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EVALUATION OF METHODS FOR ANALYZING GENE-GENE INTERACTION
DATA FOR SURVIVAL OUTCOMES

By

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B.E., China Pharmaceutical University, 2007
M.S. program, China Pharmaceutical University, 2009

A Thesis
Submitted to the Faculty of the
Graduate School of the University of Louisville
in Partial Fulfillment of the Requirements
for the Degree of

Master of Science

Department of Bioinformatics and Biostatistics
School of Public Health and Information Sciences
University of Louisville
Louisville, Kentucky

August 2011

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A Thesis Approved on

May 12th, 2011

by the following Thesis Committee:

Dr. Guy Brock (Thesis Director)

Dr. Somnath Datta

Dr. LaCreis Kidd

DEDICATION

This thesis is dedicated to my parents

Mr. Chuansheng Zhang

and

Mrs. Xueping Wang

who have given me invaluable educational opportunities.

ACKNOWLEDGMENTS

I would like to thank all the people who made this work possible. First, I would like to thank my advisor, Dr. Guy Brock, for his moral and financial support during my course work at University of Louisville. I thank Dr. Brock for being very patient and supportive in guiding me throughout this project. I would also like to thank Dr. LaCreis Kidd for giving me an opportunity to work on the related project for 9 months. I appreciate the guidance and financial support I received from her. Finally, I would like to convey my sincere thanks to Dr. Somnath Datta, for his presence and comments.

ABSTRACT

EVALUATION OF METHODS FOR ANALYZING GENE-GENE INTERACTION DATA FOR SURVIVAL OUTCOMES

Jie Zhang

May 12, 2011

In recent years, a number of computational and statistical problems for identifying SNP-SNP interactions in high dimensional survival data have been studied, and several data mining approaches have been proposed. However, the relative performance of these methods to detect SNP-SNP interactions has not been thoroughly investigated.

In this study, we directly compared the performance of the four techniques to detect gene-gene interactions in a recently conducted study of genetic polymorphisms associated with breast cancer survival and recurrence. Four methods were evaluated for their ability to detect SNP-SNP interactions: Survival Multifactor Dimensionality Reduction, Cox regression with L_1 (Lasso) and L_1 - L_2 (Elastic Net) penalties, and Random Survival Forest (RSF). Methods were contrasted on the basis of which SNPs they selected.

The results of this study demonstrate how the methods perform in detecting gene-gene interactions for survival data, and are useful in informing researchers about choosing an analysis tool for their own real data applications.

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CHAPTER I

INTRODUCTION

Technological advances are rapidly improving geneticist's ability to detect genetic factors, which influence individual susceptibility to common human diseases. Research has shown that genes don't function alone; rather, they constantly interact with one another. Given the complexity and robustness of biological networks such diseases are unlikely to be the result of single genetic variants, but instead arise from the joint interaction of two or more variants acting together.

When we think about the whole biological networks, it is important to consider epistasis, which refers to the situation where interacting genes, as opposed to a single gene, influence a trait. The methods to detect and characterize epistasis are critical to understanding complex diseases, such as Alzheimer's disease, diabetes, cardiovascular disease, and cancer.

One traditional approach to modeling the relationship between discrete predictors such as genotypes and discrete clinical outcomes is logistic regression modeling (Lecessie and Vanhouwelingen, 1992). Logistic regression is a parametric statistical approach for relating one or more independent or explanatory variables (e.g. disease status) that follows a binomial distribution. However, detecting and characterizing epistatic interactions in datasets containing large numbers of SNPs is challenging. The

number of possible interaction terms grows exponentially as each additional main effect is included in the logistic regression model. Thus, logistic regression is limited in its ability to deal with interaction data.

In response to this limitation, the multifactor dimensionality reduction (MDR) (Ritchie et al., 2001) has been developed as a method for reducing the dimensionality of multi-locus information, to improve the identification of polymorphism combinations associated with disease risk. This approach is nonparametric, is free of a specified genetic model, and is directly applicable to the analysis of case-control and discordant sib-pair study designs. Both empirical and theoretical studies suggest that MDR has excellent power for identifying high-order gene-gene interactions and is a promising new approach for overcoming some of the limitations of logistic regression.

Another machine learning method that has attracted considerable interest is Random Forests (RF) (Breiman, 2001). It is a technique that builds a forest of classification trees. Each single tree is grown from a bootstrap sample of the data, and the final outcome predictions are determined by all trees in the forest. There are several advantages of the RF method. It can handle a large number of input variables. It is fairly robust in dealing with genetic data in the presence of high amounts of missing data.

Recently, many researchers have been interested in identifying gene-gene interactions in high dimensional survival data. Thus, the above methods have been extended to survival analysis. Survival analysis involves the modeling of time to event data; in this context, death or failure is considered an “event”. Survival analysis attempts to answer questions such as “What is the fraction of a population that will survive past a certain time?”. To model survival data, there are a few basic tools such as the Kaplan-

Meier estimate of the survival function (Kaplan and Meier, 1958), the log-rank test for differences between survival functions (Breslow, 1975), and the Cox proportional hazards model for modeling the effect of multiple covariates on the hazard rate (Cox, 1972). Although numerous computational and statistical methods for identifying SNP-SNP interactions in case-control studies have been studied extensively, the analysis of SNP-SNP interactions associated with survival data is relatively uninvestigated in the literature.

In the case that survival outcomes associated with genomic data, we have two statistical objectives. The first is to identify which of the features (e.g. genes or SNPs) in the genomic data is individually most associated with the survival outcome, and the second is build a model which can accurately predict survival times. Several methods have recently been proposed in the literatures that address the two objectives mentioned above. One approach is to use penalized Cox regression (Benner et al., 2010) in such situations. This method determines a subset of covariates that are the most important ones for predicting the survival outcome by shrinking unimportant regression coefficients to zero using penalties on the magnitude of the regression coefficients. Another parametric approach is the accelerated failure time model, which has been applied in conjunction with partial least squares and the least absolute shrinkage and selection operator (Lasso) to predict patient survival times from high-dimensional microarray data (Datta et al., 2007). Other, nonparametric approaches include the Survival Multifactor Dimensionality Reduction algorithm (Survival-MDR) (Gui et al., 2011), and Random Survival Forests (RSF) (Ishwaran et al., 2008). Survival-MDR uses a multifactor dimensionality reduction algorithm that reduces the N -dimensional space for N -way interactions between SNPs to

one dimension with two levels (“low risk” and “high risk”). Random Survival Forest is a technique that builds a forest of classification trees, wherein each single tree is grown from a bootstrap sample of the data, and predictions of patients with unknown survival times are determined by aggregation of all the trees in the forest.

In the current thesis, we evaluate the use of penalized Cox regression models, Survival-MDR, and RSF for detecting gene-gene interactions associated with breast cancer (BrCa) survival and recurrence. Each method is used to identify important single nucleotide polymorphisms (SNPs) which contribute to one-, two-, and three-way interaction models for BrCa survival and recurrence. The rest of this thesis is organized as follows. In chapter 2, we describe some background methodology for survival analysis including the Kaplan-Meier estimator, the log-rank test, and the Cox proportional hazards model. Chapter 3 describes two penalized Cox regression models, Survival-MDR, and RSF, and also describes the breast cancer dataset used in this research and the data management technique. Chapter 4 summarizes the findings from using the above four methods. Chapter 5 gives some concluding remarks and discusses future research.

CHAPTER II

BACKGROUND

2.1 Kaplan-Meier Estimator

The Kaplan-Meier estimator is a nonparametric estimator of the survival function for right-censored data. It is also known as product-limit estimator. Let $S(t)$ be the probability that an item from a given population will have a lifetime exceeding t . The Kaplan-Meier estimator is the nonparametric maximum likelihood estimate of $S(t)$. It is a product of the form

$$\hat{S}(t) = \prod_{t_i \leq t} \left(\frac{n_i - d_i}{n_i} \right),$$

where t is the i^{th} distinct observed failure time, $i=1,2,\dots,D$, D is the number of distinct observed failure times, d_i is the number of individuals who experience the event of interest at time t_i , and n_i is the number of individuals at risk just before time t_i .

