Lecture 10: Gene Environment Interactions, Meta Analysis, Emerging Issues

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Lecture Outline

Yet more on rare variants...

- Gene-Environment Interaction Testing
- Meta-analysis
- Additional Concerns

Gene-Environment Interactions ($G \times E$)

Complex diseases are caused by interplay of genes & environment.
Identification of $G \times E$ aids in disease prevention.

GxE Association Testing

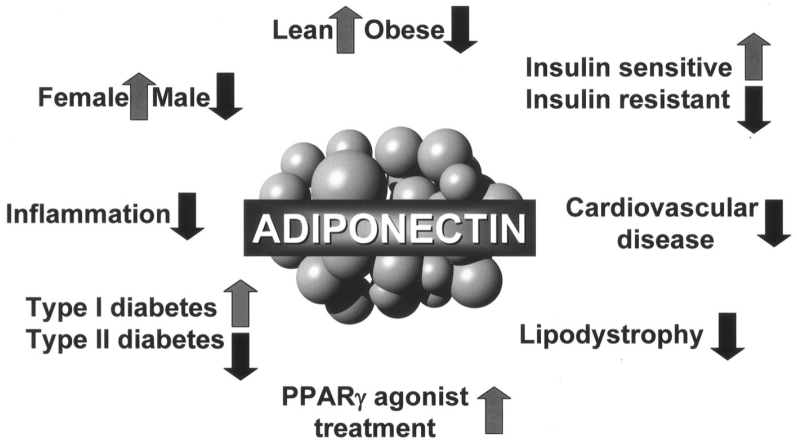
Objective: Identify statistical interactions (synergism/antogonism) between environmental variable and rare variants in sequencing studies

Standard Approach:

- Test SNV individually
- Regress outcome on single variant, environment, and interaction
- Under-powered for rare variant analysis! (possibly worse than main effects)

How do we conduct region based analysis of GxE interactions?

Motivation



- Circulating levels of adiponectin are highly heritable and associated with many conditions.
- SNVs at the adiponectin-encoding gene, ADIPOQ, are associated with adiponectin levels.
- Dataset consists of adiponectin levels and rare SNVs (MAF $< 5\%$) within the ADIPOQ gene.

Notation

Consider the following generalized linear model:

$$\begin{aligned} g(\mu_i) &= \mathbf{X}_i^\mathsf{T}\boldsymbol{\alpha}_1 + \alpha_2 E_i + \mathbf{G}_i^\mathsf{T}\boldsymbol{\alpha}_3 + E_i\mathbf{G}_i^\mathsf{T}\boldsymbol{\beta} \\ &= \widetilde{\mathbf{X}}_i^\mathsf{T}\boldsymbol{\alpha} + \mathbf{S}_i^\mathsf{T}\boldsymbol{\beta}. \end{aligned}$$

- Outcome: Y_i, has distribution from exponential family and $\mu_i = E(Y_i|\widetilde{\mathbf{X}}_i)$.
- q non-genetic covariates: $\mathbf{X}_i$.
- environmental factor: E_i.
- group of p variants: $\mathbf{G}_i = (G_{i1}, \cdots, G_{ip})^\mathsf{T}$.
- p $G \times E$ interaction terms: $\mathbf{S}_i = (E_i G_{i1}, \cdots, E_i G_{ip})^\mathsf{T}$.

We are interested in testing if there is any $G \times E$:

$$H_0 : \boldsymbol{\beta} = \mathbf{0}.$$

Collapsing tests

Intuition behind collapsing tests

- General problem: p is large and $G_1, \cdots, G_p$ are rare.
- Solution: for each individual i, summarize rare SNV-set $(G_{i1}, \cdots, G_{ip})$ using a single summary variable and conduct inference using this single summary variable.
- For example, define "collapsing" variable as $G_i^* = \sum_{k=1}^{p} G_{ik}$ = Total No. of rare alleles.

