# 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-analyize
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

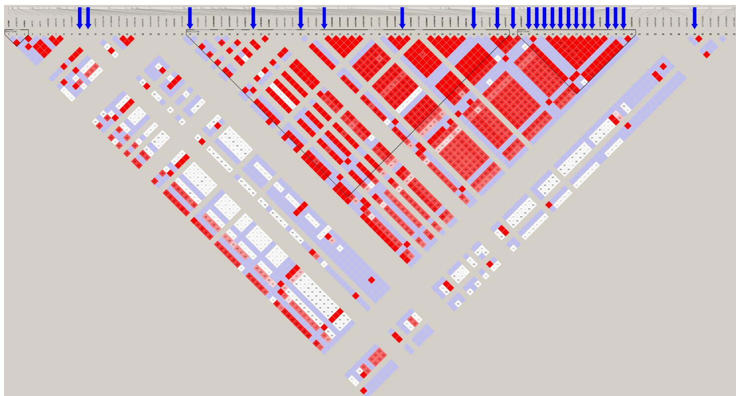
- Idea: Group SNPs to form SNP sets and test them as a unit
- Forming SNP sets:
  1. Genes
  2. Pathways (many SNPs)
  3. Evolutionarily conserved regions
  4. Moving window
  5. Any group of SNPs selected w/o using outcome data

# Advantages to Gene and Pathway Level Analysis

- Reduced multiple testing burden
  - Millions of SNPs $\rightarrow$ 20,000 genes
  - A few candidate pathways and candidate genes
- 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

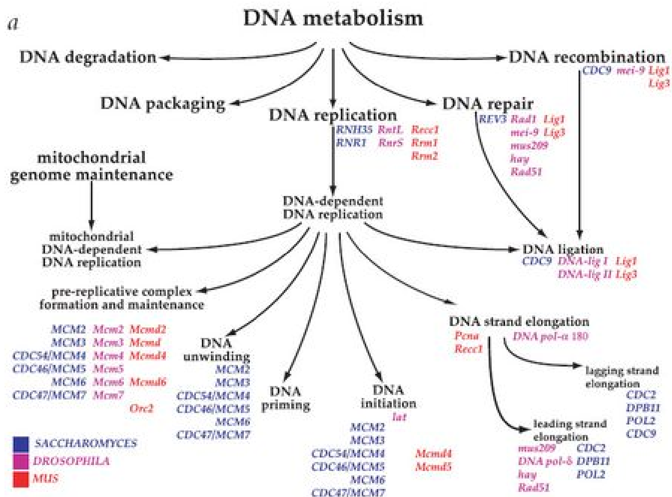
Beyond gene level (or a single region) analysis:

- 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. Ingenuity
  2. MetaCore
  3. KEGG
  4. Gene Ontology (GO) Consortium
  5. etc...
- Note: Gene sets and functional groupings are NOT the same as Pathways.

# Gene Ontology Consortium Taxonomy

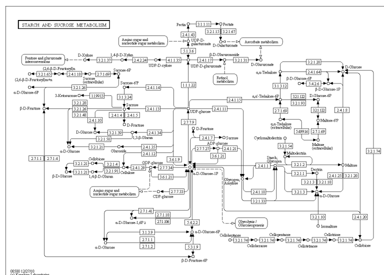
- Three principal ontologies: Biological Processes, Cellular Components, and Molecular Function
- Each ontology is a directed acyclic graph
- The graph has a hierarchy of terms (GO terms) from very broad (metabolism) down to more narrow levels (GTP biosynthesis)
- Each ontology and GO term has a comprehensive 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



# 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 than GO, but much smaller: emphasis on metabolic pathways though there are also disease and other trait related pathways.



# Question...

Suppose we know that a bunch of SNPs are inside of (genes in) a pathway. How do we test if the 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, Collapsing, Combined p-value, VC Tests
- Graphical methods ← 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.
  - Compute the effective number of tests.
  - Permutation.

# 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
- Challenge: Most p-value combination approaches require independent p-value (i.e., no LD)
  - Permutation
- Variations include taking only top few p-values

# 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)
  - Supervised approaches.
- Test effect of gene by regressing outcome on $C_i$

# 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 $\beta$'s are iid with mean 0 and variance $\tau^2$, 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
- $\chi^2$-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:

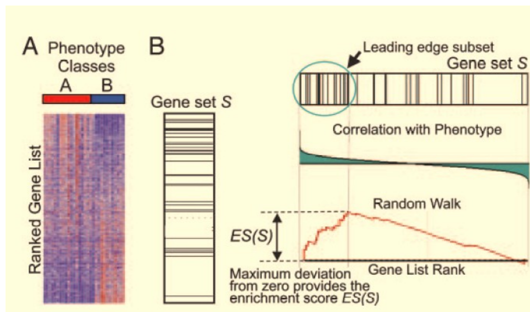
1. Rank all $N$ SNPs (or genes) based on their $p$-values to obtain $L$, the SNP/gene list
2. Calculate an Enrichment Score (ES) for the data set:
   For $G_i$ (the $i$-th gene in $L$), let:

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

   $\mathrm{ES}(S) = max_{1 \leq j \leq N} |\sum_{i=1}^{j} X_i|$
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

# Gene Set Enrichment Analysis (GSEA)

- Start from the full list of genes
- Order the genes according to differential expression between classes to obtain $L$
- Look to see of genes in the gene set $S$ are randomly distributed throughout $L$ or primarily at the top or bottom.



# 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 differentially expressed 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 differentially expressed

- 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 $H_0^{\text{self}}$ generally implies $H_0^{\text{comp}}$. Under the competitive setup significance is penalized in experiments with many differentially expressed genes.
- 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:

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

SNP Sampling:

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

# 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 gene/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.
- Broadly speaking, SNP sampling is wrong!
- How to look out for SNP sampling:
  - Words: "enrichment", "over-representation", "fisher's exact test", "hypergeometric test"
  - Software: DAVID, Ingenuity (IPA), GeneGo, most commercial stuff.
  - 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" (though then the $p$-value calculation is a waste of time)
- 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

# References

- Wang, Kai, Mingyao Li, and Maja Bucan. "Pathway-based approaches for analysis of genomewide association studies." *The American Journal of Human Genetics* (2007): 1278-1283.
- Yu, Kai, et al. "Pathway analysis by adaptive combination of Pvalues." *Genetic epidemiology* (2009): 700-709.
- Wu, Michael C., et al. "Powerful SNP-set analysis for case-control genome-wide association studies." The American Journal of Human Genetics 86.6 (2010): 929-942.
- Wu, Michael C., and Xihong Lin. "Prior biological knowledge-based approaches for the analysis of genome-wide expression profiles using gene sets and pathways." *Statistical methods in medical research* (2009): 577-593.
- Goeman, Jelle J., and Peter Bhlmann. "Analyzing gene expression data in terms of gene sets: methodological issues." *Bioinformatics* (2007): 980-987.