Lecture 2: Rare Variant Analysis: Collapsing Tests, Kernel (Variance Component) Tests and Omnibus Tests

Instructors: Andrey Ziyatdinov and Timothy Thornton

The Unidad de Medicina Experimental, Faculty of Medicine, National Autonomous University of Mexico

Lecture Overview

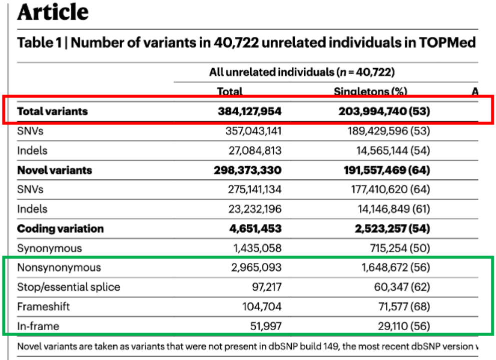
1. Limitations of GWAS
2. Rationale for Rare Variant Analysis
3. Challenges
4. Collapsing/Burden Tests
5. Variance Component Tests
6. Omnibus Tests

GWAS: Missing Heritability

- GWAS primarily focus on common variants (MAF $\geq$ 5%) whose effects are small.
- **Missing heritability:** Significant GWAS SNPs explain a small proportion of disease heritability.
- Possible reasons:
  - GxG and GxE interactions?
  - Many common causal variants: Each with a small effect?
  - Epigenetics?
  - **Rare variants?**

Why rare variants?

- Most of human variants are rare.
- Functional variants tend to be rare.



Article

Table 1 | Number of variants in 40,722 unrelated individuals in TOPMed

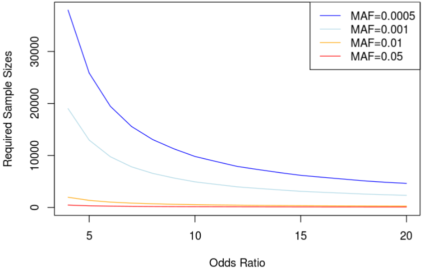
	All unrelated individuals (n = 40,722)		
	Total	Singletons (%)	A
Total variants	**384,127,954**	**203,994,740 (53)**	
SNVs	357,043,141	189,429,596 (53)	
Indels	27,084,813	14,565,144 (54)	
Novel variants	**298,373,330**	**191,557,469 (64)**	
SNVs	275,141,134	177,410,620 (64)	
Indels	23,232,196	14,146,849 (61)	
Coding variation	**4,651,453**	**2,523,257 (54)**	
Synonymous	1,435,058	715,254 (50)	
Nonsynonymous	2,965,093	1,648,672 (56)	
Stop/essential splice	97,217	60,347 (62)	
Frameshift	104,704	71,577 (68)	
In-frame	51,997	29,110 (56)	

Novel variants are taken as variants that were not present in dbSNP build 149, the most recent dbSNP version v

Talium et al., *Nature* 2021

Challenges in Association Studies for Rare Variants

- Compared to common variant studies, **individual SNP analysis in rare variant studies is seriously underpowered.** → How many subjects are needed to achieve 80% of power ($\alpha = 10^{-6}$) by single variant test?



- A lot more rare variants than common variants → larger multiple testing burden

Challenges in Association Studies for Rare Variants

- Individual rare variant tests are underpowered
- Need **cost-effective study designs** to genotype a large number of individuals
- Need **powerful statistical methods and strategies** to test for associations
  - Region based analysis: genes, moving windows, networks/pathways
  - Integrate with bioinformatics: Incorporate functional information

Region Based Analysis of Rare Variants

- Gene (or Region) based tests
- Strategy:
  - Identify all observed variants within a sequenced (sub)-region.
  - Regions: gene, regulatory region, ...
  - Test the joint effect of rare variants.

Regression Models

- p variants in a certain region.
- SNPs in a region $\mathbf{G_i} = (g_{i1}, g_{i2}, \ldots, g_{ip})'$, $(g_{ij} = 0, 1, 2)$
- Covariates $\mathbf{X}_i$: age, gender, PC scores (for population stratification).
- Continuous/binary traits:

$$\begin{aligned} g(\mu_i) &= \alpha_0 + \mathbf{X}_i'\boldsymbol{\alpha} + \mathbf{G_i}'\boldsymbol{\beta} \\ &= \alpha_0 + \mathbf{X}_i'\boldsymbol{\alpha} + \sum_j g_{ij}\beta_j \end{aligned}$$

- Joint test of no genetic effect in region:

$$H_0 : \boldsymbol{\beta} = (\beta_1, \ldots, \beta_p) = 0$$

Major Classes of Tests

- Burden/Collapsing tests
- Supervised/Adaptive Burden/Collapsing tests
- Variance component (similarity) based tests
- Omnibus tests

