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Regularized multiple mediation analysis*

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Mediation analysis is used to explore how an established exposure-outcome relationship is influenced by a third variable (mediator). Multiple mediation analysis refers to the mediation analysis with multiple mediators. We propose to use the elastic net regularized linear regression in multiple mediation analysis when the number of potential mediators is large. In exploring the exposure-mediator-outcome relationship, we regularize coefficients of mediators in predicting the outcome. The penalization on the coefficient is inversely proportional to the association between the exposure variable and each mediator. Therefore, in estimating the effect of a mediator, the exposure-mediator and the mediator-outcome associations are jointly considered. An R package, *mmabig*, is compiled for the proposed method. We perform a series of sensitivity and specificity analysis to examine factors that can influence the power of identifying important mediators. Further, we illustrate how to consider potential nonlinear associations among variables in the mediation analysis. Simulation studies have shown that the proposed mediation analysis method consistently obtain larger power when compared with its main competitors. The method is used with a real data set to explore factors that contribute to the racial disparity in survival rates among breast-cancer patients.

Keywords and phrases: Elastic net, High-dimensional data set, Multiple mediation analysis, Penalized likelihood, R package *mmabig*.

1. BACKGROUND

Mediation analysis refers to the analysis process of identifying and quantifying the mechanism of a third variable (know as a mediator) that underlies an observed relationship between an independent variable (or exposure variable) and an outcome variable. This concept has been used widely in many fields such as social science, prevention study, behavior research, and epidemiology. Investigators are interested in discovering not only the relationship between the exposure variable and response variable but also the mechanism of other risk factors that intervening the relationship [11].

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Many mediation analysis are based on linear models. Within the linear model setting, according to [2], three conditions are required to establish a mediation effect: (a) the exposure variable (X) is significantly associated with the response variable (Y); (b) the presumed mediator (M) is significantly related to X; and (c) M significantly relates to Y controlling for X. When there is only one mediator, the relationships among variables are modeled through the following linear regressions:

$$\begin{aligned} m_i &= \alpha_0 + \alpha_1 x_i + \epsilon_{i1}; \\ y_i &= \beta_0 + \beta_1 m_i + \beta_2 x_i + \epsilon_{i2}; \\ \epsilon_{ij} &\overset{ind}{\sim} N(0, \sigma_j^2), \quad j = 1, 2;\ i = 1, \ldots, n. \end{aligned}$$

In such setting, the mediation effect of M, also called indirect effect, is typically measured by $\alpha_1\beta_1$, the product of the coefficient of X when it is regressed on M, and the coefficient of M in explaining Y controlling for X [12]. When X changes by a unit, M changes by α_1 unit, and in turn, Y changes by $\alpha_1 \times \beta_1$ units through M. This method is generally called the product-of-coefficients method ("CP" for abbreviation). However, CP is not easily adaptable to separate multiple mediation effects when Y or M is not continuous or when the relationships cannot be fitted with linear regressions [9]. For example, when M is binary and the relationship between X and M is fitted by

$$logit(Pr(M_i = 1)) = \alpha_0 + \alpha_1 X_i,$$

the *CP* method cannot be used directly since α_1 denotes the change of $logit(M = 1)$ with X, but not the change of M itself.

Counterfactual framework is the other popular setting to implement mediation analysis [14, 13, 16, 1]. Let $Y_i(X)$ denote the post-treatment potential outcome if subject i is exposed to X. To compare the change in outcome when the exposure changes from x to x^* (e.g., 0 or 1 for binary X), the causal effect of treatment on the response variable for subject i is defined as $Y_i(x) - Y_i(x^*)$. It is impossible to estimate the individual causal effect since only one of the responses, $Y_i(x)$ or $Y_i(x^*)$, is observed. [5] proposed, instead of estimating causal effect on a specific subject, to estimate the average causal effect over a pool of subjects — $E(Y_i(x) - Y_i(x^*))$. If the subjects are randomly assigned to control ($X = x^*$) or treatment ($X = x$) groups, the average causal effect equals the expected conditional causal effect, $E(Y_i|X = x) - E(Y_i|X = x^*)$. The counterfactual framework fits best when the exposure variable is binary. It is difficult to choose a referral exposure level, x, when the exposure variable is multi-categorical or continuous [18, 19]. For example, [20] proposed a method that uses weights to control for other covariates in calculating mediation effects. Their proposed method can be used to consider joint effects of multiple mediators, but it is limited to binary exposures or exposures with a small number of levels since a start and an ending value of the exposure variable have to be set up for the analysis. Recently, mediation analysis has been further developed by [7] and [8] such that more general models, for example generalized linear models and generalized additive models, can be used to fit relationships among variables. By their method, general models are fitted for the outcome and mediators, based on which potential values for outcome and mediators can be simulated, and in turn to estimate the mediation effects. Common as in the counterfactual framework, the method focuses mainly on computing mediation effect between two treatment values of the exposure. [6] presented a mediation model that deals with continuous mediators, in which mediators are transformed to be orthogonal to each other. Therefore, high-dimensional mediation analysis can be performed with models, each of which contains only one orthogonal mediator. [6] also proposed a Monte-Carlo procedure to identify significant mediators. Dimensional reduction can be achieved through the transformation of mediators. However, the transformation may make the interpretation of mediation effects difficult. Moreover, the method can deal with only continuous mediators.

[21] proposed a more general definition of mediation effect, which is in terms of the "changing rate", instead of the "changing volume", of the outcome variable with the exposure variable. The motivation has three folds. First, neither the starting point (e.g. the x in the counterfactual framework) nor the changing unit of X (e.g. $x^* - x$) has to be preset; second, the definition allows for potential nonlinear relationship among variables; and third, more general predictive models are allowed to fit the relationships, therefore the mediation analysis is extended to deal with multiple and different types (binary/categorical/time-to-event/continuous) of exposure, risk, and outcome variables [22, 24].

