Association Testing with Quantitative Traits: Common and Rare Variants

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Summer Institute in Statistical Genetics 2014
Module 10
Lecture 5

Introduction to Quantitative Trait Mapping

- In the previous session, we gave an overview of association testing methods when the trait of interest is binary (e.g. 1/0, affected/unaffected, dead/alive),
- Phenotypes of interest are often quantitative, and in this session we focus on the topic of genetic association testing with quantitative traits.
- The field of **quantitative genetics** is the study of the inheritance of continuously measured traits and their mechanisms.
- Vast amounts of literature on this topic!

Introduction to Quantitative Trait Mapping

- Quantitative trait loci (QTL) mapping involves identifying genetic loci that influence the phenotypic variation of a quantitative trait.
- QTL mapping is commonly conducted with GWAS using common variants, such as variants with minor allele frequencies $\geq 1\% - 5\%$
- There generally is no simple Mendelian basis for variation of quantitative traits
- Some quantitative traits can be largely influenced by a single gene as well as by environmental factors

Introduction to Quantitative Trait Mapping

- Influences on a quantitative trait can also be due to a number of genes with similar (or differing) effects
- Many quantitative traits of interest are complex where phenotypic variation is due to a combination of both multiple genes and environmental factors
- Examples: Blood pressure, cholesterol levels, IQ, height, weight, etc.

Quantitative Genetic Model

- The classical quantitative genetics model introduced by Ronald Fisher (1918) is $Y = G + E$, where Y is the phenotypic value, G is the genetic value, and E is the environmental deviation.
- G is the combination of all genetic loci that influence the phenotypic value and E consists of all non-genetic factors that influence the phenotype
- The mean environmental deviation E is generally taken to be 0 so that the mean genotypic value is equal to the mean phenotypic value, i.e., $E(Y) = E(G)$

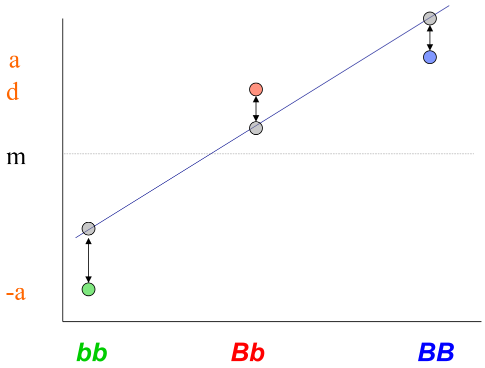
Quantitative Genetic Model

- Consider a single locus. Fisher modeled the genotypic value G with a linear regression model (least squares) where the genotypic value can be partitioned into an additive component (A) and deviations from additivity as a result of dominance (D), where

$$G = A + D$$

Linear Regression Model for Genetic Values

Falconer model for single biallelic QTL



Var (X) = Regression Variance + Residual Variance
= Additive Variance + Dominance Variance

Components of Genetic Variance

- From the properties of least squares, the residuals are orthogonal to the fitted values, and thus $Cov(A, D) = 0$. So we have that

  $$Var(G) = Var(A) + Var(D)$$

  or

  $$\sigma_G^2 = \sigma_A^2 + \sigma_D^2$$

- σ_A^2 is the **additive genetic variance**. It is the genetic variance associated with the average additive effects of alleles
- σ_D^2 is the **dominance genetic variance**. It is the genetic variance associated with the dominance effects.

Heritability

- The heritability of a trait is written in terms of the components of variances of the trait.
- Remember that $Y = G + E = A + D + E$
- The following ratio of variance components

$$h^2 = \frac{\sigma_A^2}{\sigma_Y^2}$$

  is defined to be the **narrow-sense heritability** (or simply heritability)
- h^2 is the proportion of the total phenotypic variance that is due to additive effects.
- Heritability can also be viewed as the extent to which phenotypes are determined by the alleles transmitted from the parents.

Heritability

- The **broad-sense heritability** is defined to be

$$H^2 = \frac{\sigma_G^2}{\sigma_Y^2}$$

- H^2 is the proportion of the total phenotypic variance that is due to all genetic effects (additive and dominance)
- There are a number of methods for heritability estimation of a trait.
- Module 12 (Mixed Models in Quantitative Genetics) and Module 17 (Human Complex Traits) cover the topic of heritability in more detail.

