Overlapping Group Screening for Detection of Gene-environment Interactions with Application to TCGA High-dimensional Survival Genomic Data

Jie-Huei Wang (✉ jhwang@mail.fcu.edu.tw)

Feng Chia University

Kang-Hsin Wang

Feng Chia University

Yi-Hau Chen

Academia Sinica

Research Article

Keywords: gene-environment interaction, joint model, Lasso, overlapping group screening, survival prediction, TCGA

Posted Date: January 27th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1283374/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Overlapping Group Screening for Detection of Gene-environment Interactions

with Application to TCGA High-dimensional Survival Genomic Data

Jie-Huei Wang^{1*}, Kang-Hsin Wang¹ and Yi-Hau Chen²

jhwang@mail.fcu.edu.tw, kkwang92066@gmail.com, and yhchen@stat.sinica.edu.tw

Department of Statistics, Feng Chia University, Seatwen, Taichung 40724, Taiwan¹ and Institute of
Statistical Science, Academia Sinica, Nankang, Taipei 11529, Taiwan²

Abstract

Background: In the context of biomedical and epidemiological research, gene-environment (G-E) interaction is of great significance to the etiology and progression of many complex diseases. In high-dimensional genetic data, two general models, marginal and joint models, are proposed to identify important interaction factors. Most existing approaches for identifying G-E interactions are limited owing to the lack of robustness to outliers/contamination in response and predictor data. In particular, right-censored survival outcomes make the associated feature screening even challenging. In this article, we utilize the overlapping group screening (OGS) approach to select important G-E interactions related to clinical survival outcomes by incorporating the gene pathway information under a joint modeling framework.

Results: Simulation studies under various scenarios are carried out to compare the performances of our proposed method with some commonly used methods. In the real data

applications, we use our proposed method to identify G-E interactions related to the clinical survival outcomes of patients with head and neck squamous cell carcinoma, esophageal carcinoma, and lung adenocarcinoma in The Cancer Genome Atlas clinical survival genetic data, and further establish corresponding survival prediction models. Both simulation and real data studies show that our method performs well and outperforms existing methods in the G-E interaction selection, effect estimation, and survival prediction accuracy.

Conclusions: The OGS approach is useful for selecting important environmental factors, genes and G-E interactions in the ultra-high dimensional feature space. The prediction ability of OGS with the Lasso penalty is better than existing methods. The same idea of the OGS approach can apply to other outcome models, such as the proportional odds survival time model, the logistic regression model for binary outcomes, and the multinomial logistic regression model for multi-class outcomes.

Keywords: gene-environment interaction, joint model, Lasso, overlapping group screening, survival prediction, TCGA

Background

It is believed that in the development of complex diseases such as cancer, diabetes, and so on, gene-environment (G-E) interaction plays a critical role beyond the main genetic (G) or environmental (E) factors ([1-2] and so on). For example, Batchelor et al. [3] showed that the

interaction between the gene TP53 and age affects the prognosis of glioblastoma. As a consequence, incorporating significant G-E interaction factors into a survival prediction model would enhance the performance of the later.

In the setting of high-dimensional genetic data analysis, there exist two ways to identification of important G-E interactions: the marginal and joint analyses [4]. The marginal analysis considers only one gene at a time, and fits a model consisting of multiple E factors, this gene, and its interaction with E factors. the other performs joint analysis and considers all genes in a single model.

In the framework of marginal analysis of high-dimensional genetic data, for each gene, a model consisting of multiple E factors, a single gene itself, and its interaction with E factors is fitted. Specifically, the conceptual marginal model is "Outcome~Es+G+G*(Es)", where the outcome variable can be a continuous, categorical, or survival time phenotype, Es represents a set of environmental factors such as environmental exposures, demographic, clinical, and socioeconomic variables, and G*(Es) represents the interaction between the G factor and all E factors under consideration. The significant G-E interactions can be selected based on the corresponding marginal p-values. Since the marginal model is low-dimensional, its main advantage is its computational stability and conceptual simplicity. Therefore, marginal programs are popular in the fields of bioinformatics and biomedicine. However, a common limitation of traditional methods of marginal analysis is its lack of robustness. In practical genetic studies, Xu et al. [5] pointed out that long-tailed distributions and contamination in

prognosis response and predictors are not uncommon. In addition, human input errors may
 also lead to long-tailed distributions and contamination. In Fig. 1, we analyzed The Cancer
 Genome Atlas (TCGA) clinical survival data for esophageal carcinoma (ESCA) to show the
 long-tailed distribution phenomenon. The long-tailed distributions of TCGA clinical survival
 data for head and neck squamous cell carcinoma (HNSCC) and lung adenocarcinoma (LUAD)
 are displayed in Appendix S.1. Moreover, censored survival outcomes make the relevant
 feature screening difficult.

Fig. 1 The long-tailed distribution of clinical survival data for esophageal carcinoma (the TCGA ESCA)

On the other hand, models in the framework of joint analysis better describe disease
 biology given the fact that complex diseases are related to the combined effects of multiple
 genetic biomarkers. The conceptual joint model is "Outcome~Es+Gs+(Gs)*(Es)", where Gs
 represents a set of G factors, including gene expressions, SNPs and other types of molecular

measurements, and $(Gs)*(Es)$ represents the interactions between all G and E factors. In this article, we focus on the joint analysis framework. A common challenge of joint analysis is its high dimensionality, which makes it difficult to identify significant interaction effects. Moreover, right-censored survival outcomes and contaminated biomarker data make the task even challenging.

