

# exposomeShiny User's Guide

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# Chapter 1

## Overview



exposomeShiny is a data analysis toolbox with the following features:

- Data handling: imputation, LOD, transformation, ...
- Exposome characterization
- Exposome-wide association analysis
- Multivariate association
- Omic data integration
- Omic data association
- Post-omic data analysis: CTD database
- Post-omic data analysis: Enrichment analysis

To do so, exposomeShiny relies on previously existent Bioconductor packages (rexosome, omicReXosome and CTDquerier among others), it uses them in a seamless way so the final user of exposomShiny can perform the same studies that would conduct using the Bioconductor packages but without writing a single line of code.



# Chapter 2

## Setup

### 2.1 R, RStudio and Packages Versions

If the user chooses to install and use exposomeShiny using RStudio instead of Docker, the list of package versions used for the development of the application is provided for stability purposes. When using the Docker version of the application, all of the following is bundled on the image so the user does not have to deal with the installation of any package.

Software:

R software	Version
R	4.0.2
RStudio	1.4.1103

R packages:

R Packages	Version
shiny	1.5.0
shinyBS	0.61
rexposome	1.12.2
omicRexposome	1.12.0
MultiDataSet	1.18.0
mice	3.11.0
DT	0.16
ggplot2	3.3.2
data.table	1.13.2
truncdist	1.0
shinyalert	2.0.0

R Packages	Version
shinydashboard	0.7.1
shinyjs	2.0.0
TxDb.Hsapiens.UCSC.hg19.knownGene	3.2.2
org.Hs.eg.db	3.12.0
GenomicRanges	1.42.0
CTDquerier	1.4.3
shinycssloaders	1.0.0
pastecs	1.3.21
shinyWidgets	0.5.4
clusterProfiler	3.18.1
enrichplot	1.10.2
ggupset	0.3.0
imputeLCMD	2.0
mixOmics	6.14.0

There are two different ways of setting up and using exposomeShiny

## 2.2 Downloading the source files, installing the libraries and running the application

The user can choose to download the source code of the shiny application and install all the required libraries on their local R installation. Make sure Rtools is installed to use this method.

```
# Set working directory
setwd(dir = "/some/path/")

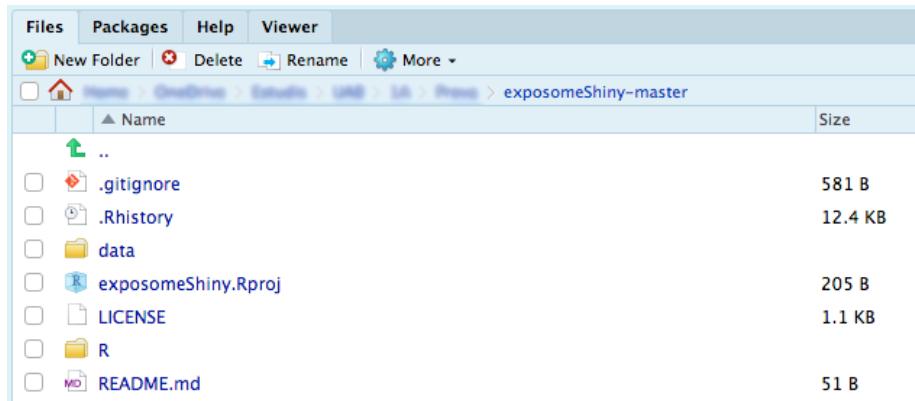
# Download zip
download.file(url = "https://github.com/isglobal-brge/exposomeShiny/archive/master.zip")

# Unzip the .zip to the working directory
unzip(zipfile = "master.zip")

# Set the working directory inside the downloaded folder
setwd(dir = "/some/path/exposomeShiny-master")
```

Now all the source files are downloaded to the location of chose and the working directory moved to the correct folder, to start the project, open the Rproj file by clicking it on the Files explorer of RStudio.

## 2.2. DOWNLOADING THE SOURCE FILES, INSTALLING THE LIBRARIES AND RUNNING THE APPLICATION



Once the project is loaded, the file found on the source folder called `installer.R` has to be sourced and run. This will install the newest versions of the packages required by Exposome Shiny on this R session. To do so, run the following code on the RStudio console.

```
source("installer.R")
```

This is only needed on the first run, once completed it doesn't need to be done prior to launching the application itself any other time.

