Gene-level aggregate methods to evaluate genetic associations and gene-by-environment interactions in cross-sectional and longitudinal data

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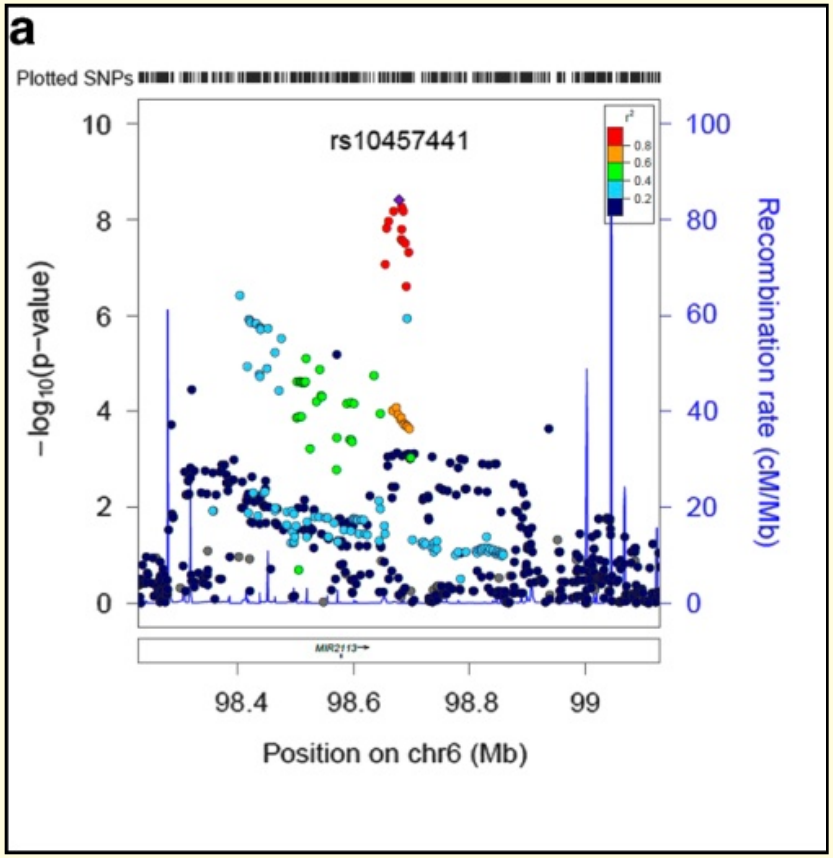
The genome is a big place with lots of variation!

Number of variants in HRS imputed data (2006-2012)

Minor Allele Frequency in HRS	Number of Variants
Common (≥ 5%)	28 million
1% to 5%	10 million
Rare (< 1%)	9 million
Total	**47 million**

Cognition GWAS results for *MIR2113 genomic* region



Davies G, et al. (2015) Mol Psych, 20:183-192.

Gene-level aggregate tests

- Test the **aggregate effect** of multiple SNPs/variants within a gene or genomic region on an outcome of interest

- **Burden tests**
  - Collapse variants to test for cumulative effects
  - Most powerful when all variants have **similar magnitude and directions of effect**
  - Can include any number of variants effectively without losing power

- **Non-burden tests**
  - Tests the distribution of the aggregated **association statistics** across variants
  - Optimal performance when variants have **differing magnitudes and directions of effect**
  - Can use both **common and rare variants**
  - Can lose power when variants > sample size
  - Examples: SKAT and LGEWIS

Sequence Kernel Association Test (SKAT)

$$\boldsymbol{Y}_i = \alpha_0 + \boldsymbol{\alpha_1}'\boldsymbol{X}_i + \boldsymbol{\beta}'\boldsymbol{G}_i + \varepsilon_i$$

where:

$\boldsymbol{Y}_i$ = outcome for subject i

α_0 = intercept term

$\boldsymbol{X}_i$ = vector of non-genetic covariates (e.g., age, sex, genetic PCs)

