

Data-adaptive SNP-set-based Association Tests of Longitudinal Traits

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- Longitudinal data analysis strategy in GWAS

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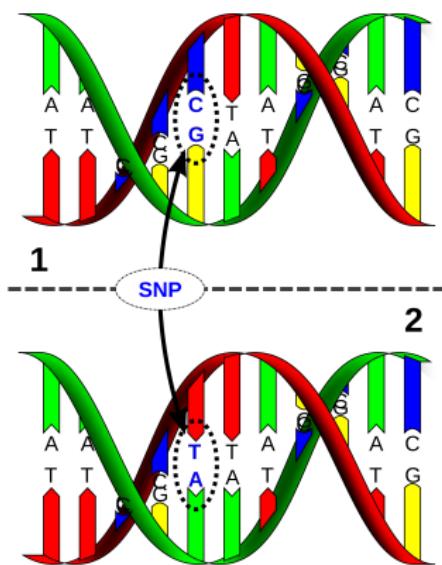
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Introduction to GWAS

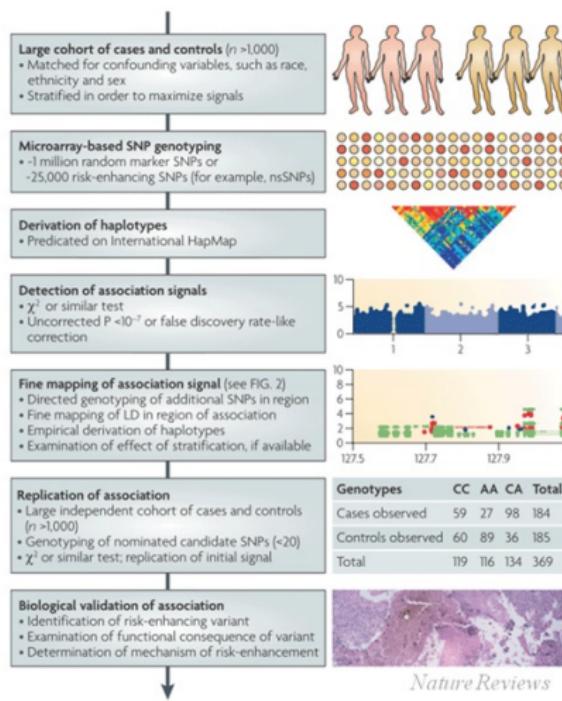
What is SNP?



A Single Nucleotide Polymorphism (SNP) is a DNA sequence variation occurring commonly within a population (e.g. 1%) in which a single nucleotide A, T, C or G in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes.

Introduction to GWAS

A flowchart of GWAS



Introduction to GWAS

How does GWAS result look like?

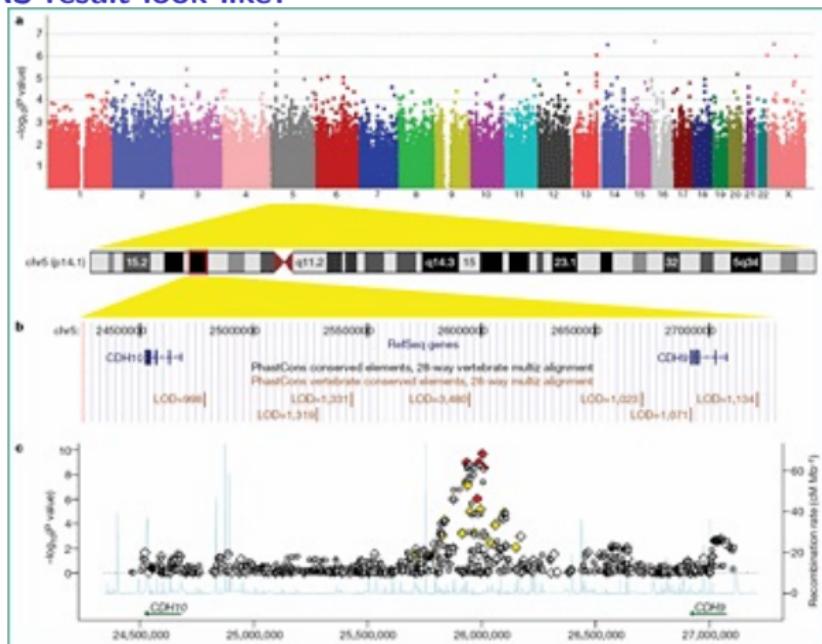


Figure: Common genetic variants on 5p14.1 associate with autism spectrum disorders [WZM⁺09]

Introduction to GWAS

GWAS Catalog

Published Genome-Wide Associations through 07/2012
Published GWA at $p \leq 5 \times 10^{-8}$ for 18 trait categories

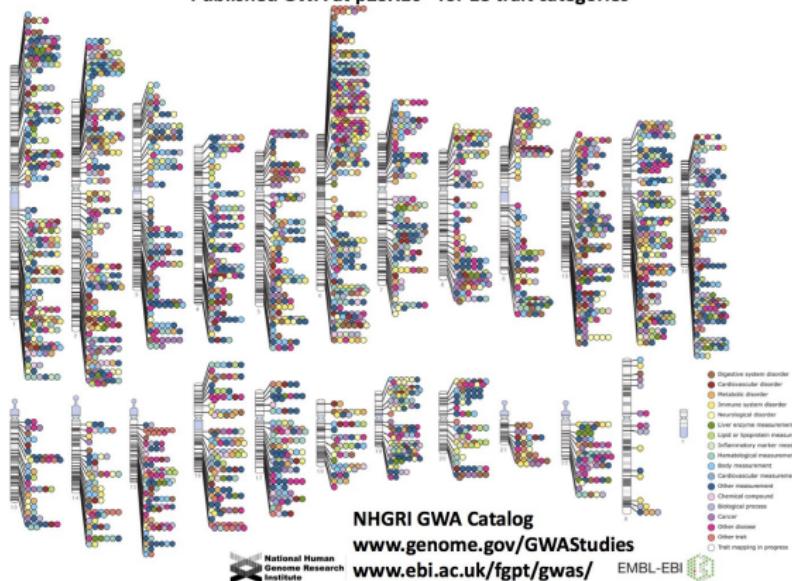


Figure: Published GWAS results for 18 trait categories

Introduction to GWAS

Common variants and rare variants

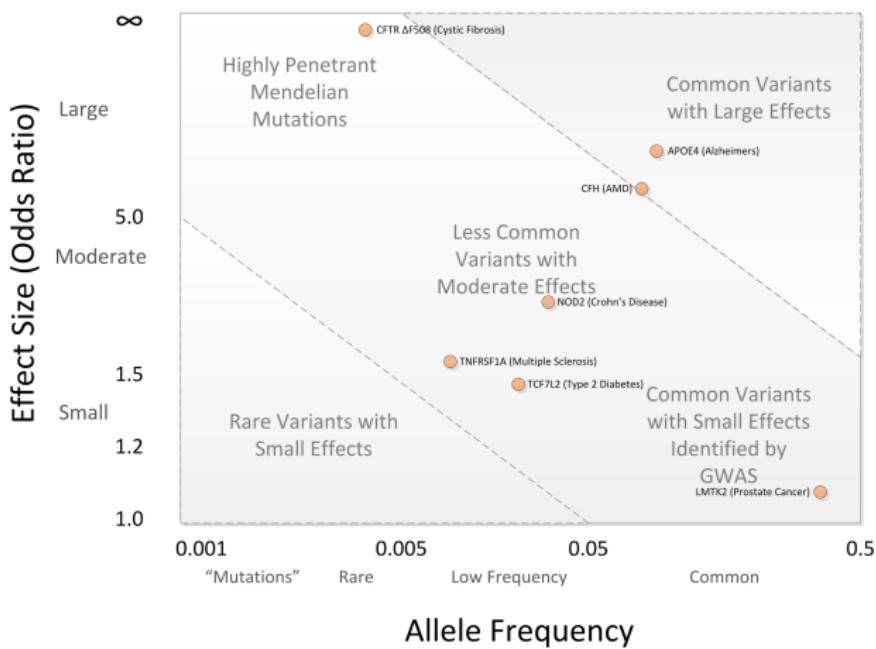


Figure: effect size of Single Nucleotide Variant [BM12]

