Lecture 4: Gene and Pathway Level Analysis of Genetic Association Studies

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Lecture Overview

1. Rationale and Background
2. Some Popular Methods for Gene and Pathway Level Testing
3. Statistical Issues: What's the null hypothesis?
   - 3.1 Competitive vs. Self-contained Hypotheses
   - 3.2 SNP-sampling vs. Subject Sampling
4. Remarks and References

Standard Analysis Strategy

Individual Variant Analysis:

1. For each SNP, compute a statistic measuring association
2. Compute a p-value for significance
3. Adjust for multiple comparisons:
   - FWER
   - FDR
4. Follow-up
   - Directly report results
   - Meta-analyze
5. Auxiliary analyses

Focus of traditional analyses is on a handful of SNPs that meet criteria for significance.

Limitations of the traditional approach:

Biggest problem: What if we don't find anything???

1. Genome Wide Significance: Stringent and difficult to reach. After correcting for multiple hypotheses testing, no SNPs are statistically significant.
2. An untyped causal SNP is in LD with multiple typed SNPs: Typed SNPs may only show moderate effects.
3. Most common diseases are complex: multi-SNP effects
   - Most individual SNPs have only modest effects
   - Joint effect of several, individually moderate, SNPs is important.
4. Reproducibility: Without strict thresholds: a large number of false positives!
5. Who Cares?: What's the biological or mechanistic interpretation of what you've found?

Alternative: Multi-SNP Analysis

Operationally Equivalent Terms: multi-SNP testing, multi-locus testing, gene based analysis, pathway analysis

Multi-SNP Analysis

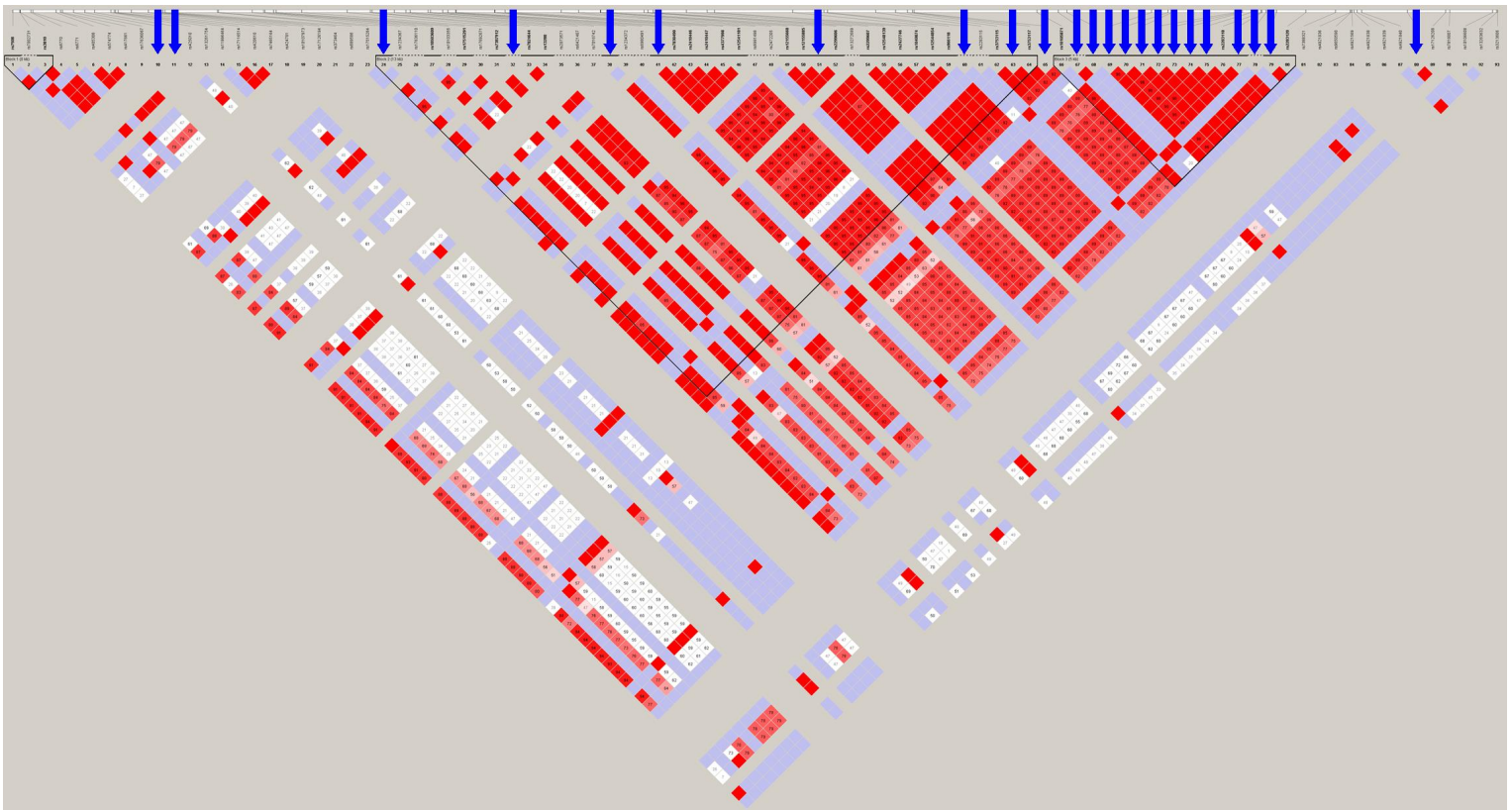
- Idea: Group SNPs to form SNP sets and test them as a unit
- Forming SNP sets:
  1. Genes
  2. Pathways (many SNPs)
  3. Evolutionarily conserved regions
  4. Moving window
  5. Any group of SNPs selected w/o using outcome data