$\boldsymbol{G}_i$ = vector of genotypes for variants,

ε_i = error term; follows a distribution with mean 0 and variance σ^2

- Assume $\boldsymbol{\beta}_j$, j=1,…,p, follows an arbitrary distribution with mean 0 and variance $w_j\tau$
  - w_j = pre-specified weight for each variant
  - τ = variance component
- Testing H_0: $\boldsymbol{\beta}$ = 0 is equivalent testing H_0: τ = 0

Wu MC, et al. (2011) AJHG, 89:82-93

Sequence Kernel Association Test (SKAT)

- H_0: $\tau = 0$
  - No association between the variants and the outcome
  - Assesses whether there is **any variance** in the set of β_js from the mean of 0
  - Can be **in any direction** (+/-)
- Variance component score test
- **Q statistic** is the sum of a mixture of χ^2 distributions based on genotype covariance matrix
- Null hypothesis is evaluated explicitly and used as a reference distribution to compute **a gene-level p-value** using bootstrapping

Wu MC, et al. (2011) AJHG, 89:82-93

SKAT variant weights

- Good choice of variant weights can improve power
- Functional vs. non-functional (e.g., PolyPhen score)
- Common vs. rare
  - Up-weighting rare variants is supported by evolutionary theory
  - Authors suggest weighting by the *Beta* function using minor allele frequency (MAF)

where:

w_j = weight for variant$_j$

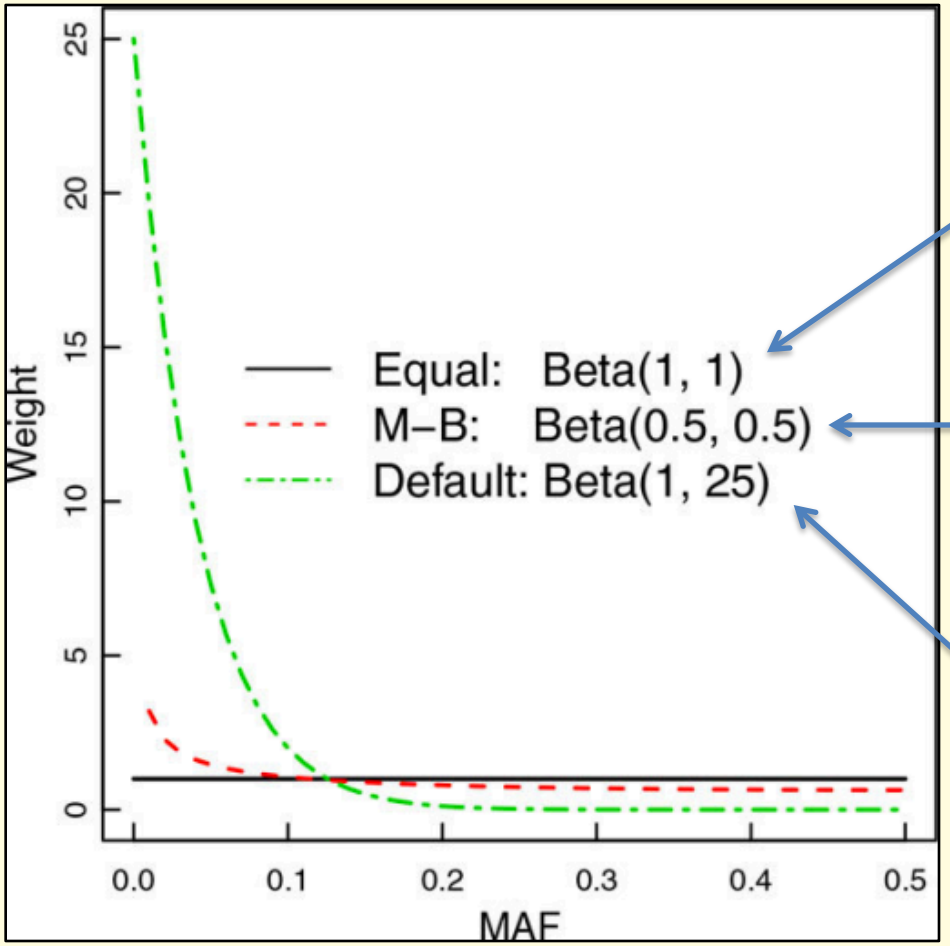
MAF$_j$ = minor allele frequency for variant$_j$

α_1 and α_2 = shape parameters for the beta function

$$\sqrt{w_j} = Beta(MAF_j; \alpha_1, \alpha_2)$$

Wu MC, et al. (2011) AJHG, 89:82-93

SKAT variant weights: Minor allele frequency



- All variants weighted equally
- Low power for rare variants

- Rare variants slightly up-weighted
- Can detect signals for both common and rare variants
- Lower power if both types of variants are not contributing

- Rare variants highly up-weighted
- Low (if any) power for common variants

Mallaney and Sung (2014) BMC Proc.