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single-SNP based association tests

the classical method

For individual i with SNP j coded as x_{ij} ($x_{ij} = 0, 1, 2$ representing copies of minor alleles) and a vector of covariates φ_i ,

$$g(\mu_i) = \beta_0 + x_{ij}\beta_j + z_i\varphi_i,$$

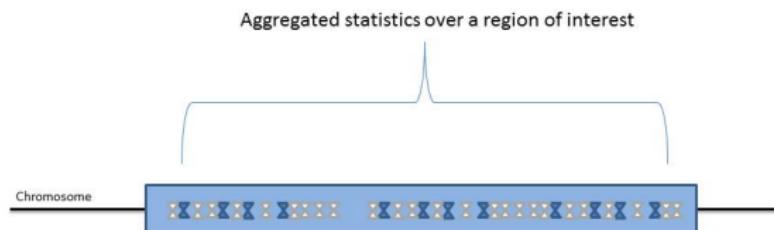
However, this method suffers from at least two disadvantages:

- 1), it will generate millions of tests thus increase the multiple test error correction burden;
- 2), the coefficient estimate of SNP j will become unstable or even the estimation algorithm cannot converge when SNP minor allele frequency (MAF) becomes smaller, e.g. MAF < 0.01.

SNP-set based association tests I

A brief review

By pooling multiple low MAF SNVs together, the SNP-set based association test can detect the signal(s) from a region (such as a gene) instead of from a single SNV.



SNP-set based association tests II

A brief review

Major categories of SNP-set based association tests:

- the so-called "burden test", which used MAF based weighting scheme to combine the sum statistics from multiple SNVs in a region [LL08, MB09];
- the variance-component test, which includes SKAT, C-alpha, SSU, etc [Pan09, NRV⁺11, WLC⁺11].
- the Lasso and group-penalized regression based methods [ZSSL10, KPS14].
- the functional linear model and functional principal component analysis based methods [LZX12b, LZX12a, LBX11, FWM⁺13].
- the adaptive test combines statistics of burden test and variance-component test, such as SKAT-O, aSum, aSSU, aScore, an exponential combination (EC) framework for set-based association tests, a robust and powerful test using Fisher's method to combine linear and quadratic statistics, a unified mixed-effect model, etc [HP10, PS11, LEB⁺12, LWL12, CHG⁺12, DLS13, SZH13].

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How do longitudinal data look like?

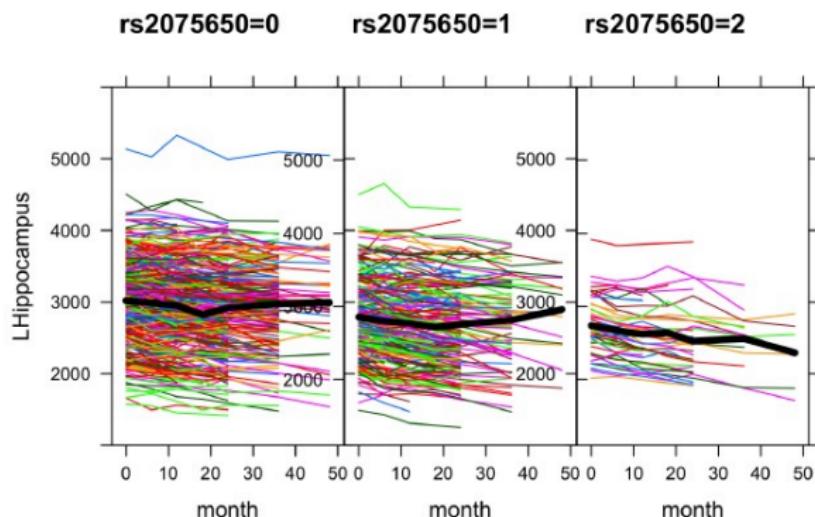


Figure: Trajectories of phenotype left hippocampus volume over time (in months) in three allele groups of SNP rs2075650 [XSP⁺14]

Why longitudinal? I

In a cross-sectional study ($n_i = 1$) we are restricted to the model

$$Y_{i1} = \beta_C x_{i1} + \epsilon_{i1}, \quad i = 1, \dots, m,$$

where β_C represents the difference in average Y across two sub-populations (samples) which differ by one unit in x . With repeated measurements, the above linear model can be extended to

$$Y_{ij} = \beta_C x_{i1} + \beta_L(x_{ij} - x_{i1}) + \epsilon_{ij}, \quad i = 1, \dots, m; j = 1, \dots, n_i$$

[WDL⁺90].

Based on above formula, we can more obviously explain the merits of longitudinal studies over cross-sectional studies.

- ① Longitudinal studies allow us to estimate both the cross-sectional difference (β_C) and the rate change over time (β_L).
- ② Even when $\beta_C = \beta_L$, longitudinal studies tend to be more powerful than cross-sectional studies. This is due to the fact that in longitudinal studies, each person can be thought of serving as his/her own control.

Why longitudinal? II

- ③ Another merit of the longitudinal study is its ability to distinguish the degree of variation in Y across time for one subject from the variation in Y across subjects.
- ④ With longitudinal studies, we can estimate a person's current and future outcome (behavior trend).

Why longitudinal? III

Longitudinal study in GWAS

A recent study by Xu et al [XSP⁺14] demonstrates the power gain from longitudinal data analysis over traditional cross-sectional data analysis used in GWAS.