Advantages to Gene and Pathway Level Analysis

- Reduced multiple testing burden
  - Millions of SNPs $\rightarrow$ 20,000 genes
  - A few candidate pathways
- Capture multi-SNP effects:
  - Aggregate modest signals
  - Capture effects of untyped SNPs
  - Possibly capture complex (e.g. interactive) effects
- Biologically meaningful unit

Example: ASAH1 Gene

LD plot (correlation structure)



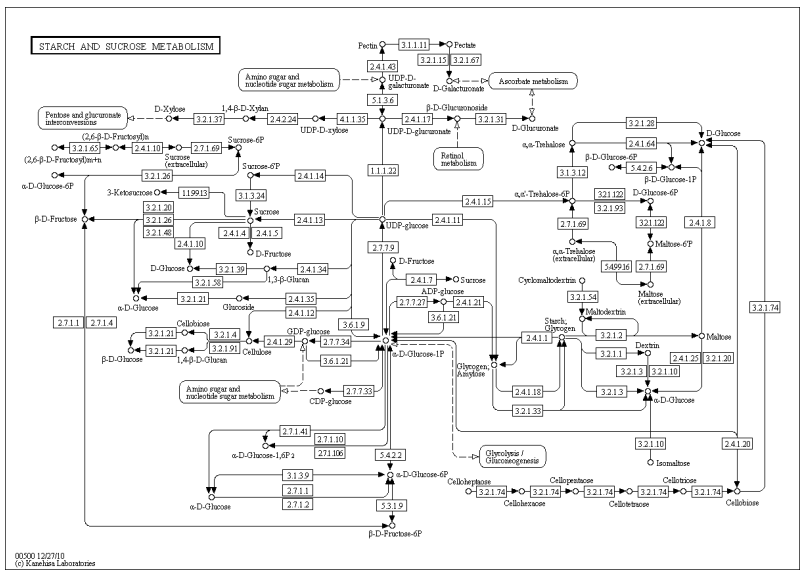
Pathways and Gene Sets

Beyond gene level (or a single region) analysis:

- Most biological phenomena occur through the concerted expression of multiple genes (signaling pathways or functional relationships)
- Use our prior knowledge of what SNPs belong to various genes which in turn belong to pathways or functional groups
- Numerous databases organizing genes into groups exist:
  1. Pathways: KEGG
  2. Functional Groups: Gene Ontology (GO), MSigDB, etc.
  3. Paid Databases: Ingenuity
  4. etc...
- Note: Functional groupings are NOT the same as Pathways.