For survival outcomes, popular models include the accelerated failure time (AFT) model and Cox's model. Based on the AFT model, several robust joint regression methods have been proposed. The Penalized trimmed regression (PTReg) method [6] uses the trimmed regression to account for long-tailed distribution/contamination in prognosis response and predictors, and Wu et al. [7] incorporates the G structure into the joint modeling. These methods conduct regularized estimation and selection based on the minimax concave penalty (MCP) penalty and utilize a decomposition technique to explain the interaction hierarchy. Their main potential disadvantage is that the model size is much larger than the sample size, and the statistical power under the penalized regression frameworks may be suboptimal [8]. In addition, since the gene expression data is often contaminated, the traditional Pearson correlation or Gaussian graphical models may not be a suitable measure to quantify the correlation among genes [9].

Based on the above rationale, we plan to adopt a two-step screening approach to detect G-E interactions by incorporating biological pathways information. The proposed method uses annotated gene sets collected in the molecular signatures database [10], which can be downloaded from the website <http://www.broadinstitute.org/gsea/msigdb>. Wang and Chen [11]

described the idea of an overlapping group screening procedure, called the OGS method, for survival prediction based on the Cox model. In this work, we extend the OGS method to detect G-E interactions, and show that OGS has several advantages: (i) it can alleviate the collinearity problem in regression analysis due to the correlation between biomarkers in the same gene/pathway; (ii) it can significantly reduce the search space for interaction effects by using the feature grouping structure; and (iii) it can significantly improve the model selection performed by penalized regression in an ultra-high dimensional feature space.

Simulation studies under various scenarios reveal that our method works well and outperforms existing methods in the model selection, estimation, and prediction accuracy. In the real data application, we combine gene expression profile data with prior pathway information from the Gene Ontology biological process (GO-BP) database and use the OGS approach to select several important environmental factors, genes, and G-E interactions that are associated with clinical survival outcomes of patients with HNSCC, ESCA, and LUAD using TCGA clinical survival genetic data [12]. Using the pathway information available from the GO-BP database to group genes into several pathways, we further conduct accurate survival predictions based on the selected main and interacting biomarkers.

Methods

We consider a study with N independent subjects. For a subject i , suppose that there are q environmental/clinical variables $\mathbf{e}_i = (e_{i1}, \dots, e_{iq})'$, and p genes $\mathbf{x}_i = (x_{i1}, \dots, x_{ip})'$ assigned to G possibly overlapping pathways; that is, a given gene may belong to multiple

113 pathways. The pathway information accounts for the natural hierarchical structure of genes,
 114 and the overlapping pathways commonly exist in the gene expression data. Our aim is to
 115 determine the main features (genes and environment) and their interactions related to clinical
 116 survival outcomes, while taking into account the pathway information.

117 For a subject i , assume the survival outcome t_i is related to the environmental/clinical
 118 variables $\mathbf{e}_i$, gene expression covariates $\mathbf{x}_i$, and their component-wise interactions $\mathbf{w}_i =$
 119 $(e_{i1}x_{i1}, \dots, e_{i1}x_{ip}, e_{i2}x_{i1}, \dots, e_{iq}x_{ip})'$ through the Cox regression model. In the Cox
 120 regression framework, the hazard function at time t for subject i 's survival given the
 121 covariates is modeled as

$$122 \quad \lambda(t|\mathbf{e}_i, \mathbf{x}_i, \mathbf{w}_i) = \lambda_0(t) \exp(\mathbf{e}_i' \boldsymbol{\alpha} + \mathbf{x}_i' \boldsymbol{\beta} + \mathbf{w}_i' \boldsymbol{\eta}),$$

123 where $\lambda_0(t)$ is a non-negative deterministic baseline hazard function and $(\boldsymbol{\alpha}, \boldsymbol{\beta}, \boldsymbol{\eta})$ are
 124 corresponding parameters. Usually the survival outcome is subject to censoring, and we use
 125 δ_i to denote whether subject i 's survival time is observed or censored.

126 Incorporating the grouping (pathway) information into the modeling process may improve
 127 the interpretability and prediction accuracy of the model. When groups overlap with each other,
 128 special techniques are required to account for the overlapping grouping information.
 129 According to Jacob et al. [13], we decompose the original coefficient vector into the sum of
 130 group-specific potential effects, that is, $\boldsymbol{\beta} = \sum_{j=1}^G \boldsymbol{\gamma}^j$ where $\boldsymbol{\gamma}^j = (\gamma_1^j, \dots, \gamma_p^j)'$ is the latent
 131 coefficient vector for group j . For $j = 1, \dots, G$ and $k = 1, \dots, p$, we set $\gamma_k^j = 0$ if gene k
 132 does not belong to group j . Redefine the latent coefficient $\boldsymbol{\gamma}^j$ by removing the zero elements

therein, and form the latent coefficient vector $\boldsymbol{\gamma}$ by stacking the vectors $\boldsymbol{\gamma}^1, \dots, \boldsymbol{\gamma}^G$. Let u be the length of $\boldsymbol{\gamma}$. We can then rewrite $\boldsymbol{\beta} = \boldsymbol{S}\boldsymbol{\gamma}$, where $\boldsymbol{S}$ is a $p \times u$ matrix whose elements are 1 or 0. A simple example for illustration is given in Appendix S.2.

On the basis of the coefficient decomposition, the original regression model can be transformed into a new model, that is, $\boldsymbol{X}_{N \times p} \boldsymbol{\beta}_{p \times 1} = \boldsymbol{X}_{N \times p} \boldsymbol{S}_{p \times u} \boldsymbol{\gamma}_{u \times 1} = \tilde{\boldsymbol{X}}_{N \times u} \boldsymbol{\gamma}_{u \times 1}$, where $\boldsymbol{X} = (\boldsymbol{x}_1, \dots, \boldsymbol{x}_N)'$. Equivalently, this new model can be constructed by duplicating the columns of overlapping variables in the original design matrix. For the new transformed model, the hazard function for subject i in the Cox regression model is re-expressed as

$$\lambda(t|\boldsymbol{e}_i, \tilde{\boldsymbol{x}}_i, \boldsymbol{w}_i) = \lambda_0(t) \exp(\boldsymbol{e}_i' \boldsymbol{\alpha} + \tilde{\boldsymbol{x}}_i' \boldsymbol{\gamma} + \boldsymbol{w}_i' \boldsymbol{\eta}).$$

The Method (OGS) for G-E interaction selection

We apply the OGS method to the environment and gene expression profile data with clinical survival trait to detect important main effects as well as interactions by incorporating prior pathway information. The steps of the OGS algorithm for G-E interaction selection are described as follows.