2.2 Log-rank test

The log-rank test statistic is widely used to compare two independent survival functions in the presence of censored observations. The test statistic is based on a normalized sum of the differences between the observed failures and the expected

failures in either of the treatment groups, and has an asymptotic chi-square distribution with one degree of freedom.

Let $j = 1, \dots, J$ be the set of distinct times of observed events in either group. Let N_{1j} and N_{2j} be the number of subjects which have not yet had an event or been censored at time j for groups 1 and 2, respectively, and define $N_j = N_{1j} + N_{2j}$. For each time j , let O_{1j} and O_{2j} be the observed number of events and $O_j = O_{1j} + O_{2j}$. Under the null hypothesis of equal survival functions for subjects in groups 1 and 2, the expected number of failures at time j is expressed as

$$E_{1j} = O_j \frac{N_{1j}}{N_j}.$$

The log rank statistic Z^2 compares each O_{1j} to its expectation E_{1j} under the null hypothesis, and is defined as

$$Z^2 = \frac{\left(\sum_{j=1}^J (O_{1j} - E_{1j}) \right)^2}{\sum_{j=1}^J \left(\frac{O_j (N_{1j} / N_j) (1 - N_{1j} / N_j) (N_j - O_j)}{N_j - 1} \right)}.$$

The statistic Z^2 has an asymptotic chi-squared distribution with one degree of freedom, under the assumption of the null hypothesis.

2.3 Cox proportional hazard model

The Cox proportional hazards model is commonly used to model survival data. It is non-parametric in that the baseline hazard function can take an arbitrary form. The model is as follows:

$$\lambda(t | x_i) = \lambda_0(t) \exp\left(\sum_{j=1}^p x_{ij} \beta_j\right),$$

where x are covariates, $\lambda(t | x_i)$ is the hazard at time t for the i th observation, $\lambda_0(t)$ is the unspecified baseline hazard function, and β is a vector of regression coefficients. The partial likelihood is given by

$$L(\beta) = \prod_{i: C_i=1} \frac{\exp(x_i \beta)}{\sum_{j: Y_j \geq Y_i} \exp(x_j \beta)},$$

where Y_i denotes the observed time (either censoring time or event time) for subject i , and C_i is the indicator that the time corresponds to an event ($C_i = 1$ if the event occurred and $C_i = 0$ if the time is a censoring time). The log partial likelihood is given by

$$l(\beta) = \sum_{i: C_i=1} (\beta x_i - \log \sum_{j: Y_j \geq Y_i} \exp(x_j \beta)).$$

Parameter estimates $\hat{\beta}$ in the Cox proportional hazard model are obtained by maximizing the partial likelihood.

CHAPTER III

METHODOLOGY

3.1 Penalized likelihood methods

Penalized likelihood methods (Benner et al., 2010) can be used for determining a subset of covariates that are the most important for predicting survival times, when we fit models for survival data on the basis of the Cox proportional hazards model. The penalized log partial likelihood is given by

$$l(\beta) - \sum_{j=1}^p p_{\lambda}(|\beta_j|)$$

where $l(\beta)$ denotes the log partial likelihood, p is the dimension of the parameter β , j is the index with parameter estimates unequal to zero, and $p_{\lambda}(|\beta_j|)$ is the penalty function with a tuning parameter λ . The penalty term on the coefficients will shrink them towards zero, thus reducing the dimensionality of the problem and increasing the stability of the solution.

We describe k -way ($k=1, 2, 3$) interaction models that capture the relationship between k genes and the survival outcome. For the one way model, the penalized Cox regression model will include just main effects. For the two-way model, both main and two-way interaction effects are considered. For the three-way model, main effects, two-, and three-way interaction effects are all used to fit the model.

In order to determine the most important SNPs for the penalized Cox regression models, we rank the coefficients by their absolute value. The larger the absolute value, the more important the SNP.

3.1.1 L₁-penalized cox regression

Based on the penalized likelihood methods, Tibshirani (Tibshirani, 1997) proposed to use the L₁-penalized Cox regression model and described a technique, called the Lasso for “Least absolute shrinkage and selection operator”, for parameter estimation. The L₁-penalized Cox model has the following penalty,

$$p_{\lambda}(|\beta_j|) = \lambda |\beta_j|, \quad j=1, \dots, p.$$

By penalizing the coefficients associated with each of the covariates during model fitting, unimportant coefficient will be shrunk towards zero. Thus, the Lasso can be considered as a version of automated variable selection, particularly useful for high-dimensional data.

3.1.2 L₂-penalized cox regression

L₂-penalized Cox regression with the ridge penalty

$$p_{\lambda}(|\beta_j|) = \lambda \beta_j^2, \quad j=1, \dots, p,$$

was introduced by Verweij and Van Houwelingen (Verweij and Van Houwelingen, 1994). The ridge regression shrinks the regression coefficients in a manner similar to lasso regression, but penalizes the square values instead of the absolute values. Regression coefficients are not shrunk completely to zero, and thus ridge regression is not a variable selection algorithm. Rather, the procedure is particularly useful for stabilizing the beta coefficients when the set of regression variables are highly correlated.

3.1.3 Elastic Net

Elastic net, which employs a combination of the L_1 - and L_2 -penalties, was introduced by Zou and Hastie (Zou and Hastie, 2005)

$$p_{\lambda_1, \lambda_2}(|\beta_j|) = \lambda_1 |\beta_j| + \lambda_2 \beta_j^2, \quad j=1, \dots, p.$$

The Elastic Net performs variable selection similarly to the Lasso. But the additional L_2 -penalty distributes the weight to more variables, such that the Elastic Net tends to select more variables than the Lasso.

3.1.4 Cross-validation and optimization

The penalized regression models use cross-validation of the log partial likelihood to assess its predictive ability and determine the optimal values of the tuning parameters λ_1 and λ_2 . The tuning parameters are chosen by maximizing the k -fold cross-validated log partial likelihood. In our study, we used 10-fold cross-validation. The tuning parameter λ_1 was chosen by maximizing 10 fold cross-validated log likelihood, while the tuning parameter λ_2 for Elastic Net was chosen from the set $\{0.0001, 0.001, 0.01, 0.1, 1, 10\}$.

3.2 Survival Multifactor Dimensionality Reduction (Survival MDR)

Survival MDR is a nonparametric and genetic model-free approach that reduces the dimensionality of the data when analyzing gene-gene interactions. It is an extension of the MDR approach developed for case-control data, and is specifically designed to characterize gene-gene interactions in the presence of right-censored data. The goal of Survival MDR is to change the representation of the data using a constructive induction

algorithm, to make interactions easier to detect. The procedure will be used for both feature selection and model validation as described next.

3.2.1 Feature selection

The Survival MDR procedure for feature selection is illustrated in Figure 1. For determining an N -way interaction amongst a pool of SNPs, the algorithm proceeds through the following three steps for all possible N -way combinations of SNPs.

Fig. 1 Survival MDR attribution construction

First, N SNPs are selected from the pool of all SNPs. In the second step, the log-rank test statistics comparing the survival time distributions with and without each of the multilocus genotype combinations in the N -way interaction table are calculated. In the third step, each multifactor cell in the N -dimensional space is classified as either “high risk,” if the log-rank test statistic is positive, or as “low risk,” if the log-rank statistic is negative. Once all the genotype are labeled as either “high risk” or “low risk”, the dimensionality of multi-locus genotype information is reduced to one dimension with two levels (“low risk” and “high risk”).

3.2.2 Model validation

Survival MDR uses the log rank test to compare the survival time distributions between high and low risk groups and uses Z^2 as the score to choose the best model amongst all possible N -way combinations of SNPs. A ten-fold cross-validation procedure is used to determine the best overall model, involving three steps.