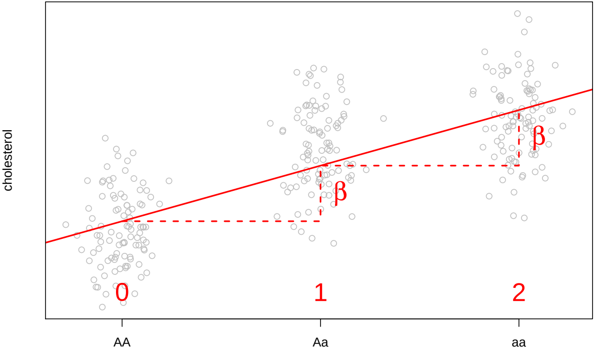
QTL Mapping

- For traits that are heritable, i.e., traits with a non-negligible genetic component that contributes to phenotypic variability, identifying (or mapping) QLT that influence the trait is often of interest.
- Linear regression models are commonly used for QTL mapping
- Linear regression models will often include a single genetic marker (e.g., a SNP) as predictor in the model, in addition to other relevant covariates (such as age, sex, etc.), with the quantitative phenotype as the response

Linear regression with SNPs

Many analyses fit the ‘additive model’

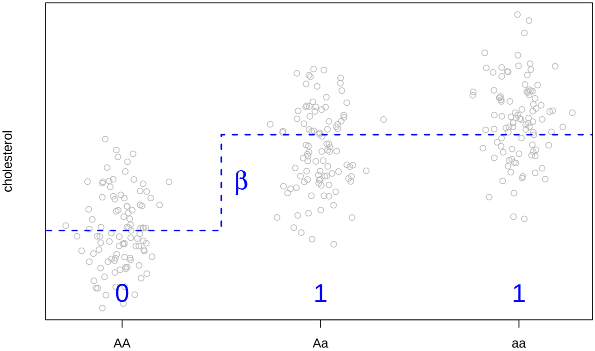
$$y = \beta_0 + \beta \times \#\text{minor alleles}$$



Linear regression, with SNPs

An alternative is the 'dominant model';

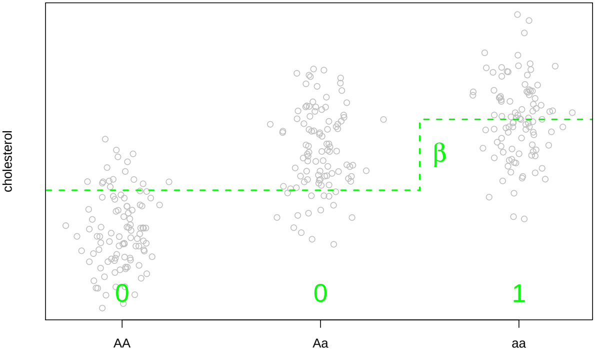
$$y = \beta_0 + \beta \times (G \neq AA)$$



Linear regression, with SNPs

or the 'recessive model';

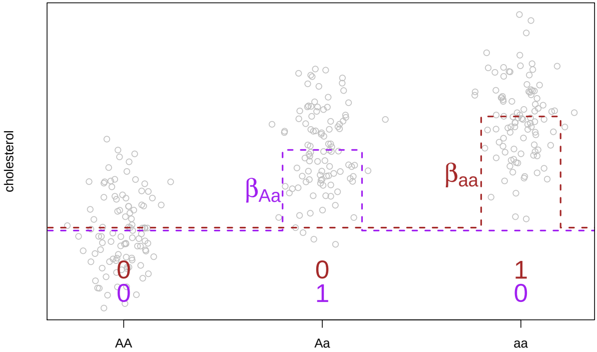
$$y = \beta_0 + \beta \times (G == AA)$$



Linear regression, with SNPs

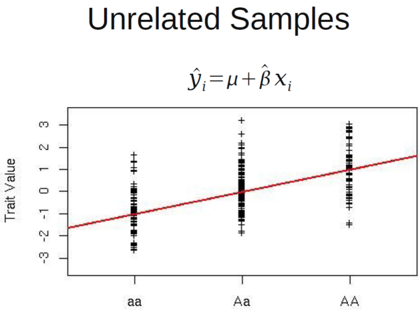
Finally, the ‘two degrees of freedom model’;

$$y = \beta_0 + \beta_{Aa} \times (G == Aa) + \beta_{aa} \times (G == aa)$$



Additive Genetic Model

- Most GWAS perform single SNP association testing with linear regression assuming an additive model.