Step1: We utilize the overlapping group Cox regression model to identify the candidate pathways based on the latent effect approach, which can be performed by the R package "*grpregOverlap*" [14]. We define $\hat{M}_{main}$ as the selected set of pathways, and $A = |\hat{M}_{main}|$ as the size of $\hat{M}_{main}$.

Step 2: We utilize the sequence kernel association test (SKAT) to obtain the group-specific significance, where each group is formed by the interaction between the genes of each

153 candidate pathway selected in the first step and the environmental factors in E_s , where E_s is a
 154 set of environmental factors. Following Chen et al. [15], the SKAT statistic under the Cox
 155 regression model is defined as

$$156 \quad Q_k = \mathbf{r}' \mathbf{R}_{(k)} \mathbf{W}_{(k)} \mathbf{W}_{(k)} \mathbf{R}_{(k)}' \mathbf{r}, k = 1, \dots, A$$

157 Here, $\mathbf{r}$ is the vector of martingale residuals estimated from the null model by regressing
 158 survival outcomes on only the environmental covariates E_s without considering the gene
 159 expression data; $\mathbf{R}_{(k)} = [r_{(k)ij}]_{N \times l}$, where l is the number of G-E interaction pairs in the
 160 candidate pathway group k , $r_{(k)ij}$ is the j -th G-E interaction pair of i -th subject in the
 161 candidate pathway group k , and $\mathbf{W}_{(k)}$ is a diagonal weight matrix that contains the weights
 162 of the l interaction pairs in the candidate pathway group k . Suitable weights can improve the
 163 testing power [16]. We utilize the penalized Cox partial likelihood approach with the Ridge
 164 penalty to estimate effect sizes for G-E interaction pairs in each candidate pathway group, and
 165 take the square root of the absolute estimated coefficients as our weights. Based on the null
 166 model by regressing survival outcomes on only the environmental covariates E_s without gene
 167 covariates, let $\mathbf{E}$ is an $N \times q$ design matrix for the q environmental covariates, and $\mathbf{V} =$
 168 $\text{diag}(c_1, \dots, c_N) - \mathbf{P}\mathbf{P}'$, where $\mathbf{P}$ is an $N \times \nu$ matrix with element p_{ij} the baseline hazard
 169 for individual i at ordered failure time $t_{(j)}$, $j = 1, \dots, \nu$, and c_i the cumulative hazard for
 170 individual i at observed time t_i .

171 Let $\Sigma_{(k)} = \mathbf{W}_{(k)} \mathbf{R}_{(k)}' (\mathbf{V} - \mathbf{V}\mathbf{E}(\mathbf{E}'\mathbf{V}\mathbf{E})^{-1} \mathbf{E}'\mathbf{V}) \mathbf{R}_{(k)} \mathbf{W}_{(k)}$ be the covariance matrix of the
 172 vector $\mathbf{W}_{(k)} \mathbf{R}_{(k)} \mathbf{r}$ under the null hypothesis of all gene-environment interaction pairs in the

173 candidate pathway group k having null effects. Under the null hypothesis, the SKAT statistic
 174 follows a mixture chi-square distribution:

$$175 \quad Q_{(k)} \sim \sum_{j=1}^l \lambda_{(k)j} \chi_{1,j}^2,$$

176 where $\lambda_{(k)j}, j = 1, \dots, l$ are the eigenvalues of $\Sigma_{(k)}$, and $\chi_{1,j}^2$'s are independent 1-df central
 177 chi-square random variables.

178 We use the Davies method [17] to approximate the tail probability of the mixture chi-
 179 square distribution, which can be calculated by the R package "*CompQuadForm*" [18].
 180 Generally speaking, the Davies method is accurate [19]. The p-values $\{p_1, \dots, p_A\}$ are used as
 181 our group screening measure; a smaller p-value corresponds to a higher group importance and
 182 therefore leads to a higher priority of selection.

183 Step 3: In the third step, we select significant G-E interactions based on the permutation
 184 procedure with the cutoff point determined by the soft-thresholding rule, where the
 185 permutation is applied to the covariate matrix consisting of both genes and environmental
 186 covariates. We randomly permute the original data $\{Y_i, \mathbf{e}_i, \mathbf{x}_i\}$ to form the permuted data
 187 $\{Y_i, \mathbf{e}_{\pi(i)}, \mathbf{x}_{\pi(i)}\}$ following the null model, where $Y_i = (t_i, \delta_i)$ is the survival outcome,
 188 and $\{\pi(1), \dots, \pi(N)\}$ is a random permutation of the index. Then we apply again the SKAT
 189 test for each of the candidate pathway groups with the permuted data to obtain the group
 190 screening measures (p-values) $\{p_1^*, \dots, p_A^*\}$. We adopt $C_{int} = \min\{p_1^*, \dots, p_A^*\}$ as a cutoff
 191 point to select candidate pathway groups, i.e.

$$192 \quad \hat{M}_{int} = \{b: p_b < C_{int}, b = 1, \dots, A\},$$

is our selected set of candidate pathway groups.

