathbf{n_1}, \mathbf{n_2}) = 1 - F_{\chi^2}(T(\mathbf{a}, \mathbf{x}, \mathbf{n_1}, \mathbf{n_2}))$$

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FAIS and FastCMH architecture in brief

$\mathbf{g}([\![t_e, t_s]\!])$ represents a meta-marker n-vector.

Two steps:

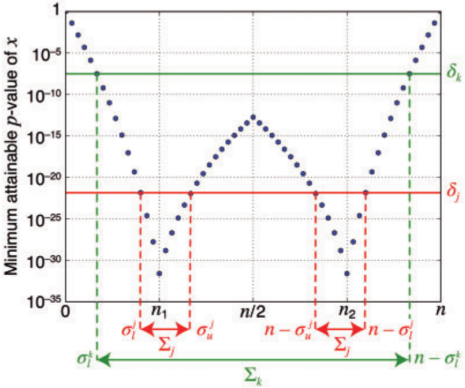
Input: Dataset of meta-markers $\mathcal{G} = \{\hat{\mathbf{g}}, \mathbf{y}, \mathbf{c}\}$, desired FWER α.
Output: Set of non-overlapping (conditionally) associated genomic regions $\mathcal{R}_{sig,filt} = \{[\![t_s, t_e]\!] \mid p([\![t_s, t_e]\!]) \leq \delta_{tar}\}$ and Tarone significance threshold δ_{tar}.

1. $(\delta_{tar}, \mathcal{R}_T(\delta_{tar})) \leftarrow \texttt{get\_significant\_regions}(\mathcal{G}, \alpha)$
2. $\mathcal{R}_{sig,filt} \leftarrow \texttt{filter\_overlapping\_regions}(\mathcal{R}_T(\delta_{tar}))$

Return: $\mathcal{R}_{sig,filt}$

1. Routine `get_significant_regions`: initialization

- $\delta \leftarrow 1$, $\mathcal{I}_T(\delta) \leftarrow \{\}$



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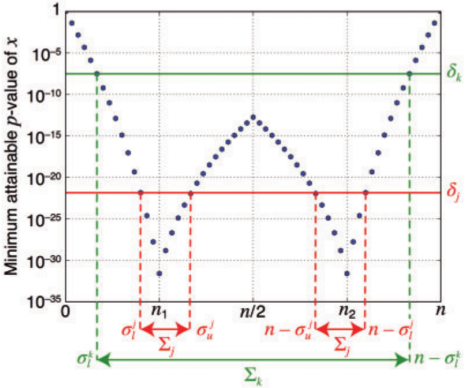
For all $[\![t_s, t_e]\!] \in \mathcal{R}_{cand}$, in increasing order of starting position t_s, and then length $t_e - t_s$:



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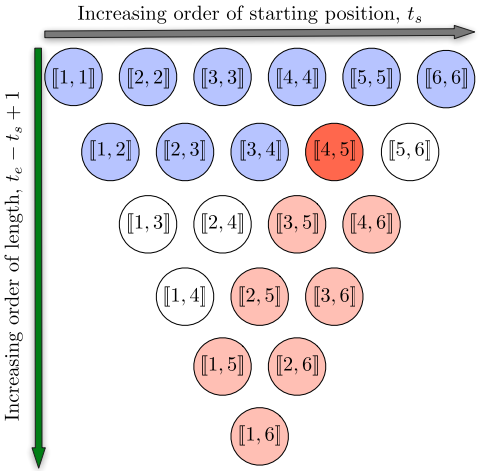
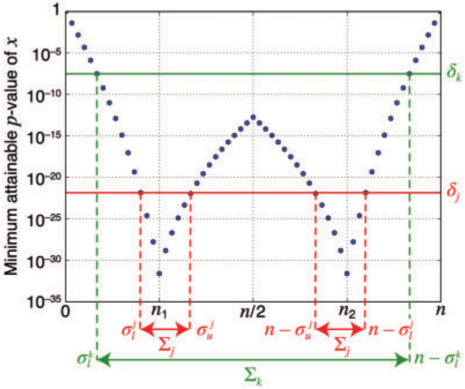


1. Routine `get_significant_regions`: interval processing

- $\delta \leftarrow 1$, $\mathcal{I}_T(\delta) \leftarrow \{\}$

For all $[\![t_s, t_e]\!] \in \mathcal{R}_{cand}$, in increasing order of starting position t_s and then length $t_e - t_s$:

- Compute $x_{[\![t_s,t_e]\!]}$



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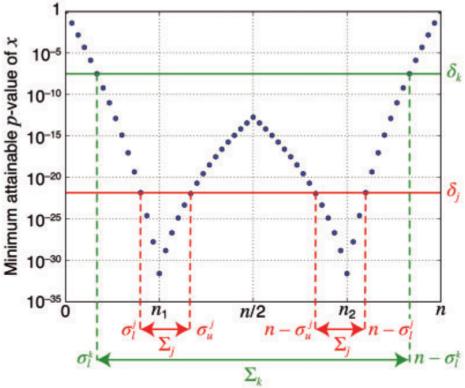
- If $\Phi(x_{[\![t_s,t_e]\!]}) \leq \delta$: $\rightarrow$ Tarone's testability criterion



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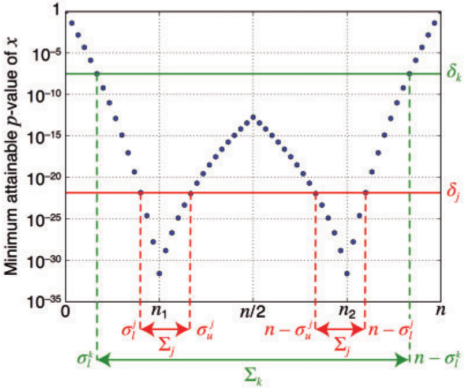
As a reminder:
$\Phi(\mathbf{x}_{[\![t_s,t_e]\!]}) = \min_{\mathbf{a} \in [\![0, \mathbf{x}_{[\![t_s,t_e]\!]}]\!]} \Psi(a, \mathbf{x}_{t_s,t_e})$ is the minimum attainable p-value.



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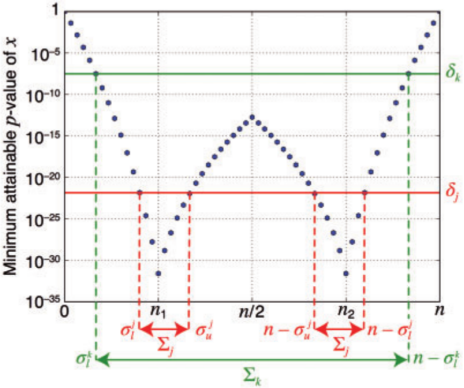

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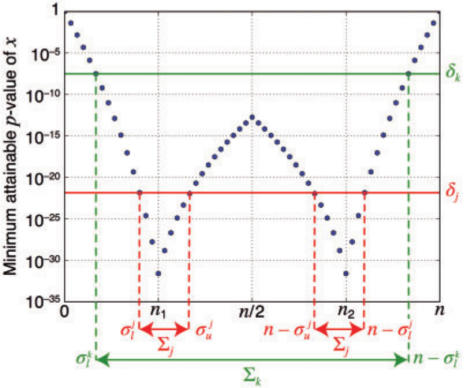
  - While $\delta\,|\mathcal{I}_T(\delta)| > \alpha$: $\rightarrow$ check $\widehat{FWER}$



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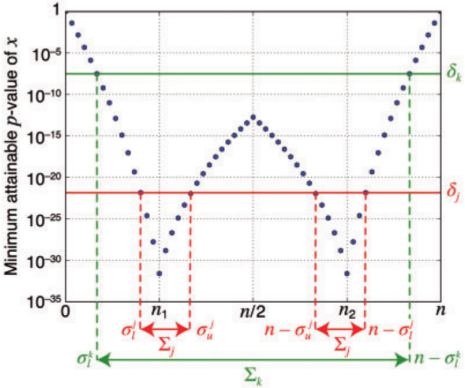
    - Decrease δ