Additive Genetic Model

- The additive linear regression model also has a nice interpretation, as we saw from Fisher's classical quantitative trait model!
- The coefficient of determination (r^2) of an additive linear regression model gives an estimate of the proportion of phenotypic variation that is explained by the SNP (or SNPs) in the model, e.g., the "SNP heritability"

Additive Genetic Model

- Consider the following additive model for association testing with a quantitative trait and a SNP with alleles A and a:

  $$Y = \beta_0 + \beta_1 X + \epsilon$$

  where X is the number of copies of the reference allele A.
- What would your interpretation of ϵ be for this particular model?

Association Testing with Additive Model

$$Y = \beta_0 + \beta_1 X + \epsilon$$

- Two test statistics for $H_0 : \beta_1 = 0$ versus $H_a : \beta_1 \neq 0$

$$T = \frac{\hat{\beta}_1}{\sqrt{var(\hat{\beta}_1)}} \sim \mathbf{t}_{N-2} \approx N(0,1) \text{ for large } N$$

$$T^2 = \frac{\hat{\beta}_1^2}{var(\hat{\beta}_1)} \sim \mathbf{F}_{1,N-2} \approx \chi_1^2 \text{ for large } N$$

where

$$var(\hat{\beta}_1) = \frac{\sigma_\epsilon^2}{S_{XX}}$$

and S_{XX} is the corrected sum of squares for the X_i's

Statistical Power for Detecting QTL

$$Y = \beta_0 + \beta_1 X + \epsilon$$

- We can also calculate the power for detecting a QTL for a given effect size β_1 for a SNP.
- For simplicity, assume that Y has been a standardized so that with $\sigma_Y^2 = 1$.
- Let p be the frequency of the A allele in the population

$$\sigma_Y^2 = \beta_1^2 \sigma_X^2 + \sigma_\epsilon^2 = 2p(1-p)\beta_1^2 + \sigma_\epsilon^2$$

- Let $h_s^2 = 2p(1-p)\beta_1^2$, so we have $\sigma_Y^2 = h_s^2 + \sigma_\epsilon^2$
- Interpret h_s^2 (note that we assume that trait is standardized such that $\sigma_Y^2 = 1$)

Statistical Power for Detecting QTL

- Also note that $\sigma_\epsilon^2 = 1 - h_s^2$, so we can write $Var(\hat{\beta}_1)$ as the following:

$$var(\hat{\beta}_1) = \frac{\sigma_\epsilon^2}{S_{XX}} \approx \frac{\sigma_\epsilon^2}{N\left(2p(1-p)\right)} = \frac{1-h_s^2}{2Np(1-p)}$$

- To calculate power of the test statistic T^2 for a given sample size N, we need to first obtain the expected value of the non-centrality parameter λ of the chi-squared (χ^2) distribution which is the expected value of the test statistic T squared:

$$\lambda = [E(T)]^2 \approx \frac{\beta_1^2}{var(\hat{\beta}_1)} = \frac{Nh_s^2}{1-h_s^2}$$

since $h_s^2 = 2p(1-p)\beta_1^2$

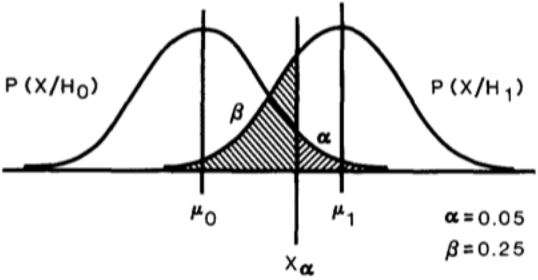
Required Sample Size for Power

- Can also obtain the required sample size given type-I error α and power $1-\beta$, where the type–II error is β :