KEGG

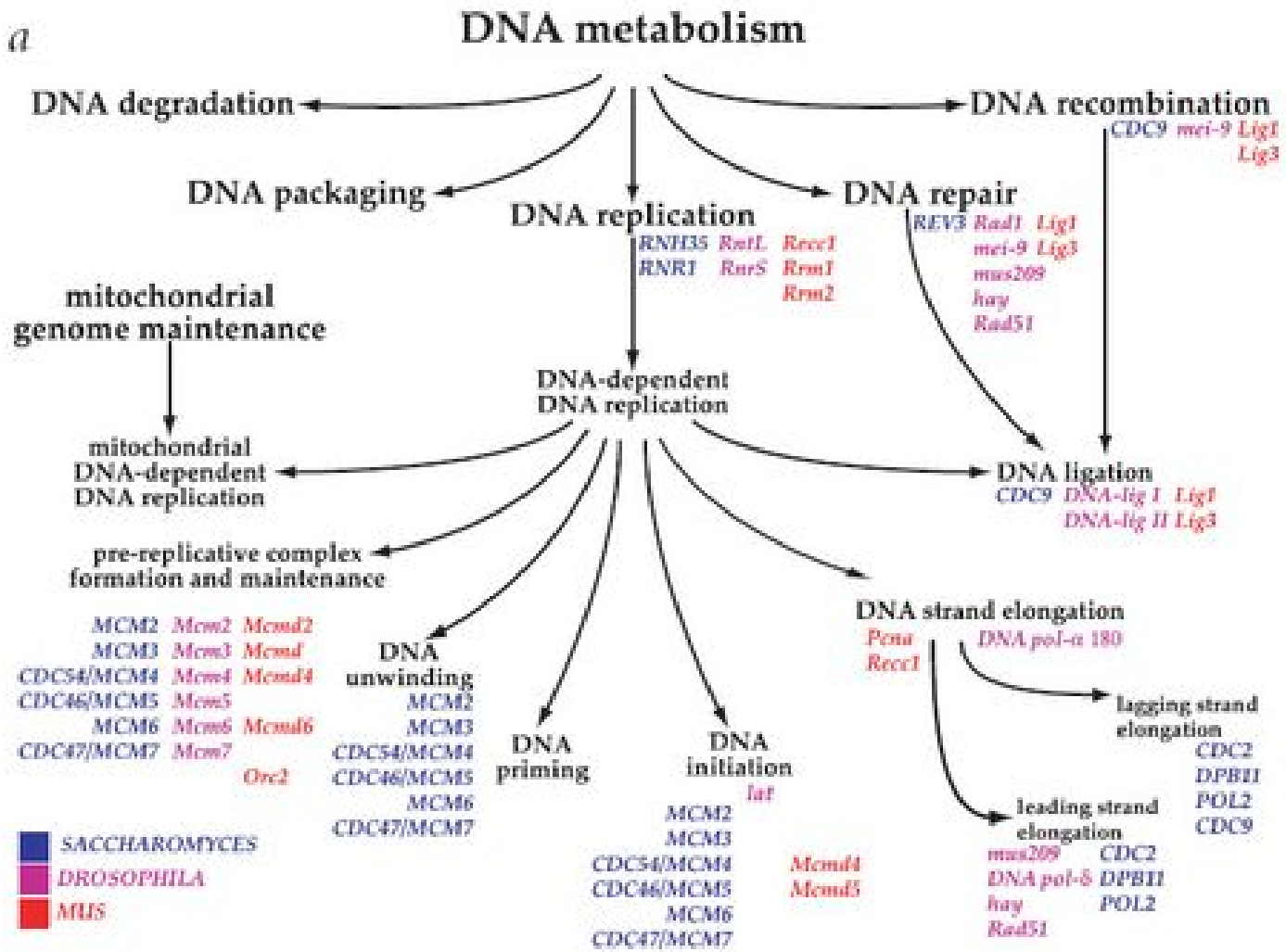
- "Collection of online databases dealing with genomes, enzymatic pathways, and biological chemicals" - Wiki
- KEGG Pathways is network of gene pathways
- Cleaner set of pathways, but much smaller: emphasis on metabolic pathways though there are also disease and other trait related pathways.



Gene Ontology (Function Groupings)

- Three principal ontologies: Biological Processes, Cellular Components, and Molecular Function
- Each ontology is a directed acyclic graph
- The graph has a hierarchy of terms (GO terms) from very broad (metabolism) down to more narrow levels (GTP biosynthesis)
- Each ontology and GO term has a comprehensive list of genes previously demonstrated to be associated with that ontology or GO term.
- Contains a lot of JUNK! Filtering is necessary.
- A wide variety of packages in R can provide many basic tools for mining gene ontology information

Gene Ontology



Question...