In the first step, the whole dataset is randomly divided into ten equal parts. Nine-tenths of the data are used as the training set, and the remaining one-tenth of the data are set aside as an independent testing set. Then Z^2 statistic is computed for each N -way interaction model in the training set.

In the second step, an attribute is created for the independent testing set using the N SNPs that have the best Z^2 score. To reduce the possibility of poor estimates of the predictive ability due to chance divisions of the data set, the entire procedure is performed 10 times so that each sample is included in the testing set at least once.

Fig 2. Ten-fold cross-validation.

In the third step, for each 10-fold cross-validation, the number of times the same best set of loci/factors was identified across the 10 data subsets is recorded. The cross-validation consistency (CVC) is a measure of the number of times a particular set of loci and/or factors is identified across the cross-validation subsets. There are 10 possible values of the CVC ranging from 1 to 10, where 10 indicate strong evidence in favor of a multifactor association. The best Survival MDR model was selected as that with the maximum testing accuracy averaged over the 10-fold cross validation, and also has the maximum cross-validation consistency (CVC) as described previously. For all of the selected models, the CVC ranged from 8 to 10 are considered as a highly predictive model.

3.3 Random Survival Forests

Random Survival Forests(RSF) are an ensemble tree method for the analysis of right censored survival data. Standard analyses often rely on restrictive assumptions such as proportional hazards. This property is especially helpful in survival analysis, and makes RSFs highly data adaptive. The procedure only requires three parameters to be set (the number of randomly selected predictors, the number of trees grown, and the splitting rule used), thus making it quite user-friendly.

3.3.1 The Algorithm

Each tree in the forest is constructed as follows:

In the first step, draw B bootstrap samples, with replacement, from the entire data set. B is the total number of trees in the forest. Each bootstrap sample excludes on average 37% of the data, called the out-of bag data (OOB data).

In the second step, a survival tree grows for each bootstrap sample. At each node in the tree, randomly select m candidate variables for splitting on. The absolute magnitude of m is a function of the number of variables in the data set and remains constant throughout the forest building process.

In the third step, split on a variable using one of several survival splitting rules. A node is split on that variable which maximizes the survival differences between daughter nodes.

Computation proceeds by iterating the second and third steps until the tree is fully grown (no pruning).

3.3.2 Splitting rules

Four different splitting rules are available for use with RSF(Ishwaran et al., 2008).The log-rank splitting rule, the default splitting rule, splits nodes by maximizing the log-rank test statistic. In this study, we the analysis under this splitting rule since is a well-established concept, having been shown to be robust in both proportional and non-proportional hazards settings. Variants on this procedure include the log-rank score rule, which splits nodes on the basis of a standardized log-rank statistic, and the random log-rank splitting rule, which is a fast approximation to the log-rank splitting rule. As an alternative to the log-rank splitting rules, node splitting can be based instead on the

conservation-of-events principle. This node splitting rule may help to reduce the potential bias in the other splitting rules to favor continuous predictors and uneven splits.

3.3.3 Variable importance (VIMP) selection

Variables can be selected by filtering on the basis of their variable importance (VIMP). For each bootstrap sample, 37% of the data are excluded on average. Thus, for a given tree, certain individuals will have been left out of the training data. Prediction error and variable importance is estimated from these ‘out-of-bag’ (OOB) data.

The OOB data is used to estimate the importance of particular variables by randomly permuting the values of that variable and testing whether these permutations adversely affect the predictive ability of trees in classifying the OOB data. If randomly permuting values of a particular variable does not affect the predictive ability of trees on OOB samples, that variable is assigned a low importance score. Conversely, if randomly permuting the values of a particular variable drastically impairs the ability of trees to correctly predict the survival time of the OOB samples, then the importance score of that variable will be high.

3.3.4 Minimal depth selection

Minimal depth variable selection method differs from the traditional method of variable selection in RSF, which has been based on variable importance measures (Ishwaran et al., 2011). Minimal depth assesses the predictive ability of a variable by its depth relative to the root node of a tree. To determine the minimal depth, first define the maximal subtree for a variable v as the largest subtree whose root node is split using v (the

first-order maximal subtree). The shortest distance from the root of the tree to the root of the closest maximal subtree of v is the minimal depth of v . The minimal depth can be considered as the minimal number of nodes crossed from the root node, until a split involving v is encountered. A smaller value corresponds to a more predictive variable.

In high-dimensional settings, the distribution of the minimal depth can be used as a means to screen unimportant variables. The mean of the minimal depth distribution represents a threshold value for selecting variables, such that those variables with forest averaged minimal depth exceeding the mean minimal depth threshold are classified as noisy and removed from the final model.

The concept of maximal subtrees can be extended to second-order subtrees, to provide a powerful tool for identifying relationships between variables. A second-order maximal (w, v) -subtree is a maximal w -subtree within a maximal v -subtree for a variable v . In this way, we can quantify the association between two variables. Two-way interactions can be estimated by using forest averaged second-order maximal subtrees.

3.4 Breast Cancer Data

The genetic data used in this thesis come from 441 specimens collected between 1989 and 1998 from Caucasian women selected from the Hormone Receptor Laboratory (HRL) Biorepository and Tumor Marker Database (TMD) (Kidd et al., 2010). Human tissue specimens were collected from 235 node-negative and 206 node-positive patients.

The HRL Biorepository and the TMD contain de-identified specimens of breast carcinoma with associated tumor clinical outcome with up to 15 years of follow-up. Clinico-pathological data, such as tumor-based properties (e.g., pathology, grade, stage,

size, tumor marker status), patient-related characteristics (e.g., age, race, menopausal status, family history, nodal status), and clinical follow-up (e.g., treatment regimen, DFS, OS) was available. Biochemical data, such as estrogen/progesterone receptor, epidermal growth factor receptor, and human epidermal growth factor receptor status were obtained from TMD.

DNA was isolated from this tissue using the AllPrep DNA/RNA/Protein Mini Kit (Qiagen, Valencia, CA) or QIAamp DNA Mini Kit (Qiagen). Thirteen angiogenesis-related SNPs with minor allele frequency >0.05 were selected for our genetic analysis. In Table 1, we list the gene names, RefSNP (rs) numbers, and nucleotide change information for these thirteen SNPs.

TABLE 1. Selected SNPs in the angiogenesis pathway and their nucleotide change

Gene	rs Number	Nucleotide change
IL10_1082	rs1800896	G>A
IL10_819	rs1800871	C>T
IL10_592	rs1800872	C>A
IL-10R_109	rs9610	G>A
TGFb1_896	rs1982073	T>C
TGFbR1_195	rs868	A>G
VEGF_2578	rs699947	C>A
VEGF_1154	rs1570360	A>G
VEGF_634	Rs2010963	G>C
VEGFR_889	rs2305948	G>A
VEGFR_1416	rs1870377	T>A
VEGFR.IVS25_92	rs1531289	G>A
VEGFR.IVS6_54	rs7692791	A>G

3.5 Missing values

Our approach for treating missing data in Lasso, Elastic Net, and Survival MDR methods was to plug in the most frequent genotype for each SNP for the subjects with

missing values. RSF uses an adaptive tree imputation algorithm to adaptively impute missing values as the tree is grown. Missing data are imputed by using a set of randomly drawn observations from the set of non-missing in-bag data within the current node (Ishwaran et al., 2008).

CHAPTER IV

RESULTS

4.1 Penalized Cox regression models

In order to fit penalized Cox regression models, we treat the 13 SNPs as categorical factors. For each of the 13 SNPs there are three different genotypes each with associated indicator variables, resulting in a total of 39 potential main effect terms. We describe k -way ($k=1,2,3$) interaction models that capture the relationship between k genes and the survival outcome. One-way interaction models include only main effect terms, while two-way interaction models include both main effects and all two-way interaction terms, and three-way interaction models include all main effect, two-way interaction, and three-way interaction terms. These full models are used as starting points for fitting the penalized regression models, which will eliminate some proportion of the terms depending on the magnitude of the shrinkage coefficient(s).