Collapsing/Burden Tests - Principle

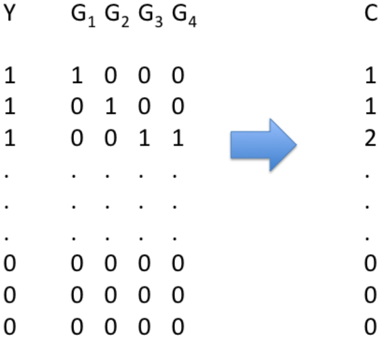
- If p is large, multivariate test $\boldsymbol{\beta} = 0$ is not powerful (df=p).
- Collapsing: Suppose $\beta_1 = \cdots = \beta_p = \beta$

$$\begin{aligned} g(\mu_i) &= \alpha_0 + \mathbf{X}_i' \boldsymbol{\alpha} + \sum_j g_{ij}\beta_j \\ &= \alpha_0 + \mathbf{X}_i^T \boldsymbol{\alpha} + C_i \beta \end{aligned}$$

- $C_i = g_{i1} + \cdots + g_{ip}$: **genetic burden/score**
- Test $H_0 : \beta = 0$ (df=1)
- **Key assumption**: all rare variants in region are causal variants with the same effect sizes and association directions.

Burden Tests

- Collapse rare variants



Y	G_1	G_2	G_3	G_4	C
1	1	0	0	0	1
1	0	1	0	0	1
1	0	0	1	1	2
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0

Burden Tests

- Many different types of tests exist based on different aggregation rules to get C_i
  - Reflects assumptions on genetic architecture
- **Existence of any rare variants can cause loss of function of a region** (e.g. CAST)

$$C_i = \begin{cases} 1 & \text{if} \quad \sum_{j=1}^{p} g_{ij} > 0 \\ 0 & \text{if} \quad \sum_{j=1}^{p} g_{ij} = 0 \end{cases}$$

- **Dominant genetic model** (e.g.. MZ-test)

$$C_i = \sum_{j=1}^{p} I(g_{ij} > 0)$$

Weighted Burden

- Assume that **rarer variants have larger effects**
- Suppose $\beta_j = w_j\beta$, where $w_j = w(MAF_j)$.
    - Ex: $w(MAF_j) = 1/\sqrt{MAF_j(1-MAF_j)}$ (Madsen and Browning).
- Weighted count of rare variants

$$C_i = w_1 g_{i1} + \cdots + w_p g_{ip}$$

Power of Burden Tests

- Power of burden tests depends on
  - Number of associated variants
  - Number of non-associated variants
  - Direction of the effects.
- **Powerful if most variants are causal and have effects in the same direction.**

Variance component test

- Burden tests are not powerful, if
  - there exist variants with different association directions
  - many non-causal variants
- Variance component tests have been proposed to address this limitation.

Sequence Kernal Association Test (SKAT)

- Recall the original regression models:

$$g(\mu_i) = \alpha_0 + \mathbf{X}_i^T \boldsymbol{\alpha} + \mathbf{G}_i^T \boldsymbol{\beta}$$

- Assume $\beta_j \sim \mathit{dist}.(0, w_j^2 \tau)$.
- $H_0 : \beta_1 = \cdots = \beta_p = 0 \iff H_0 : \tau = 0$.
- 1df test!

Sequence Kernel Association Test (SKAT)

- Score test statistic for $\tau = 0$:

$$Q_{SKAT} = (\mathbf{y} - \hat{\boldsymbol{\mu}}_0)'\mathbf{K}(\mathbf{y} - \hat{\boldsymbol{\mu}}_0),$$

- $\mathbf{K} = \mathbf{GWWG}'$: weighted linear kernel (where $\mathbf{W} = diag[w_1, \dots, w_p]$).
- It is a $N \times N$ similarity matrix

SKAT

- Q_{SKAT} is a **weighted sum of single variant score statistics**

$$\begin{aligned} Q_{SKAT} &= (\mathbf{y} - \hat{\boldsymbol{\mu}}_0)'\mathbf{GWWG}'(\mathbf{y} - \hat{\boldsymbol{\mu}}_0) \\ &= \sum_{j=1}^{p} w_j^2[\mathbf{g}_j'(\mathbf{y} - \hat{\boldsymbol{\mu}}_0)] = \sum_{j=1}^{p} w_j^2 S_j^2 \end{aligned}$$