Collapsing Tests for Interactions

To test for main effects:

$$H_{1m} : g(\mu_i) = \alpha_1^* + \alpha_2^* E_i + \alpha_3^* G_i^*$$
$$H_{0m} : \alpha_3^* = 0$$

Can we use it to test for interactions?

$$H_{1x} : g(\mu_i) = \alpha_1^* + \alpha_2^* E_i + \alpha_3^* G_i^* + \beta^* E_i G_i^*$$
$$H_{0x} : \beta^* = 0$$

Bias analysis for Collapsing $G \times E$ tests

Intuition

Null model has to be correctly specified for valid inference.
Collapsing $G \times E$ tests may not give valid inference as main effects of the SNVs may not be sufficiently accounted for.

Continuous Outcome: No, even if $\boldsymbol{G} \perp E$.

- $\boldsymbol{G}$ and E are independent:
  Model for mean of Y is valid;
  Model for variance of Y is $\boxed{not}$ valid.
- $\boldsymbol{G}$ and E not independent:
  Model for mean of Y is not valid;
  Model for variance of Y is $\boxed{not}$ valid.

Bias analysis for Collapsing $G \times E$ tests

Binary Outcome: Yes if disease is rare and $\boldsymbol{G} \perp E$.

- $\boldsymbol{G}$ and E are independent:
  Model for mean of Y is valid;
  Model for variance of Y is valid approximately.
- $\boldsymbol{G}$ and E not independent:
  Model for mean of Y is $\boxed{\textit{not}}$ valid;
  Model for variance of Y is valid approximately.

iSKAT: Model

To test if there is any $G \times E$ ($H_0 : \boldsymbol{\beta} = \mathbf{0}$):

$$H_0 : \text{logit}\left[P(Y_i = 1|E_i, \mathbf{X}_i, \mathbf{G}_i)\right] = \mathbf{X}_i^\intercal \boldsymbol{\alpha}_1 + \alpha_2 E_i + \mathbf{G}_i^\intercal \boldsymbol{\alpha}_3$$

$$H_A : \text{logit}\left[P(Y_i = 1|E_i, \mathbf{X}_i, \mathbf{G}_i)\right] = \mathbf{X}_i^\intercal \boldsymbol{\alpha}_1 + \mathbf{G}_i^\intercal \left(\boldsymbol{\alpha}_3 + E_i \boldsymbol{\beta}\right) + \alpha_2 E_i$$

In principle, we can do the same thing as with SKAT, but ...

Difficulties

Need to fit null model:

- Need to estimate main effect of variants
- Lots of variants
- LD and rarity make fitting difficult

Modifications are necessary.

iSKAT: Extension of SKAT for GxE

iSKAT: Test Statistic

- Assume $(\beta_1, \cdots, \beta_p)^\intercal$ are random and independent with mean zero and common variance τ.
- Testing H_0 reduces to testing $H_0 : \tau = 0$.
- Following Lin (1997), the score test statistic is

$$T = (\boldsymbol{Y} - \widehat{\boldsymbol{\mu}})^\intercal \boldsymbol{S}\boldsymbol{S}^\intercal (\boldsymbol{Y} - \widehat{\boldsymbol{\mu}}) = [\boldsymbol{Y} - \boldsymbol{\mu}(\widehat{\boldsymbol{\alpha}})]^\intercal \boldsymbol{S}\boldsymbol{S}^\intercal [\boldsymbol{Y} - \boldsymbol{\mu}(\widehat{\boldsymbol{\alpha}})].$$

- $\widehat{\boldsymbol{\mu}} = \boldsymbol{\mu}(\widehat{\boldsymbol{\alpha}})$ is estimated under the null model,

$$g(\mu_i | \boldsymbol{X}_i, E_i, \boldsymbol{G}_i) = \boldsymbol{X}_i^\intercal \boldsymbol{\alpha}_1 + \alpha_2 E_i + \boldsymbol{G}_i^\intercal \boldsymbol{\alpha}_3 = \widetilde{\boldsymbol{X}}_i^\intercal \boldsymbol{\alpha}.$$

- Use ridge regression to estimate $\boldsymbol{\alpha}$, impose a penalty only on $\boldsymbol{\alpha}_3$.
- Under H_0, $T \sim \sum_{v=1}^{p} d_v \chi_1^2$ approximately.
- Invert characteristic function to get p-value (Davies, 1980).