Step 4: Finally, in the framework of joint modeling, based on environmental covariates, and selected genes and G-E interactions, a penalized regression with an appropriate penalty is used to establish the final survival prediction model. Therefore, we apply the penalized Cox's regression together with the Ridge or Lasso penalty to build the final prediction model based on all environmental variables, genes in $\hat{M}_{main}$ and G-E interactions in $\hat{M}_{int}$. Please note that when applying the Ridge penalty, all candidate biomarkers will be retained, and when applying the Lasso penalty, some candidate biomarkers may be removed by estimating their corresponding coefficients to be 0. The penalized Cox regression model with the Ridge or Lasso penalty can be obtained through the R package "*glmnet*" [20].

Results

Comparison with alternative methods in variable selection, estimation, and prediction

In the following simulations, we study the performances of the proposed OGS approach in variable selection, estimation and prediction, and compare them with the performance of the "Oracle", "Univariate Selection" and "Ordinary Lasso" methods. The "Oracle" method is based on the underlying true model, which is known in the simulations but unknown in real applications. The "Univariate Selection" method uses univariate regression to select environmental variables, genes, and G-E interactions one by one, with a controlled false discovery rate (<0.2), and then includes the selected variables in a multivariate Cox regression model to form the final prediction model. The "Ordinary Lasso" method is the penalized Cox

213 regression model with the Lasso penalty considering all environmental variables, genes, and
 214 G-E interactions in the model.

215 For performance comparison, we adopt the root mean squared error (RMSE) to measure
 216 estimation accuracy, defined as

$$217 \quad RMSE = \sqrt{\frac{1}{S} \sum_{j=1}^S (\theta_j - \hat{\theta}_j)^2}$$

218 where S is the size of the full model including all main and interaction covariates and $\theta' =$
 219 (α', β', η') .

220 To evaluate the estimation performance, we report RMSE.M, the mean of the root mean
 221 square errors of 200 simulations. To evaluate the performance of the selection accuracy, we
 222 consider various criteria: T.model is the proportion of the selected models over 200 simulations
 223 containing all the underlying effective variables, including both the main and interaction terms;
 224 Tint.model is the proportion of the selected models over 200 simulations containing all the
 225 underlying effective G-E interaction terms; Sen. is the sensitivity, defined as the proportion of
 226 the underlying effective variables being selected; Spe. is the specificity, defined as the
 227 proportion of the underlying ineffective variables not being selected. We also report the
 228 average size of the selected models, S.model, in 200 simulations. To evaluate the performance
 229 of survival prediction, we consider two measures of prediction accuracy: the deviance and the
 230 c-index proposed by Harrell et al. [21]; smaller deviance or larger c-index corresponds to better
 231 prediction accuracy. The mean values of deviance and c-index over 200 simulations are
 232 reported.

Let $\hat{\theta}' = (\hat{\alpha}', \hat{\beta}', \hat{\eta}')$ an estimator of the (penalized) Cox regression parameter in a prediction model obtained from the training dataset. Let $(t_i^*, \delta_i^*, \mathbf{e}_i^*, \mathbf{x}_i^*, \mathbf{w}_i^*)$ be the survival and covariate data of subject i in the test data. Define $(\mathbf{e}_i^{*'}, \mathbf{x}_i^{*'}, \mathbf{w}_i^{*'})\hat{\theta}$ as the prognosis index (PI) value for subject i in the test data. The Cox test is defined as the p-value of PI when PI is used as the covariate in the univariate Cox model for survival outcomes in the test data. Similarly, the LR-test is the p-value of the log-rank test for the null hypothesis of equal survival between the “good” and “poor” prognostic groups in the test data, where the “good” and “poor” prognostic groups are classified according to whether the PI value is higher or lower than the median PI value in the test data. Smaller Cox-test and LR-test values correspond to better predictive power.

In simulations we consider survival data with a cohort size 300 in the training set, where each subject’s survival time follows the Cox proportional hazards model

$$\lambda_0(t|\mathbf{e}, \mathbf{x}, \mathbf{w}) = 10\exp(\mathbf{e}'\boldsymbol{\alpha} + \mathbf{x}'\boldsymbol{\beta} + \mathbf{w}'\boldsymbol{\eta}),$$

with the covariates $\mathbf{e}$ and $\mathbf{x}$ jointly following a multivariate standard normal distribution with correlation $\text{corr}(\mathbf{e}_{\cdot j}, \mathbf{e}_{\cdot k}) = 0.3^{|j-k|}$ and $\text{corr}(\mathbf{x}_{\cdot j}, \mathbf{x}_{\cdot k}) = 0.5^{|j-k|}$, and $\text{corr}(\mathbf{e}_{\cdot j}, \mathbf{x}_{\cdot k}) = 0$ for all j, k . The censoring time distribution follows a uniform distribution. We then generate survival data, independent of the training data, with a cohort size 100 as the test data to assess the prediction accuracy for different methods.

In this simulation study, we consider 5 environmental variables and assume that the first 4 are related to the survival outcome, and the corresponding effects are 1.5, 2.25, 3, -1.5. On

the other hand, the gene covariates considered contain 25 groups that have different group sizes (the numbers of genes) and may share with each other some of the genes. The group sizes and the overlapping structure (i.e. the number of the shared genes between two overlapping groups) are shown in Table 1, where the overlapping groups are shown side by side. For example, group 1 contains 3 genes, as group 2 does, but the two groups contain only 5 unique genes, and 1 gene is shared between the two groups. As a result, there are a total of 500 genes and 632 group-specific latent effects (see Section 2) in this example. Fig. 2 displays the gene network structure. Groups 1, 7, 13, and 19 are set to be effective, and genes in each of them have constant latent effects of 3, 3, 2, and -2, respectively. In addition, effective interactions (E1*G22, E1*G24, E2*G26) with the corresponding effects (4.5,4.5,6) and (E2*G78, E3*G83, E3*G88) with the corresponding effects (-3, -4.5, -6) are in group 7 and group 13, respectively. The number of effective environment, gene, or G-E interaction factors is 91 among a total of 3,005 such factors. We examine the performances of different methods under a censoring rate of 30%, 50%, or 70%. We also conduct further simulations to demonstrate the performances of the new proposal, whose details and results can be seen in Appendix S.3.