In this paper, we use the definitions of mediation effects by Yu et al. [22, 24] and propose a multiple mediation analysis method to deal with high-dimensional data sets. A high-dimensional data set includes a large number of variables and/or observations. Within high-dimensional mediation analysis, the number of potential mediators is large. For example, in explaining the racial disparity in breast cancer survival, there are a lot of risk factors need to be considered such as individual behaviors, environmental factors, social economic status, treatment effects, diagnosis characters, and even gene expression variables. We propose to use a regularized mediation analysis method that identify significant mediators among a large set of potential mediators and estimate their indirect effects simultaneously.

The rest paper is organized as follows: Section 2 proposes a novel mediation analysis algorithm and the corresponding computational method for high dimensional data sets. We also introduce the R package, *mmabig*, that is created for big data analysis. in Section 3, sensitivity and specificity of the proposed method in identifying mediators are discussed in 3.1. Section 3.2 illustrates the proposed method in dealing with nonlinear relationship with simulations and 3.3 presents the analysis of a real data set. And finally, we make conclusions, discuss limitations of the method, and point out future research directions in Section 4.

2. METHODS

2.1 Regularized mediation analysis in linear regression setting

Consider the general linear regression setting that a data set has n observations with p predictors, $(\mathbf{x}_1, \ldots, \mathbf{x}_p)$. The purposes of the analysis are to select important predictors in predicting the outcome, $\mathbf{y}$, and to estimate coefficients for the selected covariates. Usually, given $(\mathbf{x}_1, \ldots, \mathbf{x}_p)$, the response $\mathbf{y}$ is predicted by

$$\hat{\mathbf{y}} = \hat{\beta}_0 + \hat{\beta}_1 \mathbf{x}_1 + \ldots + \hat{\beta}_p \mathbf{x}_p.$$

[27] proposed a regularization and variable selection method, called elastic net, that for any fixed non-negative λ and $\gamma \in [0, 1]$, the coefficients of the predictors are the minimizer of the penalized function:

$$L(\lambda, \gamma, \beta) = \sum_{i=1}^{n} (y_i - \mathbf{x}_i \beta)^2 + \frac{\lambda(1-\gamma)}{2} \sum_{j=1}^{p} \beta_j^2 + \lambda\gamma \sum_{j=1}^{p} |\beta_j|.$$

When $\gamma = 0$, the loss function is the ridge penalty [4] and when $\gamma = 1$, the loss function is the lasso penalty [17]. The elastic net produces a sparse model while improves the prediction accuracy over the ordinary least squares estimates. We would like to use elastic net in mediation analysis so that mediators are identified and their effects estimated at the same time.

The conceptual model of the multiple mediation analysis is presented in Figure 1. We assume there are one exposure variable, denoted as $\mathbf{x} = \{x_1, \ldots, x_n\}$, where n is the number of observations; p mediators: $\mathbf{m} = \{\mathbf{m}_1, \ldots, \mathbf{m}_p\}$, where $\mathbf{m}_j^T = (m_{j1}, \ldots, m_{jn})$ and $j = 1, \ldots, p$; and one outcome, $\mathbf{y} = \{y_1, \ldots, y_n\}$. In Figure 1, the lines (with no arrows) among mediators indicate that given the exposure variable, mediators can be associated with each other. Other covariates, $\mathbf{z}$, are variables that are significantly related with the outcome but not with the exposure variable. For simplicity, we ignore $\mathbf{z}$ for now. In addition, the exposure variable and the outcome can be extended to multivariate, but our

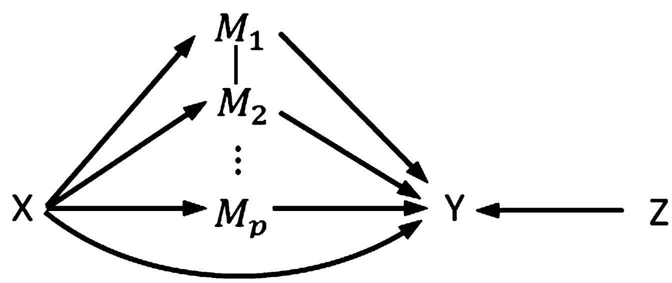

Figure 1. The Conceptual model of mediation analysis.

discussion in this section focuses on one exposure and one outcome only.

The assumptions needed for general mediation analysis [21] are that:

A1 No-unmeasured-confounder for the exposure-outcome relationship;
A2 No-unmeasured-confounder for the mediator-outcome relationship;
A3 No-unmeasured-confounder for the exposure-mediator relationship.

Usually there is a fourth assumption for mediation analysis: Any mediator is not causally prior to other mediators. This assumption is not required for the general mediation effects defined by [22, 24, 26]. The three assumptions require that no unobserved confounders exist in the triangle relationships among the exposure, mediators and the outcome. A confounder for the $A-B$ relationship refers to a factor other than A (e.g., the exposure) that may affect B (e.g., the outcome of interest), and that can be related to A [10]. The difference between a confounder and a mediator lies in its relationship with A. The mediation effect assumes a causal relationship from A to the mediator while a confounding effect only requires an association between A and the confounder without the causal assumption. When there are confounders, the confounders have to be put in the analysis at their correct formats (as covariates for the outcome or mediators). If any of the assumptions is violated, the estimated mediation effects can be biased. For a discussion of the assumptions, readers are referred to the paper by [3]. In the paper, the authors discussed in detail how the estimation can be influenced in terms of estimation bias, variance, specificity and sensitivity if any of the assumption is violated. It is difficult to check whether all assumptions are satisfied or not. Mostly, a complete random design can be used to control confounders. But for observational studies, it is possible to have unobserved confounders that can influence the estimation of mediation effects. We recommend to use observational studies to explore relationships among variables and whenever possible, design and implement experiments to establish the potential causal effect.