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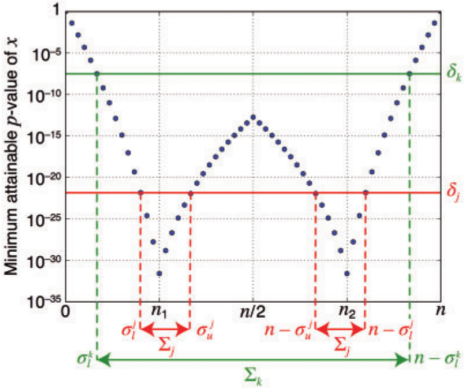
  - While $\delta\,|\mathcal{I}_T(\delta)| > \alpha$: $\rightarrow$ check $\widehat{FWER}$
    - Decrease δ
    - Remove newly untestable intervals from $\mathcal{I}_T(\delta)$



1. Routine `get_significant_regions`: interval processing

- $\delta \leftarrow 1$, $\mathcal{I}_T(\delta) \leftarrow \{\}$

For all $[\![t_s, t_e]\!] \in \mathcal{R}_{cand}$, in increasing order of starting position t_s and then length $t_e - t_s$:

- Compute $x_{[\![t_s,t_e]\!]}$
- If $\Phi(x_{[\![t_s,t_e]\!]}) \leq \delta$: $\rightarrow$ Tarone's testability criterion
  - $\mathcal{I}_T(\delta) \leftarrow \mathcal{I}_T(\delta) \cup \{[\![t_s, t_e]\!]\}$
  - While $\delta\,|\mathcal{I}_T(\delta)| > \alpha$: $\rightarrow$ check $\widehat{FWER}$
    - Decrease δ
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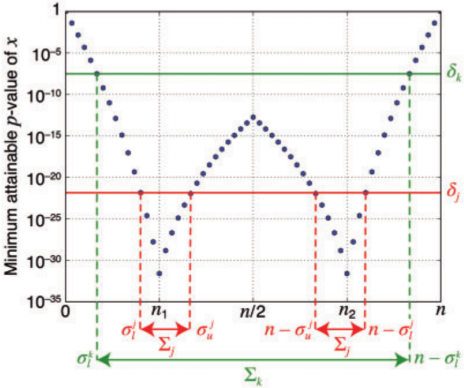
- If **pruning_condition**$(x_{[\![t_s,t_e]\!]})$ then: $\Rightarrow$ depends on the test statistic



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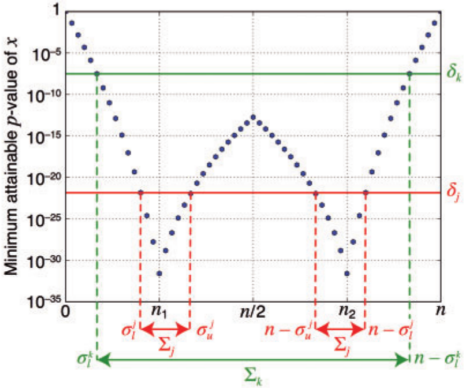
  - Prune all intervals $[\![t'_s, t'_e]\!] \supset [\![t_s, t_e]\!]$ from $\mathcal{R}_{cand}$



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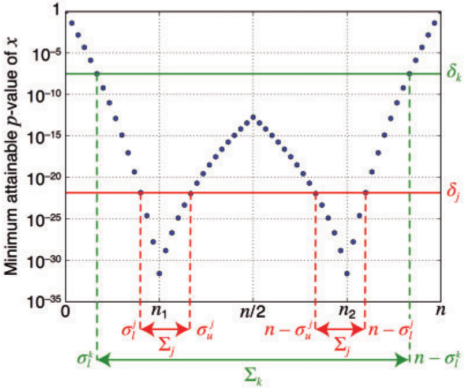
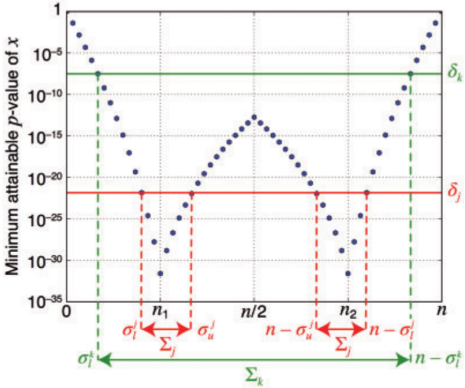
Return: δ_{tar} and $\mathcal{R}_T(\delta_{tar})$

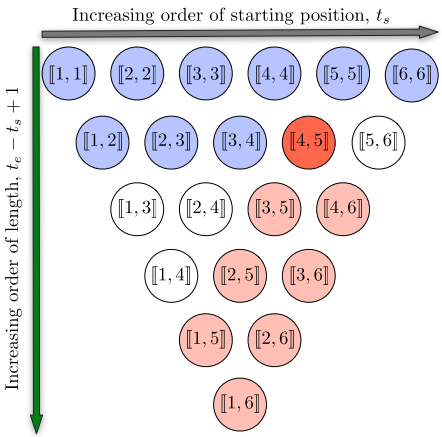


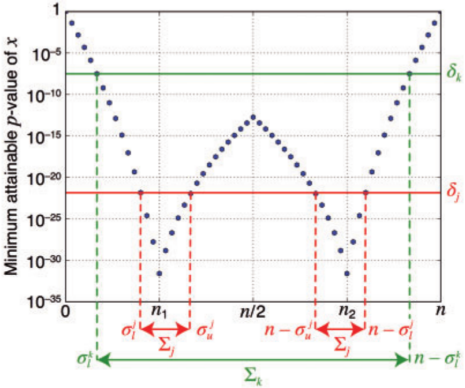
Pruning conditions for FAIS

- FAIS: χ^2, Fisher exact test.
- The minimum attainable p-value is monotonically increasing as x increases in $\mathcal{R}_{cor} = [\max(n_1, n_2), n]$.
- The pruning condition is straight forward:
  $x_{[\![t_s,t_e]\!]} \geq \max(n_1, n_2)$ and
  $\Phi(x_{[\![t_s,t_e]\!]}) > \delta$



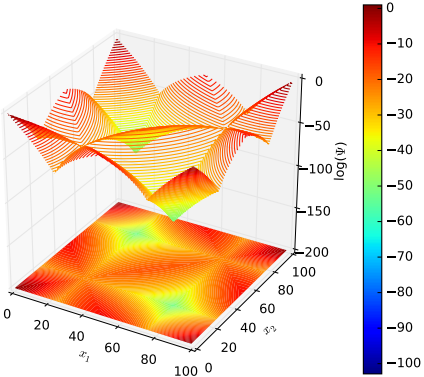
1. Routine `get_significant_regions`: interval pruning





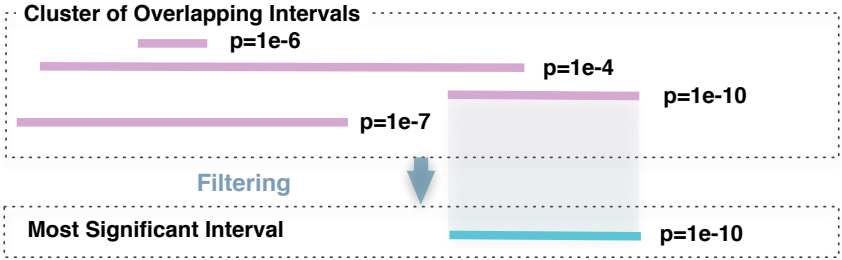
Pruning conditions for `FastCMH`

- `FastCMH`: CMH-test.
- The minimum attainable p-value $\Phi(x_{[\![t_s,t_e]\!]})$ is not monotonic for $\mathbf{x}_{[\![t_s,t_e]\!]} \in \mathcal{R}_{cor} = [\![\max(n_{1,h}, n_{2,h}), n]\!]_{h=1}^{k}$.
  - We compute a monotonic lower bound to the p-value surface in the prunable search space $\mathcal{R}_{cor}$.
  - Runtime scales as $O(k \log(k))$



2. Routine `filter_overlapping_regions`

- Selection of the interval with the smallest *p*-value



- Advantage: Corrects for redundancy, LD partly;
- Limitation: Dependent statistical tests:
  - Solution: Permutation testing, implemented with FAIS-WY but not with FastCMH.