- S_j is a score test statistic in the SNP j only model:

$$g(\mu_i) = \alpha_0 + \mathbf{X}_i^T\boldsymbol{\alpha} + g_{ij}\beta_j$$

- Under H_0, Q_{SKAT} (asymptotically) follows a **mixture of** χ^2 **distribution** $\sum_{j=1}^{p} \lambda_j \chi_{1,j}^2$

SKAT: P-value calculation

- P-values can be computed by inverting the characteristic function using Davies' method (1973, 1980)
  - Characteristic function

$$\varphi_x(t) = E(e^{itx}).$$

  - Characteristic function of $\sum_{j=1}^{p} \lambda_j \chi^2_{1,j}$

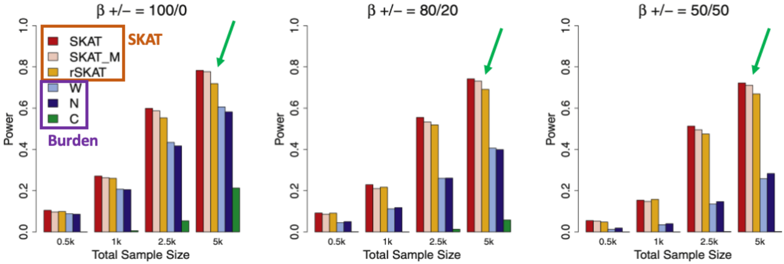
$$\varphi_x(t) = \prod_{i=j}^{p} (1 - 2\lambda_j it)^{-1/2}.$$

  - Inversion Formula

$$P(X < u) = \frac{1}{2} - \frac{1}{\pi} \int_0^\infty \frac{Im[e^{-itu}\varphi_x(t)]}{t} \,\mathrm{d}t.$$

Burden vs SKAT

- Power simulations: 5% of the variants in region are causal & vary the directions of effects
- SKAT remains powerful even if variants have different effect directions



Lee et al., *Am J Hum Genet* 2011

SKAT vs. Collapsing

- Collapsing tests are more powerful when a large % of variants are causal and effects are in the same direction.
- SKAT is more powerful when a small % of variants are causal, or the effects have mixed directions.
- Both scenarios can happen when scanning the genome.
- Best test to use depends on the underlying biology.

$\rightarrow$ Difficult to choose which test to use in practice.

SKAT vs. Collapsing

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  → Difficult to choose which test to use in practice.

We want to develop a unified test that works well in both situations → Omnibus tests

Combine Test Statistics: Unified Test Statistics

Lee (2012). *Biostatistics*

- Combined Test of Burden tests and SKAT

$$Q_\rho = (1-\rho)Q_{SKAT} + \rho Q_{Burden}, \quad 0 \le \rho \le 1.$$

- Q_ρ includes SKAT and burden tests.
  - $\rho = 0$: SKAT
  - $\rho = 1$: Burden

SKAT-O

- Model:

  $$g(\mu_i) = \alpha_0 + \mathbf{X}_i^T \boldsymbol{\alpha} + \mathbf{G}_i^T \boldsymbol{\beta}$$

  where β_j / w_j follows any arbitrary distribution with mean 0 and variance τ and the correlation among β_j's is ρ.
- SKAT-O considers $0 \leq \rho \leq 1$
- Special cases:
  - SKAT: $\rho = 0$
  - Burden: $\rho = 1$

SKAT-O

- Set a grid of values for ρ in $[0, 1]$ and pick ρ which maximizes power
  - Use the smallest p-value from different ρs:

    $$T = \inf_{0 \leq \rho \leq 1} P_\rho.$$

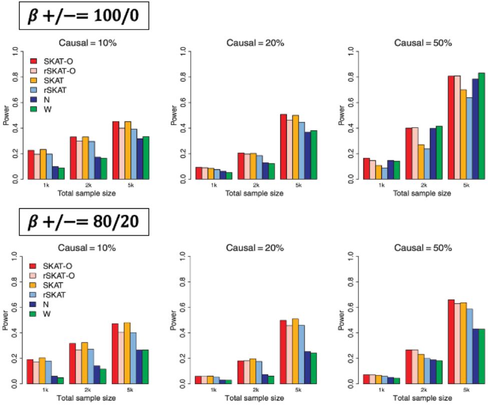
    where P_ρ is the p-value of Q_ρ for given ρ.
  - Test statistic:

    $$T = min\, P_{\rho_b}, \quad 0 = \rho_1 < \ldots < \rho_B = 1.$$

- SKAT-O p-value is obtained through numerical integration

SKAT-O vs Burden/SKAT

- SKAT-O remains powerful across all scenarios



Lee et al., *Biostatistics* 2012

Aggregated Cauchy Association Test: ACAT

- Based on the Cauchy combination method to combine a set of p-values $\{p_j\}$:

$$T_{ACAT} = \sum_j w_j \tan\{\pi(0.5 - p_j)\}$$

- Computing p-value is extremely fast

$$\text{p-value} \approx 0.5 - \frac{\arctan\{T_{ACAT}/w\}}{\pi}, \quad w = \sum_j w_j$$