Table 1 Gene group structure in the simulation study

Pathway	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Gene Size	3	3	3	6	6	6	9	9	9	15	15	15	24	24	24	36	36	36	45	45	45	60	60	60	38
Overlapping	1	1	0	2	2	0	3	3	0	5	5	0	8	8	0	12	12	0	15	15	0	20	20	0

Fig. 2 gene network structure

Summary of simulation results

From the simulation results shown in Table 2 and Table S.3 in Appendix S.3 where the gene network structure is complex, we see that the OGS method using the Lasso or Ridge penalty performs substantially better than the "Univariate Selection" and the "Ordinary Lasso" methods in variable selection, effect estimation, and survival prediction. On the other hand, simulation results shown in Table S.4 of Appendix S.3 where a simpler gene structure is considered, the performance of OGS with Lasso or Ridge penalty is worse than that of the "Ordinary Lasso" method when the censoring rate is 30% or 50%; while when the censoring rate is higher (70%), the OGS with the Lasso or Ridge penalty performs better than the "Ordinary Lasso".

Furthermore, further simulation studies with a small cohort size are conducted under the scenario where all simulated settings are the same as those in the previous simulation study

except for a cohort size defined as 150/50 (training/testing). We still obtain similar numeric results patterns; these corresponding results are shown in Table S.5~S.7 in Appendix S.3.

Table 2 The average of the performance measures out of 200 simulation replications for different approaches

	Oracle	Uni. Sel.	Ordinary Lasso	OGS Ridge	OGS Lasso
Censoring rate = 30%					
RMSE	0.4039	0.4196	0.4128	0.4106	0.3830
T.model	1.0000	0.0000	0.0000	0.3650	0.3050
Tint.model	1.0000	0.0000	0.2250	0.3900	0.3900
Sen.	1.0000	0.2507	0.6956	0.9723	0.9114
Spe.	1.0000	0.9978	0.9829	0.9488	0.9920
C.model	91.0000	29.1600	113.1650	237.8050	106.1550
Deviance	-120.1966	-24.9825	-93.6840	-106.4978	-191.8980
C-index	0.8661	0.7092	0.8430	0.8835	0.9126
Censoring rate = 50%					
RMSE	0.3946	0.4179	0.4159	0.4117	0.4021
T.model	1.0000	0.0000	0.0000	0.3250	0.0100
Tint.model	1.0000	0.0050	0.0450	0.3800	0.2850
Sen.	1.0000	0.2577	0.5524	0.9671	0.8172
Spe.	1.0000	0.9970	0.9838	0.9403	0.9933
C.model	91.0000	32.1550	97.4050	262.0400	94.0050
Deviance	-91.7132	-17.1172	-55.4557	-79.1409	-108.8120
C-index	0.8803	0.7278	0.8194	0.8883	0.8925
Censoring rate = 70%					
RMSE	0.3898	0.4198	0.4188	0.4138	0.4114
T.model	1.0000	0.0000	0.0000	0.1800	0.0000
Tint.model	1.0000	0.0000	0.0000	0.2800	0.0500
Sen.	1.0000	0.1666	0.3847	0.8668	0.5627
Spe.	1.0000	0.9973	0.9855	0.9503	0.9955
C.model	91.0000	23.1000	77.1300	223.6400	64.3250
Deviance	22.7686	-1.6320	-24.1135	-46.6483	-45.5560
C-index	0.8517	0.7008	0.7826	0.8709	0.8459

292 Real data application: TCGA HNSCC data

293 The TCGA HNSCC RNA-Seq expression data, which were collected using the IlluminaHiseq
 294 RNAseq V2 platform, together with the phenotype data containing the survival time and
 295 censoring status data for 484 subjects, can be downloaded from the R package “*GEInter*” [22].
 296 The censoring rate of the survival time is about 58%, and gene expression measurements for
 297 a total of 18,409 genes are available in this data. As the number of cancer-related genes is not
 298 expected to be large, we conduct prescreening using marginal Cox models, which can also
 299 improve stability for feature selection. The top 2,000 genes with the smallest p-values in the
 300 marginal (univariate) Cox models are selected for downstream analysis. The five E factors
 301 analyzed including age, gender, AJCC pathologic stage nodes, AJCC pathologic stage tumor,
 302 and ICD O3 site. Summary information for these clinical variables is reported in the Table 3.

303 **Table 3**

304 The selected clinical variables information of the TCGA HNSCC

Variable	Coding	Missing status	Continuous(EC) /Discrete(ED)
age		No	EC
gender	female=0, male=1	No	ED
AJCC pathologic nodes	n0=0, n1=1, (n2, n2a, n2b, n2c)=2, n3=3, nx=4	No	ED
AJCC pathologic tumor	t0=0, t1=1, t2=2, t3=3, (t4, t4a, t4b)=4, tx=5	No	ED
ICD O3 site	(C00.9, C01.9, C02.1, C02.9)=0, (C03.0, C03.1, C03.9, C04.0, C04.9)=1,	No	ED

(C05.0, C05.9
C06.0, C06.2,
C06.9)=2,
(C09.9, C10.3,
C10.9)=3,
(C13.9, C14.8)=4,
and 5 for others.

The PTReg method [5] was developed to conduct robust joint analysis using penalized trimmed regression with the MCP penalty under the AFT model for the right-censored survival outcome. We are interested in comparing the PTReg approach with our proposed OGS approach in the real data application. The whole 12,005 main and G-E interaction predictors are considered for the "Univariate Selection", "Ordinary Lasso", and "PTReg" methods. For the OGS method, among the 2,000 preselected genes, prior pathway information for 1,637 genes, which are mapped into 4,651 pathways based on the GO biological process database, is utilized. The 363 genes that are not mapped into any pathways in the GO biological process database are either discarded or put together as a group for the latent effect analysis in the OGS method, leading to a total of 9,827 or 12,005 main and G-E interaction effects considered.