iSKAT

Consider a GLMM framework:

$$H_0 : g(\mu_i) = \alpha_1 + \alpha_2 E_i + \boldsymbol{G}_i^\mathsf{T} \boldsymbol{\alpha}_3$$

$$H_1 : g(\mu_i) = \alpha_1 + \alpha_2 E_i + \boldsymbol{G}_i^\mathsf{T} \boldsymbol{\alpha}_3 + \boxed{E_i \boldsymbol{G}_i^\mathsf{T} \boldsymbol{\beta}}$$

- Let $\beta_j \sim F(0, w_j^2 \tau)$ and let $\boldsymbol{\beta}$ have exchangeable correlation structure with pairwise correlation ρ.
- $\rho = 0$ and $\rho = 1$ correspond to H_{1b} and H_{1a} respectively.
- For a fixed ρ, a score test statistic for testing $H_0 : \tau = 0$ is:

$$\begin{aligned} Q_\rho &= (\boldsymbol{Y} - \widehat{\boldsymbol{\mu}})^\mathsf{T} \boldsymbol{SW} \left[\rho \mathbf{1}\mathbf{1}^\mathsf{T} + (1-\rho)\boldsymbol{I}\right] \boldsymbol{WS}^\mathsf{T} (\boldsymbol{Y} - \widehat{\boldsymbol{\mu}}) \\ &= \boxed{\rho Q_{1a} + (1-\rho) Q_{1b}}. \end{aligned}$$

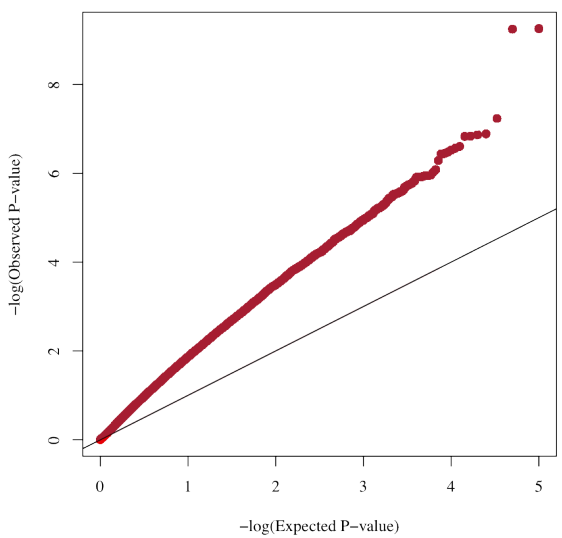
- Find optimal ρ to maximize Q_ρ: $\boxed{Q_{\text{iSKAT}} = \min_{0 \le \rho \le 1} p_\rho}$,

  where p_ρ is the p-value computed based on Q_ρ.

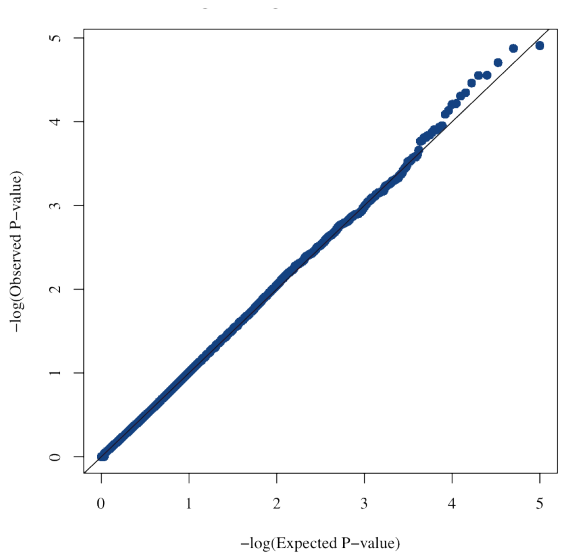
Size Simulations: iSKAT vs. Collapsing $G \times E$ test

- Bootstrap dataset samples to obtain genotypes/covariates.
- Main effects of SNVs chosen to mimic dataset.
- Simulate outcome under null hypothesis to investigate the size.

Collapsing $G \times E$ test



iSKAT



Data Application: Adiponectin Levels

- Adiponectin levels are associated with many diseases.
- SNVs at the adiponectin-encoding gene, ADIPOQ, are associated with adiponectin levels.
- Adiponectin levels from 1945 individuals.
- 11 rare SNVs within the exon region of ADIPOQ.
- Test for $G \times E$.

	p-value
iSKAT:	0.037
$\rho = 0$:	0.23
$\rho = 1$:	0.022

Rare variant Meta-analysis

- Meta-analysis is an effective approach to combine data from multiple studies.
- Rare variant meta-analysis: desirable properties
  - Use summary statistics
  - Same power as mega-analysis (joint analysis)
  - Account for varying levels of heterogeneity of genetic effects across studies.