SKAT kernels

The more general SKAT model:

$$\boldsymbol{Y}_i = \alpha_0 + \boldsymbol{\alpha_1}'\boldsymbol{X}_i + f(\boldsymbol{G}_i) + \varepsilon_i$$

Where $f(\boldsymbol{G}_i)$ can specify different kernels:

- **Linear**
- Identity-by-state (IBS)
- Epistatic (variant*variant) interactions
- 2-way interaction (product kernel consisting of both variant main effects and variant*variant interactions)

Optimal Unified Sequence Kernel Association Test (SKAT-O)

- Optimally combines **non-burden test (SKAT)** with **burden test**
- Test statistic (Q_ρ):

$$Q_\rho = (1 - \rho)Q_S + \rho Q_B$$

where

  - Q_S is the test statistic from SKAT
  - Q_B is the test statistic from a burden test
  - ρ is a parameter calculated to optimize the test statistic

- Output includes the **Q statistic, p-value, and ρ**
- Allows the user to evaluate both types of effects simultaneously, and boosts power by performing the optimal tests
- "SKAT" package in R, includes a simulation function to calculate power

Lee S, et al. (2012) AJHG, 91:224-237

Example: HRS memory performance and decline

Use gene-based methods to assess whether **common** or **rare variants** in **41 genes** previously shown to be associated with cognition and related phenotypes (like AD):

1) Are associated with **episodic memory performance and decline** in non-Hispanic whites (N=9,914) and African Americans (N=2,223) from the Health and Retirement Study

- **Memory score** = total number of words correctly recalled immediately plus 5 minutes later (range: 0-20), assessed at multiple time points
- A **growth curve model** with random effects was used to extract features of memory performance and decline over time
- **Gene regions** included all variants in the gene +/- 5kb

Variant weights matter

- Whites (N=9,914)
- Memory decline (slope) = sex + college education + 4 genetic PCs + performance (age 65 intercept) + variants (1000G imputed data)
- SKAT
  - Beta(1,1): equal weights for all variants
  - Beta(1,25): rare variants up-weighted

Gene Name	Chromosome	Start Position	Gene Size (kb)	Number of Variants	P-value Beta (1,1)	P-value Beta (1,25)
XIRP1	3	39224701	9	101	**0.0228**	0.5635
CELF1	11	47487496	100	532	0.7159	**0.0498**
MS4A6A	11	59939081	13	165	0.1993	**0.0013**
DPH6	15	35509546	329	2132	**0.0197**	**0.0413**
PVRL2	19	45349432	43	401	**0.0043**	**0.0003**
TOMM40	19	45393826	13	116	**1.52E-08**	**1.55E-10**
APOE	19	45409011	4	30	**2.38E-11**	**2.09E-09**
APOC1	19	45417504	5	88	**1.64E-14**	**2.43E-11**

Results for genes with SKAT p<0.05 for at least one weighting scheme are shown

Inclusion of burden test matters

- Same analysis

- SKAT/SKAT-O
  - Beta(1,1): equal weights for all variants
  - Beta(1,25): rare variants up-weighted
  - ρ = 0 if SKAT, 1 if burden score