We take ten random splits of the whole data into 387:97 training/test sets to evaluate the performances of all the methods considered in the TCGA HNSCC data application. Table 4 reports the median of the survival prediction results over the ten folds when the 363 ungrouped genes are discarded from analysis. We see that the performance of the OGS method with Ridge or Lasso penalty is better than the "Univariate Selection", "Ordinary Lasso", and "PTReg" methods. The OGS approach putting the 363 ungrouped genes together as an additional group

results in the same prediction model as the one discarding the ungrouped genes. Also, the OGS analysis results based on the pathway information obtained from other annotated gene set databases, including GO cellular component (GO-CC), GO molecular function (GO-mf), KEGG, and Biocarter, are compared with the other methods for survival prediction in the TCGA HNSCC data, as shown in Table S.8. These additional results based on pathway information from alternative gene set databases still reveal that the OGS approach performs better than the other methods.

Table 4
Results (median of prediction accuracy of different methods in the TCGA HNSCC data over 10 random splits of 387:97 training /test sets based on GO-BP database)

	Uni. Sel.	Ordinary_Lasso	OGS_Ridge	OGS_Lasso	PTReg
Cox-test	0.2181	0.0844	0.0056	0.0074	0.0261
LR-test	0.1411	0.0999	0.0200	0.0301	0.1320
Deviance	1501.3094	14.0777	9.0998	9.5429	57.4644
C-index	0.5570	0.5658	0.6452	0.6354	0.5665

Based on one random split of the data, Fig. 3 displays the Kaplan-Meier survival curves of the “good” and “poor” prognosis groups in the test data. It can be seen that the OGS method separates the two groups better than other methods. When applying the OGS with the Lasso penalty to the entire data based on the GO biological process database, we identify several significant main and G-E interaction effects, and obtain the corresponding parameter estimates, as shown in Table S.9. We note that the clinical variable "AJCC pathological stage tumor" interacts with several genes, and most of

these genes, such as LHX1 [23] and SZT2 [24-25], have been shown to be related to HNSCC.

Fig. 3

Kaplan-Meier curves for the 97 subjects in the TCGA HNSCC testing data. Good and poor groups are identified by the median of the PI scores in the test dataset

Real data application: TCGA ESCA data

The TCGA ESCA RNA-Seq expression data, together with the phenotype data containing the survival time and censoring status data, can be downloaded from the R package 'TCGAbiolinks' [26], or 'UCSCXenaTools' [27]. After excluding patients with missing survival time data, our analysis is focused on the subset of the TCGA ESCA data with

351 368 patients and 20,501 gene expression variables. The censoring rate of the survival time in
 352 the data is about 58%. The TCGA ESCA clinical information data can be obtained from
 353 the 'FireBrowse' database [28].

354 Since the number of cancer-related genes is expected to be limited, when applying the
 355 proposed OGS method, the top 2,000 genes with the smallest p-values based on the marginal
 356 (univariate) Cox models are selected for downstream analysis. The seven clinical variables
 357 whose E effects are analyzed include age, gender, esophageal tumor central location, peson
 358 neoplasm cancer status, race, BMI, and AJCC pathologic stage, and their summary
 359 information is reported in the Table 5. Some of the clinical variables contain missing values,
 360 and we use the sparse boosting method [29] in the R package "*GEInter*" to perform multiple
 361 imputation for the missing values in the clinical variables. Based on the GO biological process
 362 database, 1,440 genes among the top 2,000 genes are mapped into 4,290 pathways and such
 363 prior pathway information is utilized in the OGS method. Excluding the genes without being
 364 mapped into any pathway, there are a total of 11,527 main and G-E interaction covariates in
 365 the proposed OGS method. On the other hand, a total of 16,007 main and G-E interaction
 366 predictors are considered in the "Univariate Selection", "Ordinary Lasso", and "PTReg"
 367 methods.

368 **Table 5**

369 The selected clinical variables information of the TCGA ESCA data

Variable	Coding	Missing status	Continuous(EC) /Discrete(ED)
esophageal tumor central	proximal=1, mid=2,	Yes	ED

location	distal=3		
person neoplasm cancer status	tumor free=1, with tumor=2,	Yes	ED
race	white=1, asian=2, black or African american=3	Yes	ED
BMI	weight/height^2	Yes	EC
AJCC pathologic stage	(stage i , stage ia, stage ib) =1 (stage ii, stage iia, stage iib)=2 (stage iii, stage iiia, stage iiib, stage iiic)=3 (stage iv, stage iva)=4	Yes	ED
age	days_to_birth	No	EC
gender	female=0, male=1	No	ED

We take ten random splits of the whole TCGA ESCA data into 294:74 training/test sets to evaluate the performances of all methods for survival prediction in the TCGA ESCA data. Table 6 reports the median of the survival prediction results among the ten folds. We see that the performance of the OGS method with the Ridge or Lasso penalty is better than the "Univariate selection", "Ordinary Lasso", and "PTReg" methods. In addition to the OGS analysis discarding the 560 genes without mapped pathways in the GO biological process database, we also perform the OGS analysis putting the unmapped genes together as an additional group, and the two different implements of the OGS method result in the same prediction model. Also, different annotated gene sets databases, including GO-CC, GO-MF, KEGG, and Biocarter are also used in the OGS approach to catch pathway information. As

shown in Table S.10. the OGS method still outperforms than the other methods using such alternative pathway information.