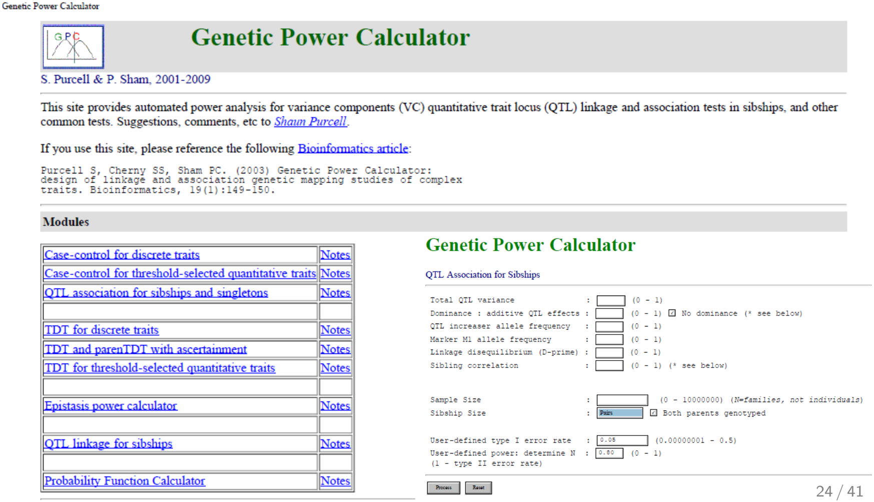
$$N = \frac{1-h_s^2}{h_s^2}\left(z_{(1-\alpha/2)} + z_{(1-\beta)}\right)^2$$

where $z_{(1-\alpha/2)}$ and $z_{(1-\beta)}$ are the $(1-\alpha/2)$th and $(1-\beta)$th quantiles, respectively, for the standard normal distribution.

Statistical Power for Detecting QTL



Genetic Power Calculator (PGC)
http://pngu.mgh.harvard.edu/~purcell/gpc/



Genetic Power Calculator

Genetic Power Calculator

S. Purcell & P. Sham, 2001-2009

This site provides automated power analysis for variance components (VC) quantitative trait locus (QTL) linkage and association tests in sibships, and other common tests. Suggestions, comments, etc to Shaum Purcell.

If you use this site, please reference the following Bioinformatics article:

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Purcell S, Cherny SS, Sham PC. (2003) Genetic Power Calculator:
design of linkage and association genetic mapping studies of complex
traits. Bioinformatics, 19(1):149-150.
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Modules

Case-control for discrete traits	Notes
Case-control for threshold-selected quantitative traits	Notes
QTL association for sibships and singletons	Notes
TDT for discrete traits	Notes
TDT and parenTDT with ascertainment	Notes
TDT for threshold-selected quantitative traits	Notes
Epistasis power calculator	Notes
QTL linkage for sibships	Notes
Probability Function Calculator	Notes

