SOFTWARE TOOL ARTICLE

GAC: Gene Associations with Clinical, a web based application

[version 1; peer review: 1 approved with reservations]

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Abstract

We present GAC, a shiny R based tool for interactive visualization of clinical associations based on high-dimensional data. The tool provides a web-based suite to perform supervised principal component analysis (SuperPC), an approach that uses both high-dimensional data, such as gene expression, combined with clinical data to infer clinical associations. We extended the approach to address binary outcomes, in addition to continuous and time-to-event data in our package, thereby increasing the use and flexibility of SuperPC. Additionally, the tool provides an interactive visualization for summarizing results based on a forest plot for both binary and time-to-event data. In summary, the GAC suite of tools provide a one stop shop for conducting statistical analysis to identify and visualize the association between a clinical outcome of interest and high-dimensional data types, such as genomic data. Our GAC package has been implemented in R and is available via <http://shinygispa.winship.emory.edu/GAC/>. The developmental repository is available at <https://github.com/manalirupji/GAC>.

Keywords

SuperPC, binary outcome, continuous, time-to-event, forest plot

This article is included in the **RPackage** gateway.

Open Peer Review

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Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Heterogeneity in terms of tumor characteristics, prognosis, and survival among cancer patients has been a persistent problem for many decades. Currently, prognosis and outcome predictions are solely based on clinical factors. However, inaccurate prognosis and prediction may result from using only these. For example, based on clinical factors only, two tumor patients may have the same prognosis, but may not respond to the same treatment as the tumors may have a completely different molecular composition¹⁻⁵.

One way to improve prognosis and clinical outcome predictions in cancer is to examine such associations using a tumor's genomic profile. To achieve this goal, Bair and Tibshirani⁶ proposed a supervised principal component (SuperPC) method to identify subtypes of cancer. The method uses both gene expression and clinical data to diagnose future patients. This approach was applied to several publicly available datasets, showing that it was able to accurately predict the clinical outcome of interest based on a given gene expression profile. Since its inception, SuperPC has been introduced as a powerful tool for cancer diagnosis and treatment.

Currently, the SuperPC method has been developed as an R package, 'superpc' but as it stands, it is unable to address the following:

- 1) clinical association based on a binary outcome (e.g. responders versus non-responders);
- 2) ease of use for clinicians and researchers with limited programming skills; and
- 3) a visual summary of results.

To address these limitations, we developed GAC: Gene Association with Clinical, an interactive, GUI-based web-based application for analysis of gene associations with various clinical outcomes of interest. We developed GAC based on the R packages 'shiny' and 'superpc'. Our GAC tool enables the user to perform a SuperPC analysis for three types of outcomes: time-to-event, continuous, and binary, and provides a summary of results using forest plots that may be readily exported into a file.

Methods

Supervised principal component analysis

SuperPC is a generalization of principal component analysis, which generates a linear combination of the features or variables of interest that capture the directions of largest variation in a dataset. Instead of using the whole dataset directly, SuperPC defines a list of genes based on their association with an outcome of interest. To select the list of genes, a univariate score for each gene is calculated and those features (a.k.a., genes) whose score exceeds a threshold are retained as input into a principal component analysis, based on the retained features. For details, refer to Bair and Tibshirani⁶.

Time-to-event outcome

SuperPC for time-to-event was conducted using the 'superpc' package in R. Depending on the sample size of the original dataset; the researcher selects what proportion of the dataset to split into training and testing. The researcher can also specify how many numbers to test to check which the optimal threshold is. The number of folds for cross validation to determine the threshold also needs to be determined. There is also an option to run the analysis randomly, or upload fold IDs to replicate an analysis that was previously carried out. The association between the time-to-event outcome and the predicted principal component may be represented in a KM plot by dichotomizing the principal component using the median (Figure 1).