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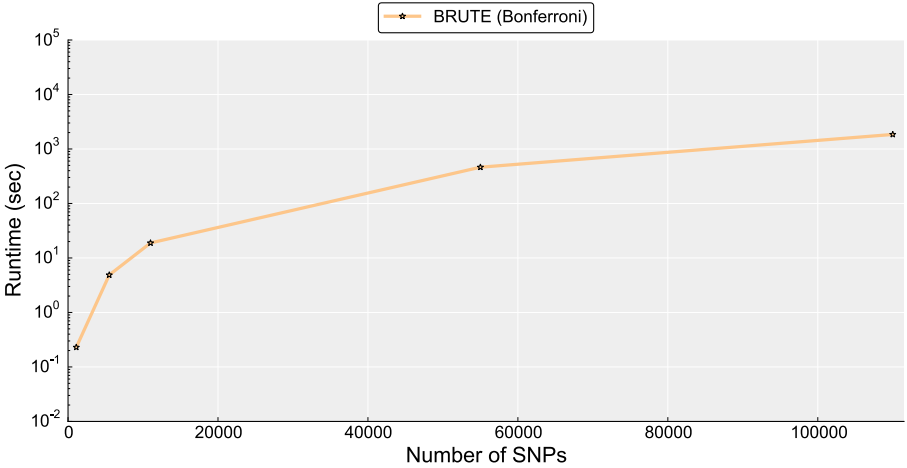
FAIS: runtime simulation



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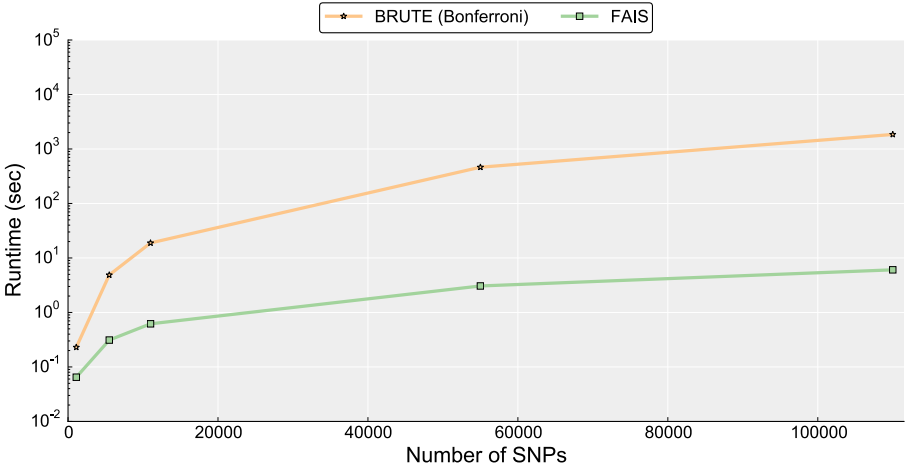
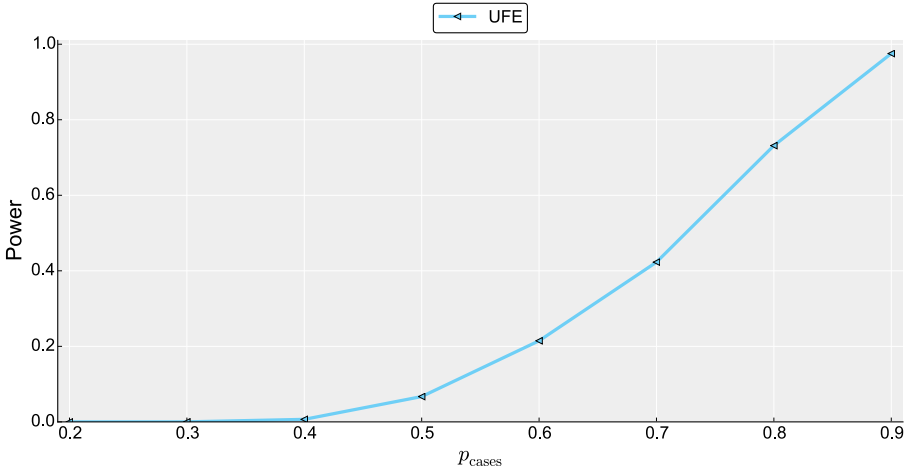


FAIS: power simulation



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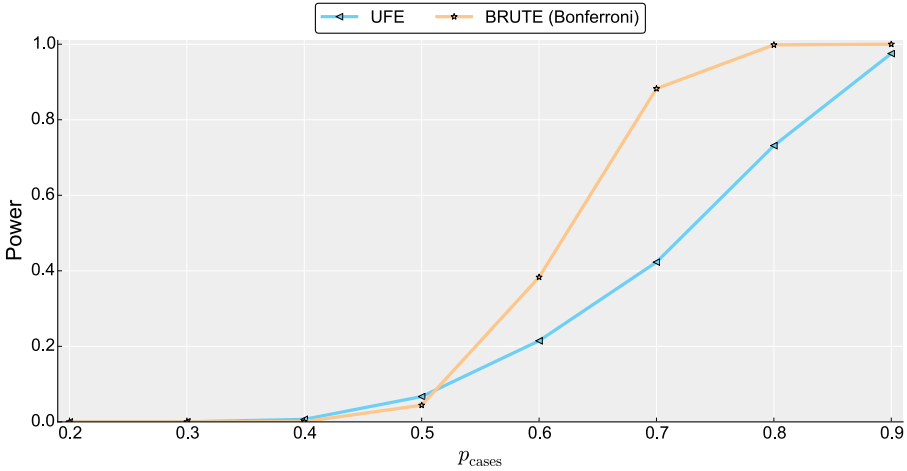

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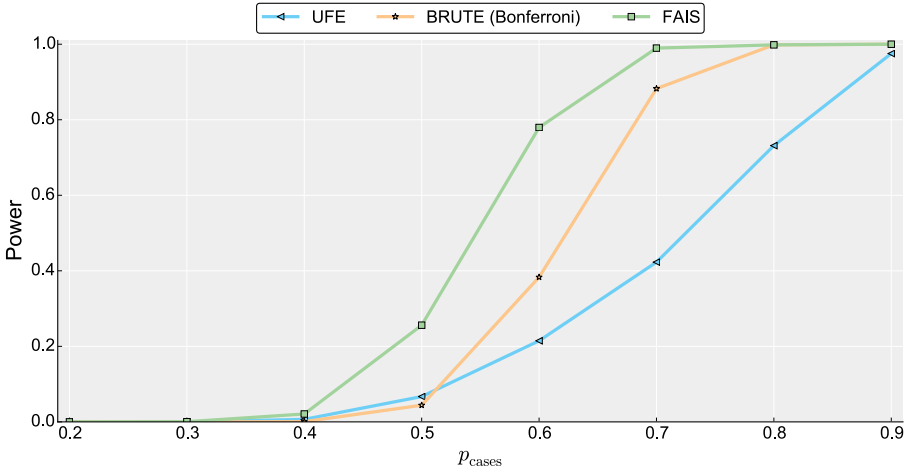