4.1.1 L_1 -penalized Cox regression

Table 2 summarizes the optimal tuning parameter λ_1 and corresponding cross-validated log likelihood value based on the Lasso method, for the main effect, two-way,

and three-way interaction models. As these results show, for each model there's an optimal tuning parameter λ_1 and a cross-validated log likelihood value.

TABLE 2. Optimal tuning parameter for Lasso method

	λ_1	CV_{LL}
Main effect	14.741	-813.657
Two-way	10.802	-812.737
Three-way	10.510	-812.471

CV_{LL} , cross-validated log likelihood

In Table 3, we summarize the selected one-, two-, and three-way models based on the Lasso method by listing the corresponding coefficients. To determine the most important SNPs for each model, we rank the coefficients by their absolute magnitude, with the largest magnitude coefficients ranked the highest. For the main effect model, no SNPs were included in the model. An interaction between IL10R_109_G/A and TGFBR1_195_A/A is selected as the most important coefficient from the two-way interaction model, and an interaction among VEGF_2578C/C, VEGFR_889G/G and VEGFR_1416T/T is the strongest coefficient from the three-way interaction model.

TABLE 3. Selected models based on Lasso method

SNP	MainEffect	Two-way	Three-way
VEGF_2578C/C	--	0.026	--
IL10R_109G/A:TGFBR1_195A/A	--	-0.235	-0.247
VEGF_2578C/C:VEGFR_1416T/T	--	0.048	--
VEGFR.IVS25_92G/G:VEGFR.IVS6_54A/G	--	-0.140	-0.149
IL10R_109G/G:VEGF_1154G/G:VEGFR_1416T/T	--	--	0.024
VEGF_2578C/C:VEGFR_889G/G:VEGFR_1416T/T	--	0.026	--

Results are presented as coefficients.

In Figure 3, we plot the survival curves for the high-risk versus low-risk genotypes based on the Lasso two-way and three-way models. For this method, we divide genotypes into high or low risk groups based on linear predictors. For each linear predictor, if its value exceeds the median of all linear predictors, then it is considered high-risk. Otherwise, it is considered low-risk.

Fig.3 Kaplan-Meier survival curve for high and low risk groups based on Lasso two-way and three-way models

4.1.2 Elastic Net

Table 4 summarizes the optimal tuning parameters λ_1 and λ_2 , and the corresponding cross-validated log likelihood values based on the Elastic Net method for the main effect, two-way, and three-way interaction models.

In Table 5, we summarize the top one, two, and three-way models identified by the Elastic Net. VEGF_2578_C/C is selected as the most important for main effects. A

combination of IL10R_109_G/A and TGFBR1_195_A/A is selected as the most important two-way model. This combination is also selected as the most important three-way model.

TABLE 4. Optimal tuning parameter for Elastic Net method

	λ_1	λ_2	CV_{LL}
Main effect	8.031	0.01	-813.413
Two-way	11.068	0.0001	-813.461
Three-way	13.531	0.0001	-813.561

CV_{LL} , cross-validated log likelihood

TABLE 5. Selected models based on Elastic Net method

SNP	MainEffect	Two-way	Three-way
IL10R_109G/A	-0.063	--	--
IL10R_109G/G	0.027	--	--
VEGF_2578C/C	0.167	0.023	--
VEGFR.IVS25_92G/G	-0.047	--	--
VEGFR.IVS6_54A/G	-0.047	--	--
IL10R_109G/A:TGFBR1_195A/A	--	-0.224	-0.132
VEGF_2578C/C:VEGFR_1416T/T	--	0.035	--
VEGFR.IVS25_92G/G:VEGFR.IVS6_54A/G	--	-0.131	-0.045

Results are presented as coefficients.

In Figure 4, we plot the survival curves for the high-risk versus low-risk genotypes defined by Elastic Net method. We divide genotypes into high or low risk groups based on linear predictors.

Fig.4 Kaplan-Meier survival curves for high and low risk groups based on Elastic Net one-, two-, and three-way models.

4.2 Survival MDR

For the main effects, two-way and three-way interaction combinations, the best models are shown in Table 6. The VEGF_2578 has the highest cross-validation consistencies (60%) of the one factor model, and is therefore selected as the final best models. A combination between IL10R_109 and TGFBR1_195 serves as the best two factor predictors, with a cross validation consistency of 50%. Lastly, a combination among IL10R_109, TGFBR1_195 and VEGF_2578 serve as the best three factor model with a cross validation consistency of 40%. One curious result is that a main effect model (VEGFR.IVS25_92) appears amongst the list of models for both the two-way and three-way interaction evaluations, with a CVC of 10% in each case. We are unable to determine why this single factor model appeared in each case.

TABLE 6. Selected models based on Survival MDR method

SNP	MainEffect	Two-way	Three-way
VEGF_2578	60%	--	--
VEGFR.IVS25_92	20%	10%	10%
VEGFR.IVS6_54	20%	--	--
IL10R_109:TGFBR1_195	--	50%	--
IL10R_109:VEGF_1154	--	40%	--
IL10R_109:TGFBR1_195:VEGF_2578	--	--	40%
IL10R_109:TGFBR1_195:VEGF_1154	--	--	20%
IL10R_109:VEGF_1154:VEGFR_1416	--	--	10%
VEGF_2578:VEGFR.IVS25_92:VEGFR.IVS6_54	--	--	10%
VEGF_1154:VEGFR.IVS25_92:VEGFR.IVS6_54	--	--	10%

Results are presented as cross-validated consistency (cvc).

Every genotype is assigned into an appropriate group (high / low risk) by Survival MDR's constructive induction algorithm. Based on this, we constructed Kaplan-Meier plots of the high and low risk groups determined by the best one-, two-, and three-way models (Figure 5).

Fig.5 Kaplan-Meier survival curve for high and low risk groups based on Survival MDR one-, two-, and three-way models.

4.3 Random Survival Forest

Table 7 gives the average VIMP scores and minimal depth thresholds for each SNP based on 50 independent runs of RSF with *mtry* equal to 1000. Both the VIMP and minimal depth selection methods select IL10R_109 as the most important main effect. The second order minimal depth values for each SNP relative to the other SNPs were calculated, and these values were used to define a distance matrix between the SNPs. Figure 6 plots the resulting dendrogram based on hierarchical clustering of the data. SNPs IL10_1082 and IL10R_109 are joined together first based on the hierarchical clustering, and indicated a possible strong interaction between the SNP pair.

TABLE 7. Selected models based on Random Survival Forest method

VIMP			Minimal Depth		
Variable	Average	Rank	Variable	Average	Rank
IL10R_109	0.035	1.00	IL10R_109	0.043	1.00
VEGF_2578	0.014	2.68	VEGF_2578	0.015	3.07
VEGF_634	0.011	3.54	VEGF_634	0.014	3.23
VEGF_1154	0.009	4.42	TGFBR1_195	0.013	3.44
TGFBR1_195	0.009	4.84	TGFB1_896	0.009	4.83
TGFB1_896	0.006	5.96	VEGF_1154	0.005	6.45
IL10_592	0.003	7.46	IL10_592	0.002	7.32
VEGFR.IVS6_54	0.002	7.54	VEGFR_1416	0.002	7.56
VEGFR.IVS25_92	0.000	8.94	VEGFR.IVS6_54	-0.002	9.52
VEGFR_1416	-0.002	9.92	IL10_1082	-0.004	10.69
IL10_1082	-0.004	10.92	IL10_819	-0.005	11.13
VEGFR_889	-0.005	11.46	VEGFR_889	-0.005	11.25
IL10_819	-0.007	12.32	VEGFR.IVS25_92	-0.005	11.51

Fig.6 Variables clustered by second-order minimal depth

4.4 Comparison of Results

As shown in Tables 8, 9, and 10, we list the two most important SNPs from the one, two, and three-way models identified by Lasso, Elastic Net, and Survival MDR. Also given are the most important SNPs based on the VIMP and minimal depth from RSF, and the most significant two-way interaction identified by minimal depth using second-order maximal subtrees. In Figure 8, we put the Kaplan-Meier survival curves based on the Lasso, Elastic Net and Survival MDR methods together.