Genetic Power Calculator

QTL Association for Sibships

```
Total QTL variance               :       (0 - 1)
Dominance : additive QTL effects :       (0 - 1) ☑ No dominance (* see below)
QTL increaser allele frequency   :       (0 - 1)
Marker M1 allele frequency       :       (0 - 1)
Linkage disequilibrium (D-prime) :       (0 - 1)
Sibling correlation              :       (0 - 1) (* see below)


Sample Size                      :          (0 - 10000000) (N=families, not individuals)
Sibship Size                     : Pairs  ☑ Both parents genotyped

User-defined type I error rate   : 0.05     (0.00000001 - 0.5)
User-defined power: determine N  : 0.80  (0 - 1)
(1 - type II error rate)
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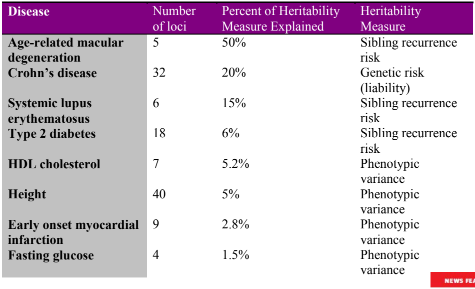
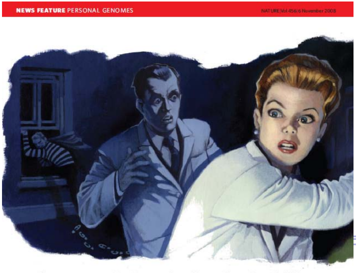
Missing Heritability



Disease	Number of loci	Percent of Heritability Measure Explained	Heritability Measure
Age-related macular degeneration	5	50%	Sibling recurrence risk
Crohn's disease	32	20%	Genetic risk (liability)
Systemic lupus erythematosus	6	15%	Sibling recurrence risk
Type 2 diabetes	18	6%	Sibling recurrence risk
HDL cholesterol	7	5.2%	Phenotypic variance
Height	40	5%	Phenotypic variance
Early onset myocardial infarction	9	2.8%	Phenotypic variance
Fasting glucose	4	1.5%	Phenotypic variance

- GWAS works
- Effect sizes are typically small
  - Disease: OR ~1.1 to ~1.3
  - Quantitative traits: % var explained <<1%



Genetic Power Calculator (Shaun Purcell)

http://pngu.mgh.harvard.edu/~purcell/gpc/

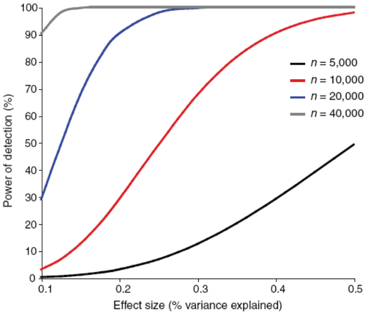
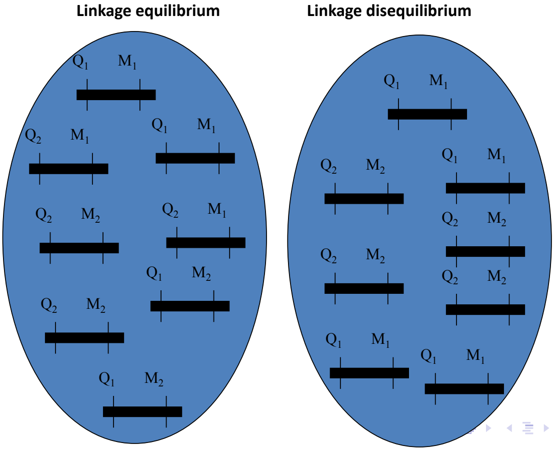


Figure 1 Statistical power of detection in GWAS for variants that explain 0.1–0.5% of the variation at a type I error rate of 5×10^{-7} (calculated using the Genetic Power Calculator[15]). Shown is the power to detect a variant with a given effect size, assuming this type I error rate, which is typical for a GWAS with a sample size of n = 5,000–40,000.

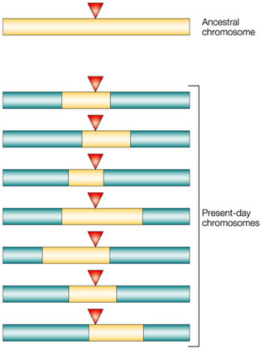
LD Mapping of QTL

- For GWAS, the QTL generally will not be genotyped in a study



LD Mapping of QTL

Linkage disequilibrium around an ancestral mutation



LD Mapping of QTL

- $r^2 =$ LD correlation between QTL and genotyped SNP
- Proportion of variance of the trait explained at a SNP $\approx r^2 h_s^2$
- Required sample size for detection is

$$N \approx \frac{1 - r^2 h_s^2}{r^2 h_s^2} \left(z_{(1-\alpha/2)} + z_{(1-\beta)}\right)^2$$

- Power of LD mapping depends on the experimental sample size, variance explained by the causal variant and LD with a genotyped SNP

Rare Variants and Sequencing Studies

- The uncovered variants from GWAS have largely been of small effect and explain only a small fraction of trait heritability.
- Rare variants, defined here as variants with minor allele frequencies less than $1\% - 5\%$, likely play a significant role in many complex traits
- Some of the missing heritability not explained by the common variants identified through GWAS potentially can be explained by causal rare variants

Uncovering Rare Variants through Sequencing

- Detecting rare variant associations from GWAS data is difficult due to rare variants having low LD with common variants SNP genotyping arrays
- Advancements in high-throughput sequencing technologies allow for rare variants to be identified from sequence data
- Whole-genome and whole-exome sequencing studies are now routinely conducted for the identification of rare-variants that are associated with complex traits.

Association Testing with Rare Variants

- The single-variant association tests previously discussed have essentially no power for detecting associations with rare variants due to their low frequencies.
- A popular strategy for detecting rare variant associations from sequencing data is to jointly consider all rare variants in a genetic region or gene in the association analysis.

Association Testing with Rare Variants

- Consider a sample with n individuals with quantitative phenotype data available and that have been sequenced in a genetic region with m variant sites.
- For individual i in the sample, let y_i denote the quantitative trait value for individual i in the sample.
- Let $\mathbf{G_i} = (g_{i1}, \ldots, g_{im})$ denote the genotypes for individual i at the m variant sites where g_{ij} takes values of 0, 1, or 2 according to the minor allele count at the j-th variant site.
- Most association methods for rare variants from sequencing data can be classified into two groups: burden association tests and kernel association tests