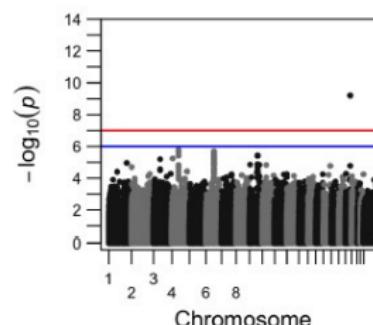
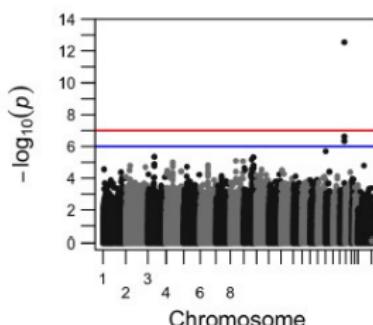


Figure: Comparison of the Manhattan plots for genome-wide p-values for phenotype left hippocampus volume from longitudinal analysis (left) and from cross-sectional analysis (right) [XSP⁺14]

Why longitudinal? IV

Power

Model	rs2075650			rs439401		
	$P = 10^{-8}$	$P = 10^{-10}$	$P = 10^{-15}$	$P = 10^{-8}$	$P = 10^{-10}$	$P = 10^{-15}$
LME-RSI	1	1	0.859	0.872	0.677	0.148
GEE-Robust	1	1	0.857	0.871	0.676	0.170
Baseline	0.736	0.448	0.038	0.077	0.015	0

LME-RSI: a linear mixed-effects model with random slope and intercept; LME-RI: a linear mixed-effects model with only a random intercept term; GEE-Robust: GEE with the sandwich covariance estimator; GEE-Naive: GEE with the model-based covariance estimator; Baseline: a linear model at the baseline testing for the main effects of an SNP.

doi:10.1371/journal.pone.0102312.t008

Figure: Simulation results at significance level P with different methods [XSP⁺14]

A brief review of major longitudinal data analysis methods I

Major categories of longitudinal data analysis methods:

- random effect models

Random effect model is a two-stage models, which treat probability distributions for the response vectors of different individuals as a single family and the random-effects parameters which hold the same for the same individual as another distribution [LW82].

- marginal effect models

Marginal effect model is an extension to quasi-likelihood method. Rather than giving subject-specific(SS) estimates as in random effect models, marginal effect models by Generalized Estimating Equation (GEE) give population-averaged (PA) estimates.

- transitional (Markov) models

The transitional (Markov) model, describes the conditional distribution of each response y_{ij} as an explicit function of first q prior observations $y_{ij-1}, \dots, y_{ij-q}$ from history response vector: $H_{ij} = \{y_{ik}, k = 1, \dots, j - 1\}$ and covariates x_{ij} . The integer q is referred as the order of the Markov models.

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Research Aims

- Aim 1: Data-adaptive SNP-set-based association tests (aSPU) for longitudinal data analysis within GEE framework;
- Aim 2: Longitudinal aSPU family tests on Rare Variants

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Public Health Significance I

- ① Due to the **complexity** in genetics association with phenotype, e.g. specific association effect directions and sizes, a given test favoring one scenario may or may not perform well in other scenarios [Pan09, DLS13, PKZ⁺14, SZH13]. In other words, there is **no single test** the most powerful among all testing scenarios.

Therefore, a few data-adaptive tests were developed as an ad hoc strategy, e.g. some tests tried to combine the advantage of burden test and variance-component test; some other tests tried to use a set of pre-determined weights for individual RVs.

Compared to the previous limited sense data-adaptive tests, our proposed method will be more extensive and generalized in **data adaptability**. The new tests will provide a relative high power in almost all data scenarios;

Public Health Significance II

- ② There is not yet a **SNP-set** based **data-adaptive** association test method for **longitudinal** data analysis in GWAS: we will propose such a new method to fill in this gap;
- ③ CVs and RVs are **both** important in finding the missing heritability of human complex disease. Our proposed new method will have the ability to handle both of them (either CVs or RVs);

In conclusion, this research work will provide useful methods/tools for identifying the underlying genetic factors explaining the heritability of human complex disease, and in the long run this will contribute to the prevention, diagnosis and cure of complex diseases.

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Aim 1

To develop a data-adaptive longitudinal association test within GEE framework for **common variants**, which will be done in either sliding-window based or gene-based manner for real GWAS data.

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Aim 1 |

Methods: introduction to notation and formula

Suppose for each subject $i = 1, \dots, n$, we have k total longitudinal measurements

$$\mathbf{y}_i = (y_{i1}, y_{i2}, \dots, y_{ik})'$$

with y_{im} as a element, p SNPs of interest as a row vector

$$\mathbf{x}_i = (x_{i1}, x_{i2}, \dots, x_{ip})$$

with x_{ij} coded as 0,1 or 2 for the count of the minor allele, and

$$\mathbf{z}_i = (z_{i1}, z_{i2}, \dots, z_{iq})$$

as a row vector for q variates.

Thus, we have:

$$\mathbf{X}_i = \begin{pmatrix} x_{i1} \\ x_{i2} \\ \vdots \\ x_{iq} \end{pmatrix}, \mathbf{Z}_i = \begin{pmatrix} 1 & z_{i1} \\ 1 & z_{i2} \\ \vdots & \vdots \\ 1 & z_{iq} \end{pmatrix}$$

\mathbf{X}_i is a $k \times p$ matrix, and \mathbf{Z}_i is a $k \times (q + 1)$ matrix.

Aim 1 II

Methods: introduction to notation and formula

We then have the GLM equation as,

$$g(\mu_i) = \eta_i = Z_i\varphi + X_i\beta = H_i\theta$$

The consistent and asymptotically normal estimates of β and φ can be obtained by solving the GEE [LZ86]:

$$U(\varphi, \beta) = \sum_{i=1}^n U_i(\varphi, \beta) = \sum_{i=1}^n \left(\frac{\partial \mu_i}{\partial \theta'} \right)' V_i^{-1} (Y_i - \mu_i) = 0,$$

with

$$\frac{\partial \mu_i}{\partial \theta'} = \frac{\partial g^{-1}(H_i\theta)}{\partial \theta'}, \quad V_i = \phi A_i^{\frac{1}{2}} R_w A_i^{\frac{1}{2}},$$

and

$$A_i = \begin{bmatrix} v(\mu_{i1}) & 0 & \cdots & 0 \\ 0 & v(\mu_{i2}) & 0 & 0 \\ \vdots & 0 & \ddots & \vdots \\ 0 & 0 & \cdots & v(\mu_{ik}) \end{bmatrix}$$

Aim 1 III

Methods: introduction to notation and formula

With a canonical link function and a working independence model, we have a closed form of the U vector with **two parts** corresponding to SNPs and covariates, and its covariance estimator:

$$U = \left(U'_{.1}, U'_{.2} \right)' = \sum_i (Z_i, X_i)' (Y_i - \mu_i)$$

$$\tilde{\Sigma} = \widehat{\text{Cov}}(U) = \sum_i (Z_i, X_i)' \widehat{\text{var}(Y_i)} (Z_i, X_i) = \sum_i (Z_i, X_i)' (Y_i - \hat{\mu}_i)(Y_i - \hat{\mu}_i)' (Z_i, X_i) = \begin{pmatrix} V_{11} & V_{12} \\ V_{21} & V_{22} \end{pmatrix} \quad (1)$$

Aim 1 IV

Methods: introduction to notation and formula

Quantitative traits

We use the identity link, i.e. $g(\mu_{im}) = \mu_{im}$ and $v(\mu_{im}) = \phi \times 1 = \phi$. Then we have:

$$\begin{aligned} U &= \sum_i (Z_i, X_i)' R_w^{-1} (Y_i - \mu_i) \\ \tilde{\Sigma} &= \sum_i (Z_i, X_i)' R_w^{-1} (Y_i - \hat{\mu}_i) (Y_i - \hat{\mu}_i)' R_w^{-1} (Z_i, X_i) \end{aligned} \quad (2)$$

if the assumption of a common covariance matrices across Y_i for i is valid, e.g. for quantitative continuous traits study [Pan01], we can adopt a more efficient covariance estimator:

$$\tilde{\Sigma} = \sum_{i=1}^n (Z_i, X_i)' \widehat{\text{var}(Y_i)} (Z_i, X_i) = \sum_{i=1}^n (Z_i, X_i)' \left(\sum_{i=1}^n \frac{(Y_i - \hat{\mu}_i)(Y_i - \hat{\mu}_i)'}{n} \right) (Z_i, X_i) = \begin{pmatrix} V_{11} & V_{12} \\ V_{21} & V_{22} \end{pmatrix}$$

which is used by default for its better finite-sample performance [Pan01].

