Testing for the Effect of a Genetic Pathway in Longitudinal/Clustered Data

with Application to DNA Methylation Data

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Outline

1. Introduction and Motivation
2. Kernel Machine Based Approach
3. Simulation and Data Results

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Longitudinal Data: Normative Aging Study (NAS)

Objective

Test association between the methylation of pro-inflammatory genes and cardiovascular biomarkers.

NAS is a ongoing cohort study of older men in Boston, MA.

- 277 men ages 50-100 living in the greater Boston area
- Physical evaluations every 3 years
- Measurements of gene specific methylation at 2 visits

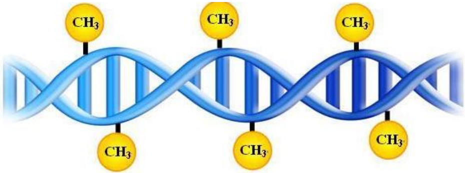
Outcome: C-reactive protein (CRP)

- Biomarker of inflammation and is predictive of cardiovascular events

DNA Methylation

DNA Methylation

- Addition of methyl groups to CpG sites in the DNA
- Modifies genome function without changing the DNA sequence



DNA Methylation

- An epigenetic regulator of gene expression.
- Methylation status or patterns are involved in determining cardiovascular diseases (CVD).
- Modifiable in response to environmental factors.
- De-methylation of DNA in a particular gene is expected to increase the expression and activity of the protein coded by the gene.

Genes of interest

- Toll-like receptor 2 (TLR2)
- Interferon gamma (IFN-γ)
- Intercellular adhesion molecule 1 (ICAM-1)
- Interleukin 6 (IL-6)

Scientific Questions

Outcome: C-reactive protein (CRP)

1. Are Toll-like receptor 2 (TLR2) and Interferon gamma (IFN-γ) associated with CRP levels?
    - Function: TLR2 regulates IFN-γ

2. Are Intercellular adhesion molecule 1 (ICAM-1) and Interleukin 6 (IL-6) associated with CRP levels?
    - Function: on same inflammatory pathway

Objective

- Scientific goal: test for genetic effects on disease outcome
  - A set of genetic covariates may be on the same biological pathway
  - Genes within a pathway may be correlated, and may interact in functional ways
  - Outcome/genes between visits are correlated

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1. Introduction and Motivation
2. **Kernel Machine Based Approach**
3. Simulation and Data Results

Longitudinal Modeling Framework

For each subject $i = 1, \ldots, n$ at time $j = 1, \ldots, J$,

- Y_{ij} is a continuous response
- $\mathbf{Z}_{ij} = (Z_{ij1}, \ldots, Z_{ijM})^T$ are M genetic covariates
- $\mathbf{X}_{ij}$ is a set of clinical covariates

We assume the following model:

$$Y_{ij} = \mathbf{X}_{ij}^T \beta + h(\mathbf{Z}_{ij}) + \epsilon_{ij}$$

where,

- β is a unknown parameter vector
- $h(\cdot)$ is an unknown function
- $\epsilon_i = (\epsilon_{i1}, \ldots, \epsilon_{iJ}) = Normal\{0, R(\tau)\}$

Framework

Test for $H_0 : h(\mathbf{z}) = h(z_1, \ldots, z_M) = 0$

- This formulation covers a broad range of models
  - Main effects only model:

$$h(z_1, \ldots, z_M) = z_1\eta_1 + \ldots + z_M\eta_M$$

$$H_0 : \eta_1 = \ldots = \eta_M = 0$$

  - First-order interaction model:

$$h(z_1, \ldots, z_M) = \sum_{j=1}^{M} z_j\eta_j + \sum_{j<k} z_j z_k \gamma_{jk}$$

$$H_0 : \eta_1 = \ldots = \eta_M = \gamma_{12} = \ldots = \gamma_{M-1,M} = 0$$

  - Nonparametric formulation of $h(\cdot)$ is also allowed

Parametric Problem

- Assume that $h(\cdot)$ has a parametric form, e.g., the first order interaction model
- Usual test for H_0 is the F-test with $M(M+1)/2$ degrees of freedom

Drawbacks of the usual F-test

- Uses large degrees of freedom resulting in power loss
- The parametric assumption on $h(\cdot)$ might be too strong and not flexible
- Power loss due to possible correlation among the genes or correlation among the visits