Table 6

Results (median of prediction accuracy of different methods in the TCGA ESCA data over 10 random splits of 394:74 training /test sets based on GO-BP database)

	Uni. Sel.	Ordinary_Lasso	TS-OGS Ridge	TS-OGS Lasso	PTReg
Cox-test	0.0190	2.0732e-08	1.4088e-08	2.5767e-09	0.0023
LR-test	0.1561	2.1075e-05	6.8139e-08	1.2715e-07	0.0070
Deviance	256.2041	-14.0819	-19.9024	-27.2311	63.8834
C-index	0.5773	0.7935	0.8551	0.8564	0.7194

Based on one random split of the data, Fig. 4 displays the Kaplan-Meier survival curves for the “good” and “poor” prognosis groups in the test data. It is seen that the two survival curves are better separated by the OGS approach than other methods. When applying the OGS with the Lasso penalty for whole data based on the GO biological process database, we identify and estimate several significant main and G-E interaction effects, which are shown in Table S.11. We find that the two specific genes TSPYL2 (Testis Specific Protein Y-Linked 2) and TSPYL4 (Testis Specific Protein Y-Linked 4) interact with the clinical variables “BMI”, “AJCC pathologic stage”, and “age”.

Fig. 4

Kaplan-Meier curves for the 74 subjects in the TCGA ESCA testing data. Good and poor groups are identified by the median of the PI scores in the test dataset

In addition to the previous analyses based on TCGA data, the analysis results for TCGA LUAD data are provided in Appendix S.4.3. We find that the four specific genes, DKK1, VAX1, EPGN, and EREG, interact significantly with most of the clinical variables mentioned above. In fact, the proposed OGS approach performs consistently well across these different cancer datasets.

Conclusion

In this article, we propose a two-stage overlapping group screening procedure to identify important main and gene-environment (G-E) interaction effects efficiently for survival prediction. In the first stage, the new proposal utilizes the latent effect approach to identify candidate gene pathways for survival prediction, adjusting for the E and G-E interaction factors. Different gene pathways are allowed to overlap with each other, i.e., to share common genes. In the second stage, we utilize the SKAT approach [15], which is a popular group testing approach, to obtain the group-level p-value of each candidate gene pathway as well as the associated G-E factors, adjusting for the E factors. A pathway as well as the associated G-E factors is then selected when their group-level p-value is smaller than the one under covariate (both G and E factors) permutation. The final survival prediction model is constructed by a Cox model based on the E factors, the selected gene pathways as well as the associated G-E factors, subject to the Ridge or Lasso penalty. Simulation and real data studies demonstrate that, compared with the analysis that ignores pathway information, the new proposal can significantly improve the accuracy of gene and gene-environment interaction selection, as well as the resulting survival predictions.

Discussion

The OGS method is flexible. Although we focus on survival prediction based on the Cox proportional hazards model, the same idea can straightforwardly apply to other outcome models, such as the proportional odds survival time model, the logistic regression model for

binary outcomes, and the multinomial logistic regression model for multi-class outcomes. For example, the SKAT statistic involved in the OGS method can be modified simply by using the residuals from the alternative model under consideration.

The OGS method employs the latent effect approach to extract gene network structure information in terms of gene pathways. This requires a pre-designated gene group (pathway) structure and is limited to genes that can be assigned to at least one group (pathway). It is interesting to study how to relax these restrictions to improve the performance of feature selection and survival prediction in the presence some covariate network structure.

Abbreviations

AFT: Accelerated failure time; AJCC: American joint committee on cancer; BMI: Body mass index; ESCA: Esophageal carcinoma; GEInter: Gene–environment interaction; G-E: Gene-environment; GO-BP: Gene Ontology biological process; GO-CC: Gene Ontology cellular component; GO-MF: Gene Ontology molecular function; HNSCC: Head and neck squamous cell carcinoma; Lasso: Least absolute shrinkage and selection operator; LR-test: Log-rank test; LUAD: Lung adenocarcinoma; MCP: Minimax concave penalty; OGS: Overlapping grouped screening; PTReg: Penalized trimmed regression; PI: Predictor index; RMSE: Root mean squared error; SKAT: Sequence kernel association test; SNPs: Single-nucleotide polymorphisms; TCGA: The Cancer Genome Atlas.

Declarations

Author's contributions

444 Conceived and designed the experiments: JH. Analyzed the data: JH, KH. Wrote the first draft
445 of the manuscript: JH. Contributed to the writing of the manuscript: JH, YH. Agree with
446 manuscript results and conclusions: JH, KH, YH. Jointly developed the structure and
447 arguments for the paper: JH, KH, YH. Made critical revisions and approved final version: JH,
448 YH. All authors reviewed and approved of the final manuscript.

449 **Acknowledgements**

450 None

451 **Funding**

452 This research is supported by the grant MOST 110-2118-M-035-001- MY2 from the Ministry
453 of Science and Technology of Republic of China (Taiwan).

454 **Availability of data and materials**

455 The TCGA ESCA, and LUAD genomic data with survival traits and pathway information
456 database analyzed during this study are all available at figshare website
457 https://figshare.com/articles/dataset/TCGA_cancer_genomic_data/16816654. The TCGA
458 HNSCC genomic data can be downloaded from the R package “*GEInter*” [22]. R codes for
459 the simulation studies and real data are available at figshare website
460 https://figshare.com/articles/software/R_codes_for_simulation_and_real_data/16816303.

461 **Ethics approval and consent to participate**

462 Not applicable

463 **Consent for publication**

Not applicable

Competing interests

The authors declare that they have no competing interests.