Aim 1 V

Methods: introduction to notation and formula

Binary traits

For binary traits (trait value coded as 0 and 1), we use the logit link function so that

$g(\mu_{im}) = \log \frac{\mu_{im}}{1 - \mu_{im}}$ and $v(\mu_{im}) = \mu_{im}(1 - \mu_{im})$. Additionally the (m, l) th element of $\frac{\partial \mu_i}{\partial \theta'}$ is $H_{i,ml}\mu_{im}(1 - \mu_{im})$ with $H_{i,ml}$ as the (m, l) th element of H_i , which is the short notation for (Z_i, X_i) .

Then we have:

$$\begin{aligned} U &= \sum_{i=1} (\frac{\partial \mu_i}{\partial \theta'})' V_i^{-1} (Y_i - \mu_i) \\ &= \sum_{i=1} (\frac{\partial \mu_i}{\partial \theta'})' \phi A_i^{-\frac{1}{2}} R_w^{-1} A_i^{-\frac{1}{2}} (Y_i - \mu_i) \end{aligned}$$

and

$$\begin{aligned} \tilde{\Sigma} &= \sum_i \left(\frac{\partial \mu_i}{\partial \theta'} \right)' \phi A_i^{-\frac{1}{2}} R_w^{-1} A_i^{-\frac{1}{2}} (Y_i - \hat{\mu}_i)(Y_i - \hat{\mu}_i)' \phi A_i^{-\frac{1}{2}} R_w^{-1} A_i^{-\frac{1}{2}} \left(\frac{\partial \mu_i}{\partial \theta'} \right) \\ &= \begin{pmatrix} V_{11} & V_{12} \\ V_{21} & V_{22} \end{pmatrix} \end{aligned}$$

Aim 1 VI

Methods: introduction to notation and formula

In this research, I will **focus on** the case with quantitative traits, since they are most typical traits used as the response variable in longitudinal data analysis. In general, the only difference lies in which canonical link we will use, with all other equations/formulas remaining the same.

Aim 1 VII

Methods: introduction to notation and formula

Our goal is to detect whether there is any association between the longitudinal trait and the SNPs via testing on hypothesis

$$H_0 : \beta = (\beta_1, \beta_2, \dots, \beta_p)' = 0$$

We have under the null hypothesis with $g(Y_i) = Z_i\varphi$ to obtain φ and predict $\hat{\mu} = g^{-1}(Z\hat{\varphi})$. We hereby have score vector under the null hypothesis, with a working independence model, is:

$$U(\hat{\varphi}, 0) = (U'_{.1}, U'_{.2})' = \sum_{i=1}^n (U'_{i1}, U'_{i2})'$$

where

$$U_{.1} = \sum_i Z'_i (Y_i - \hat{\mu}_i), \quad U_{.2} = \sum_i X'_i (Y_i - \hat{\mu}_i)$$

As U asymptotically follows a multivariate normal distribution under H_0 , then the score vector for β also has an asymptotic normal distribution:

$$U_{.2} \sim N(0, \Sigma_{.2}), \quad \Sigma_{.2} = \widehat{\text{Cov}}(U_{.2}) = V_{22} - V_{21}V_{11}^{-1}V_{12}$$

, where V_{xx} are defined in Equation 1.

Aim 1 VIII

Methods: introduction to notation and formula

Several classical tests:

- **The Wald Test:** The Wald Test known as $T = \hat{\beta}'\text{cov}(\hat{\beta})\hat{\beta}$ is most commonly used, where $\hat{\beta}$ is the estimate of β after fitting the full GEE model with $g(\mu_i) = Z_i\varphi + X_i\beta$. Under H_0 , we have $T \sim \chi_p^2$. The Wald test is more time consuming by fitting full model, may fail to converge with many SNPs put on RHS of the regression-like equation to test, and more importantly, the type I error tends to inflate in such case [PKZ⁺14, ZXSP14].
- **The Score Test:** $T = U_{.2}'\Sigma_{.2}^{-1}U_{.2}$, where $U_{.2}$ and $\Sigma_{.2}$ are discussed above; the statistic is asymptotically equivalent to the Wald test with the same null distribution $T \sim \chi_p^2$. Since we only need to fit the null model with covariates, it is computationally easier and less likely to have numerical convergence problems. More importantly, the score test controls the type I error well [PKZ⁺14, ZXSP14].
- **The UminP Test:** $T = \max_j \frac{U_{2,j}^2}{\Sigma_{2,jj}}$ for $j \in 1, 2, \dots, p$, of j th SNP effect. The $\Sigma_{2,jj}$ is the j th entry on the diagonal of $\Sigma_{.2}$. With $\max_j T$, we can get minimal p-value accordingly. A simulation method based on the asymptotic normal distribution of the score vector can be used to calculate its p-value [PKZ⁺14, ZXSP14]. An asymptotic multivariate normal distribution numerical integration based method provided an alternative to calculate its p-value [PHS09, Pan09].

Aim 1 I

Methods: A new class of tests and a data-adaptive test in longitudinal data settings

A general form of score-vector-based statistic can be generalized as:

$$T_w = W' U = \sum_{j=1}^p W_j U_j$$

where $W = (W_1, \dots, W_p)'$ is a vector of weights for the p SNVs [LT11].
with special cases:

$$T_{Sum} = 1' U = \sum_{j=1}^p U_j, \quad T_{SSU} = U' U = \sum_{j=1}^p U_j^2,$$

These two tests are called Sum test and SSU test [Pan09].

Aim 1 II

Methods: A new class of tests and a data-adaptive test in longitudinal data settings

If we choose weight to be

$$W_j = U_{.2,j}^{\gamma-1}$$

for a series of integer value $\gamma = 1, 2, \dots, \infty$, leading to the sum of powered score (U) tests called **SPU** tests:

$$T_{SPU(\gamma)} = \sum_{j=1}^P U_{.2,j}^{\gamma-1} U_{.2,j}$$

When $\gamma \rightarrow \infty$ as an extreme situation, where ∞ is assumed to be an even number, we have

$$T_{SPU(\gamma)} \propto \|U\|_\gamma = \left(\sum_{j=1}^P |U_{.2,j}|^\gamma \right)^{\frac{1}{\gamma}} \rightarrow \|U\|_\infty = \max_{j=1}^P |U_{.2,j}| \equiv T_{SPU(\infty)}.$$

In our experience, SPU(γ) test with a large $\gamma > 8$ usually gave similar results as that of SPU(∞) test [PKZ⁺14], thus we will only use $\gamma \in \Gamma = \{1, 2, \dots, 8, \infty\}$ for the whole dissertation work.