In the linear-model setting, to make inferences on mediation effects, $p+1$ linear regressions are needed. The first model is on $\mathbf{y}$ given $\mathbf{x}$ and $\mathbf{m}$. The linear regression has the following format:

$$(1)\qquad y_i = \beta_0 + \beta_x x_i + \beta_1 m_{1i} + \ldots + \beta_p m_{pi} + \epsilon_{0i},\quad \epsilon_{0i} \overset{iid}{\sim} N(0, \sigma_0^2).$$

In addition, there are p linear regressions, each modeling the relationship between a mediator, $\mathbf{m}_j$, and the exposure variable such that

$$(2)\qquad m_{ji} = \alpha_{0j} + \alpha_j x_i + \epsilon_{ji}, \quad j = 1, \ldots, p;$$

where $\epsilon_i^T = (\epsilon_{1i}, \ldots, \epsilon_{pi})$ has an independent multivariate normal distribution with the mean vector $\mathbf{0}_p$ and variance-covariance matrix Σ, for $i = 1, \ldots, n$. Note that Σ does not have to be a diagonal matrix, indicating that given x, mediators are allowed to be associated with each other. Assumption 3 only prohibits a mediator to be causally preceding any other mediators.

Under the above setting, the general multiple mediation analysis proposed by [21] generates the same mediation effect estimates as the CP method where the direct effect from the exposure variable is β_x, the indirect effect from the jth mediator is $\alpha_j\beta_j$, and the total effect is $\beta_x + \sum_{j=1}^p \alpha_j\beta_j$. The purpose of mediation analysis is to identify mediators that have significant indirect effects and estimate those effects. We propose to estimate the coefficients of regression model (1), and therefore the mediation effects, by minimizing the penalized function, $L(\lambda, \gamma, \hat{\alpha}, \beta)$:

$$(3)\qquad \sum_{i=1}^n \left(y_i - \beta_0 - \beta_x x_i - \sum_{j=1}^p \beta_j m_{ji} \right)^2 + \frac{\lambda(1-\gamma)}{2} \left[\sum_{j=1}^p (\hat{\alpha}_j \beta_j)^2 + \beta_x^2 \right] + \lambda\gamma \left[\sum_{j=1}^p |\hat{\alpha}_j \beta_j| + |\beta_x| \right],$$

for some $\lambda \geq 0$ and $\gamma \in [0, 1]$, where $\hat{\alpha}s$ are the coefficient estimates for models (2). Inheriting the good properties of elastic net, the proposed method can identify significant mediators, whose mediation effects, $\alpha\beta$ or β_x, are significantly different from 0, and estimate their mediation effects at the same time.

2.2 Computation: the algorithm to estimate mediation effects with general models

There are established methods to estimate the coefficients from the elastic net regularized generalized linear models. We use the *glmnet* package [15] for the proposed mediation analysis. *glmnet* can deal with Gaussian, Binomial, Poisson, Multinomial, and time-to-event (using cox model) type of outcomes.

We propose an algorithm to use the elastic net computational method to estimate mediation effects of interests.

In the algorithm, we also allow the use of general predictive models to fit relationship between the exposure variable and mediators, therefore enable the fitting of potential nonlinear relationship and allow the mediators to be of different type. Model (2) is generalized to have the following format

$$E(m_{ji}|x_i) = l_j^{-1}\left(g_j(x_i)\right), \quad j = 1, \ldots, p; \tag{4}$$

where l_j is the link function and g_j is any predictive model that predicts m_j using x. For example, to use the generalized spline models to fit the relationship between the exposure variable and mediators, model (4) is

$$E(m_{ji}|x_i) = l_j^{-1}\left(\alpha_{0j} + \sum_{k=1}^{K} \alpha_{kj} h_k(x_i)\right), \quad j = 1, \ldots, p;$$

where h_k is the spline basis function, and K basis functions are used to fit m_j with x. Using the general predictive models, Algorithm 1 presents the procedure of estimating mediation effects.

Algorithm 1. *Algorithm to estimate mediation effects.*

1. *For each mediator, fit the general model (4) that predicts m_j using x.*
2. *Based on the models fitted from step 1, for $j = 1, \ldots, p$:*
   - *If x is continuous, calculate the average changing rate in m_j when x changes by a margin mg,*

$$\Delta M_j = \frac{1}{n}\sum_{i=1}^{n} \frac{E(m_{ji}|x_i = x_i + mg) - E(m_{ji}|x_i = x_i)}{mg}.$$

   - *If x is binary, calculate the change in the mean of m_j when x changes from 1 to 0,*

$$\Delta M_j = E(m_{ji}|x_i = 1) - E(m_{ji}|x_i = 0).$$

3. *Transform all mediators such that $m^*_{ij} = m_{ij}/\Delta M_j$, for $i = 1, \ldots, n$ and $j = 1, \ldots, p$.*
4. *Fit a linear regression model where y_i is the response variable, x_i and $m^*_{ij}, j = 1, \ldots, p$ are covariates, using the elastic net penalized function to estimate coefficients.*
5. *The average mediation effect for each variable is its estimated coefficient from elastic net regression: direct effect for x and indirect effect for M_j.*

We have the following comments for the proposed method and calculation algorithm:

- ΔM_j measures the average changing rate in M_j when x changes by an margin, mg. Throughout the paper, we set $mg = 1$. The $\hat{\alpha}_j$ in the penalization function (3) is ΔM_j. The potential nonlinear relationship between mediators and the exposure variable can be fitted through different predictive models. As is shown in the algorithm, ΔM_j can be calculated with any predictive models. In this paper, we use the generalized spline models.
- Algorithm 1 provides the estimate of average indirect effect for each of the mediators. However, for continuous mediators, due to the potential nonlinear relationship between X and M_j, the indirect effect for M_j at different values of x can be variant from the average indirect effect. The indirect effect of M_j at different x is also estimable. Denote the average indirect effect of M_j as AIE_j, the indirect effect of M_j at $X = x$ is $\frac{AIE_j}{\Delta M_j} \times \frac{E(m_j|X=x+mg)-E(m_{ji}|X=x)}{mg}$.
- In step 4, a linear regression model is fitted with elastic net method. This is easily extended to any generalized linear models to deal with any types (e.g. categorical, continuous, time-to-event) of response variable(s).
- For elastic net regression, covariates need to be standardized to have the same mean and standard deviation, so that all coefficients are shrunk at the same scale. For mediation effect estimation, the standardization of covariates is not required since the mediation effects are calculated at the same scale: the changing rate in Y when X changes. Hence the estimated mediation effects are scale invariant.
- The parameter λ in elastic net (Equation (3)) is the penalty parameter for coefficients. Large value of λ will shrink coefficients (the estimated mediation effects) toward 0. γ is used to balance between the l_1 and l_2 regularization. The parameters can be tuned together through cross-validation.
- Although we discussed only the binary and continuous exposures in the algorithm, the method is readily extended to multivariate exposure variables. Therefore, a multi-categorical exposure variable can be handled easily by transforming it to multiple binary exposures.
- Bootstrap method are used with the algorithm to estimate uncertainties.