FAIS: genetic heterogeneity detection in *Arabidopsis thaliana*

Dataset (Atwell 2010)

- 21 defense and development binary phenotypes

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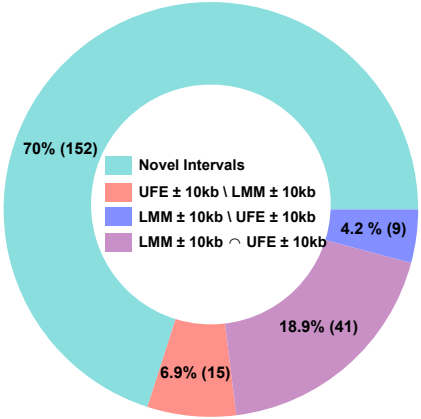

Sources for intervals found

- True genetic heterogeneity

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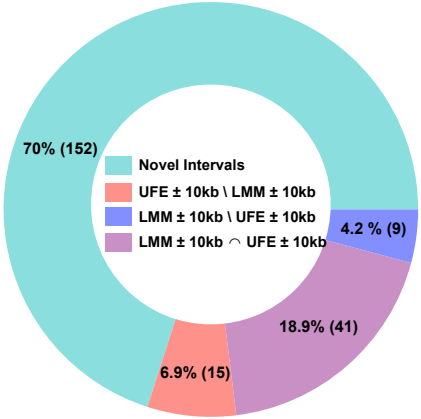

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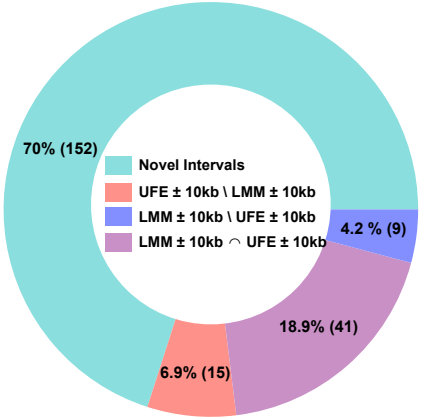
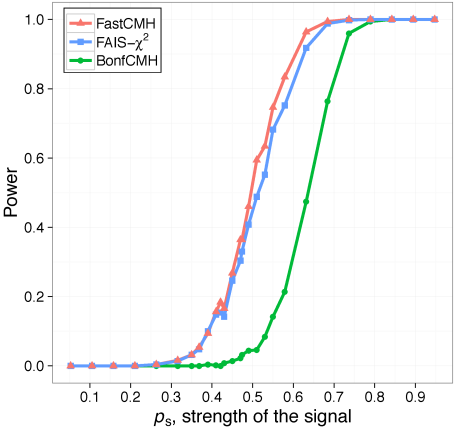


Sources for intervals found

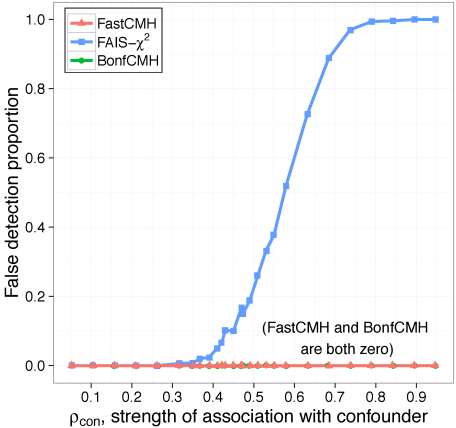
- True genetic heterogeneity
- Linkage to causal SNPs
- Structural variation in the region

FastCMH: simulations show a high power, low false detection proportion and high-speed detection



(a)

FastCMH: simulations show a high power, low false detection proportion and high-speed detection



(b)

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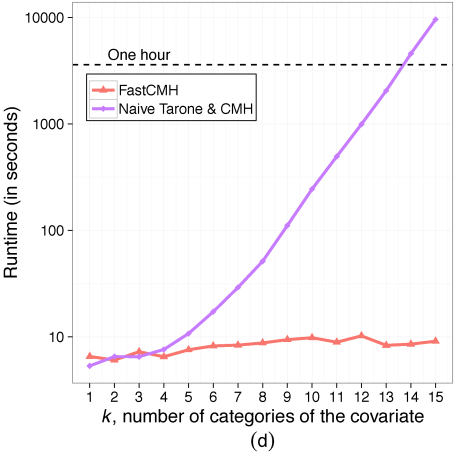

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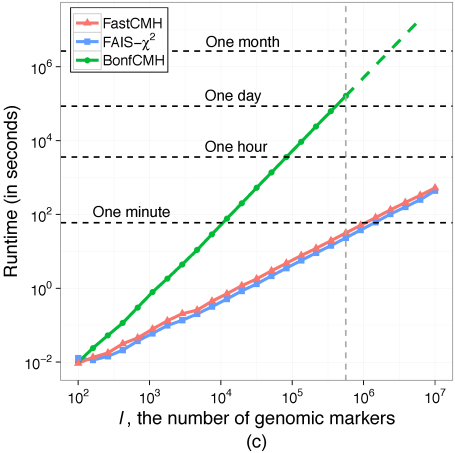


(c)

Datasets

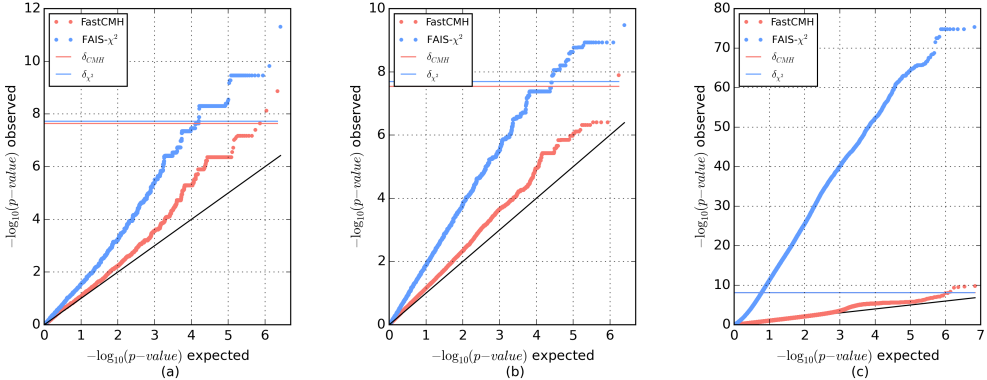
COPD case/control study

- Binary phenotype: COPD cases vs. controls.
- 8,011 samples, 3,633 are cases and 4,378 are controls.
- Approximately 615,906 SNPs, binarized using a dominant encoding, to study the risk factor of any minor-allele
- 2,665 African-American and 5,346 non-Hispanic whites.

Arabidopsis thaliana dataset

- 5 binary phentoypes
- 2-5 geographical origins (Eigenstrat, Price 2006).

FastCMH: correcting for confounders in COPD and Arabidopsis thaliana case/control studies



QQplots for: (a) LES phenotype, (b) LY phenotype, (c) COPD study

FastCMH reports novel genomic regions

COPD case/control study

- Each of the 3 reported regions overlaps with a gene in: CHRNA5-CHRNA3-CHRNB4, a nicotine receptor (nAChR).
- None of the SNPs alone shows an association with COPD.
- Separated studies (AA and NHW alone) do not find those three significant hits.

A. thaliana studies

- `FastCMH` reports 33 genomic regions and `FAIS-`χ^2 reports 81
- Decrease of the genomic inflation factor.
- 45% of the total number of reported SNPs are not into genes.