Aim 1 III

Methods: A new class of tests and a data-adaptive test in longitudinal data settings

Simulation-based P-value estimation of $T_{SPU(\gamma)}$

Suppose T is short notation of $T_{SPU(\gamma)}$ for a specific γ and $\hat{\Sigma}_{.2}$ is the covariance matrix of the score vector $U_{.2}$ based on original data (see Equation 1). We draw B samples of the score vector from its null distribution:

$$U_{.2}^{(b)} \sim MVN\left(0, \hat{\Sigma}_{.2}\right),$$

with $b = 1, 2, \dots, B$, and thus obtain a statistics under null hypothesis: $T^{(b)} = \sum_{j=1}^p U_{.2,j}^{(b)} \gamma$. We then can calculate the p-value of $T_{SPU(\gamma)}$ as

$$P_{SPU(\gamma)} = \sum_{b=1}^B \frac{I(T^{(b)} \geq T^{obs}) + 1}{B + 1}.$$

Aim 1 IV

Methods: A new class of tests and a data-adaptive test in longitudinal data settings

The aSPU test

Although we have a list of $SPU(\gamma)$ statistics and p-values, we are not sure which one is **the most powerful** in a specific data situation. Thus, it will be convenient to have a test which data-adaptively and automatically **select/combine the best** $SPU(\gamma)$ test(s).

We hereby propose an adaptive SPU (aSPU) test to achieve such purpose. Accordingly, we will have the aSPU test statistic:

$$T_{aSPU} = \min_{\gamma \in \Gamma} P_{SPU(\gamma)},$$

Aim 1 V

Methods: A new class of tests and a data-adaptive test in longitudinal data settings

Simulation-based P-value estimation of T_{aSPU}

Similarly,

$$P_{SPU(\gamma)}^{(b)} = \sum_{b_1 \neq b}^B \frac{I(T_{SPU(\gamma)}^{(b_1)} \geq T_{SPU(\gamma)}^{(b)}) + 1}{(B - 1) + 1}$$

for every γ and every b . Then, we will have $T_{aSPU}^{(b)} = \min_{\gamma \in \Gamma} P_{SPU(\gamma)}^{(b)}$, and the final p-value of aSPU test is:

$$P_{aSPU} = \sum_{b=1}^B \frac{I(T_{aSPU}^{(b)} \leq T_{aSPU}^{obs}) + 1}{B + 1}.$$

It is worth noting again that the same B simulated score (U) vectors have been used in calculating the P_{aSPU} .

Aim 1 VI

Methods: A new class of tests and a data-adaptive test in longitudinal data settings

The "data-adaptive" genome wide scan strategy

In practice for genome wide scan purpose, we can use a "data-adaptive" aSPU test strategy that is:

- ① we first start with a smaller B , say $B = 1000$
- ② we increase B to say 10^6 for just a few groups of SNVs, which passed an pre-determined significance cutoff (e.g. p-value $\leq 5/B$) in 1
- ③ repeat 2 until a pre-determined B number reached

In this "data-adaptive" way of implementing the simulation based p-value calculating method for aSPU test, we will be able to apply the aSPU test to GWA data.

Aim 1 VII

Methods: A new class of tests and a data-adaptive test in longitudinal data settings

Other versions of aSPU test

- **aSPUw test**

The SPUw test is a *diagonal-variance-weighted* version of the SPU test, defined as:

$$T_{SPUw(\gamma)} = \sum_{j=1}^p \left(\frac{U_{.2,j}}{\sqrt{\hat{\Sigma}_{.2,jj}}} \right)^\gamma$$

- **aSPU(w).Score test**

$$T_{aSPU.Score} = \min \left\{ \min_{\gamma \in \Gamma} P_{SPU(\gamma)}, P_{Score} \right\},$$

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Aim 1

Methods in data simulation

Simulation of genotype data

- ① a latent vector $G_i = (G_{i1}, \dots, G_{ip})'$ was first drawn from a **multivariate Normal distribution** $N(0, R)$, where R had a AR(1) correlation structure with its (i, j) th element in terms of purely correlation $r_{ij} = \text{Corr}(G_{if}, G_{ig}) = \rho^{|f-g|}$ between any two latent components, G_{if} and G_{ig} for $f \neq g$. In our simulations we set $\rho = 0.8$;
- ② the latent vector G_i was dichotomized to yield a haplotype with each latent element G_{ij} dichotomized to 0 or 1 with probability $\text{Prob}(G_{ij} = 1) = \text{MAF}$ of j th SNP; the MAFs were randomly drawn from a uniform distribution: for causal SNPs the MAFs were set between 0.3 and 0.4; for null SNPs the MAFs were set between 0.1 and 0.5;
- ③ we combined two independent haplotypes to form the genotype $X_i = (X_{i1}, \dots, X_{ip})'$ for subject i . The haplotypes for different subject were generated independently.

Aim 1 II

Methods in data simulation

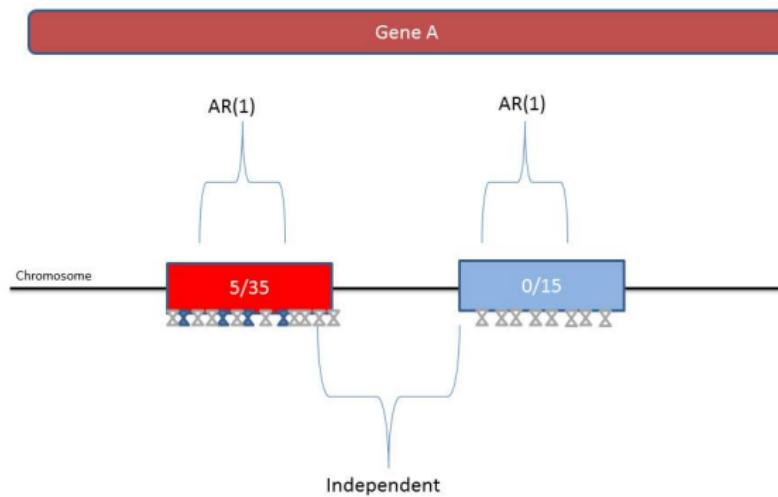


Figure: Demo graph of genotype simulation

Aim 1 III

Methods in data simulation

Simulation of phenotype data

We setup the mixed effect model to achieve the AR(1) correlation structure as:

$$y_{im} = \mu_i + b_i + \underbrace{\rho e_{i,m-1} + s_{i,m}}_{e_{i,m}}, \quad (3)$$

with $m = 1, \dots, k$ indexes the longitudinal measurements within subject i ;

$$\mu_i = Z_i \varphi + X_i \beta = H_i \theta$$

as in quantitative trait case; b_i is the random intercept representing the subject-level random effect, and

$$\rho e_{i,m-1} + s_{i,m} = e_{i,m},$$

where ρ is lag-one autocorrelation coefficient, so we can plugin our estimate from real data here by setting up $\rho = 0.7$. We assume the following distribution:

Aim 1 IV

Methods in data simulation

$$b_i \sim N(0, \sigma_b^2)$$

$$e_{i,m} \sim N(0, \sigma_e^2)$$

$$s_{i,m} \sim N(0, (1 - \rho^2)\sigma_e^2)$$

Under this assumption, the variance-covariance matrix across longitudinal measurements becomes (assuming $k = 4$ for the number of longitudinal measurements):

$$\Sigma_{4 \times 4} = \text{Var} \begin{pmatrix} b_i + e_{i1} \\ b_i + \rho e_{i1} + s_{i2} \\ b_i + \rho e_{i2} + s_{i3} \\ b_i + \rho e_{i3} + s_{i4} \end{pmatrix} = \sigma_b^2 \begin{pmatrix} 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \end{pmatrix} + \sigma_e^2 \begin{pmatrix} 1 & \rho & \rho^2 & \rho^3 \\ \rho & 1 & \rho & \rho^2 \\ \rho^2 & \rho & 1 & \rho \\ \rho^3 & \rho^2 & \rho & 1 \end{pmatrix} \quad (4)$$