2.3 The R package: mmabig

The *mmabig* package [23], available on the Comprehensive R Archive Network (CRAN), was generated based on the above algorithm for mediation effect inferences. In the package, *glmnet* by [15] is used for the elastic net estimates. The package *mmabig* provides a step-by-step process for mediation analysis. The function *data.org.big* identifies potential mediators and covariates through multiple tests. A potential mediator is identified if it is significantly associated with both the exposure and the outcome variables. In this function, two methods can be chosen to test whether the mediator is significantly associated with the outcome: univariate tests or elastic net regression that jointly considers all variables together. A covariate is identified if it is significantly related with the outcome but not with the exposure variable. Then the organized data set from the function *data.org.big* is read into the function *med.big* to select mediators and estimate their mediation effects (indirect effect (IE), total effect (TE), direct effect (DE), and relative effects (defined as IE/TE or DE/TE, denoted as RE)). Finally, the function *mma.big* uses the bootstrap method to estimate variances and generate confidence intervals for the estimated mediation effects.

The R package *glmnet* is used in the *mmabig* package for the elastic net regression. The tuning parameters λ and γ (see Equation (3))can be selected through cross-validation or other established methods. In the *mmabig* package, γ is chosen by the user after it is tuned, while λ can be tuned in the embedded functions by assigning a sequence of potential values. The function *cv.glmnet* is called within *mmabig* to tune the value of λ.

The *mmabig* package provides generic functions to help explain results from the mediation analysis. The results from *data.org.big* can be summarized to identify potential mediators and covariates, and show test results (p values) for each association of interests. Moreover, the outputs from the function *mma.big* can be summarized to show the inference results on mediation effects (estimates, standard deviations, and confidence intervals). The function *joint.effect* makes inferences on the joint effect of multiple mediators from the *mmabig* object created by *mma.big*. The graphic function, plot, helps researchers visualize the complicated relationships and explain the directions of mediation effects. The readers are referred to the help menu of the *mmabig* package for instructions and examples that implement the functions.

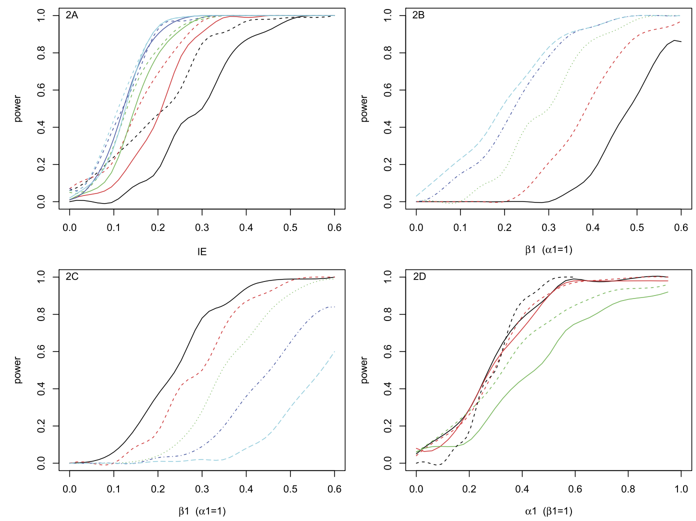

Figure 2. Sensitivity in identifying important mediators. For 2A, solid lines are when $\alpha = 1$ and the small dash lines are for $\beta = 1$. Black, red, green, dark blue, and light blue represent n at 100, 150, 200, 250 and 300 respectively. Solid, small dashes, dot, dot-dash, and dash lines represent $\beta_0 = 0.1, 0.3, 0.5, 0.7, 0.9$ respectively for 2B, and $J = 10, 20, 30, 40, 50$ for 2C. For 2D, the solid black line represents the power for the binary mediator at $c_1 = 0$, while small dashed black line represents power for the continuous mediator. The red lines are for $|c_1| = 0.5$ and green for $|c_1| = 1$, where solid is for positive and small dash is for negative c_1s.

3. RESULTS AND DISCUSSIONS

3.1 Sensitivity and specificity analysis

We did a series of analysis to check the sensitivity and specificity of the proposed method in identifying important mediators. We would like to find out how the power and the misclassification rate are influenced by factors such as the number of variables, the sample size, mediator types (continuous or categorical) and the effect size. The original data are generated as follows:

$$(5)\qquad \begin{aligned} x_i &\sim N(0,1), i = 1, \dots, n; \\ m_{ij} &\sim N(0,1), j = 1, \dots, J-2; \\ m_{ij} &\sim N(\alpha_1 x_i, 1), j = J-1, J; \\ y_i &\sim N(\beta_0 x_i + \beta_1 m_{i1} + \beta_1 m_{iJ}, 1), i = 1, \dots, n. \end{aligned}$$