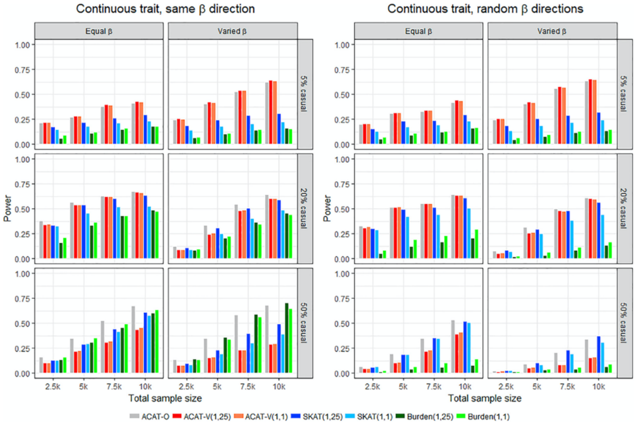
- Very accurate for small p-values
- Robust to correlation between the tests

Aggregated Cauchy Association Tests

- ACAT-V
  - Apply ACAT to single variant p-values from rare variants
  - More powerful when fewer variants are associated (i.e. sparse alternative)
  - SKAT & Burden can loose substantial power under this scenario
- ACAT-O
  - Apply ACAT to combine the p-values of SKAT, Burden and ACAT-V
  - Omnibus test which should work well whether
    - Effects are in same direction & many variants are associated
    - Effects are in different directions
    - Very few variants are causal

ACAT/SKAT/Burden

- ACAT-O remains powerful across all scenarios

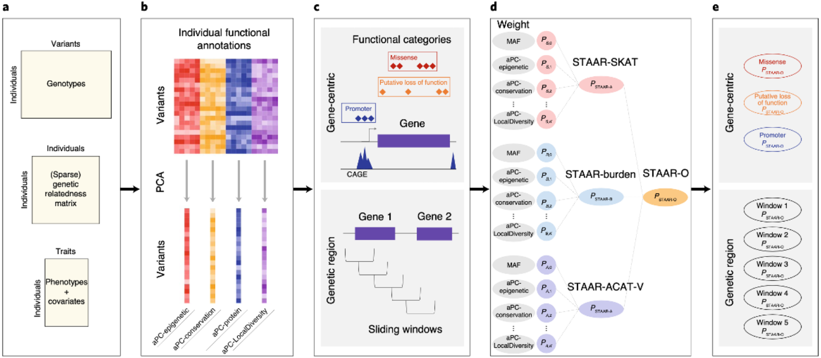


Liu et al., *AJHG* 2019

Incorporating external biological information

- What are the best variant weights to use in SKAT/Burden/ACAT-V tests?
- Using functional annotations can help improve statistical power, e.g.
  - variant effect predictor categories : loss of function, missense, ...
  - epigenetic scores (e.g. DNA methylation levels)
  - distance to coding region or transcription start/end site
- How to choose which set of variants to test jointly?
  - Within a gene
  - Sliding window

STAAR



Li et al., *Nat Gen* 2020

Summary

- Region based tests can increase the power of rare variants analysis compared to single variant tests.
- Relative performance of rare variant tests depends on underlying disease models
- Combined tests (omnibus tests), e.g, SKAT-O/ACAT-O, are more robust and powerful across different scenarios
- Can integrate functional annotation to boost statistical power

References

- Taliun, D. et al. Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program. *Nature* **590**, 290-299 (2021).
- Madsen, B.E. & Browning, S.R. A Groupwise Association Test for Rare Mutations Using a Weighted Sum Statistic. *PLoS Genetics* **5**, e1000384 (2009).
- Wu, M.C. et al. Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet* **89**, 82-93 (2011).

References

- Lee, S., Wu, M.C. & Lin, X. Optimal tests for rare variant effects in sequencing association studies. *Biostatistics* **13**, 762-75 (2012).
- Liu, Y. et al. ACAT: A Fast and Powerful p Value Combination Method for Rare-Variant Analysis in Sequencing Studies. *Am J Hum Genet* **104**, 410-421 (2019).
- Li, X., Li, Z., Zhou, H., Gaynor, S. M., Liu, Y., Chen, H., et al. Dynamic incorporation of multiple in silico functional annotations empowers rare variant association analysis of large whole-genome sequencing studies at scale. *Nature Genetics*, **52**, 969-983 (2020).