Reference

1. McAllister K, Mechanic LE, Amos C, et al. Current Challenges and New Opportunities for Gene-Environment Interaction Studies of Complex Diseases. *Am J Epidemiol*. 2017;186(7):753-761. doi:10.1093/aje/kwx227
2. Thomas D. Gene--environment-wide association studies: emerging approaches. *Nat Rev Genet*. 2010;11(4):259-272. doi:10.1038/nrg2764
3. Batchelor TT, Betensky RA, Esposito JM, et al. Age-dependent prognostic effects of genetic alterations in glioblastoma. *Clin Cancer Res*. 2004;10(1 Pt 1):228-233. doi:10.1158/1078-0432.ccr-0841-3
4. Zhou F, Ren J, Lu X, Ma S, Wu C. Gene-Environment Interaction: A Variable Selection Perspective. *Methods Mol Biol*. 2021;2212:191-223. doi:10.1007/978-1-0716-0947-7_13
5. Xu Y, Wu M, Zhang Q, Ma S. Robust identification of gene-environment interactions for prognosis using a quantile partial correlation approach. *Genomics*. 2019;111(5):1115-1123. doi:10.1016/j.ygeno.2018.07.006
6. Xu Y, Wu M, Ma S, Ahmed SE. Robust gene-environment interaction analysis using

penalized trimmed regression. *J Stat Comput Simul.* 2018;88(18):3502-3528.
doi:10.1080/00949655.2018.1523411

7. Wu M, Zhang Q, Ma S. Structured gene-environment interaction analysis. *Biometrics.* 2020;76(1):23-35. doi:10.1111/biom.13139

8. Fan J, Lv J. Sure independence screening for ultrahigh dimensional feature space (with discussion). *J. R. Stat. Soc. B.* 2008;70(5):849–911. <https://doi.org/10.1111/j.1467-9868.2008.00674.x>

9. Wang JH, Chen YH. Network-adjusted Kendall's Tau Measure for Feature Screening with Application to High-dimensional Survival Genomic Data [published online ahead of print, 2021 Jan 29]. *Bioinformatics.* 2021;btab064. doi:10.1093/bioinformatics/btab064

10. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A.* 2005;102(43):15545-15550. doi:10.1073/pnas.0506580102

11. Wang, JH., Chen, YH. Overlapping group screening for detection of gene-gene interactions: application to gene expression profiles with survival trait. *BMC Bioinformatics* **19**, 335 (2018). <https://doi.org/10.1186/s12859-018-2372-2>

12. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature.* 2008;455(7216):1061-1068. doi:10.1038/nature07385

13. Jacob L, Obozinski G, Vert JP. Group lasso with overlap and graph lasso. In: Proceedings

of the 26th annual international conference on machine learning. Montreal: ACM; 2009. p. 433–40.

14. Zeng Y, Breheny P. Overlapping Group Logistic Regression with Applications to Genetic Pathway Selection. *Cancer Inform.* 2016;15:179-187. doi:10.4137/CIN.S40043

15. Chen H, Lumley T, Brody J, et al. Sequence kernel association test for survival traits. *Genet Epidemiol.* 2014;38(3):191-197. doi:10.1002/gepi.21791

16. Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X. Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet.* 2011;89(1):82-93. doi:10.1016/j.ajhg.2011.05.029

17. Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X. Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet.* 2011;89(1):82-93. doi:10.1016/j.ajhg.2011.05.029

18. Davies RB, Algorithm AS. 155: The distribution of a linear combination of x^2 random variables. *J R Stat Soc Ser C Appl Stat.* 1980;29(3):323–33.

19. Duchesne P, Lafaye De Micheaux P. Computing the distribution of quadratic forms: Further comparisons between the Liu-Tang-Zhang approximation and exact methods. *Comput Stat Data Anal.* 2010;54(4):858–62.

20. Wu B, Guan W, Pankow JS. On Efficient and Accurate Calculation of Significance P-Values for Sequence Kernel Association Testing of Variant Set. *Ann Hum Genet.* 2016;80(2):123-135. doi:10.1111/ahg.12144

21. Simon N, Friedman J, Hastie T, Tibshirani R. Regularization Paths for Cox's Proportional Hazards Model via Coordinate Descent. *J Stat Softw.* 2011;39(5):1-13. doi:10.18637/jss.v039.i05
22. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med.* 1996;15(4):361-387. doi:10.1002/(SICI)1097-0258(19960229)15:4<361::AID-SIM168>3.0.CO;2-4
23. Wu M, Qin X, Ma S. GEInter: an R package for robust gene-environment interaction analysis. *Bioinformatics.* 2021;37(20):3691-3692. doi:10.1093/bioinformatics/btab318
24. Zhang, B., Wang, H., Guo, Z. *et al.* A panel of Transcription factors identified by data mining can predict the prognosis of head and neck squamous cell carcinoma. *Cancer Cell Int* **19**, 297 (2019). <https://doi.org/10.1186/s12935-019-1024-6>
25. Tan FH, Bai Y, Saintigny P, Darido C. mTOR Signalling in Head and Neck Cancer: Heads Up. *Cells.* 2019;8(4):333. doi:10.3390/cells8040333
26. Sakai A, Ando M, Fukusumi T, et al. Aberrant expression of CPSF1 promotes head and neck squamous cell carcinoma via regulating alternative splicing. *PLoS One.* 2020;15(5):e0233380. doi:10.1371/journal.pone.0233380
27. Colaprico A, Silva TC, Olsen C, et al. TCGAAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res.* 2016;44(8):e71. doi:10.1093/nar/gkv1507

543 28. Wang S, Liu X. The UCSCXenaTools R package: a toolkit for accessing genomics data
544 from UCSC Xena platform, from cancer multi-omics to single-cell RNA-seq. *J. Open*
545 *Source Softw.* 2019;4(40):1627. <https://doi.org/10.21105/joss.01627>

546 29. Deng M, Brägelmann J, Kryukov I, Saraiva-Agostinho N, Perner S. FirebrowseR: an R
547 client to the Broad Institute's Firehose Pipeline. *Database (Oxford)*. 2017;2017:baw160.
548 Published 2017 Jan 6. doi:10.1093/database/baw160

549 30. Wu M, Ma S. Robust semiparametric gene-environment interaction analysis using sparse
550 boosting. *Stat Med*. 2019;38(23):4625-4641. doi:10.1002/sim.8322

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [BMCOGSAppendix.docx](#)