Figure 2 shows how the power changes with different factors, where power is defined as the proportion of times that the 95% confidence interval of the average indirect effect does not include 0. For each scenario, we simulate the dataset 100 times and calculate the proportion of times that the important mediator m_J is successfully identified. In Figure 2A, the small dash lines show how the power changes with α_1 when β_1 is set at 1. In comparison, when α_1 is set at 1, the solid lines show how the power changes with β_1. Note that when one of the values of β_1 or α_1 is fixed at 1, the indirect effect of M_J is α_1 or β_1 respectively, since theoretically, the actual indirect effect is $\alpha_1\beta_1$. The different sample sizes, n, are shown by different colors: black, red, green, purple, and blue represent n at 100, 150, 200, 250 and 300 respectively. In general, we found that at the same sample size and effect size, fixing β_1 at 1 while changing α_1 is related with a little higher power than changing α_1 but fixing β_1 at 1. This is due to the fact that in estimating α_1, the regularization method is not used, therefore, the effect of α is not shrunk. We also found that when sample size increases, the difference in power becomes undetectable. Figure 2B shows how the power changes with β_0, the intercept in predicting y, when α_1 is fixed at 1 and β_1 changes. We see that as β_0 increases, the power of detecting the indirect effect also increases. Figure 2C shows how the power changes with the number of potential mediators, J. As J increases, the power decreases as expected. Finally, Figure 2D is to check how the type of mediator can influence the power of detecting an indirect effect. To create binary mediators, for $j = J-1$ or J, we let the new mediator $\tilde{m}_{ij}$ be 0 if m_{ij} is less than $c1$, otherwise be 1. Changing $c1$ will alter the original distribution of m_j by changing the probability $p(m_{ij} = 1)$. We found that first, when α_1 is small, the power is higher to identify binary mediator than the continuous mediator (solid vs. small dash black lines respectively). However when α_1 becomes larger, it is easier to identify the continuous mediator. This is because the indirect effect for the binary mediator is roughly $E[P(m=1|x+1) - P(m=1|x)]\beta_1$ versus $\alpha_1\beta_1$ for the continuous mediator. Second, we found that as c_1 moves away from 0, $P(m=1)$ moves away from 0.5, i.e. the original distribution of m becomes more uneven, the power of detecting the important binary mediator reduces.

Another interesting finding is that when α_1 is set at 1, while β_1 changes, the type-I error is controlled well under 0.05 (the solid lines in 2A of Figure 2). While if β_1 is fixed at 1 but α_1 changes, the type-I error is not controlled as well in the mediation analysis, ranging from 0.05 to 0.07. The same happens when mediators are binary (Panel 2D). This is due to that α_1 is estimated and fixed in the first step of Algorithm 1. In the simulations, β_1 has a relative big value. The elastic net regression in the proposed mediation analysis tends to shrink more based on the value of β than on that of α. A solution to control the type-I error rate is that when testing the relationship between the exposure and potential mediators, reduce the significance level to consider the multiple comparisons – since there are multiple mediators.

Figure 3 shows the misclassification rates, $(1 - specificity)$, at the same scenario as in Figure 2. The left panel, false positive rate 1, shows the probabilities of taking m_1 as an important mediator (where $\alpha_1 = 0$ while $\beta_1 \neq 0$) and the right panel, the false positive rate 2, for identifying m_{J-1} (where $\beta_1 = 0$ while $\alpha_1 \neq 0$) as an important mediator. Figure 3A shows that the false positive rate 1 is generally bigger than the false positive rate 2. In addition, the the false positive rates increase as the sample size increases. In Figure 3B, we found that both false positive rates increase as β_0 increases. 3C shows that the specificity decreases with the number of potential mediators. Figure 3D shows that the false positive rates do not change a lot with the original distribution of $P(m=1)$. The false positive rate for m_1 is zero for different α_1s when m_J is binary. Overall, the type I error is controlled under 0.05.

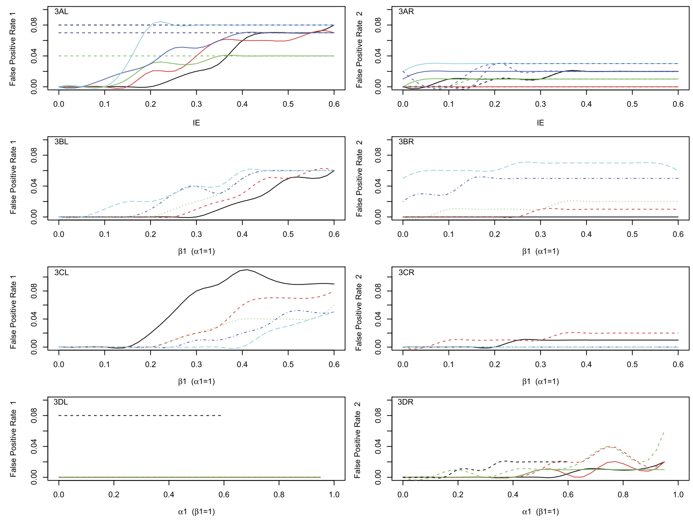

Figure 3. 1-specificity in identifying mediators. The left column, the false positive rate 1, shows the probabilities of taking m_1 as an important mediator, and the right column, the false positive rate 2, for picking m_{J-1}. For 3A, black, red, green, dark blue, and light blue represent n at 100, 150, 200, 250 and 300 respectively. Solid, small dashes, dot, dot-dash, and dash lines represent $\beta_0 = 0.1, 0.3, 0.5, 0.7, 0.9$ respectively for 3B, and $J = 10, 20, 30, 40, 50$ for 3C. For 3D, the solid black line represents the power for the binary mediator at $c_1 = 0$, while small dashed black line represents misclassfication rate for the continuous mediator. The red lines are for $|c_1| = 0.5$ and green for $|c_1| = 1$, where solid is for positive and small dash is for negative c_1s.

3.2 Simulation studies

The purpose of simulation studies in this section is to illustrate the proposed mediation method when there are nonlinear relationship or co-linearity among variables. In the following, we demonstrate the method in different situations.