Table 8. Comparison table of significant variables selected by different methods based on main effect models.

Method	Main Effect
Lasso	-- --
Elastic Net	VEGF_2578C/C IL10R_109G/G
SurvivalMDR	VEGF_2578 / VEGFR.IVS25_92 VEGFR.IVS6_54
VIMP	IL10R_109 VEGF_2578
MinimalDepth	IL10R_109 VEGF_2578

Table 9. Comparison table of significant variables selected by different methods based on two-way models.

Method	Two-way
Lasso	IL10R_109G/A:TGFBR1_195A/A VEGFR.IVS25_92G/G:VEGFR.IVS6_54A/G
Elastic Net	IL10R_109G/A:TGFBR1_195A/A VEGFR.IVS25_92G/G:VEGFR.IVS6_54A/G
SurvivalMDR	IL10R_109: TGFBR1_195 IL10R_109: VEGF_1154
VIMP	-- --
MinimalDepth	IL10_1082:IL10R_109 VEGF_634:VEGFR.IVS6_54

Table 10. Comparison table of significant variables selected by different methods based on three-way models.

Method	Three-way
Lasso	IL10R_109G/A:TGFBR1_195A/A VEGF_2578C/C:VEGFR_889G/G:VEGFR_1416T/T
Elastic Net	IL10R_109G/A:TGFBR1_195A/A VEGFR.IVS25_92G/G:VEGFR.IVS6_54A/G
SurvivalMDR	IL10R_109: TGFBR1_195: VEGF_2578 IL10R_109:TGFBR1_195:VEGF_1154
VIMP	-- --
MinimalDepth	-- --

Fig. 8 Kaplan-Meier survival curve for high and low risk groups based on Lasso, Elastic Net, and Survival MDR methods.

Fig. 9 Kaplan-Meier survival curve for high and low risk groups based on VIMP and minimal depth one-way models

In Figure 9, we plot the Kaplan-Meier survival curves for high and low risk groups based on the VIMP and minimal depth models. In this situation, we separate high or low risk groups according to VIMP score and mean minimal depth threshold. We use median as the threshold. Note that, as opposed to the other methods, there are no additional two- and three-way interaction models for the RSF method. This is because RSF already implicitly includes interactions, in the sense that second-order maximal (w, v) -subtrees identify interactions between two variables w and v .

CHAPTER V

DISCUSSION

In the current study, we directly compared the performance of four variable selection techniques for detecting SNP-SNP interactions in survival genetic association studies: the Lasso and Elastic Net penalized Cox regression models, Survival Multifactor Dimensionality Reduction, and Random Survival Forest. As shown in the previous section, we demonstrate that all these methods can detect the presence of multiplicative interaction models even when the main effects are not statistically significant. All of the plots shown in the previous section indicate the significant SNPs chosen by these four methods separate the two survival curves, although the statistical significance of this separation was not evaluated.

Among the four methods, the Lasso and Elastic Net are semi-parametric methods. The Elastic Net performed very similarly to the Lasso in our study, particularly for the two-, and three-way interaction models. Both methods select the same top two most important terms in each case. But the additional L_2 -penalty for the Elastic Net distributes the weight to more variables, so that the Elastic Net selected more variables overall compared to the Lasso. This is especially useful in the situation of high correlation. When high correlation exists, the Lasso will only pick one of the correlated variables, whereas the Elastic Net will select the entire set of correlated variables. Thus, it is not surprising

to see that the Lasso selected no main effect terms, whereas five SNPs were selected using the Elastic Net. A limitation of these two methods is that they constructed and selected variables corresponding to individual genotypes, rather than selecting the set of genotypes corresponding to a particular SNP. This shortcoming can be overcome by extending the group Lasso to survival time data (Meier et al., 2008; Winham et al., 2011).

In contrast, Survival MDR and Random Survival Forest are non-parametric methods. The potential advantage of Survival MDR over parametric methods lies in determining high and low risk groups for genotype combinations regardless of whether linear relationships exist between survival time and SNP effects. A limitation of Survival MDR is that it does not have a way to adjust for covariate effects such as gender, family history, and smoking status. In terms of Random Survival Forest, it is highly data adaptive and its computation is fast even for very large data sets. It can adaptively impute missing data as a tree is grown using its own missing data algorithm. This might cause difference between the result of RSF and other methods since they are using different ways in imputing missing values. As shown in the previous section, both the Elastic Net and Survival MDR select VEGF_2578 C/C as the most important main effect term. However, both the VIMP and minimal depth choose IL10R_109 as the most important main effect. Different results also show up in the situation of the most important two-way interactions.

In recent years, some researchers have attempted to evaluate the performance of different approaches to analyze high-dimensional data with respect to a survival endpoint. Benner and Zucknick (Benner et al., 2010) implemented the Lasso, Elastic Net, adaptive Lasso, and smoothly clipped absolute deviation (SCAD) methods into the model building

process when analyzing high-dimensional data with the Cox proportional hazards model. They demonstrated that the Lasso and also Elastic Net performed the best in terms of prediction accuracy, and thus recommended to use those approaches in actual data application. Winham (Winham et al., 2011) compared the performance of MDR, the traditional Lasso with L_1 penalty, and the group Lasso for categorical data with group-wise L_1 penalty to detect gene-gene interactions. Unlike Benner, they evaluated the predictive performance using a broad range of simulations. They found that the performance of each method is context dependent. Thus, they concluded that each approach might be best suited for detecting and characterizing interactions with different mechanisms. Similarly to Winham, we found differences between the methods when identifying the most prognostic relevant covariates.

While this study is useful for informing researchers about different analysis tools for identifying SNP-SNP interactions, it is not a fully comprehensive comparison. In order to definitely guide researchers in how to choose an analysis method for real data application, prediction error for each method on independent test data should be provided. In addition, various difficulties in real data such as missing values and genetic heterogeneity should be incorporated into the comparison. Additional methods not investigated here may prove even more fruitful for identifying SNP-SNP interactions. For example, the adaptive Lasso has proved a promising technique with attractive theoretical properties. We anticipate that these methods will be used to find interactions among covariate effects, in addition to genotypes. We also anticipate comparing the performance of these methods to detect gene-gene interactions through simulations.

In summary, we compared and contrasted the performance of four variable selection approaches to identify epistatic interactions between SNPs using survival data for breast cancer patients. While we have not conducted a comprehensive comparison in order to determine which method(s) performed the best for indentifying interactions, each method has potential for application to high-dimensional genetic interaction data with survival outcomes..