Aim 1 V

Methods in data simulation

Connect phenotype data with genotype data

Let we first introduce the below splitting of the phenotype variance:

$$\text{Var}(y_{im}) = \text{Var}(X_{ij})\beta_j^2 + \sigma_{oth}^2 = 2f(1-f)\beta_j^2 + \sigma_{oth}^2 \quad (5)$$

Now let we look at the relationship between genetic heritability (narrow-sense heritability) and equation (5):

$$h^2 = \frac{\text{Var}(A)}{\text{Var}(P)} \quad (6)$$

In our situation for j th SNP, this can be extended to:

$$h_j^2 = \frac{\text{Var}_j(A)}{\text{Var}(P)} = \frac{\text{Var}(X_{ij})\beta_j^2}{\text{Var}(y_{im})} = \frac{\text{Var}(y_{im}) - \sigma_{oth}^2}{\text{Var}(y_{im})} \approx \frac{\text{Var}(y_{im}) - \sigma_b^2 - \sigma_e^2}{\text{Var}(y_{im})} \quad (7)$$

Aim 1 VI

Methods in data simulation

Summary of parameter setup in simulation studies

After this point, by systematically solving the equations (5) and (7), we can easily calculate the β_j for j th SNP once we have determined the value of h_j^2 , σ_b^2 , σ_e^2 and f . Usually a h_j^2 for a single SNP j will not be high for complex disease and we used $h_j^2 = 0.001$ in our simulation study to control β_j . We summarize the parameters used in simulation studies here:

- $h_j^2 = 0.001$
- $\sigma_b^2 = 1$
- $\sigma_e^2 = 1$
- n varies between 500 and 3000
- $k = 4$
- 1000 replicates of simulated dataset
- $\alpha = 0.05$
- $\rho_y = 0.7$
- $\rho_x = 0.8$
- $R = AR(1)$
- $Rw = I$

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Aim 1

Simulation results

- Tests under default simulation settings with varying sample size

n	Score	UminP	SumP	SumP.w	SSU	aSPU	aSPUw	aSPU.sco	aSPUw.sco
500	0.038	0.056	0.058	0.053	0.044	0.052	0.051	0.050	0.048
1000	0.047	0.054	0.048	0.049	0.065	0.065	0.064	0.059	0.057
2000	0.055	0.041	0.053	0.053	0.059	0.052	0.055	0.058	0.058
3000	0.055	0.054	0.057	0.060	0.065	0.063	0.054	0.056	0.059

Table: Type I error under using working independence R_w

Aim 1 II

Simulation results

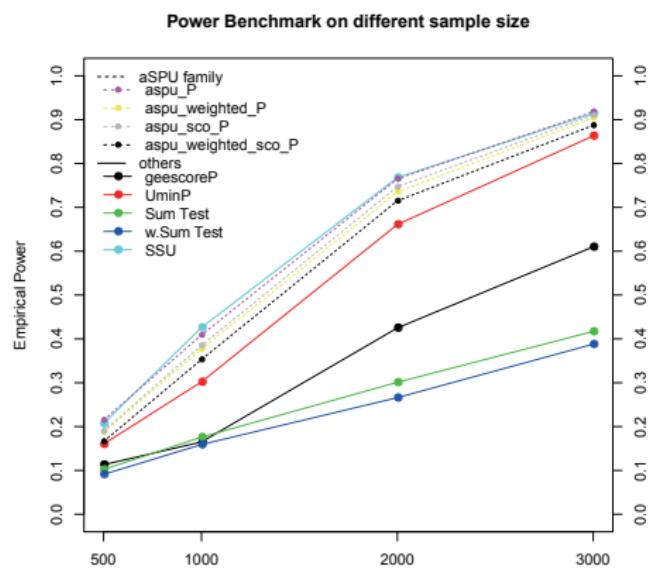


Figure: Empirical power benchmark under different n using working independence R_w

Aim 1 III

Simulation results

- **Tests with half number of SNPs in opposite effect direction**

In 5 causal SNPs, we set 2 of them to have opposite effect direction to the left 3 SNPs. The other settings kept the same as the above. We have the empirical power benchmark result as below:

Aim 1 IV

Simulation results

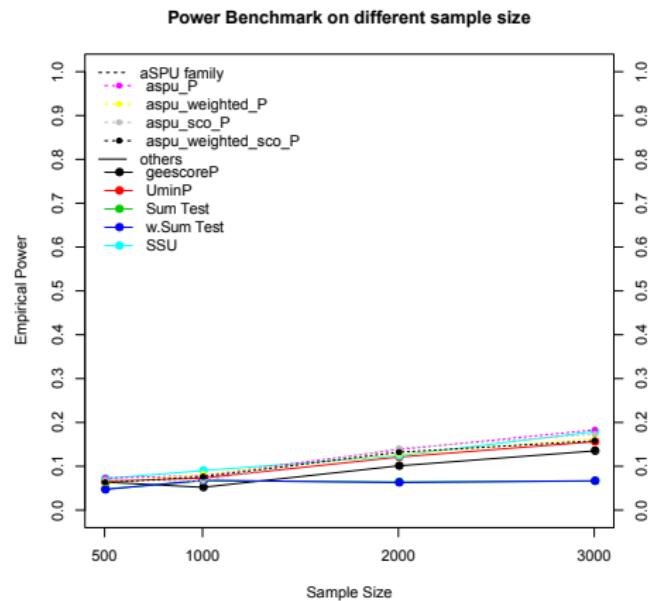


Figure: Empirical power benchmark under a mixed SNP effects

Aim 1 V

Simulation results

- Tests with growing number of Null SNPs

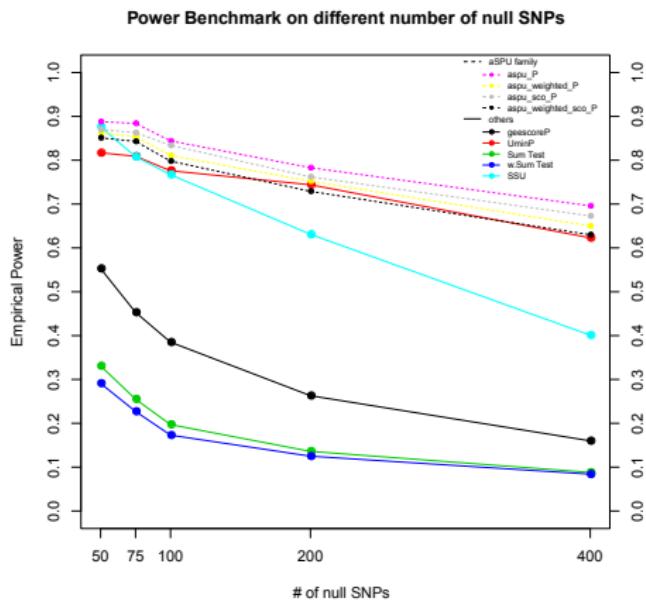


Figure: Empirical power benchmark under an increasing number of Null SNPs

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Aim 2

Extend the data-adaptive longitudinal association test within GEE framework to work for **rare variants** in a gene-based manner.