3.2.1 X-M relationship is nonlinear

The data are simulated similarly as equations (5) except that the exposure variable is simulated with a mean of c_2, and when $j = J-1$ or J, m_{ij} is simulated by

$$m_{ij} \sim N(\alpha_1 x_i^2, 1). \quad (6)$$

That is, the exposure-mediator relationship is quadratic. If a linear model is forced to fit the relationship between x and m_J, the coefficient for x would be insignificantly different from 0. Therefore it would be very difficult to identify the important mediator m_J. A smoothing spline to model the relationship would solve the problem. This is shown in Figure 4, the simulation with $c_2 = 0$, $J = 20$ and $n = 100$. The solid line indicates the power of identifying M_J when $E(M_J|x) = \alpha_1 x$, i.e. when M_J has a linear relationship with x. When the mediator has a quadratic relationship with x as in Equation (6), the small dash line is the power of identifying it using a linear model to fit the $x-m$ relationship, and the dot-dash line is the power for using a smoothing spline. For both the linear model for the linear association and the smoothing spline for the quadratic association, the type-I error rates are controlled well under 0.05.

If a linear model is fitted for the $x-m$ relationship, as c_2 moves to the right of 0, it is more likely to fit a positive coefficient for x. If c_2 moves to the left, a negative coefficient is more likely to fit for x. Therefore the important mediator can be found with more power but with a misleading explanation of the direction of the indirect effect. Using the smoothing spline to fit the relationship, the actual $E(\Delta M_j)$ is $2\alpha c_2 + \alpha$. c_2 still influences the power of detecting m_J, but in a very different way. Figure 5 shows

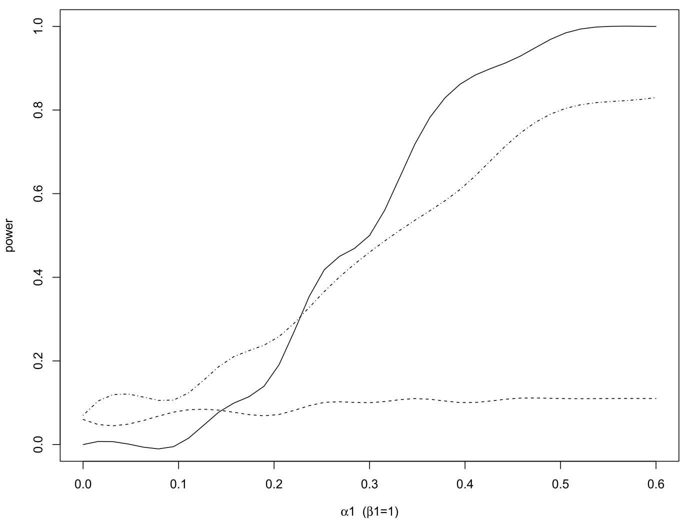

Figure 4. The power of identifying a mediator that is linearly related with x (solid), quadratic related and fitted with linear model (small dashes), quadratic related and fitted with smoothing spline (dot-dashes).

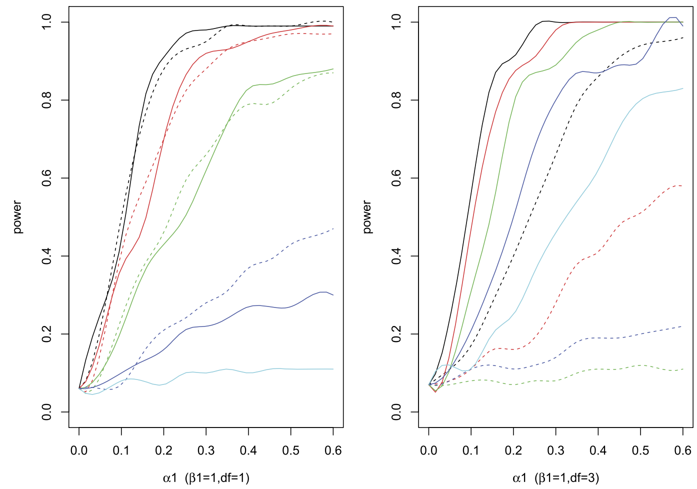

Figure 5. The power of identifying a mediator using linear model (left) or smoothing spline (right) as c_2 changes. Dark blue, light blue, green, red, and black represent $|c_2|$ be $0, 0.25, 0.5, 0.75,$ and 1 respectively. Solid lines are for positive and small-dashes are for negative c_2.

how the power changes with c_2 using linear model (left) or smoothing spline (right). We see that for the linear model, the power increases as c_2 moves away from 0 almost symmetrically. For smoothing spline, the power is the smallest when $c_2 = -0.5$, at which $E(\Delta M_j)$ is 0. As c_2 moves away from -0.5, the power increases. The type-I error rates are controlled a little above 0.05 – the maximum value is 0.07 for all simulations for reasons that have been discussed in Section 3.1. Figure 6 shows the interpretation of the mediation effect when $\alpha_1 = 1$ and $c_2 = 0$. The upper panel shows the boxplots of the coefficients for m^*_{20} from the 100 repeats. The lower panel shows the estimated $\Delta M_{i,20}$ vs. x_i from the first iteration. For the left panel, ΔM is calculated using linear models, and the right using smoothing splines. Smoothing spline method helps to interpret the direction of mediation effect correctly.

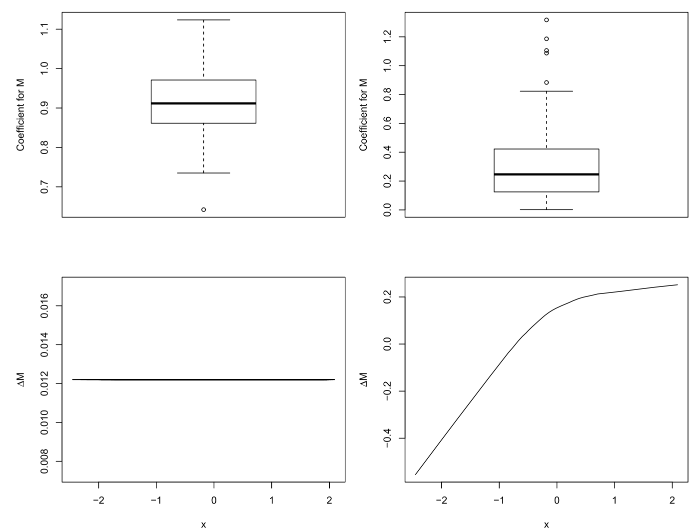

Figure 6. The upper panel shows the boxplots of the coefficients for m^*_{20} from the 100 repeats. The lower panel shows the average estimated ΔM with x. For the left panel, ΔM is calculated using linear model, and the right with smoothing splines.