Continuous outcome

SuperPC for continuous outcomes is implemented using the 'superpc' package in R, with the same options as time-to-event analysis. The predicted principal component is presented visually as continuous values through a scatter plot along with Pearson's correlation (Figure 2). The predicted principal component could also be presented as binary groups (cutoff at median) through a boxplot, with a t-test applied.

Binary outcome

SuperPC for binary outcome follows the same analysis workflow as the other two outcomes. The predicted principal component can be visualized as either a continuous variable through a box plot, with a t-test to summarize the statistical association (Figure 3), or as binary groups (cutoff at median) using a bar plot, with a chi-square test to summarize the statistical association between the predicted and the observed outcome.

Forest plot

A forest plot is a graphical display of point estimates of association widely used in meta-analysis. It has become popular for displaying the associations between clinical and genomic data. With our GAC tool, users have the option to generate a forest plot to display results (Figure 4).

Implementation

The GAC tool is written in R and tested using version 3.3.0. The interactive plots and data tables are made available using the shiny R package (www.rstudio.com/shiny).

Operation

Using a windows 7 Enterprise SP1 PC with a 32.0 GB RAM and a 3.30 GHz Intel® Xeon® Processor E5 Family, the time-to-event and regression analysis with 45 patients from the TCGA BRCA dataset was completed in 2.54 s and 1.80 s, respectively. The binary outcome analysis using the CoMMPass data of 135 patients was completed in 65.05 s. Source code is available at <https://doi.org/10.5281/zenodo.803309>.

Figure 1. SuperPC time-to-event outcome. The interface shows an example SuperPC for time-to-event outcome. The left panel allows the user to select the various options such as data split, the optimal threshold, number of folds and a choice of generating new fold IDs or using a pre-existing set to replicate results. The right panel includes the results of the analysis. The KM plot displays the association of the outcome with the predicted principal component by the median. In addition, the univariate analysis regression scores and tables are also available for download.

Figure 2. SuperPC continuous outcome. The interface shows an example SuperPC for continuous outcome. Similar to Figure 1, the user can choose the appropriate settings using the left panel and view the results of the analysis to the right. The association of the continuous outcome with the predicted principal component is summarized using a scatter plot (as seen). The user can alternatively choose to summarize these results through boxplots by dichotomizing the predicted principal component based on the median. As in Figure 1, the univariate regression scores and plots are available for download in the left panel.

Figure 3. SuperPC binary outcome. The interface shows an example SuperPC for binary outcome. The user can opt for similar options as in the previous figures. Also, as in Figure 2, the user has a choice to display the continuous predicted principal component as a scatter plot, or divide it into binary discrete groups (using median cut-off) to represent the association through a barplot. Similar download options are available.

Figure 4. Forest plot. The interface shows an example forest plot. The left side comprises a user menu and the right includes the result plot. Users can upload their own summarized results with hazard ratio and confidence intervals for the survival outcome, or odds ratio and confidence intervals for the binary outcome. For graphical display, the researchers could choose to input different labels, font sizes and colors.

Discussion

GAC is a suite of tools that allows the user to conduct statistical analysis to identify and visualize the association between clinical outcomes of interest and genomic data using an interactive application in R.

Data and software availability

For SuperPC time-to-event and regression analysis, we used TCGA BRCA RNASeqV2 gene expression and clinical data, downloaded from the TCGA data portal (now accessed at <https://portal.gdc.cancer.gov/>)⁷. The data included 380 differentially expressed genes when favorable (patients who did not die with at least 7 years of follow up) and unfavorable (patients who died 30 months post-treatment) outcomes from 45 patients were compared.

For SuperPC binary outcome analysis, CoMMPass IA9 RNASeq expression and clinical data was downloaded from the publicly available [Multiple Myeloma Research Foundation](https://research.themmrf.org/rp/download) (MMRF) database (<https://research.themmrf.org/rp/download>). Patient cytogenetics for outcome dichotomization was obtained from the MMRF data portal's 'Analysis Tools' section (<https://research.themmrf.org/rp/explore/>). 135 patients with clinical, gene

expression and copy number data were classified as high risk based on cytogenetics and the remaining 343 as not high risk. Among these patients, the top 1450 most variable genes were used.