Rare Variant Meta-Analysis References

- Lee S, Teslovich T, Boehnke M, and Lin X (2013) General Framework for Meta-analysis of Rare Variants in Sequencing Association Studies. *AJHG* 93, 42-53
- Hu Y *et al.*(2013) Meta-analysis of Gene-Level Associations for Rare Variants Based on Single-Variant Statistics. *AJHG* 93, 236-248
- Lumley T *et al.*(2013) Meta-analysis of a rare-variant association test *manuscript*, http://stattech.wordpress.fos.auckland.ac.nz/files/2012/11/skat-meta-paper.pdf
- Liu DJ, *et al.*(2014) Meta-analysis of gene-level tests for rare variant association. *Nat Genet* 46, 200-204

Multi-Study Model

- For the k^{th} study $(k = 1, \ldots K)$,
  - Genotype $\mathbf{G}_{ki} = (g_{ki1}, \ldots, g_{kip})'$
  - Covariates $\mathbf{X}_{ki} = (x_{ki1}, \ldots, x_{kiq_k})'$
  - Model:

$$g(\mu_{ik}) = \mathbf{X}_{ki}\boldsymbol{\alpha}_k + \mathbf{G}_{ki}\boldsymbol{\beta}_k$$

  - Test H_0: $\boldsymbol{\beta}_k = 0$ $(k = 1, \cdots, K)$

Meta-Analysis for Rare Variants in Sequencing Association Studies

- Challenges
  - Jointly analyze multiple SNPs in a region.
  - Hard to estimate β_k for rare variants
  - Wald-based meta-analysis for vector β_k is challenging

Meta-Analysis for Rare Variants in Sequencing Association Studies

- Idea of Meta-SKAT family tests:
  - Work with the scores of β_k by fitting only null models.
  - Assume a distribution for β_{kj} $(j = 1, \cdots, p)$
  - Perform variance component score test by allowing homogeneous and heterogeneous genetic effects across studies.

Single study k

- Score statistic of variant j

$$S_{kj} = \sum_{i=1}^{n} g_{ijk}(y_{ij} - \widehat{\mu}_{ij})/\widehat{\phi}_k$$

- SKAT and Burden test statistics:

$$Q_{SKAT} = \sum_{j=1}^{p} (w_{jk} S_{kj})^2, \quad Q_{Burden} = \left(\sum_{j=1}^{p} w_{jk} S_{kj} \right)^2$$

Single study k

- SKAT-O (combined approach):

$$T = min_{0 \leq \rho \leq 1} P_\rho$$

where P_ρ is the p-value of

$$Q_\rho = (1 - \rho) Q_{SKAT} + \rho Q_{Burden}$$

Input Summary Statistics for meta-analysis

- Input summary statistics from each study
  - MAF
  - S_{kj}: score statistic of each marker
  - Between-variant relationship matrix $(p \times p)$

$$\Phi_k = \mathbf{G}_k' \mathbf{P}_k \mathbf{G}_k,$$

where $\mathbf{P}_k = \mathbf{V}_k^{-1} - \mathbf{V}_k^{-1}\mathbf{X}_k(\mathbf{X}_k'\mathbf{V}_k^{-1}\mathbf{X}_k)^{-1}\mathbf{X}_k'\mathbf{V}_k^{-1}$

Homogeneous genetic effects

- Assume the same SNP effects across studies.
  - $\boldsymbol{\beta}_1 = \boldsymbol{\beta}_2 = \cdots = \boldsymbol{\beta}_K = \boldsymbol{\beta}$
  - $E(\beta_j) = 0$, $\text{var}(\beta_j) = w_j\tau$ and $\text{cor}(\beta_j, \beta_{j'}) = \rho$.
- Derive the VC score test for $H_0 : \tau = 0$.
- Multivariate score-based analog of univariate fixed effect meta-analysis