3.2.2 M-Y relationship is nonlinear

When the $M - Y$ relationship is nonlinear, transformations of the mediators are needed to accurately estimate the mediation effect. Now simulations are generated as in Equations (5), except that the outcome is generated in the following way, which includes an additional important mediator m_J^2:

$$y_i \sim N(\beta_0 x_i + \beta_1 m_{i1} + \beta_1 m_{iJ} + \beta_1 m_{iJ}^2, 1), i = 1, \ldots, n.$$

In such case, m_J^2 is created as a $(J+1)^{th}$ mediator. Figure 7 shows the power of identifying m_J (solid) and m_J^2 (small dashes) as an important mediator separately. Note that the power is defined as the proportion of times that the 95% confidence interval of the average indirect effect does not include 0 and we have $E(m_J^2|x) = \alpha_1 x^2 + 1$, which decreases with x when it is negative. Therefore, when x is negative, the indirect effect from m_J^2 is negative, while the indirect effect is positive when x is positive. When x is centered at 0, the average indirect effect is around 0 even when m_J^2 is an important mediator. Therefore, the power looks to be smaller for m_J^2 than for m_J. When we shift x away from 0, as is shown in Figure 8, the power of identifying m_J^2 becomes

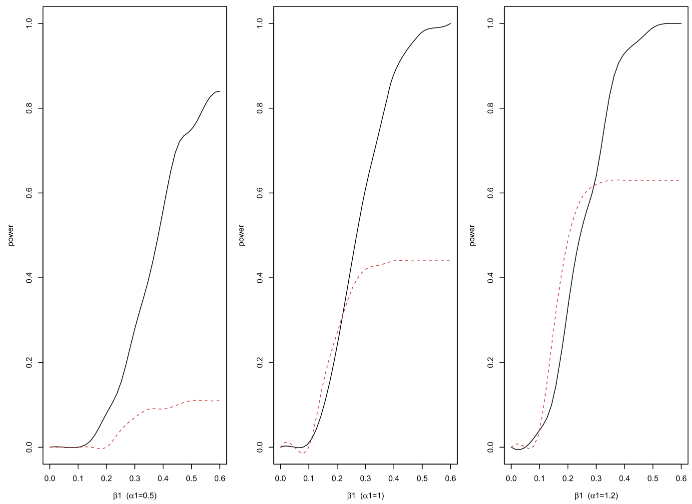

Figure 7. The power of identifying a linear mediator (solid) and a quadratic mediator (dashes).

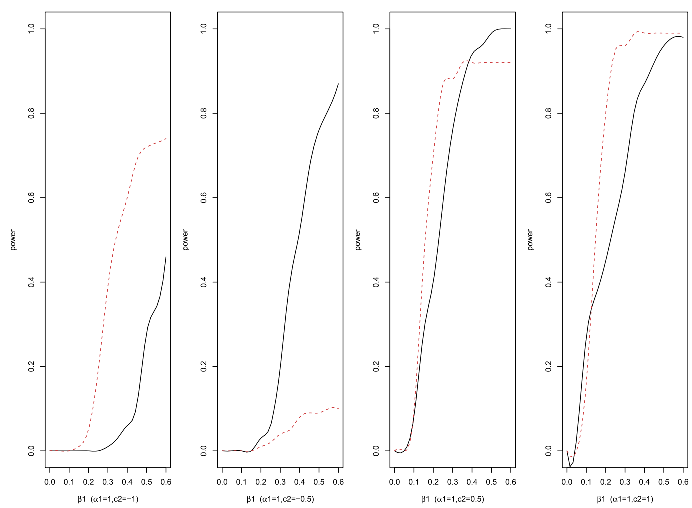

Figure 8. The power of identifying a linear mediator (solid) and a quadratic mediator (dashes) when the center of x shifts among $\{-1, -0.5, 0.5, 1\}$ from left to right.

larger. In Figure 8, the four graphs show the powers when x is centered at $-1, -0.5, 0.5$ and 1 separately from left to right. For all cases, the type-I error rates are controlled well under 0.05.

3.2.3 When mediators are highly correlated

The last simulation study is to compare the proposed method with the traditional CP method. We compare the power of detecting important mediators when mediators are correlated. In this case, we still use equations in (5) to simulate the original data. In addition, we set $J = 10$ and $n = 200$. However, in generating the mediators, we put an autocorrelation structure among the mediators such that

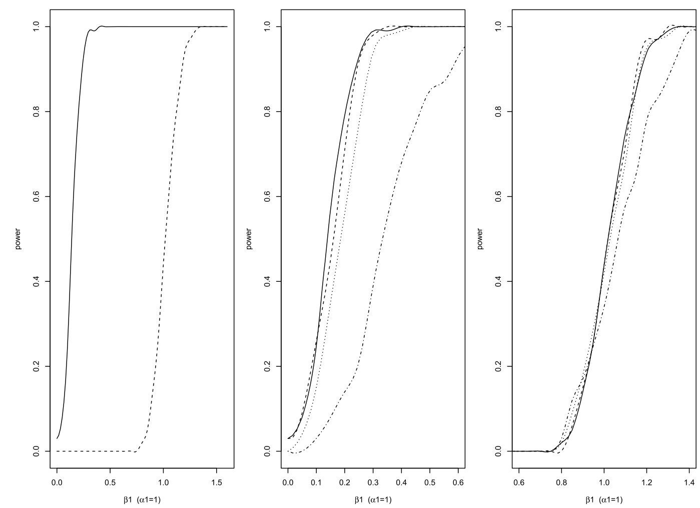

Figure 9. The left panel shows the powers in detecting m_{10} using the mmabig (solid line) and the CP method (small dashes). The middle (mmabig) and right (CP) panels show how the power changes with ρ, where ρ changes among 0 (solid), 0.3 (small dashes), 0.6 (dots), and 0.9 (dot-dashes).

the correlation coefficient between m_{j_1} and m_{j_2} is $\rho^{|j_1-j_2|}$. Figure 9 shows the comparison results. All type-I error rates are under 0.05. The left panel shows the comparison of powers in detecting m_{10} using the proposed method (solid line) and the CP method (small dashes). We found that the proposed method has a much larger power. The middle and right panels show how ρ influences the power by the proposed method (mmabig) and the CP method respectively. Note that to show the figures better, we change the range of the indirect effect for mmabig method to $(0, 0.6)$ and for CP to $(0.8, 1.4)$. We found the pattern that power decreases with ρ for both methods.