The example data used in this article is available in Zenodo (<https://doi.org/10.5281/zenodo.803309>).

License: GAC is available under the GNU public license (GPL-3).

Competing interests

No competing interests were disclosed.

Grant information

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Shengjie Yang

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The authors did a nice job to develop a web tool called GAC, which is used to explore the association between clinical and genomic data. Essentially, GAC is a GUI of R package 'superpc'. They not only provided a handy web tool but also published the source code to allow researchers to do some further expansion according to their own specific study objects. The manuscript is well written, but I have some comments:

1. Since the example data is from TCGA and this work aims to facilitate clinicians and researchers with limited programming skills, it is necessary to cite some literature to tell readers how to retrieve and process the TCGA Data, such as TCGA-Assembler (Nature methods, 2014), and it would be better if such tools can be embedded into GAC.
2. The authors stated that 'superpc' is unable to address the limitation about clinical association based on a binary outcome in the Introduction. But in the Methods section, it said 'SuperPC for binary outcome follows the same analysis workflow as the other two outcomes'. It confuses me and please explain the details.
3. I find that the calculation will be automatically triggered when changes any one option in the sidebar. That is not a user-friendly experience, especially it happens in the 'Super PC Binary Outcome' module which can not return the result immediately. Think about that if the users want to change two default options, they have to adjust the first one, wait a few seconds and then adjust the second option, which is not efficient. So adding a 'submit' button to trigger the calculation is a better way.
4. Build a user manual page to explain the details of each function and use an example to demonstrate the usage.

Is the rationale for developing the new software tool clearly explained?

Partly

Is the description of the software tool technically sound?

Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?

Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?

Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 Sep 2017

Manali Rupji, Winship Cancer Institute of Emory University, Atlanta, USA

We thank the reviewer for their thoughtful comments that have led to an improved paper. Below, we respond individually to each comment.

1. Since the example data is from TCGA and this work aims to facilitate clinicians and researchers with limited programming skills, it is necessary to cite some literature to tell readers how to retrieve and process the TCGA Data, such as TCGA-Assembler (Nature methods, 2014), and it would be better if such tools can be embedded into GAC.

Various tools are available for extracting TCGA data (now available through GDC data portal). Data can be downloaded directly from the GDC data portal (<https://gdc-portal.nci.nih.gov/>) or by use of R packages. Some of the more recent packages include TCGA Biolinks (Colaprico *et. al*, 2015) which is available through Bioconductor and the TCGA Assembler (Zhu *et. al*, 2014) available as an R package. Users may refer to the detailed tutorials of these tools for instructions on extracting TCGA data types.

2. The authors stated that 'superpc' is unable to address the limitation about clinical association based on a binary outcome in the Introduction. But in the Methods section, it said 'SuperPC for binary outcome follows the same analysis workflow as the other two outcomes'. It confuses me and please explain the details.

We have included a sentence in the methods section that clarifies how the superPC tool has been extended to include analysis of binary outcome data.

3. I find that the calculation will be automatically triggered when changes any one option in the sidebar. That is not a user-friendly experience, especially it happens in the 'Super PC Binary Outcome' module which cannot return the result immediately. Think about that if the users want to change two default options, they have to adjust the first one, wait a few seconds and then adjust the second option, which is not efficient. So adding a 'submit' button to trigger the calculation is a better way.

A 'Run Analysis' submit button is now available under each analysis option that will update results based on user-specified options to multiple parameters, as opposed the previous version which triggered a calculation after each separate change.

4. Build a user manual page to explain the details of each function and use an example to demonstrate the usage.

We have included a tutorial as a separate tab within the tool to demonstrate the usage of each function with example data.

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Competing Interests: No competing interests were disclosed.

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