Suppose we know that a bunch of SNPs are in a genes or in a pathway.

How do we test if the gene or pathway is associated with the phenotype?

Statistical Methods:

Gene Level Analysis

- Minimum p-value Tests (minP)
- Combined p-value approaches
- Averaging/Collapsing Tests
- Variance Component (VC) Tests

Pathway Level Analysis

- Over-representation Analysis (ORA)
- Gene Set Enrichment Analysis (GSEA)
- minP, Averaging, Combined p-value, VC Tests
- Graphical methods $\leftarrow$ not covered (usually like ORA)

Many tools can (technically) be used interchangeably

Minimum p-value

- Idea: let the smallest individual SNP p-value be the p-value for the entire pathway.
- Easy to run individual SNP analysis.
- How do we correct for having taken the smallest p-value?
  - Bonferroni correction. (conservative)
  - Compute the effective number of tests. (suspect)
  - Permutation. (sloooow...)

Combined p-value Approaches

- Idea: combine the p-values across the SNPs in the gene
- Operationally:
  1. Test each individual SNP for association
  2. Combine the p-value for top SNPs, e.g. via Fisher's method
- Variations include taking only top few p-values (Tail strength)
- Challenge: Most p-value combination approaches require independent p-value (i.e., no LD)
  - Permutation
  - Alternative methods claim to capture LD (most fail!)
  - **Recent Development:** Cauchy-Combination Test

Cauchy-Combination Test

Approach to overcome LD

1. Suppose we have $p_1, p_2, \ldots, p_k$ which are the p-values from k SNPs in a gene
2. We transform the p's to be Cauchy's:

$$\begin{aligned} p_1 &\rightarrow T_1 = tan\{(0.5 - p_1)\pi\} \\ p_2 &\rightarrow T_2 = tan\{(0.5 - p_2)\pi\} \\ \vdots &\quad \vdots \quad \vdots \\ p_k &\rightarrow T_k = tan\{(0.5 - p_k)\pi\} \end{aligned}$$

3. Calculate $T = \sum_{j=1}^{k} T_k$ and the final p-value:

$$p = 1/2 - arctan(T)/\pi$$

Idea: Cauchy distribution is robust to correlation in the tails.

Averaging/Collapsing

- Idea: can we collapse the SNP values down to a single value?
- We can construct a weighted average:

$$C_i = \sum_{j=1}^{p} w_j x_{ij}$$

  such that C_i is a "super-SNP". Then we can test for association between C and y.
- Common approaches to get the w_j
  - Simple average
  - Inverse of MAF
  - p-values from previous studies
  - PCA (1st or many)
  - Using other -omics/outcomes (PrediXcan)
  - Supervised approaches –¿ requires permutation
- Test effect of gene by regressing outcome on C_i

PrediXcan: "Transcriptome Wide Association Study (TWAS)"

Gamazon et al. (2015, *Nature Genetics*)

- Idea:
  - Genetic effect may go through expression regulation
  - Identify component of expression regulated by genetics and correlate 'predicted expression' with trait
- Operationally:
  - Using reference samples, regress expression on SNPs within a gene (Elastic Net)
  - Treat regression coefficients as the weights w_j
- Issues:
  - Tissue
  - Genetics often explains very little variation
  - Not all effects are through expression levels
- **Remember:** TWAS ARE JUST GENE-BASED TESTS!

Similarity Based/Variance Component Methods: "Global Test"

- Build a regression model to predict the phenotype based on the SNPs:

$$g(\mathcal{E}(y_i)) = \alpha' \mathbf{Z}_i + \beta_1 x_{i1} + \beta_2 x_{i2} + ... + \beta_p x_{ip}$$

  Where x_{ij} is the genotype value for the j^{th} SNP of the i^{th} sample, $\mathbf{Z}_i$ are covariates, and g is some link function (e.g. logit).
- Testing for the joint effect of the SNPs is equivalent to:

$$H_0 : \beta_1 = \beta_2 = ... = \beta_{N_S} = 0$$

- Assuming β's are iid with mean 0 and variance τ^2, then our null hypothesis is simply $H_0 : \tau^2 = 0$
- Can either use permutation or asymptotics to get the p-values.