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Aim 2 I

Methods

For CVs we have:

$$U_{.2}^{(b)} \sim MVN \left(0, \hat{\Sigma}_{.2} \right)$$

with $b = 1, 2, \dots, B$, and thus obtain a statistics under null hypothesis: $T^{(b)} = \sum_{j=1}^p U_{.2,j}^{(b)\gamma}$. We then calculate the p-value of $T_{SPU(\gamma)}$ as $P_{SPU(\gamma)} = \sum_{b=1}^B \frac{I(T^{(b)} \geq T^{obs}) + 1}{B+1}$.

The above algorithms will hold in RV case by large, except that the $U_{.2}^{(b)}$ may **not** follow the multivariate Normal distribution any longer. As a remedy, we propose a permutation algorithm that generates the empirical null distribution of $U_{.2}^{(b)}$ and in the same time **Maintain the relationship** between longitudinal traits and possible covariates such as age, gender, etc, for subject i . The algorithm will also be robust to **missing data** as this is a usual case in longitudinal data settings.

Aim 2 II

Methods

The permutation algorithm can be implemented as follows:

- ① identify the max k across all n subjects, which is the number of longitudinal measurements, e.g. $k = 4$.
- ② detect if the data has missing values, if yes, fill the missing value with NA to complement the data dimension (for example, subject i with $Y_i = (y_{i,1}, \dots, y_{i,4})'$ has two missing measurements at time 2 and time 3. After missing value complementing, it becomes $Y_i = (y_{i,1}, NA, NA, y_{i,4})'$). Now we should have all the subjects with each Y_i of dimension equal to $k \times 1$.
- ③ complement H_i to be of full dimension, i.e. $k \times (p + q + 1)$, for covariates and SNVs. Now we should have $(Y_i \quad H_i)$ as an augmented matrix of dimension $k \times (p + q + 2)$ for each subject i , where $H_i = (Z_i, X_i)$. For total n subjects, we have row-wise binded matrix

$$M = \begin{pmatrix} Y_1 & H_1 \\ Y_2 & H_2 \\ \vdots & \vdots \\ Y_n & H_n \end{pmatrix}$$

of dimension $nk \times (p + q + 2)$.

Aim 2 III

Methods

- ④ permute the SNV chunk among different individuals, i.e. the X_i in $(Y_i \quad Z_i, X_i)$ with the X_j in $(Y_j \quad Z_j, X_j)$, where $i \neq j$.
- ⑤ with permuted

$$M^{*(b)} = \begin{pmatrix} Y_1 & Z_1, X_1^{*(b)} \\ Y_2 & Z_1, X_2^{*(b)} \\ \vdots & \vdots \\ Y_n & Z_1, X_n^{*(b)} \end{pmatrix}$$

we refit the GEE model and get the $U_{.2}^{*(b)}$

- ⑥ repeat step 4 - 5 B times to produce $U_{.2}^{*(b)}$ with $b = 1, 2, \dots, B$.

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Aim 2 I

Methods in data simulation

The simulation strategy of RV data is almost the same with previous strategy for generating CV data , except that:

- ① the MAF of RVs, regardless of casual one or null one, are set between **0.001** and **0.01**.
- ② the casual RVs are **not** excluded from later test as we expect the whole-genome sequencing or exome sequencing/Chip platform will identify high density SNVs including the real casual ones.

We will use the same simulated longitudinal phenotype data as for CVs.

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Aim 2 I

Simulation results

If we still use the CVs' strategy on RVs, we will have

- **Simulation-based Test under default settings with varying sample size**

n	pSSU	pSSUw	pScore	pSum	mvn.UminP	UminP	SPU(1)	SPUw(1)	SPU(2)	SPUw(2)	aSPU	aSPUw	aSPU.sco	aSPUw.sco
500	0.053	0.054	0.052	0.049	0.047	0.022	0.052	0.026	0.063	0.025	0.056	0.021	0.059	0.035
1000	0.055	0.040	0.042	0.048	0.054	0.049	0.048	0.046	0.061	0.044	0.045	0.045	0.053	0.047
2000	0.054	0.050	0.048	0.049	0.046	0.045	0.053	0.044	0.063	0.061	0.066	0.062	0.062	0.062
3000	0.045	0.044	0.039	0.060	0.053	0.055	0.057	0.058	0.058	0.052	0.049	0.055	0.055	0.057

Table: Empirical type I error using simulation-based method in RV analysis. mvn.UminP: UminP method based MVN distribution; UminP: UminP method based on simulation.

Aim 2 II

Simulation results

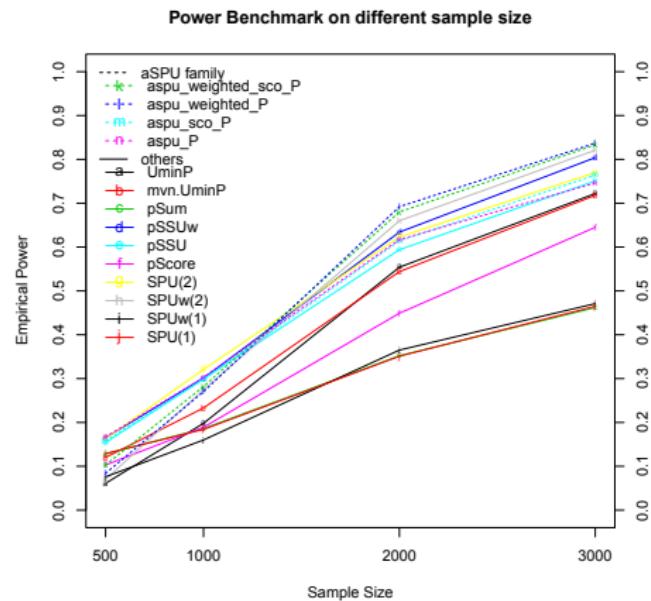


Figure: Empirical power benchmark using simulation-based method in RV analysis

Aim 2 III

Simulation results

- Permutation-based Test under default settings with varying sample size

As noted before, there are some minor issues in using simulated-based aSPU method to test RVs, we thus tested the aSPU performance based on permutation algorithm. The type I error is shown below.

n	pSSU	pSSUw	pScore	pSum	mvn.UminP	UminP	SPU(1)	SPUw(1)	SPU(2)	SPUw(2)	aSPU	aSPUw	aSPU.sco	aSPUw.sco
500	0.053	0.054	0.052	0.049	0.047	0.046	0.050	0.049	0.056	0.061	0.054	0.053	0.060	0.056
1000	0.055	0.040	0.042	0.048	0.054	0.056	0.048	0.049	0.056	0.043	0.047	0.045	0.052	0.051
2000	0.054	0.050	0.048	0.049	0.046	0.046	0.049	0.043	0.053	0.052	0.063	0.057	0.058	0.056
3000	0.045	0.044	0.039	0.060	0.053	0.050	0.058	0.058	0.047	0.048	0.049	0.053	0.049	0.053

Table: Empirical type I error using permutation-based method in RV analysis.

mvn.UminP: UminP method based MVN distribution; UminP: UminP method based on permutation.