Meta-SKAT: Homogeneous genetic effects

- Meta-SKAT assuming homogeneous genetic effects:

$$Q_{hom\_meta\_SKAT} = \sum_{j=1}^{p} \left(\sum_{k=1}^{K} w_{kj} S_{kj} \right)^2$$

- Meta-Burden:

$$Q_{meta\_Burden} = \left(\sum_{j=1}^{p} \sum_{k=1}^{K} w_{kj} S_{kj} \right)^2$$

- Meta-SKAT-O:

$$Q_{hom\_meta}(\rho) = (1-\rho) Q_{hom\_meta\_SKAT} + \rho Q_{meta\_Burden}$$

Meta-SKAT: Homogeneous genetic effects

- Test statistics are essentially identical to those of the mega analysis SKAT and burden test
  $\Rightarrow$ As powerful as mega-analysis
- P-values can be computed using the Davies method.
  $\Rightarrow$ Fast computation
- SKAT-O can be conducted with adaptively selecting ρ.

Meta-SKAT: Heterogeneous genetic effects

- Assume genetic effects vary between studies
  - $\boldsymbol{\beta}_1, \cdots, \boldsymbol{\beta}_K$ are iid
  - $E(\beta_{kj}) = 0$, $\text{var}(\beta_j) = w_{kj}\tau$ and $\text{cor}(\beta_{kj,kj'}) = \rho$.
- Multivariate score-based analog of the univariate random effect model meta-analysis.
- P-values can be calculated analytically
- Useful for meta analysis of studies of the same ethnicity or different ethnicities.

Meta-SKAT: Heterogeneous genetic effects

- Meta-SKAT assuming heterogeneous genetic effects:

$$Q_{het\_meta\_SKAT} = \sum_{j=1}^{p} \sum_{k=1}^{K} (w_{kj} S_{kj})^2$$

- Meta-SKAT-O:

$$Q_{hom\_meta}(\rho) = (1-\rho) Q_{het\_meta\_SKAT} + \rho Q_{meta\_Burden}$$

Meta-SKAT for multi-ethnicities:

- Multi-ethnic studies:
  - within-group homogeneity and between-group heterogeneity
  - $\beta_k = \beta_l$ for the same group and $\beta_k \perp \beta_l$ for the different groups
- Meta-SKAT with B ancestry groups

$$Q_{het\_meta\_SKAT} = \sum_{j=1}^{p} \sum_{b=1}^{B} \left(\sum_{k=k_{b-1}+1}^{k_b} w_{kj} S_{kj} \right)^2$$