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APPENDIX

R code for data management

```
## Reading in Breast Cancer Data
breastcancer<-
read.csv("E:/STUDY/THESIS/BRCADDataForDartmouth/BRCADDataForDartmouth.csv",
         header=T)
names(breastcancer)

## Create Partial Dataset 'breastca'
## Contains only 14 genes & survival time(OS) & event(dod.ind)

breastca<- breastcancer[,c(2:15)]
breastca$time<- breastcancer$OS
breastca$event<- breastcancer$dod.ind
names(breastca)
length(breastca[,1]) # 441

## Impute Missing Value
## Remove the rows that contain missing values in 'time' and 'event'
## Remove the rows contain missing values in 'dod.ind'
brca<- breastca
head(brca)
which(is.na(brca$event)) # 97 265 316 345
brca<- brca[-c(97,265,316,345),]

## Check result
length(breastca[,1])
length(brca[,1])
table(brca$event)

## Remove the rows contain missing values in 'time'
which(is.na(brca$time)) # 375
brca<- brca[-375,]
table(brca$time) # Check result
length(brca[,1]) # 436

## Remove the rows that time=0

which(brca$time== 0) # 123 380
brca<- brca[-c(123,380),]

length(brca[,1]) # Check result 434
```

```

## Assign all missing samples using the most common genotype
brca.mean[,1][which(is.na(brca.mean[,1]))]<-geno
table(brca.mean[,1])

## Try to use a LOOP through all the genes (1-14)
for(i in 1:14){
  tab<- table(brca.mean[,i])
  geno<- names(tab)[which.max(table(brca.mean[,i]))]
  brca.mean[,i][which(is.na(brca.mean[,i]))]<-geno
}

##brca.mean # Check the result
length(brca.mean[,1]) #434

## Convert 1st Dataset Factors to Numbers
#####
## Create separate data.frame called 'numeric brca'
## Levels should be 0, 1, 2 (1= heterozygote,0= most common homozygous)

numeric.brca.mean<- brca.mean
names(numeric.brca.mean)

## "IL10_1082"
length(which(is.na(numeric.brca.mean[,1]))) # Check again
table(numeric.brca.mean[,1])
## A/A A/G G/G
## 116 201 117

numeric.brca.mean[,1]<-
ifelse(numeric.brca.mean[,1]%in%"A/G",1,ifelse(numeric.brca.mean[,1]%in%"G/G",0,2))
table(numeric.brca.mean[,1]) # check result

## "IL10_819"
table(numeric.brca.mean[,2])
## C/C C/T T/T
## 304 109 21
numeric.brca.mean[,2]<-
ifelse(numeric.brca.mean[,2]%in%"C/T",1,ifelse(numeric.brca.mean[,2]%in%"C/C",0,2))
table(numeric.brca.mean[,2]) # check result

## "IL10_592"

table(numeric.brca.mean[,3])

## A/A A/C C/C
## 13 117 304
numeric.brca.mean[,3]<-
ifelse(numeric.brca.mean[,3]%in%"A/C",1,ifelse(numeric.brca.mean[,3]%in%"C/C",0,2))
table(numeric.brca.mean[,3]) # check result

```



```

## "IL10R_241"
table(numeric.brca.mean[,4])
## A/A A/G G/G
## 58 148 228
numeric.brca.mean[,4]<-
ifelse(numeric.brca.mean[,4]%in%"A/G",1,ifelse(numeric.brca.mean[,4]%in%"G/G",0,2))
table(numeric.brca.mean[,4]) # check result

## "IL10R_109"
table(numeric.brca.mean[,5])
## A/A G/A G/G
## 74 216 144
numeric.brca.mean[,5]<-
ifelse(numeric.brca.mean[,5]%in%"G/A",1,ifelse(numeric.brca.mean[,5]%in%"G/G",0,2))
table(numeric.brca.mean[,5]) # check result

## "TGFB1_896"
table(numeric.brca.mean[,6])
## C/C C/T T/T
## 35 236 163
numeric.brca.mean[,6]<-
ifelse(numeric.brca.mean[,6]%in%"C/T",1,ifelse(numeric.brca.mean[,6]%in%"T/T",0,2))
table(numeric.brca.mean[,6]) # check result

## "TGFB1_195"
table(numeric.brca.mean[,7])
## A/A A/G G/G
## 286 132 16
numeric.brca.mean[,7]<-
ifelse(numeric.brca.mean[,7]%in%"A/G",1,ifelse(numeric.brca.mean[,7]%in%"A/A",0,2))
table(numeric.brca.mean[,7]) # check result

## "VEGF_2578"
table(numeric.brca.mean[,8])

## A/A C/A C/C
## 85 216 133
numeric.brca.mean[,8]<-
ifelse(numeric.brca.mean[,8]%in%"C/A",1,ifelse(numeric.brca.mean[,8]%in%"C/C",0,2))
table(numeric.brca.mean[,8]) # check result

## "VEGF_1154"
table(numeric.brca.mean[,9])
## A/A A/G G/G
## 24 152 258
numeric.brca.mean[,9]<-
ifelse(numeric.brca.mean[,9]%in%"A/G",1,ifelse(numeric.brca.mean[,9]%in%"G/G",0,2))
table(numeric.brca.mean[,9]) # check result

```

```

## "VEGF_634"
table(numeric.brca.mean[,10])
## C/C G/C G/G
## 53 217 164
numeric.brca.mean[,10]<-
ifelse(numeric.brca.mean[,10]%in%"G/C",1,ifelse(numeric.brca.mean[,10]%in%"G/G",0,2))
table(numeric.brca.mean[,10]) # check result

## "VEGFR_889"
table(numeric.brca.mean[,11])
## A/A A/G G/G
## 5 81 348
numeric.brca.mean[,11]<-
ifelse(numeric.brca.mean[,11]%in%"A/G",1,ifelse(numeric.brca.mean[,11]%in%"G/G",0,2))
table(numeric.brca.mean[,11]) # check result

## "VEGFR_1416"
table(numeric.brca.mean[,12])
## A/A T/A T/T
## 17 120 297
numeric.brca.mean[,12]<-
ifelse(numeric.brca.mean[,12]%in%"T/A",1,ifelse(numeric.brca.mean[,12]%in%"T/T",0,2))
table(numeric.brca.mean[,12]) # check result

## "VEGFR.IVS25_92"
table(numeric.brca.mean[,13])
## A/A G/A G/G
## 31 127 276

numeric.brca.mean[,13]<-
ifelse(numeric.brca.mean[,13]%in%"G/A",1,ifelse(numeric.brca.mean[,13]%in%"G/G",0,2))
table(numeric.brca.mean[,13]) # check result

## "VEGFR.IVS6_54"
table(numeric.brca.mean[,14])
## A/A A/G G/G
## 133 217 84
numeric.brca.mean[,14]<-
ifelse(numeric.brca.mean[,14]%in%"A/G",1,ifelse(numeric.brca.mean[,14]%in%"A/A",0,2))
table(numeric.brca.mean[,14]) # check result
head(numeric.brca.mean) # Check Conversion

## remove snps that fail HWE "IL10R_241"

brca<- read.csv("brca.csv")
names(brca)
brca<- brca[,-c(1,5)]

```

```
brca.mean<- read.csv("brca.mean.csv")
names(brca.mean)
brca.mean<- brca.mean[,-c(1,5)]

numeric.brca.mean<- read.csv("numeric.brca.mean.csv")
names(numeric.brca.mean)
numeric.brca.mean<- numeric.brca.mean[,-c(1,5)]
```

R code for Lasso method

```
library(penalized)
library(survival)
set.seed(101)

## main effects
#####

opt1 <- optL1(Surv(brca.mean$time, brca.mean$event) ~ ., data = brca.mean[1:13], fold=10)
opt1$ lambda # 14.74060
opt1$ cvl #cross-validation likelihood= -813.6569

## Search for best one way model

fit1 <- penalized(Surv(brca.mean$time, brca.mean$event) ~ ., data = brca.mean[1:13],
                  lambda1 =14.74060, lambda2 = 0)
coefficients(fit1)

## two way interactions
#####

opt2 <- optL1(Surv(brca.mean$time, brca.mean$event) ~ .^2, data = brca.mean[1:13], fold=10)
opt2$ lambda # 10.80183
opt2$ cvl #cross-validation likelihood= -812.7365

## Search for best two way model
fit2 <- penalized(Surv(brca.mean$time, brca.mean$event) ~ .^2, data = brca.mean[1:13],
                  lambda1 = 10.80183, lambda2 = 0)
coefficients(fit2)

## three way interactions
#####

opt3 <- optL1(Surv(brca.mean$time, brca.mean$event) ~ .^3, data = brca.mean[1:13], fold=10)
opt3$ lambda # 10.51043
opt3$ cvl # -812.4712

## Search for best three way model
fit3 <- penalized(Surv(brca.mean$time, brca.mean$event) ~ .^3, data = brca.mean[1:13],
                  lambda1 = 10.51043, lambda2 = 0)
```

```

coefficients(fit3)

## Lasso KM curve based on three-way model
## divide patients into low/high risk based on linear predictor
slotNames(fit3)
lp<- linear.predictors(fit3)
lp.50 <- median(lp)
lp.low.high<- ifelse(lp<= lp.50, 0, 1)
fit.lp.2grps <- survfit(Surv(brca.mean$time, brca.mean$event) ~ lp.low.high)

plot(fit.lp.2grps, main="Lasso KM curve based on three-way model", xlab = "Months from
Diagnosis", ylab="Survival Probability", lty=1:2, cex=0.5)
legend("bottomright", c("Low risk group", "High risk group"), lty=1:2)