Similarity Based/Variance Component Methods: Kernel Machine Methods

- Generalize the variance component testing to nonparametric regression setting:

$$g(\mathcal{E}(y_i)) = \alpha' \mathbf{Z}_i + h(\mathbf{X}_i)$$

  where the effect of the SNPs are modeled non-parametrically.
- Allows for "complex" effects of SNPs on outcome: interactions, nonlinearity, etc.
- More on this when we talk about rare variants.

Over-representation Analysis (ORA)

- Start from the list of "significant" SNPs
  - Can be based on multiple comparisons criterion as mentioned earlier
  - 100 SNPs with smallest p-value
  - Top 5% of SNPs with smallest p-value
  - Many other ways...
- Look for an over-representation of the SNPs in the pathway among "most significant" SNPs (or over-representation of "most significant" SNPs in the pathway)

ORA - 2x2 Contingency Tables

With the list of "significant" SNPs (D) and the list of SNPs in the pathway (S), we can build a 2x2 table:

	Significant	Not Significant	
In pathway	N_{SD}	N_{SD^c}	N_S
Not in pathway	N_{S^cD}	$N_{S^cD^c}$	N_{S^c}
total	N_D	N_{D^c}	N

Generate a p-value for representation by using a test for independence:

- Fisher's Exact Test
- χ^2-test
- Hypergeometric Test
- Binomial proportions z-test
- Choice of test is unimportant in practice.

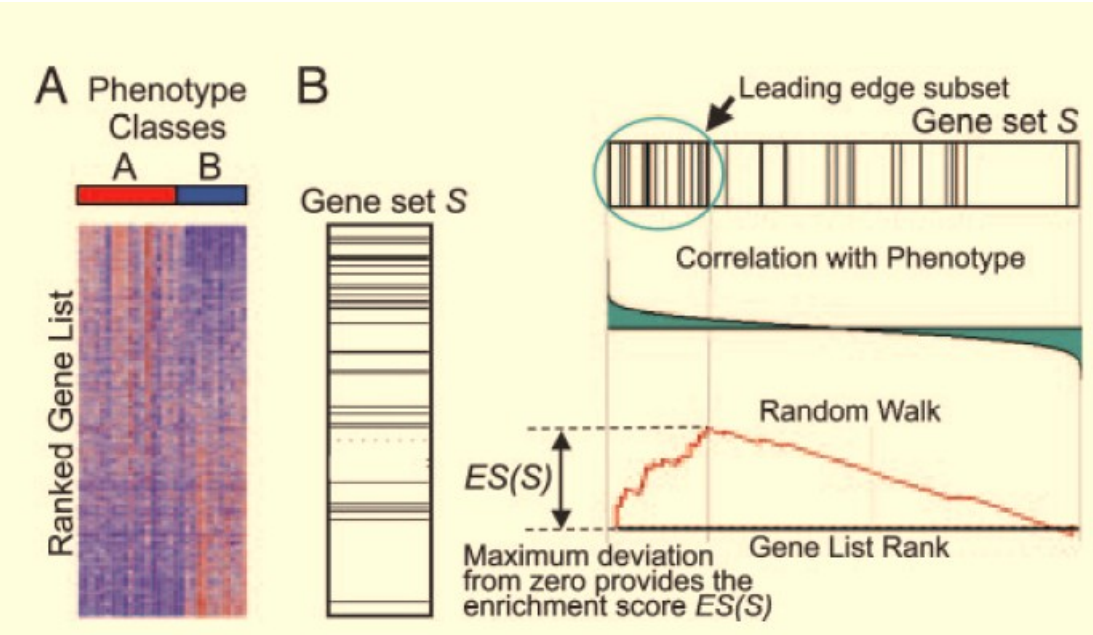
ORA - Criticism

- All of the tests on the previous slide require independence among SNPs.
- Length Bias.
- Alternative approach:
  - Conduct a gene level analysis (multiple regression) to get a p-value for all SNPs in the gene
  - Apply ORA at the gene (instead of SNP) level.
- LD and length bias are NOT the biggest problem: more on this later.

Gene Set Enrichment Analysis (GSEA)

Original GSEA Approach:

- Start from the full list of SNPs
- Order the SNPs according to association p-value to obtain L
- Look to see of SNPs in the S are randomly distributed throughout L or primarily at the top or bottom.