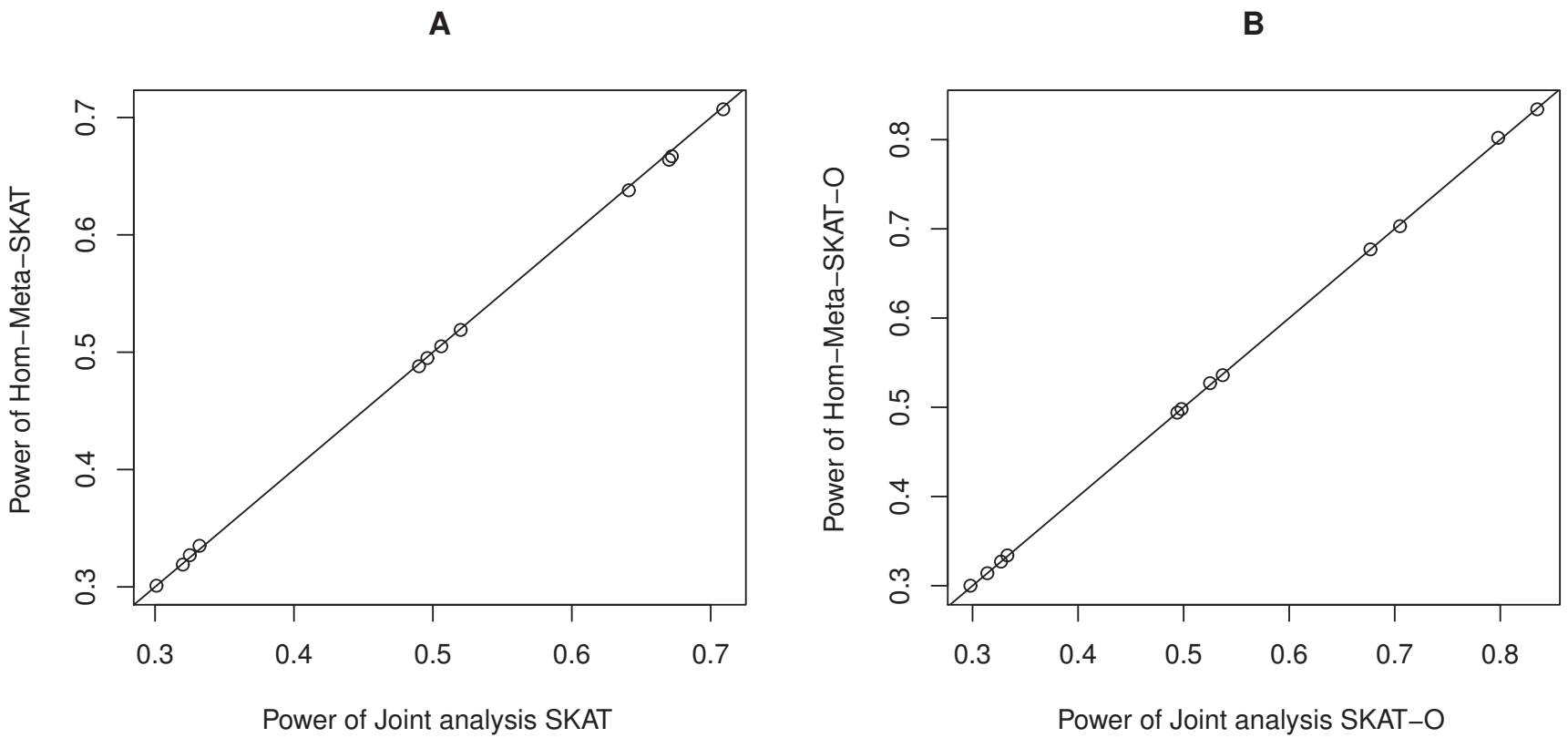
- Meta-SKAT-O:

$$Q_{hom\_meta}(\rho) = (1-\rho) Q_{het\_meta\_SKAT} + \rho Q_{meta\_Burden}$$

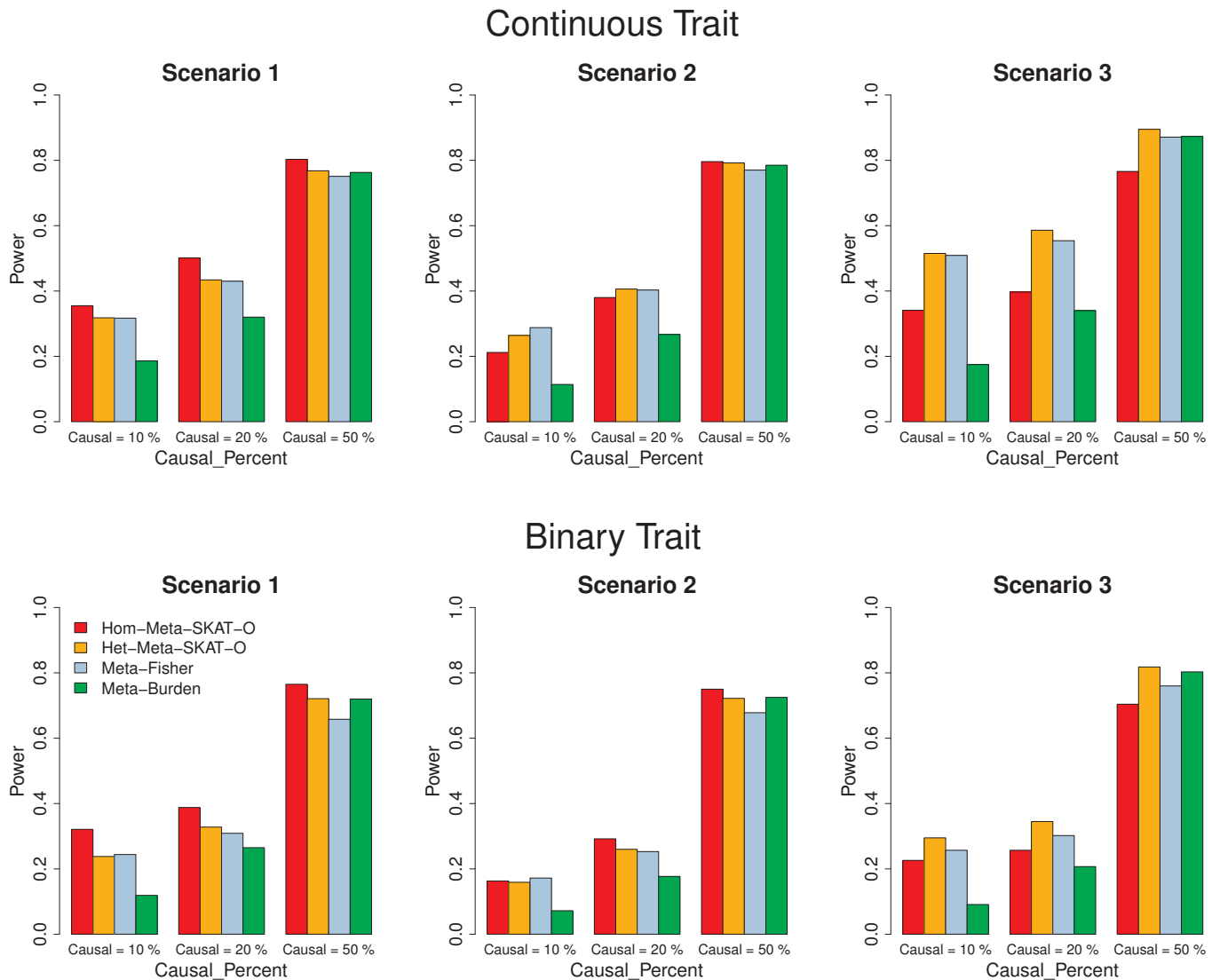
Simulation Studies

- 3kb randomly selected regions
- Three scenarios:
  - 1: homogeneous
  - 2: moderately heterogeneous (studies share 50 % of causal variants)
  - 3: two different ancestry groups (study 1,2 : EUR and study 3: AA)

Powers comparison: Meta vs Mega (Joint)



Simulation Results, $\beta +/- = 100/0$



Analysis of the lipid data (LPL gene)

- 11,000 samples from 7 studies.
- Adjusted for the index SNP
- For HDL and TG, Het-Meta-SKAT-O achieved the smallest p-values

Traits	Hom-Meta SKAT-O	Het-Meta SKAT-O	Meta-Fisher	Meta-Burden
HDL	2.5×10^{-2}	1.2×10^{-4}	1.7×10^{-2}	3.5×10^{-1}
LDL	1.00	4.0×10^{-1}	3.9×10^{-1}	2.1×10^{-2}
TG	5.3×10^{-3}	2.8×10^{-5}	6.0×10^{-4}	7.7×10^{-2}

Summary of Meta-Anlaysis for Rare Variants

- Based on study-specific summary score statistics
- As powerful as the joint analysis
- Flexible to accommodate a wide range of heterogeneity of genetic effects

Additional Concerns

- Quality control:
  - Are the observed variants really variants?
  - Batch effects
  - Some standard pipelines now in place
- Population stratification:
  - Common strategy: just use same PCs from common variant analysis to correct for PS
  - Some evidence that rare variants require special accommodation (much larger number of PCs)
- Accommodating common variants:
  - What do you do with common variants?
  - (a) Assess joint effect with rare variants
  - (b) Adjust for effect of common variants

Additional Concerns

- Prediction
  - In a new population (sample), we're unlikely to see the same variants and we're likely to see a lot of variants not previously observed
- Prioritization of individual variants
  - How to choose individual causal variants?
  - Some work on variable selection methods, but no ability to control type I error.
  - Bioinformatics and functionality tools may be useful
- Incorporation of functional information and other genomic data

Additional Concerns

- Design Choices
  - Want to enrich for variants (extreme phenotypes)
  - Some of these designs require specialized methods
  - Stuck with the design chosen
- Dealing with admixed populations
- Related individuals
- Tim: what is a "rare variant"?
- (Statistically) complex phenotypes