Burden tests: a genome-scale approach to study high-order interactions

Burden tests collapse SNPs into genes and test for the association of the entire region with the phenotypic trait (Lee 2014).
We used:

- a logistic regression model
- two encodings: (1) OR combination of SNPs inside the genes and (2) minor-allele counts.
- three covariate corrections: (1) principal components of the kinship matrix (only for *Arabidopsis th.*), (2) $k-1$ dummy variables for k classes and (3) CMH-test.

Limitations: Test a small subset of all possible regions in a genome by discriminating them on their function.

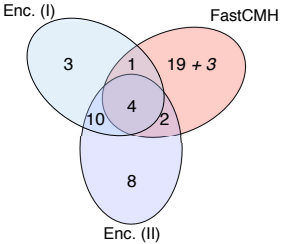
FastCMH finds genomic regions that can not be found by burden tests

COPD case/control study

- None of the three genes in CHRNA5-CHRNA3-CHRNB4 are reported by the burden tests.
- `FastCMH`'s advantage: significant regions do not span the entire genes.

Arabidopsis thaliana studies

- High variability among the hits
- Low to medium confounder correction.



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Summary

- `FastCMH` enables to discover *all* candidate genomic regions of genetic heterogeneity, efficiently, with high power and while correcting for confounders.
- Principled approach for meta-analysis.
- Code available:
  https://www.bsse.ethz.ch/mlcb/research/bioinformatics-and-computational-biology.html

Outlook

- Implementing the permutation testing version to correct for dependency between the tests.
- Extending `FastCMH` to heterozygous genotypes and continuous phenotypes.
- Including long-range interactions by enabling all combinations of SNPs (submitted work).
- Adding biological prior:
  - Differentiating between SNPs that prevent or cause a disease.
  - Detecting significant gene clusters in pathways (Part III).

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Part III
Significant Subgraph Search in Protein-Protein Interaction Networks

By Anja Gumpinger

Outline

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Motivation

Paradigm

- Univariate analysis of SNPs only account for small amount of total phenotypic variation [Manolio et al., 2009]
- Several variants, each with weak association to phenotype, orchestrate to manifest phenotype

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Paradigm

- Univariate analysis of SNPs only account for small amount of total phenotypic variation [Manolio et al., 2009]
- Several variants, each with weak association to phenotype, orchestrate to manifest phenotype

Idea

- Genes do not interact randomly with each other, but are organized in pathways
- Include biological prior knowledge into interaction search
- Use protein-protein interaction (PPI) networks
  - KEGG pathways [Kanehisa and Goto, 2000]
  - PINA [Cowley et al., 2011]

Problem statement

Initial setup:

- Dataset of n individuals that can be classified into two phenotypic groups:
  - n_1 cases
  - n_2 controls
- Protein-protein interaction network that will serve as biological prior knowledge

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- Dataset of n individuals that can be classified into two phenotypic groups:
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Problem statement: significant subgraph search

- Find subgraphs of genes within the PPI, such that the genotypes of the genes in the subgraphs are significantly associated with the phenotype
- Rigorous correction for multiply hypothesis testing by controlling the family wise error rate

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State of the art: dmGWAS

BIOINFORMATICS ORIGINAL PAPER
Vol. 27 no. 1 2011, pages 95–102
doi:10.1093/bioinformatics/btq615

Genetics and population analysis
Advance Access publication November 2, 2010

dmGWAS: dense module searching for genome-wide association studies in protein–protein interaction networks

Peilin Jia[1,2,†], Siyuan Zheng[1,3,†], Jirong Long[4], Wei Zheng[4,5] and Zhongming Zhao[1,2,3,5,6,*]

[1]Department of Biomedical Informatics, [2]Department of Psychiatry, [3]Functional Genomics Shared Resource, [4]Vanderbilt Epidemiology Center, [5]Vanderbilt-Ingram Cancer Center and [6]Bioinformatics Resource Center, Vanderbilt University, Nashville, TN 37232, USA

Associate Editor: Jeffrey Barrett

- Method [Jia et al., 2011] to identify subgraphs or genes for complex diseases
- Achieved by integrating the association signal from GWAS datasets into human protein-protein interaction networks

dmGWAS - Implementation: Input/Output

R implementation of dmGWAS available.

Input

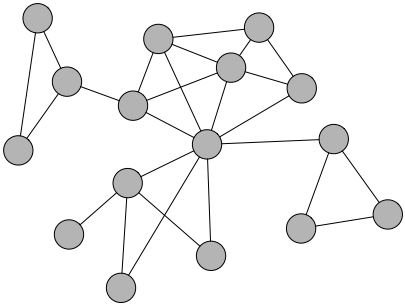
- Protein-protein interaction network
- P-values p_i for each gene in network
- User-specified parameters

Output

- List of subgraphs within the protein-protein interaction network, enriched with low p-value genes
- Subgraphs ranked by subgraph score

dmGWAS - Greedy search for subgraphs

1. Transformation of p-values, $z_i = \Phi^{-1}(1 - p_i)$



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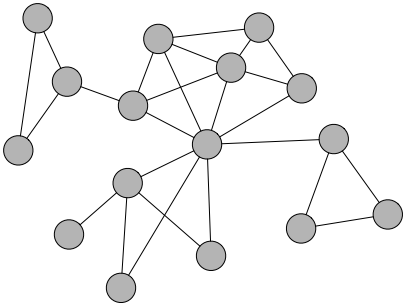

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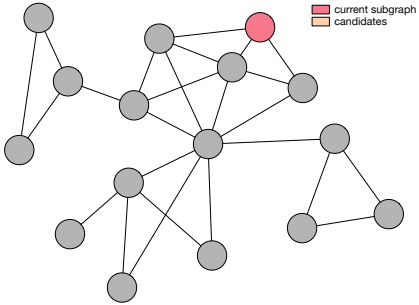

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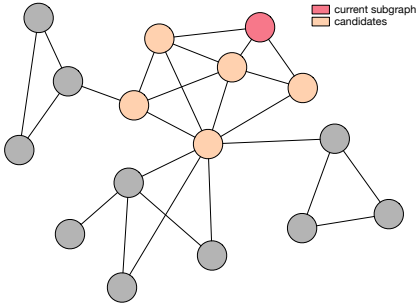

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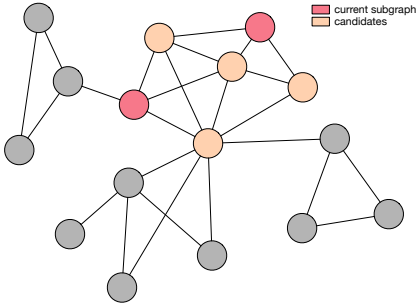

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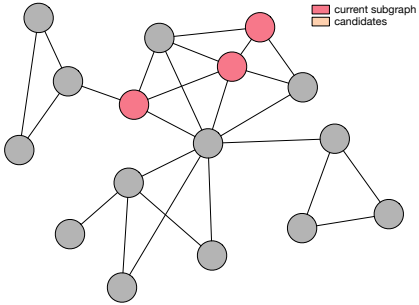

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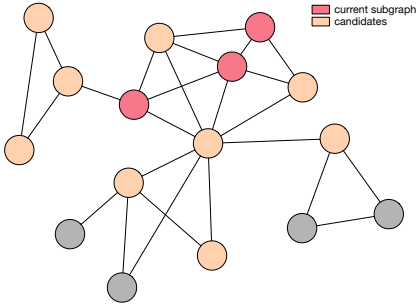

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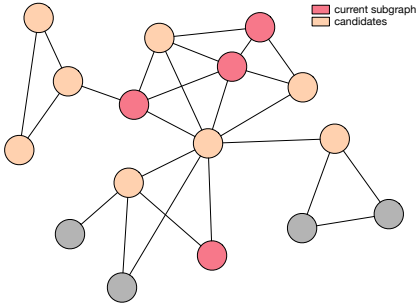