Kernel Machine Formulation

- Assume the function $h : R^M \to R$ resides in a function space $\mathcal{H}_K$ with a positive semidefinite reproducing kernel $K : R^M \times R^M \to R$

- A kernel function $K(\mathbf{z}, \mathbf{z}')$ has two arguments
  - $\mathbf{z}$: covariate vector for subject 1
  - $\mathbf{z}'$: covariate vector for subject 2

- $K(\mathbf{z}, \mathbf{z}')$ measures the 'similarity' (or 'dissimilarity') between these covariate vectors

- By Riesz representation theorem

$$h(\mathbf{z}) = \langle h, K(\mathbf{z}, \cdot) \rangle_{\mathcal{H}_K}$$

Kernel Machine Formulation

- Two ways to characterize h
  - Using basis functions (primal form): corresponds to regular regression

$$h(\mathbf{z}) = \sum_{\ell=1}^{L} \phi_\ell(\mathbf{z})\eta_\ell$$

  - Using a positive definite kernel function $K(\cdot,\cdot)$ (dual form):

$$h(\mathbf{z}) = \sum_{i,j} K(\mathbf{Z}_{ij}, \mathbf{z})\alpha_{ij}$$

Mercer's theorem (Cristianini and Shawe-Taylor, 2000)

The kernel function $K(\cdot,\cdot)$ implicitly specifies a unique function space spanned by a particular set of orthogonal basis functions.

Kernel Machine Formulation

Examples

- Linear kernel: $K(\mathbf{z}, \mathbf{z}') = 1 + z_1 z_1' + \ldots + z_M z_M'$
  - Basis representation: $\phi(\mathbf{z}) = [z_1, \ldots, z_M]$
- Quadratic kernel: $K(\mathbf{z}, \mathbf{z}') = (1 + z_1 z_1' + \ldots + z_M z_M')^2$
  - Basis representation: $\phi(\mathbf{z}) = [z_1, \ldots, z_M, z_1^2, \ldots, z_M^2, z_1 z_2, \ldots, z_{M-1} z_M]$
- Gaussian kernel: $K(\mathbf{z}, \mathbf{z}') = \exp\{-\sum_{j=1}^{M} (z_j - z_j')^2 / \delta\}$
  - Basis representation: space spanned by radial basis

Methodology

- Model: $Y_{ij} = \mathbf{X}_{ij}^T \beta + h(\mathbf{Z}_{ij}) + \epsilon_{ij}$
- Penalized log-likelihood:

$$-\sum_{i,j,k} \{Y_{ij} - \mathbf{X}_{ij}^T \beta - h(\mathbf{Z}_{ij})\} R^{jk}(\tau) \{Y_{ik} - \mathbf{X}_{ik}^T \beta - h(\mathbf{Z}_{ik})\} - \lambda^{-1} ||h||^2$$

- Kernel representation: $h(\mathbf{z}) = \sum_{i=1}^n \sum_{j=1}^J K(\mathbf{Z}_{ij}, \mathbf{z}) \alpha_{ij} = \mathbf{K}\alpha$

Kernel log-likelihood

$$-\{\mathbf{Y} - \mathbf{X}^T \beta - \mathbf{K}^T \alpha\} R^{-1}(\tau) \{\mathbf{Y} - \mathbf{X}^T \beta - \mathbf{K}^T \alpha\} - \lambda^{-1} \alpha^T \mathbf{K} \alpha$$

Normal Equations

$$\begin{bmatrix} \mathbf{X}^{\mathrm{T}}\widetilde{R}^{-1}\mathbf{X} & \mathbf{X}^{\mathrm{T}}\widetilde{R}^{-1}\mathbf{K} \\ \widetilde{R}^{-1}\mathbf{X} & \lambda\mathbf{K}^{-1}+\widetilde{R}^{-1} \end{bmatrix} \begin{pmatrix} \beta \\ \mathbf{h} \end{pmatrix} = \begin{bmatrix} \mathbf{X}^{\mathrm{T}}\widetilde{R}^{-1}\mathbf{Y} \\ \widetilde{R}^{-1}\mathbf{Y} \end{bmatrix},$$

where $\widetilde{R} = I_n \otimes R$

Mixed Model Formulation

- Penalized formulation is equivalent to mixed model

$$Y_{ij} = \mathbf{X}_{ij}^T \beta + h_{ij} + \epsilon_{ij},$$

where

$$\begin{aligned} \mathbf{h} &= (h_{11}, \ldots, h_{nJ})^T = Normal(0, \lambda K) \\ \epsilon &= (\epsilon_{11}, \ldots, \epsilon_{nJ})^T = Normal(0, I_n \otimes R(\tau)) \end{aligned}$$