```

R code for Elastic Net method

```

library(penalized)
library(survival)
set.seed(101)

## Use lambda2= {0.0001, 0.001, 0.01, 0.1, 1, 10}
## main effects
#####

## Find optimal lambda1 and lambda2
optlambda<- function(i){
  lambda2storage<- matrix(nrow = 6, ncol = 1)
  lambda2storage[,]<- c(0.0001, 0.001, 0.01, 0.1, 1, 10)
  rownames(lambda2storage)<- seq(1:6)
  cvlstorage<- matrix(nrow = 6, ncol = 2)
  set.seed(100+i)
  idx<- sample(1:434, 434, replace=FALSE)
  snps.perm<- brca.mean[idx,1:13]
  snps.perm$time<- brca.mean$time
  snps.perm$event<- brca.mean$event
  for (j in 1:6){
    opt<- optL1(Surv(time, event) ~ ., data=snps.perm, lambda2 = lambda2storage[j],
fold=10)
    cvlstorage[j,1]<- opt$cvl
    cvlstorage[j,2]<- opt$lambda
  }
  k<- which.max(cvlstorage[,1])
  lambda2<- lambda2storage[k]
  lambda1<- cvlstorage[k,2]
  return(list(lambda1=lambda1,lambda2=lambda2, cvl=cvlstorage[k,1]))
}

opt1<-optlambda(1)

```

```

opt1

fit1 <- penalized(Surv(brca.mean$time, brca.mean$event) ~ ., data = brca.mean[1:13],
                  lambda1 = 8.0312, lambda2=0.01 )
coefficients(fit1)

## Interactions: ~.^2
#####

optlambda2<- function(i){
  lambda2storage<- matrix(nrow = 6, ncol = 1)
  lambda2storage[,]<- c(0.0001, 0.001, 0.01, 0.1, 1, 10)
  rownames(lambda2storage)<- seq(1:6)
  cvlstorage<- matrix(nrow = 6, ncol = 2)
  set.seed(100+i)
  idx<- sample(1:434, 434, replace=FALSE)
  snps.perm<- brca.mean[idx,1:13]
  snps.perm$time<- brca.mean$time
  snps.perm$event<- brca.mean$event
  for (j in 1:6){
    opt<- optL1(Surv(time, event) ~ .^2, data=snps.perm, lambda2 =
lambda2storage[j], fold=10)
    cvlstorage[j,1]<- opt$cvl
    cvlstorage[j,2]<- opt$lambda
  }
  k<- which.max(cvlstorage[,1])
  lambda2<- lambda2storage[k]
  lambda1<- cvlstorage[k,2]
  return(list(lambda1=lambda1,lambda2=lambda2, cvl=cvlstorage[k,1]))
}

opt2<-optlambda2(1)
opt2

## optimal parameters: lambda1 = 9.053774, lambda2=0.0001
fit2 <- penalized(Surv(brca.mean$time, brca.mean$event) ~ .^2, data = brca.mean[1:13],
                  lambda1 = 11.06831, lambda2=0.0001 )
summary(fit2)
coefficients(fit2)

```

```

## Interactions: ~.^3
#####

optlambda3<- function(i){
  lambda2storage<- matrix(nrow = 6, ncol = 1)
  lambda2storage[,]<- c(0.0001, 0.001, 0.01, 0.1, 1, 10)
  rownames(lambda2storage)<- seq(1:6)
  cvlstorage<- matrix(nrow = 6, ncol = 2)

```

```

set.seed(100+i)
idx<- sample(1:434, 434, replace=FALSE)
snps.perm<- brca.mean[idx,1:13]

snps.perm$time<- brca.mean$time
snps.perm$event<- brca.mean$event
for (j in 1:6){
  opt<- optL1(Surv(time, event) ~ .^3, data=snps.perm, lambda2 =
lambda2storage[j], fold=10)
  cvlstorage[j,1]<- opt$cvl
  cvlstorage[j,2]<- opt$lambda
}
k<- which.max(cvlstorage[,1])
lambda2<- lambda2storage[k]
lambda1<- cvlstorage[k,2]
return(list(lambda1=lambda1,lambda2=lambda2, cvl=cvlstorage[k,1]))
}

## lambda3<- optlambda3(1)

fit3 <- penalized(Surv(brca.mean$time, brca.mean$event) ~ .^3, data = brca.mean[1:13],
lambda1 = 13.53075, lambda2=1e-04 )
coefficients(fit3)

## Elastic Net KM curve based on three-way model
#####slotNames(fit)
lp<- linear.predictors(fit3)
lp.50 <- median(lp)
lp.low.high<- ifelse(lp<= lp.50, 0, 1)
fit.lp.2grps <- survfit(Surv(brca.mean$time, brca.mean$event) ~ lp.low.high)

plot(fit.lp.2grps,main="Elastic Net KM curve based on three-way model", xlab = "Months from
Diagnosis", ylab="Survival Probability", lty=1:2, cex=0.5)
legend("bottomright", c("Low risk group", "High risk group"), lty=1:2)

```

R code for Survival MDR method

```

## Survival MDR use data 'numeric.brca.mean'
source("Surv-MDR.R")
set.seed(101)

## Search for the best one-way model
ffit1 <- ssimucross(numeric.brca.mean[1:13],numeric.brca.mean$time,
numeric.brca.mean$event,10,1)

```

```

table(ffit1$index)
## 7 12 13
## 6 2 2
colnames(numeric.brca.mean)[c(7,12,13)] # "VEGF_2578" "VEGFR.IVS25_92"
"VEGFR.IVS6_54"

## Search for the best two-way model
ffit2 <- ssimucross(numeric.brca.mean[1:13],numeric.brca.mean$time,
numeric.brca.mean$event,10,2)
table(ffit2$index)
## 12 406 408
## 1 5 4
colnames(numeric.brca.mean)[c(4,6,8)] # "IL10R_109" "TGFB1_195" "VEGF_1154"

## Search for the best three-way model
ffit3 <- ssimucross(numeric.brca.mean[1:13],numeric.brca.mean$time,
numeric.brca.mean$event,10,3)
ffit3
table(ffit3$index)
## 12 40607 40608 40811 71213 81213
## 1 4 2 1 1 1
colnames(numeric.brca.mean)[c(8,12,13)]

```

R code for RSF method

```

## VIMP variable selection, get VIMP scores
#####
## Fit RSF using 50 different seed values
## Use for loop, change seed each time, keep VIMP scores

library("randomSurvivalForest")
ntree<- 1000
set.seed(101)

## use log-rank splitting rule, which is the default splitting rule
## use data-imputing (redo 50 repeats using na.impute (but not for poster)
brca.out<- rsf(Survrsf(time, event)~.,brca,ntree=ntree, na.action="na.impute")
brca.out$importance
names(brca)
sort(brca.out$importance, decreasing=TRUE)

## VIMP KM curve based on one-way model
brca.out$mortality
q50.mort <- median(brca.out$mortality)
split.rsfc<- ifelse(brca.out$mortality> q50.mort, 1, 0)
library(survival)