Gene Set Enrichment Analysis (GSEA)

Original GSEA Approach:

1. Rank all N SNPs (or genes) based on their p-values to obtain L, the SNP/gene list
2. Calculate an Enrichment Score (ES) for the data set:
   For G_i (the i-th gene in L), let:

$$X_i = \begin{cases} \sqrt{\frac{N_{S^c}}{N_S}} & \text{if } G_i \text{ is in } S \\ -\sqrt{\frac{N_S}{N_{S^c}}} & \text{if } G_i \text{ is NOT in } S \end{cases}$$

   $\text{ES}(S) = max_{1 \le j \le N} | \sum_{i=1}^{j} X_i |$
3. Evaluate Significance:
   - 3.1 Randomly permute the class labels
   - 3.2 Re-rank the SNPs
   - 3.3 Calculate ES(S) based on the new ranked gene list
   - 3.4 Repeat the above for a bunch of times

Statistical Considerations

Goals...

Goal: Test the null hypothesis that my pathway is not associated with the outcome...

What does this even mean???

What's my Null?

Two different possible null hypotheses:

Competitive Null Hypothesis:

H_0^{comp} : The SNPs in S are at most as often associated with the outcome as the SNPs in S^c

- Over-representation analysis (2x2 contingency table methods)
- GSEA

Self-contained Null Hypothesis:

H_0^{self} : No SNPs in S are associated with the outcome

- Variance Component Tests
- Minimum P-value
- Collapsing

Competitive Null Hypotheses

- Pits one pathway against another
- Competitive tests cannot compare all of the SNPs on the chip.
- In the competitive testing framework, significant SNPs in one pathway will generally lead to larger p-values for other pathway. Thus, p-values tend to be negatively correlated which is problematic if we want to control for the FDR.

Self Contained Null Hypotheses

- Self-contained tests theoretically have more power since truth of H_0^{self} generally implies H_0^{comp}. Under the competitive setup significance is penalized in experiments with many disease associated SNPs.
- Self-contained tests are direct generalizations of individual SNP tests (they are equivalent for pathways with only a single SNP).
- Testing the global null sometimes violates the spirit of pathway analysis.
- Note: outside of SNPs, self-contained tests may be *too* powerful in data sets where many features appear to be important

What's my sampling unit?

Subject Sampling:

- (original) GSEA
- Variance Component Tests
- Averaging/Collapsing
- MinP and Combined p-value tests

SNP Sampling:

- Over-representation analysis (2x2 contingency table methods)
- (new) GSEA

SNP vs. Subject Sampling

- *Classical tests are based on experiments that sample subjects:* draw a sample of subjects, each with the same fixed set of SNPs (sample size is number of subjects)
- *SNP sampling flips the classical setup:* draw a new sample of SNPs coming from a fixed set of subjects (sample size is number of SNPs)
- Interpretation of p-value's depends on the sampling scheme:
  - *Subject Sampling:* significant p-value gives confidence that the associations found between SNPs and the outcome will be found for a new sample of subjects
  - *SNP Sampling:* significant p-value gives confidence that a for a new set of SNPs from the same subjects, there will be a similar association between being in the pathway and being called "significant"

SNP vs. Subject Sampling (continued)

- *SNP sampling fails to mimic the biological experiment performed* which always take a new sample of subjects rather than a new sample of genes.
- Both sampling schemes assume sampling units are *independent and identically distributed*. That SNPs are independent is extremely unrealistic. – this is minor relative to the interpretation of the p-value.
- How to look out for SNP sampling:
  - Words: "enrichment", "over-representation", "fisher's exact test", "hypergeometric test"
  - Software: DAVID, EASE, Ingenuity (IPA)... anything too easy
  - Tiny, tiny p-values
  - Any method that only uses individual p-values.
  - Fancy pictures.

Remarks

- Different methods give different results
- Different methods operate under different assumptions
- SNP sampling is generally not reasonable for most practical settings: "invalid"
  - Invalid statistics does not mean biology is wrong
  - Can still be useful for "interpretation"
- Self contained testing is in some ways more natural, but can be difficult to interpret as a pathway result.

Skepticism Regarding Pathway Analysis

A quote from a well known statistician regarding pathway analysis:

"... at best the authors believe it to be true."

Some Issues:

- Inappropriate or invalid methods used
- Applied when no marginal significance (i.e. run when there really isn't much going on in the data)
- Cherry-picking results: inappropriate control for multiple testing

My opinion: Still useful for interpreting results!!

References

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