Gene Name	Chr	Gene Size (kb)	Number of Variants	Beta (1,1)			Beta (1,25)		
				SKAT	SKAT-O		SKAT	SKAT-O	
				P-value	P-value	ρ	P-value	P-value	ρ
XIRP1	3	9	101	**0.02**	**0.04**	0	0.56	**0.05**	1
CELF1	11	100	532	0.72	0.22	1	**0.05**	**0.03**	1
MS4A6A	11	13	165	0.20	0.23	0	**0.001**	**0.001**	0.04
DPH6	15	329	2132	**0.02**	**0.02**	0.01	**0.04**	0.07	0
PVRL2	19	43	401	**0.004**	**$1x10^{-06}$**	1	**$3x10^{-04}$**	**$5x10^{-04}$**	0
TOMM40	19	13	116	**$2x10^{-08}$**	**$1x10^{-08}$**	0	**$2x10^{-10}$**	**$2x10^{-10}$**	0
APOE	19	4	30	**$2x10^{-11}$**	**$2x10^{-10}$**	0	**$2x10^{-09}$**	**$1x10^{-08}$**	0
APOC1	19	5	88	**$2x10^{-14}$**	**$4x10^{-16}$**	0.25	**$2x10^{-11}$**	**$2x10^{-10}$**	0.01

Results for genes with SKAT p<0.05 for at least one weighting scheme are shown

iSKAT and iSKAT-O for gene-environment interactions

$$\boldsymbol{Y}_i = \alpha_0 + \boldsymbol{\alpha_1}'\boldsymbol{X}_i + \boldsymbol{\alpha_2}'\boldsymbol{G}_i + \alpha_3 E_i + \boldsymbol{\beta}'\boldsymbol{S}_i + \varepsilon_i$$

where:

$\boldsymbol{S}_i$ = vector of GxE interaction terms

- Same idea as SKAT-O, except that the **distribution of the betas from the GxE interaction terms** are evaluated (H_0: $\boldsymbol{\beta} = 0$)
- Null hypothesis is that the interaction terms are all equal to zero
- Caveat: No gene-level joint tests have yet been developed to examine the variant main effects and GxE interaction effects simultaneously

Example: HRS memory performance and decline

Use gene-based methods to assess whether **common** or **rare variants** in **41 genes** known to be associated with cognition and related phenotypes:

1) Are associated with **episodic memory performance and decline** in non-Hispanic whites (N=9,914) and African Americans (N=2,223) from the Health and Retirement Study, and

2) Evaluate whether **interactions** between these genes and **educational attainment** are also associated with memory performance and decline

iSKAT/iSKAT-O Results

SKAT/SKAT-O (marginal variant effects)

Memory decline (slope) = sex + college education + 4 genetic PCs + age 65 intercept + **variants**

Gene Name	Chr	Gene Size (kb)	Number of Variants	Beta (1,1)			Beta (1,25)		
				SKAT	SKAT-O		SKAT	SKAT-O	
				P-value	P-value	ρ	P-value	P-value	ρ
MS4A6A	11	13	165	0.20	0.23	0	**0.001**	**0.001**	0.04

iSKAT/iSKAT-O (GxE interaction effects)

Memory decline (slope) = sex + college education + 4 genetic PCs + age 65 intercept + variants + **variants*college education**

Gene Name	Chr	Gene Size (kb)	Number of Variants	Beta (1,1)			Beta (1,25)		
				iSKAT	iSKAT-O		iSKAT	iSKAT-O	
				P-value	P-value	ρ	P-value	P-value	ρ
MS4A6A	11	13	165	0.22	0.15	1	**0.03**	**0.007**	1

Longitudinal gene-environment-wide interaction studies (LGEWIS) for repeated measures data

- New methods developed that can incorporate longitudinal measures
  - **Marginal variant effects** (optimized test)
  - **Gene-environment interactions**
- Use generalized estimating equations (GEE) instead of variance components
- Models the association with the outcome, not the longitudinal trajectory (change over time)
- Advantages
  - Increased power over using baseline or average measures
  - Allows for non-linear environmental effects
  - Robust when number variants >> sample size
  - Robust to model misspecification

He Z, et al., JASA (accepted); He, et al. (submitted)

Conclusions

- Advantages of gene-level aggregate methods
  - May be better suited for **multi-ethnic studies** than single variant analysis
  - **Increased power** due to limited number of tests, especially for rare variants
  - Allows **deeper interrogation** of the variation in genes identified through GWAS
  - Provide a platform for **integrating social science with genetics**
- Non-burden tests
  - **Less assumptions** than burden scores
  - **Flexible** in specifying genetic models
  - **Evolving** to incorporate more complicated data types

Thank you!

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