```

```

fit.km <- survfit(Surv(brca$time, brca$event) ~ split.rsf)

plot(fit.km, main="VIMP KM curve based on one-way model", xlab = "Months from
Diagnosis", ylab="Survival Probability", lty=1:2, cex=0.5)
legend("bottomright", c("Low risk group", "High risk group"), lty=1:2)

B <- 50
VIMP <- matrix(NA, nrow = 13, ncol = B)
rownames(VIMP) <- names(brca)[1:13]

for (i in 1:B) {
  brca.multi<- rsf(Survrsf(time, event)~.,brca,ntree=ntree,
seed=(100+i),na.action="na.impute")
  VIMP[,i] <- brca.multi$importance
}
VIMP.mean<- rowMeans(VIMP)
VIMP.mean
round(sort(VIMP.mean, decreasing=TRUE),3)

rank.VIMP<- apply(-1*VIMP, 2, rank)
rank.VIMP.mean<- rowMeans(rank.VIMP)
sort(rank.VIMP.mean)

## Minimal Depth variable selection
#####

library("randomSurvivalForest")
ntree<- 1000
nodesize<- 2
mtry<- 14*(3/4)
nsplit<- 10

brca.out<- rsf(Survrsf(time, event)~.,brca, ntree=ntree, nodesize=nodesize, mtry=mtry,

nsplit=nsplit, seed= 101,forest= TRUE,na.action=
"na.impute")
sort(brca.out$importance, decreasing=TRUE)

## Minimal Depth one-way Plot

brca.out$mortality
q50.mort <- median(brca.out$mortality)
split.rsf<- ifelse(brca.out$mortality> q50.mort, 1, 0)
library(survival)

```



```

fit.km <- survfit(Surv(brca$time, brca$event) ~ split.rsf)

plot(fit.km, main="Minimal Depth KM curve based on one-way model", xlab = "Months
from Diagnosis", ylab="Survival Probability", lty=1:2, cex=0.5)
legend("bottomright", c("Low risk group", "High risk group"), lty=1:2)

## Minimal Depth two-way Plot
#####
###
brca.out<- rsf(Survrsf(time, event)~.,brca, ntree=ntree, nodesize=nodesize, mtry=mtry,
               nsplit=nsplit, seed= 101,forest= TRUE,na.action=
"na.impute")

max.out2 <- max.subtree(brca.out, max.order = 2, sub.order = TRUE)
dist.mat<- as.dist(max.out2$subOrder)
clust.snps<- hclust(dist.mat)
plot(clust.snps,xlab = "Months from Diagnosis", cex=1)

B <- 50
Minimal <- matrix(NA, nrow = 13, ncol = B)
Minimal.mean<- matrix(NA, nrow = 13, ncol = 1)
rownames(Minimal) <- names(brca)[1:13]

for (i in 1:B) {
  brca.multi<- rsf(Survrsf(time, event)~.,brca,ntree=ntree,nodesize=nodesize,
mtry=mtry,
               nsplit=nsplit,seed=(100+i),forest= TRUE,na.action= "na.impute")
  Minimal[,i] <- brca.multi$importance
}

Minimal.mean<-rowMeans(Minimal)
Minimal.mean
round(sort(Minimal.mean, decreasing=TRUE),3)

Minimal.rank<- apply(-1*Minimal, 2, rank)
Minimal.rank.mean<- rowMeans(Minimal.rank)
sort(Minimal.rank.mean)

```

CURRICULUM VITAE

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EDUCATION

- 2009.08 – Current MS (candidate, expected in May 2011),
Biostatistics, GPA 3.844
University of Louisville (UofL), Louisville, Kentucky, USA
- 2007.09 – 2009.06 MS program in Pharmaceutics
China Pharmaceutical University (CPU), Nanjing, China
- 2003.09 – 2007.06 BE, Bioengineering
China Pharmaceutical University (CPU), Nanjing, China

RESEARCH EXPERIENCE

- 2010.08--- Now Graduate Student (Guy N. Brock)
Graduate Thesis—Simulation study comparing methods for
detecting gene-gene interactions for survival data.
Dept of Bioinformatics and Biostatistics,
School of Public Health and Information Sciences, UofL
- 2010.09---Now Data Analyst & Lab Assistant (LaCreis R Kidd)
'Innate immunity-related sequence variants as predictors of
prostate cancer risk using Multifactor Dimensionality Reduction
Method '
Dept of Pharmacology and Toxicology,
School of Medicine, UofL
- 2010.05---2010.09 Data Analyst & Lab Assistant (Mary Eng)
'Employment outcomes of patients following successful renal
transplantation'
Jewish Hospital Transplant Center &Uof L
- 2009.10—2010.08 Data Analyst & Lab Assistant
(G. Rafael Fernandez-Botran)
'IL-4 and IL-4-δ2 in the modulation of Th1 response to
Mycobacterium tuberculosis among HIV-infected women'
Dept of Pathology and Laboratory Medicine,
School of Medicine, UofL
- 2009.08—2009.10 Data Analyst & Lab Assistant (Eugenia Wang)
Bioinformatics Data Analysis

Gheens Center on Aging,
School of Medicine, UofL

2008.07 – 2009.06 Graduate Student (JiashengTu)
Worked on project ‘Reduction of gelatin cross-linking within capsules’
Worked as participant of compiling two guide books
<Scientific Design of Experiments –Role in Pharmaceutical Sciences > and <Guideline of Pharmaceutical Excipients >
Dept of Pharmaceutics, CPU

2007.02 - 2007.06 Bachelor Student (JiashengTu)
Worked on graduation thesis ‘The Pharmacokinetics of Polymeric micelle of Cyclosporine’
Dept of Pharmaceutics, CPU

2005.07-2005.08 Intern quality controller
Nanjing Xiaoying Pharmacy Corp.

PUBLICATIONS

1. Eng M, **Zhang J**, Cambon A, Brock G, Gleason J. **Employment outcomes of Patients following successful renal transplantation. Clinical Transplantation, 2011 Feb 23, accepted**
2. Kidd LR, Beache SC, **Zhang J**, Brock GN, Lillard JW Jr, Rudd J, Kimbro KS. Chemokine and chemokine receptor genetic variants as predictors of aggressive prostate cancer among European-American men. BMC Bioinformatics, submitted
3. Kimbro KS, Beache SC, **Zhang J**, Lillard JW Jr., Brock GN, Rudd J, Kidd LR. Chemokine and chemokine receptor genetic variants as predictors of breast cancer risk among Caucasian women. BMC Bioinformatics, submitted
4. Fernandez-Botran R, **Zhang J**, Brock G, Annapan S, Plankey M, Young M, Herold B, Durkin H, Villacres MC, Greenblatt RM, French A, Golub J, Bordon J. Cytokine expression profiles in HIV-infected women preceding tuberculosis. Cytokines in infectious diseases autoimmune and cancer meeting, Chicago, 2010
5. Fernandez-Botran R, **Zhang J**, Brock G, Annapan S, Plankey M, Young M, Herold B, Durkin H, Villacres MC, Greenblatt RM, French A, Golub J, Bordon J. Cytokine expression profiles in HIV-infected women preceding tuberculosis. Poster, Research! Louisville Poster Competition, Louisville, Oct 11-15, 2010
6. Kimbro KS, Beache SC, **Zhang J**, Lillard JW Jr., Brock GN, Rudd J, Kidd LR. Chemokine and chemokine receptor genetic variants as predictors of breast cancer risk among Caucasian women. AACR(American Association for Cancer Research) 102nd Annual Meeting, Orlando, FL, April 2-6, 2011
7. **Zhang J**, Kidd LR, Brock GN, Comparison of Approaches for Detecting Gene-Gene Interactions for Survival Data. Poster, 10th Annual UT-ORNL-KBRIN Bioinformatics Summit, Memphis, TN, April 1-3, 2011
8. **Zhang J**, Kidd LR, Brock GN, Comparison of Approaches for Detecting Gene-Gene Interactions for Survival Data. Poster, 10th Annual UT-ORNL-KBRIN Bioinformatics Summit, Memphis, TN, April 1-3, 2011

9. Lavender NA, Rudd J, **Zhang J**, Hu T, Brock GN, Kimbro KS, Moore JH, Hein DW, Kidd LR. Interaction among apoptosis-associated sequence variants and joint effects on aggressive prostate cancer. *Molecular Systems Biology*, April, submitted.