Aim 2 IV

Simulation results

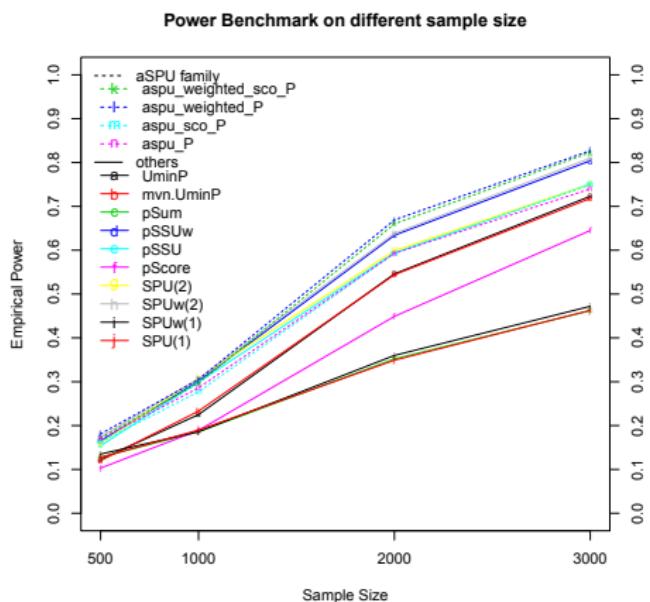


Figure: Empirical power benchmark using simulation-based method in RV analysis

Aim 2 V

Simulation results

- An effort to combine the advantages from aSPU, aSPUw and score test. The aSPU.aSPUw.Score test can save user's effort in deploying a best version of aSPU family test on a specific dataset with only a small amount of power loss in the process of compromising among different versions.

$$T_{aSPU.aSPUw.Score} = \min \left\{ \min_{\gamma \in \Gamma} P_{SPU(\gamma)}, \min_{\gamma \in \Gamma} P_{SPUw(\gamma)}, P_{Score} \right\},$$

Aim 2 VI

Simulation results

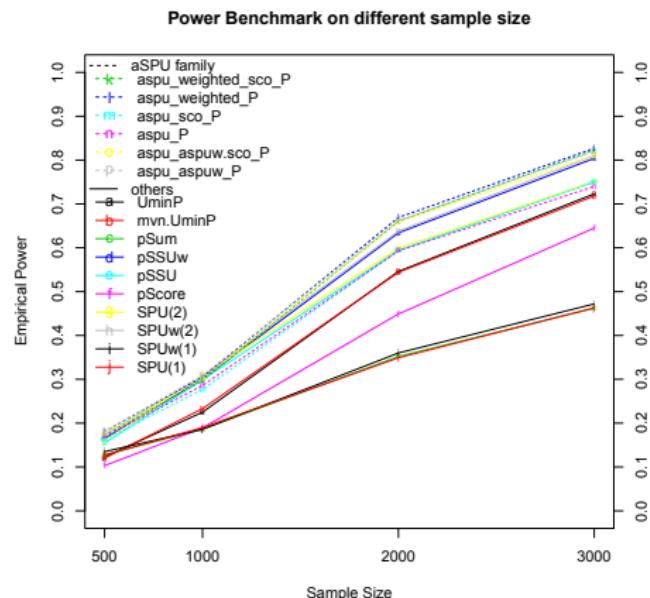


Figure: Empirical power benchmark with aSPU.aSPUw.Score test in RV analysis

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Real Data Introduction

The real data used in my dissertation will be obtained from the Atherosclerosis Risk in Communities (ARIC) Study (<https://www2.cscc.unc.edu/aric/>).

The Cohort Component of the ARIC study began in 1987. A total of 15,792 participants received an extensive examination, including medical, social, and demographic data. These participants were re-examined every three years with the first screen (baseline) occurring in 1987-89, the second in 1990-92, the third in 1993-95, and the fourth exam was in 1996-98. In 2009, the NHLBI funded a fifth exam, which is currently being conducted.

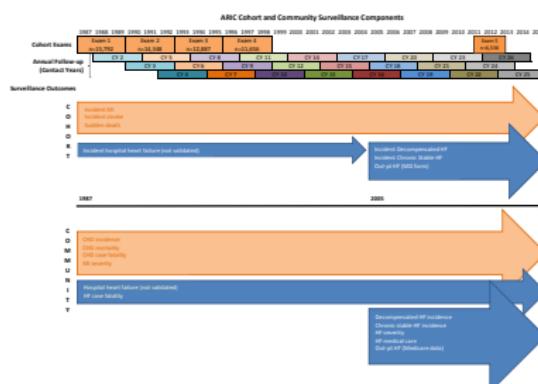


Figure: ARIC Cohort and Community Surveillance Components. Figure adopted from the ARIC website

We applied our novel method on ARIC data. Specifically, we will use the four closely cardiovascular-disease-related traits measured in ARIC cohort data, which are **total cholesterol (tch)**, **High-density lipoprotein (HDL)**, **Low-density lipoprotein (LDL)** and **triglycerides (trgs)**. We will exclusively use Caucasian samples ($n = 11478$). For the covariates, we will include but not limited to subject's demographic information such as age, gender, BMI, etc.

Real Data Result Demo

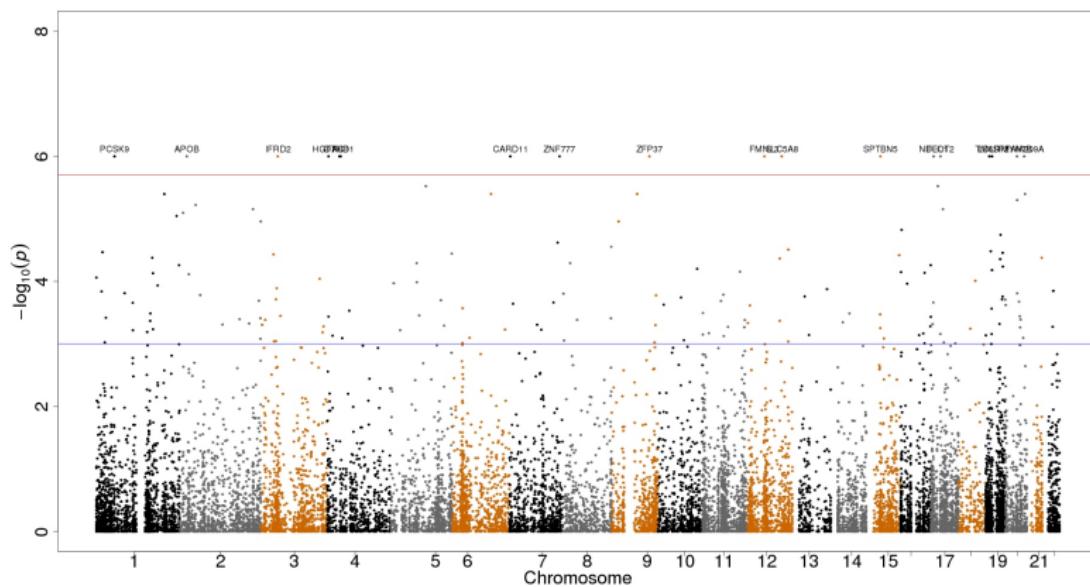


Figure: Manhattan Plot of aSPUw.score test on ARIC data Total Cholesterol trait



Thank you for your participation!

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