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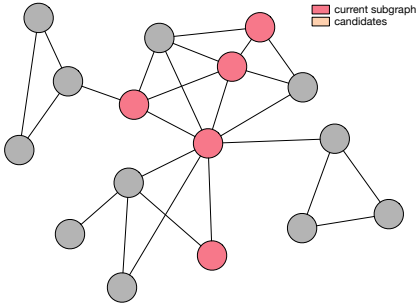

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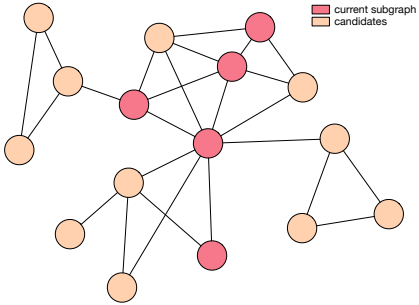

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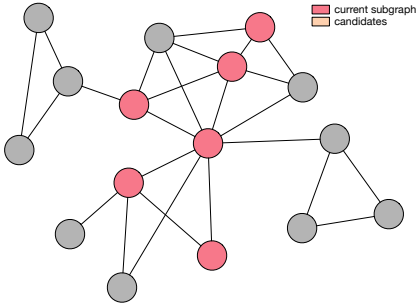

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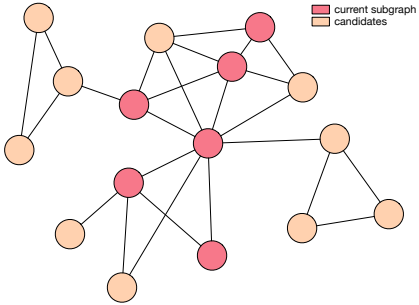

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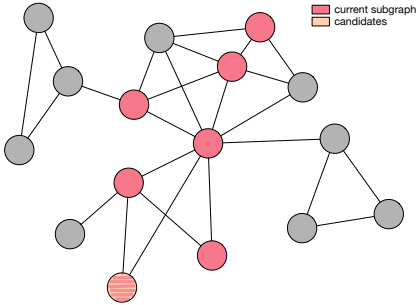

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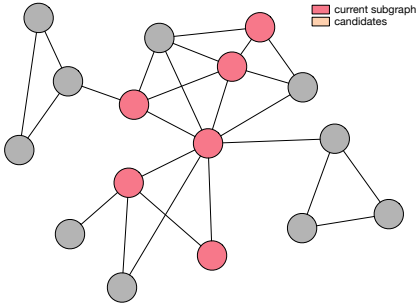

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dmGWAS - Characteristics

- Greedy approach, based on computation of gene-wise p-values
- No p-value, but ranking of subgraphs with high scores
- Outcome (number of subgraphs, sizes of subgraphs) highly dependent on setting of parameters d and r
  - Suggestions by authors:
    - $d = 2$: median distance between any two genes in PPI < 5 [Chuang et al., 2007]
    - r: test various values and take reasonable one
- Postprocessing of output:
  - Upper bound on number of reported subgraphs: number of genes in PPI
  - Suggestion of authors: use top 10% ranked subgraphs
  - Analysis of induced sugraph of top-ranked subgraphs (consensus graph)

State of the art: other methods

DAPPLE: Disease Association Protein-Protein Link Evaluator [Rossin et al., 2011]

- Network of genes associated with phenotype are more densely connected than expected by pure chance
- To show this: random permutation of underlying network

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- To show this: random permutation of underlying network

SConES: Selecting CONnected Explanatory SNPs [Azencott et al., 2013]

- Finding subgraphs in network with maximized association, connectivity and sparsity
- Can be written as optimization problem
- Code available at: https://www.bsse.ethz.ch/mlcb/research/bioinformatics-and-computational-biology/scones.html

Table of Contents

Short Revision: Tarone Trick

- Conducting high number of statistical significance test $\rightarrow$ multiple hypothesis testing problem

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- Control family-wise error rate (FWER)

$$\text{FWER} = \Pr\left(\text{FP} \geq 1\right) \leq \alpha \tag{1}$$

- Need to find the maximum significance threshold δ such that Eq. 1 holds

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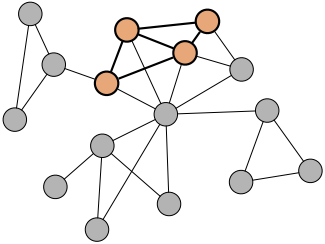
- Need to find the maximum significance threshold δ such that Eq. 1 holds
- Bonferroni correction: $\delta = \frac{\alpha}{\text{number of tests}}$
- Minimum attainable p-value: subgraphs that are not testable at a significance threshold δ cannot become false positives, thus no correction is required for those
- Tarone correction: $\delta = \frac{\alpha}{\text{number of testable subgraphs}}$

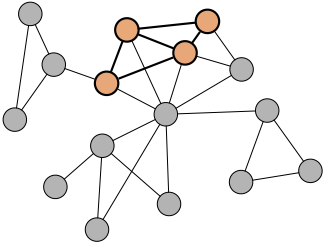
Tarone method for graphs: contingency tables

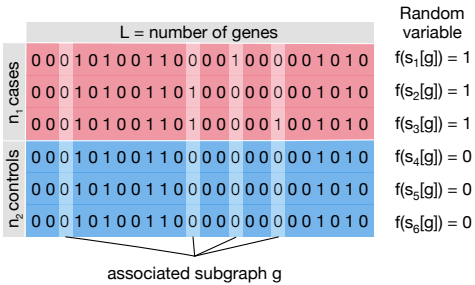


	L = number of genes	Random variable
n_1 cases	0 0 0 1 0 1 0 0 1 1 0 0 0 0 1 0 0 0 0 0 1 0 1 0	$f(s_1[g]) = 1$
	0 0 0 1 0 1 0 0 1 1 0 1 0 0 0 0 0 0 0 0 1 0 1 0	$f(s_2[g]) = 1$
	0 0 0 1 0 1 0 0 1 1 0 1 0 0 0 0 0 1 0 0 1 0 1 0	$f(s_3[g]) = 1$
n_2 controls	0 0 0 1 0 1 0 0 1 1 0 0 0 0 0 0 0 0 0 0 1 0 1 0	$f(s_4[g]) = 0$
	0 0 0 1 0 1 0 0 1 1 0 0 0 0 0 0 0 0 0 0 1 0 1 0	$f(s_5[g]) = 0$
	0 0 0 1 0 1 0 0 1 1 0 0 0 0 0 0 0 0 0 0 1 0 1 0	$f(s_6[g]) = 0$

associated subgraph g

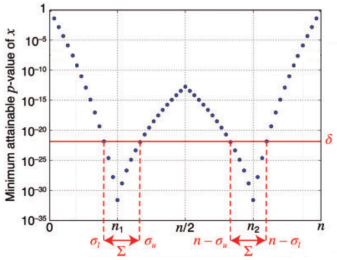
Tarone method for graphs: contingency tables





Variables	f(s[g]) = 1	f(s[g]) = 0	Row totals
y = case	α_g	$n_1 - \alpha_g$	n_1
y = control	$x_g - \alpha_g$	$n_2 - (x_g - \alpha_g)$	n_2
Col. totals	x_g	$N - x$	n

Tarone method: intervals vs. subgraphs



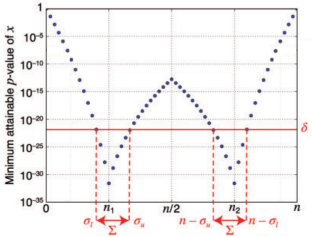
Interval search:

- Exploration of search space: subsequently combining intervals
- Pruning of search space: intervals containing non-testable intervals are non-testable

Subgraph search:

- Exploration of search space: growing subgraphs by subsequently adding nodes
- Pruning of search space: supergraphs of non-testable subgraph is non-testable

Network Tarone: growing and pruning graphs



- Subgraph g with x_g

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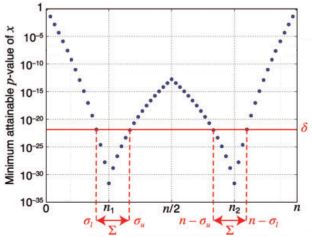

- Subgraph g with x_g
- **Monotonicity**: adding a new gene to a subgraph can only increase x_g

	current subgraph	Random variable
n_1 cases	0 1 0 1	$f(s_1[g]) = 1$
	0 0 0 1	$f(s_2[g]) = 1$
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n_2 controls	0 0 0 0	$f(s_4[g]) = 0$
	0 0 0 0	$f(s_5[g]) = 0$
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$x_g = 2$

	new subgraph		Random variable
n_1 cases	0 1 0 1	0	$f(s_1[g]) = 1$
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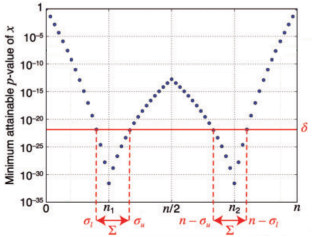
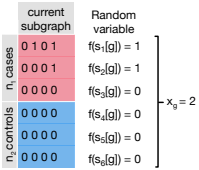
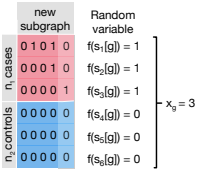
$x_g = 3$

Network Tarone: growing and pruning graphs



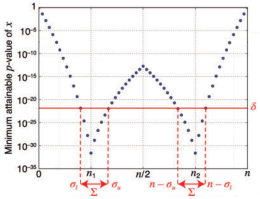




- Subgraph g with x_g
- **Monotonicity**: adding a new gene to a subgraph can only increase x_g
- Pruning: only subgraphs with $n - \sigma_l < x_g$ can be pruned from search space
  - If subgraph is non-testable: adding genes will always result in non-testable supergraph
  - Once subgraph is non-testable with $n - \sigma_l < x_g$: can stop growing graph

Network Tarone: adjusting the significance threshold

1. Compute minimum attainable p-value $\Psi(x_g)$ of current subgraph g with x_g

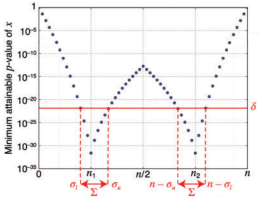


Network Tarone: adjusting the significance threshold

1. Compute minimum attainable p-value $\Psi(x_g)$ of current subgraph g with x_g
2. Subgraph is testable (i.e. $\Psi(x_g) \leq \delta$):
   1. Number of subgraphs that have to be corrected for increased
   2. Lower significance threshold δ s.t. FWER criterion is fulfilled

$$\delta * |\text{testable subgraphs}| \leq \alpha$$

   3. Add next gene to subgraph and return to step 1



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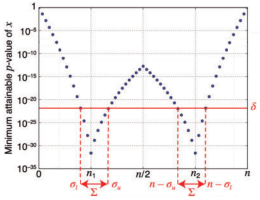
3. Subgraph is non-testable (i.e. $\Psi(x_g) > \delta$):
   1. $x_g < n - \sigma_l$: Add next gene to subgraph an return to step 1
   2. $x_g > n - \sigma_l$: Stop growing subgraph



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   - GWAS data
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   - Reporting of results and biological interpretation

Binarization of data

- Binarization depends on type of data used
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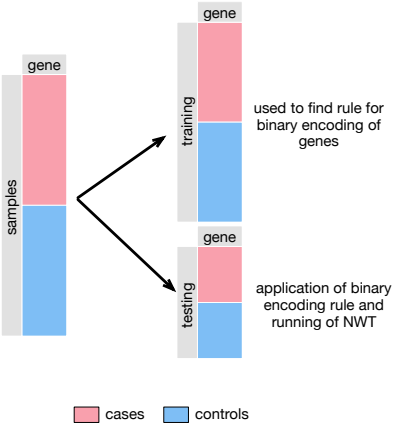
    - Machine learning approaches

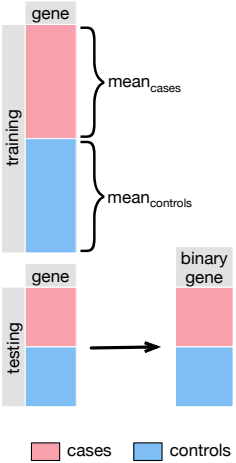
Idea: Risk gene encoding

- For one sample, binary status of a gene reflects whether sample can rather be assigned as case or control, based on only that gene
- Approaches require splitting of data into training and test set



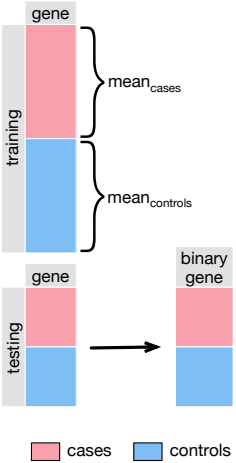
Binarization of gene-expression data

1. For each gene, compute the mean of cases mean_{cases} and controls $\text{mean}_{controls}$ in training set



Binarization of gene-expression data

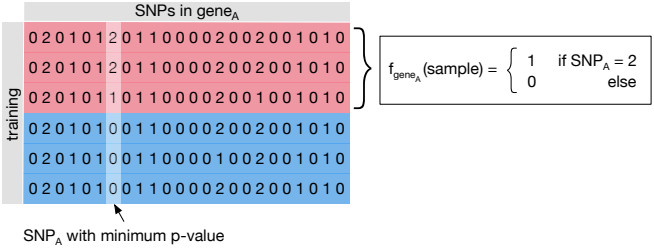
1. For each gene, compute the mean of cases mean_{cases} and controls $\text{mean}_{controls}$ in training set
2. Use data in test set to run Network Tarone:
   1. Binarize the data in test set by assigning the gene the label of the group with the smaller distance to the mean
   2. Use binary data as input for NWT



Binarization of GWAS data using allele counts

Building a classification rule

1. Represent gene by all SNPs in or near gene



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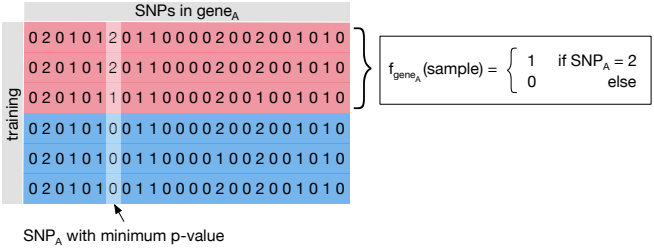
2. Compute univariate p-values for each SNP in gene (using PLINK, FaSTLMM, ...)
3. Represent gene by SNP with lowest p-value



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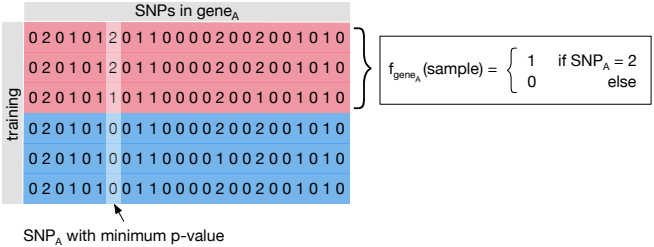
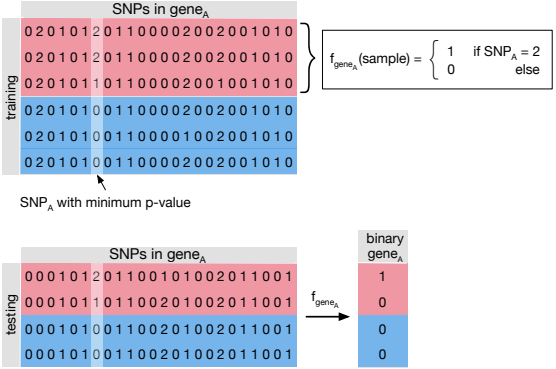
3. Represent gene by SNP with lowest p-value
4. Determine most frequent genotype of selected SNP in cases and use this as classification rule