Burden Association Tests for Rare Variants

- Burden tests aggregate all rare variants across a genetic region into a single value for each individual.
- Weights for the m variant sites, $w_1, \ldots, w_m$, can also be used:
    - variant sites can all be given the same weight
    - weighting can be done according to minor allele frequency
    - weights can be assigned based on other features of the variants, such as functionality.

Burden Association Tests for Rare Variants

- A general linear regression model for burden tests is:

$$y_i = \beta_0 + \beta_1 \sum_{j=1}^{m} w_j g_{ij} + \epsilon_i$$

- A sample individual's aggregate value across a genetic region into a single value, $\sum_{j=1}^{m} w_j g_{ij}$, which can be viewed as the individuals genetic burden score.
- Association of phenotype and burden scores (or the variant-sum across the region) can be obtained by testing the null hypothesis $H_0 : \beta_1 = 0$ versus $H_A : \beta_1 \neq 0$ using a score statistic.

Burden Association Tests for Rare Variants

- Burden tests have been demonstrated to have reasonable power when a large proportion of the rare variants in a region are causal and with effects on phenotype that are in the same direction
- Can also perform burden tests for a case-control phenotype:

  $$logit(\pi_i) = \beta_0 + \beta_1 \sum_{j=1}^{m} w_j g_{ij}$$

  where π_i is the disease probability for individual i.
- A number of burden tests have been proposed including the combined multivariate and collapsing method (Li and Leal, 2008) and the weighted sum test (Madsen and Browning, 2009) and .

Kernal Association Tests for Rare Variants

- Kernel association methods do not aggregate variants into a single value as the burden tests do.
- Kernel-based approaches aggregate individual variant statistics measuring strength of association with each variant site.
- The sequence kernel association test (SKAT) proposed by Wu et al. (2011) is a widely used kernel rare variant association test.

SKAT for rare-variant association testing

- SKAT is based on the following linear mixed model:

  $$y_i = \alpha_0 + \mathbf{G_i^T}\boldsymbol{\beta} + \epsilon_i$$

  where

  - $\mathbf{G_i} = (g_{i1}, \ldots, g_{im})^T$ is the genotype vector for individual i at the m variant sites,
  - $\boldsymbol{\beta}$ is an $m \times 1$ vector of random effects for the m variant sites with $\boldsymbol{\beta} \sim N(\mathbf{0}, \tau\mathbf{W})$
  - τ is the variance component for the variants
  - $\mathbf{W}$ is an m-dimensional diagonal matrix of pre-specified weights for the variant sites.
- The SKAT association statistic is a score statistic for testing the null hypothesis $H_0 : \tau = 0$ versus $H_A : \tau > 0$

SKAT for rare-variant association testing

- This is equivalent to testing the null hypothesis of $H_0 : \boldsymbol{\beta} = \mathbf{0}$ versus $H_A : \boldsymbol{\beta} \neq \mathbf{0}$ but without requiring an m-degree of freedom test for detecting genetic associations for all variant sites, which would have little to no power when testing multiple variants with small effect sizes.
- Note that the variance-covariance matrix of the phenotype data $\mathbf{Y} = (y_1, \ldots, y_n)$ is a function of the linear "kernel" matrix $\mathbf{K} = \mathbf{G}\mathbf{W}\mathbf{G}^{\mathbf{T}}$, where $\mathbf{G} = (\mathbf{G_1}, \ldots, \mathbf{G_n})$ is an $n \times m$ matrix of the genetic vectors for the n sample individuals at the m variant sites.
- Other non-linear kernel matrices have also been proposed.

SKAT-O: A unified rare-variant association approach

- Kernal-based methods have higher power when a large proportion of the variants are non-causal or if there is a combination of both risk and protective variants in a region that are influencing the phenotype (Wu et al., 2011).
- Lee et al. (2012) proposed the SKAT-O method (where the "O" is for "optimal")
- SKAT-O is a unified rare-variant association approach that essentially a weighted average of burden and SKAT score tests. The optimal weighting, based on the data, is chosen for increased power.
- SKAT-O has been demonstrate to work well in a variety of rare-variant association settings.

References

- Lee S, Emond MJ, Bamshad MJ, Barnes KC, Rieder MJ, Nickerson DA, Christiani DC, Wurfel MM, Lin X (2012) Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. **Am J Hum Genet** 91:224-237.
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- Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X (2011) Rare-variant association testing for sequencing data with the sequence kernel association test. **Am J Hum Genet** 89: 82-93.