- $\widehat{\beta}$: Best Linear Unbiased Estimator (BLUE)
- $\widehat{\mathbf{h}}$: Best Linear Unbiased Predictor (BLUP)

Testing

Hypothesis

$$H_0 : h(\cdot) = 0 \iff H_0 : \lambda = 0$$

- Restricted log-likelihood

$$\begin{aligned} L_{\mathrm{REML}} &= -\log|\mathbf{V}|/2 - \log|\mathbf{X}\mathbf{V}^{-1}\mathbf{X}^T|/2 \\ &\quad -(\mathbf{Y} - \mathbf{X}^T\beta)^T\mathbf{V}^{-1}(\mathbf{Y} - \mathbf{X}^T\beta)/2, \end{aligned}$$

  where $\mathbf{V} = I_n \otimes R(\tau) + \lambda\mathbf{K}$.
- Score test
  - Score statistic is a quadratic form
  - Null distribution can be computed

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Simulation

- Number of simulations: 2,000
- $n = 100$ subjects, $J = 3$ time points per subject
- Health outcome Y_{ij} depends linearly on X_{ij} and $\mathbf{Z}_{ij}$,

$$Y_{ij} = \mathbf{X}_{ij}^T \beta + \mathbf{Z}_{ij}^T \gamma + \epsilon_{ij}$$

- 3 clinical covariates $\mathbf{X}_{ij}$ simulated with some correlation within subject
- 10 genetic covariates $\mathbf{Z}_{ij}$ were continuous and simulated to induce correlation within subject-visit and within a given gene across visits
- $\beta = (0.7, 0.7, 0.7)$ and $\gamma = (c, \ldots, c)$
- ϵ_{ij} simulated with compound symmetry structure, where several values of correlation (ρ) were considered: $0, 0.03, 0.06$