Binarization of GWAS data using allele counts

Classification of samples in test set

1. Represent gene by SNP with lowest p-value in training set
2. Apply classification rule found on training set to get binary representation of gene for each sample in test set



Binarization of GWAS data using machine learning (work in progress)

Building classification rule

1. Represent gene by all SNPs in or near gene
2. Determine a classification rule for each gene using all SNPs to predict risk encoding

Classification of samples in testing set

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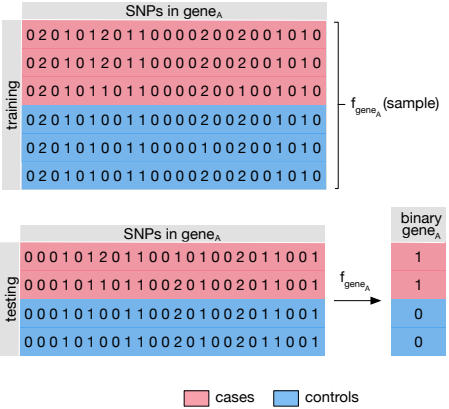
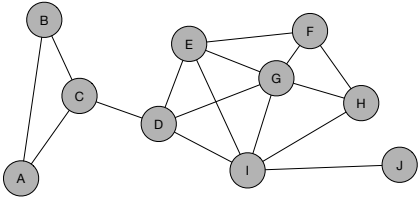
2. Apply classification rule found on training set to get binary representation of gene for each sample in test set



Exploring the network

Growing the subgraphs

Need computationally efficient way to enumerate subgraphs in order to avoid visiting same subgraphs multiple times. Approach based on [Wernicke, 2006].



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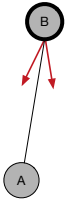

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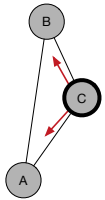

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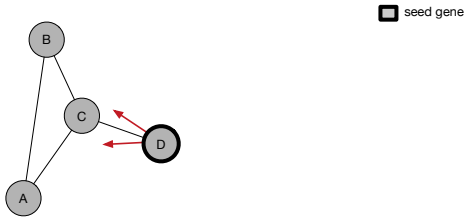

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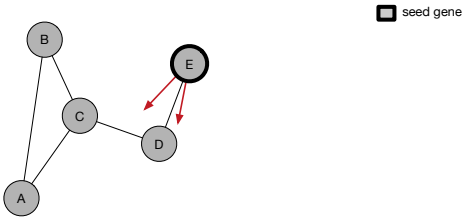

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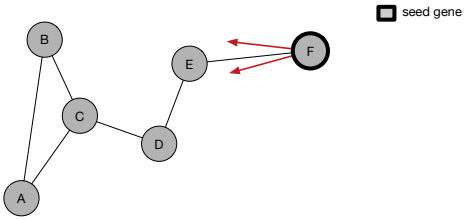

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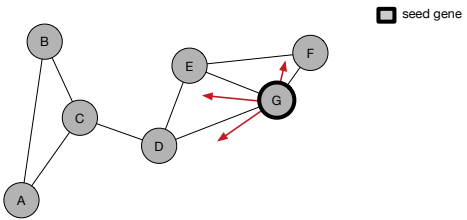

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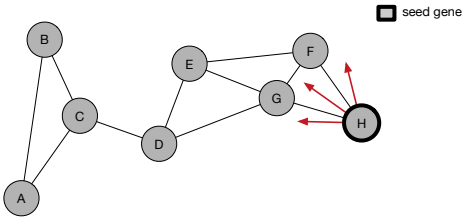

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Some results of NWT on artificial data

Artificial generation of binary data

- Generation of binary data with known ground truth (true significant subgraph)
- using R package 'bindata'
- Parameters to set:
  - Size of data set
  - Sizes of associated subgraphs
  - Risk ratio (ratio of 1/0 in binarized data)
  - Strength of association between subgraph and phenotype
- Size of underlying network: 68 nodes, 84 edges

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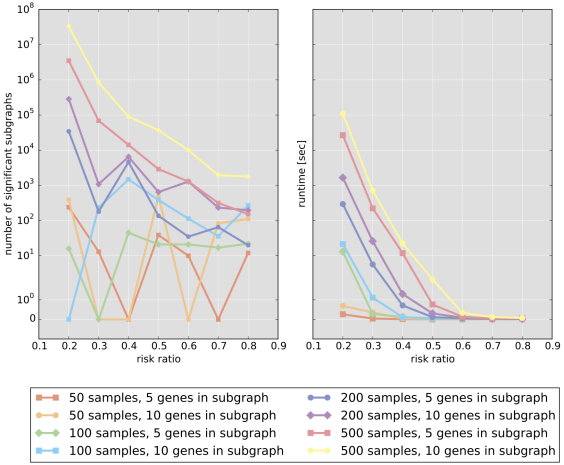

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Cluster all significant subgraphs, use subgraph with lowest p-value from each cluster as final output

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DBSCAN clustering (work in progress)

- Create graph of subgraphs, where each subgraph corresponds to node, edge weighted by Jaccard-index, correlation, ...

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Dataset:
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- Number of samples: 207
- Number of mRNAs measured: 24.385
- Patients in study are divided into two groups
  - Estrogen receptor positive (ER+)
  - Estrogen receptor negative (ER-)
- Tumors from two groups show different molecular patterns in terms of cell differentiation, proliferation, survival, invasion, angiogenesis
- In general: better prognosis and treatment of ER+ patients compared to ER- patients

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1. Binarization of data
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1. Binarization of data
   - 107 samples in test set, 100 samples in training set
   - risk ratio: 0.27
2. Application of NWT approach to 11 KEGG pathways
   - 7 signaling pathways
   - 2 pathways linked to cell adhesion
   - 2 pathways linked to cell cycle and apoptosis
3. **Results**: Found significant subgraphs in 9 KEGG pathways

Application to breast cancer mRNA profiles

KEGG pathway	Pathway description	genes in pathway	significant subgraphs	average size	runtime (in sec)
04115	p53 signaling pathway	65	2049	6.83	10.35
04150	mTOR signaling pathway	48	0		0.49
04330	Notch signaling pathway	46	93	5.42	1.73
04064	NF-kappa B signaling pathway	70	1	5	3.50
04012	ErbB signaling pathway	83	12	4.75	4.72
04010	MAPK signaling pathway	240	29063	7.79	149.34
04310	Wnt signaling pathway	127	91	5.58	176.74
04510	Focal adhesion	195	670	8.10	2824.77
04520	Adherens junction	68	0		2.19
04110	Cell cycle	114	45	6.69	33.25
04210	Apoptosis	75	21	6.33	0.96

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- Less conservative significance threshold than classical approaches, such as Bonferroni correction

Future work

- Application to more sophisticated PPI networks
  - Networks with directed edges (pathways are directed)
    - → reduces number of networks to test

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- Improve runtime for datasets with large sample sizes
  - → GPU implementation
- Include correction for covariates
  - → Analogously to CMH

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Thank you

- Felipe Llinares López
- Dean Bodenham
- Udo Gieraths
- Dominik Grimm
- Elisabetta Ghisu
- Anja Gumpinger
- Xiao He
- Laetitia Papaxanthos
- Damian Roqueiro
- Menno Witteveen
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Thank you for your patience and attention