3.3 Explore racial disparity in breast cancer survival

Mediation analysis method can be used to explore disparities in health outcomes. The data for this study comes from a Centers for Disease Control and Prevention (CDC) supported Pattern-of-Care (PoC) study to explore the racial disparity in survival among female patients with breast cancer. We use the data set collected by the Louisiana Tumor Registry for the PoC study, which re-abstract medical records of 1453 Louisiana non-Hispanic white and black women diagnosed with invasive breast cancer in 2004. We found that the 3-year death rate of breast cancer is much higher for blacks than for whites (odds ratio = 2.03, [21]). To explore the racial disparity in mortality, multivariate mediation analysis can be used. In this case, the exposure variable x is the binary race (black or white). The outcome of this analysis is time-to-event, where y_i denotes the observed time (either censoring or death time) for subject i and c_i

Table 1. Summary of Mediation Effect Estimations for Breast Cancer Survival

	Mediation Effect (95% CI)	Relative Effect (%)
molecular subtype	0.064 (0.005, 0.144)	30.27 (3.15, 56.33)
age at diagnosis	−0.033 (−0.001, −0.070)	−15.9 (−0.1, −43.0)
tumor stage	0.115 (0.041, 0.189)	54.9 (25.3, 89.5)
tumor size	0.062 (0.003,0.121)	29.5 (5.4, 53.6)
employment	0.004 (0.001, 0.051)	1.3 (0.1, 8.4)
Hormonal Therapy	0.008 (0.001, 0.052)	3.0 (0.1, 8.3)
total effect	0.210 (0.097, 0.394)	100.0

be the indicator that the time corresponds to an event (i.e. $c_i = 1$ for death and $c_i = 0$ for censoring). We consider 25 variables as potential mediators that includes demographic information of the patients, residence environment factors, tumor characteristics at the diagnosis, and treatment information.

A cox model is used to build the hazard function of death in breast cancer. We first transform the mediators to have a rough linear relationship with the outcome and then apply the proposed mediation analysis to the transformed data through the *mmabig* package. Table 1 shows the selected mediators and the estimates of mediation effects in explaining the racial disparity in breast cancer survival. In the table, relative effect is defined as the mediation effect divided by the total effect. Relative effect is explained as that if the corresponding mediator can be manipulated to distribute equally among blacks and whites, the potential change in the racial disparity in survival.

Table 1 shows that the significant mediators include the molecular cancer subtype, age at diagnosis, tumor stage, tumor size, employment status, and hormonal therapy status. The exposure variable, race, is not significant, meaning that mediators considered in the study can completely explain the racial disparity in breast cancer survival. The relative effect of age at diagnosis is negative, which can be explained by Figure 10. The left panel is the boxplot of the coefficients for age in the cox model through the bootstrap samples. The coefficients are mostly positive, meaning that the hazard rate increases with age at diagnosis. The right panel shows the average ΔM for age, which are negative, meaning that compared with whites, blacks were diagnosed at an average younger age. Therefore, if the age at diagnosis can be manipulated so that the age distributions are equivalent among blacks and whites, the racial disparity in breast cancer survival would increase by 15.9%. Rather than explain the disparity, adjust for the age would enlarge the disparity. Thus the relative effect is negative. The identified significant mediators and their estimated indirect effects are similar to [25], which used the nonlinear predictive model, multivariate additive regression trees, for the mediation analysis. The R package *mmabig* provides the plot function to generate similar pictures for each potential mediators in explaining the directions of mediation effects.

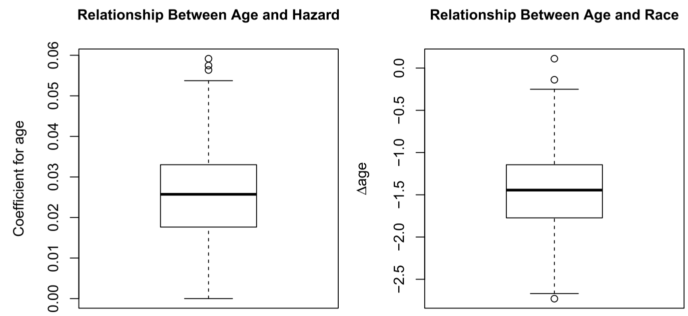

Figure 10. The left panel is the boxplot of the coefficients for age in the cox model through the bootstrap samples. The right panel shows the average ΔM for age.

4. CONCLUSIONS

With the motivation of dealing with large data sets in mediation analysis, we propose a method that use elastic net in mediation analysis. In exploring the exposure-mediator-outcome relationship, we regularize the coefficient of a mediator in predicting the outcome with a weight that is inversely proportional to the association measurement between the exposure variable and the mediator. Therefore, the indirect effects are estimated considering both the exposure-mediator and the mediator-outcome associations. The selection and estimation process were performed simultaneously. We performed a series of sensitivity and specificity analysis to check the factors that can influence the power of identifying important mediators. Further, we illustrate how to deal with potential nonlinear associations in the mediation analysis. The method is used with a real data set to explore factors that explain the racial disparity in survival rates among breast-cancer patients using an elastic net regularized cox hazard function. In the real analysis, five risk factors were identified as important mediators and all racial disparity was explained by the identified factors. As future research, we are going to apply other predictive models for the relationships among variables, and also apply the regularized mediation analysis method with different predictive models to handle various types of outcomes.

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