Simulation

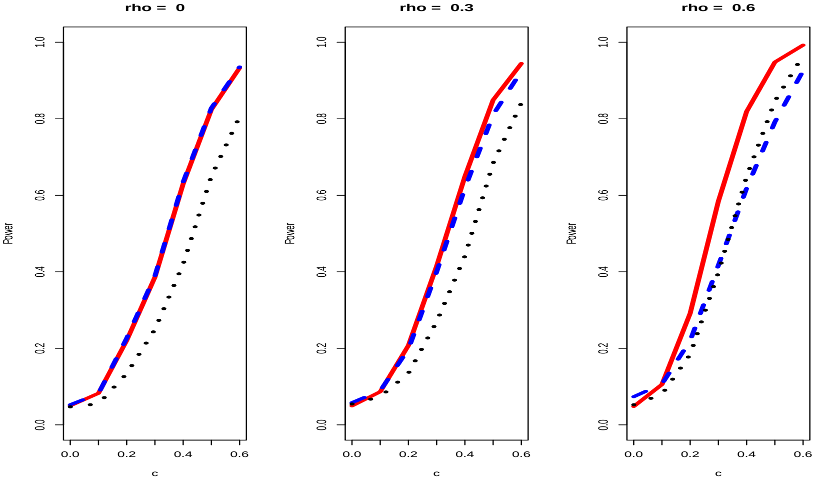
For each set of simulations,

- Size: $c = 0$ (no dependence on Z)
- Power: $c = 0.1, 0.2, \ldots, 0.6$

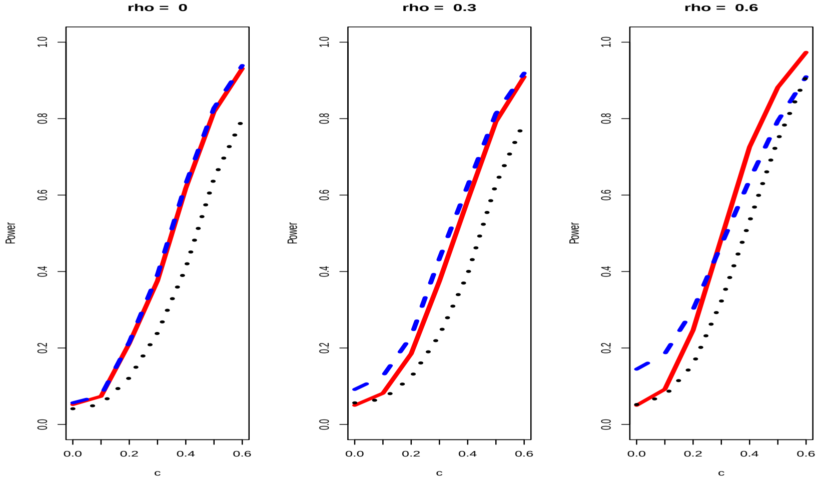
We considered 3 simulation cases with different correlations in the genes:

1. $Corr(Z_{ijm}, Z_{ij'm}) = 0.17$, $Corr(Z_{ijm}, Z_{ijm'}) = 0.17$
2. $Corr(Z_{ijm}, Z_{ij'm}) = 0.4$, $Corr(Z_{ijm}, Z_{ijm'}) = 0.4$
3. $Corr(Z_{ijm}, Z_{ij'm}) = 0.75$, $Corr(Z_{ijm}, Z_{ijm'}) = 0.33$

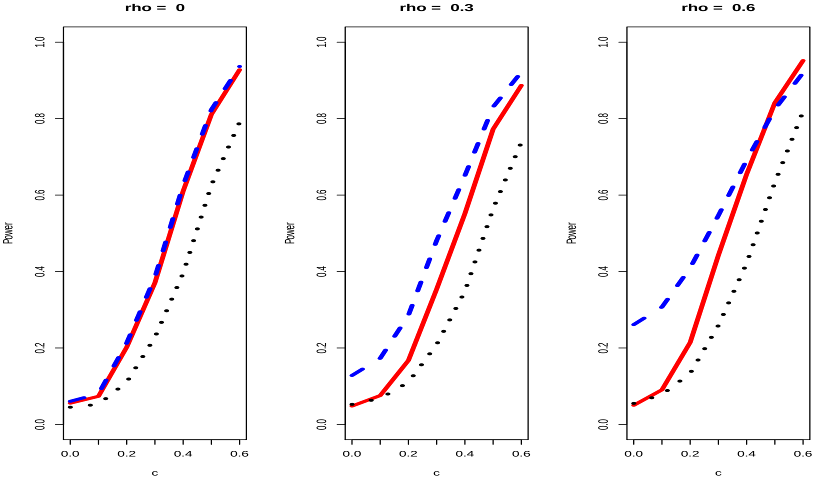
Results for Case 1: Low correlation



Results for Case 2: Moderate correlation



Results for Case 3: High correlation



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Gene-set of interest

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- Intercellular adhesion molecule 1 (ICAM-1) and Interleukin 6 (IL-6)

NAS Data Analysis

Toll-like receptor 2 (TLR2) and Interferon gamma (IFN-γ)

- Function: TLR2 regulates IFN-γ
- TLR2 measured at 5 positions
- IFN-γ measured at 2 positions

Results

- F test: 7 df, p-value= 0.005
- Independent KM test: 1.972 df, p-value= 0.00000932
- Longitudinal KM test: 2.047 df, p-value= 0.00001937 (corr = 0.17 in residual errors)

NAS Data Analysis

Intercellular adhesion molecule 1 (ICAM-1) and Interleukin 6 (IL-6)

- Function: on same inflammatory pathway
- ICAM-1 measured at 3 positions
- IL-6 measured at 2 position

Results

- F test: 5 df, p-value= 0.1411
- Independent KM test: 1.29 df, p-value= 0.0849
- Longitudinal KM test: 1.35 df, p-value= 0.0610 (corr = 0.23 in residual errors)

Summary

- In simulations, we see better performance when accounting for correlation in both the genetic data and in the residual errors
- Preliminary analysis of data suggests methylation of pro-inflammatory genes may be associated with CRP levels.

Next steps:

- Testing for individual genes
- Different correlation structures
- More in-depth analysis of data