Now everything is ready to launch the Shiny application. To do so there are two approaches, one is to open the `ui.R` or the `server.R` files that are inside the `R` folder and press `Run App`.

The screenshot shows the RStudio code editor with the file `ui.R` open. The code contains the following library imports:

```
1 library(shiny)
2 library(shinyBS)
3 library(rexposome)
4 library(omicRexposome)
5 library(MultiDataSet)
6 library(mice)
7 library(DT)
8 library(ggplot2)
9 library(ggiraph)
10 library(data.table)
11 library(truncdist)
```

Or the other option is to input the following command on the console.

```
shiny::runApp('R')
```

## 2.3 Pulling the official Docker image from DockerHub

If there's any trouble downloading the required R packages to make exposomeShiny work, there's the option of using Docker. It has the disadvantage of being a little bit difficult to install on a Windows machine, however, it's extremely simple on a Mac OS X / Linux environment. For the Windows users refer to the following links for instructions on how to install Docker and setup your machine to run WSL2 and launch bash commands on Windows 1, 2, 3.

To download and launch exposomeShiny, execute the following command on a bash terminal(make sure Docker is running, if not search for the Docker Desktop app and launch it).

```
docker run --rm -p 80:80 brgelab/exosome-shiny
```

This command will download the Docker image of exposomeShiny (be aware it weights ~ 3 GB, so if your internet connection is slow it may take a while) and run a container with it. The container will be exposed on the local port 80 and it will render on that port the application itself, so to start using exposomeShiny open your web browser and go to the site

```
localhost:80
```

At the beginning it may take some time for the application to render, this is because all the needed R libraries are being loaded, to be sure the container is actually working, take a look at the terminal where you inputed the Docker command, there you will see all the R verbose stating the libraries are being loaded.

Once the user has finished using exposomeShiny, the container needs to be stopped to avoid wasting CPU resources, to do so, input the following command on a bash terminal (the command needs to be inputted on a new bash window):

```
docker container ls
```

This will prompt all the running containers, find the one with the NAMES `brgelab/exosome-shiny` and copy its CONTAINER ID, then input the following bash command:

```
docker stop xxxxxxxxxxxx
```

Where `xxxxxxxxxxxx` is the CONTAINER ID.

To run the application again, just enter the first bash command (`docker run --rm -p 80:80 brgelab/exosome-shiny`), since it has already been downloaded, the application is cached on the computer and it will launch straight

### *2.3. PULLING THE OFFICIAL DOCKER IMAGE FROM DOCKERHUB*

away. If the user wants to remove the Docker image from the computer, input the following bash command:

```
docker image rm brgelab/exosome-shiny
```



# Chapter 3

## Data sets

### 3.1 Exposome dataset

The exposome is composed of three different files (in `*.csv`, `*.tsv` or `*.txt` format). Those files are referred inside the Shiny as exposures, description and phenotypes. Their content is the following:

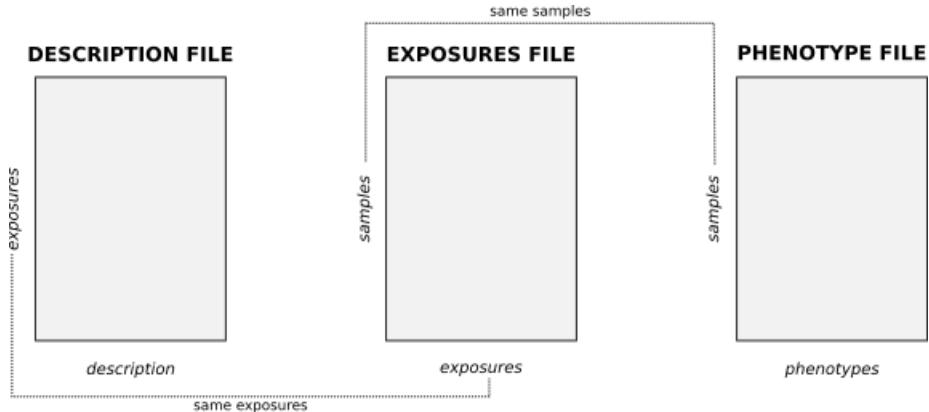
- The `exposures` file contains the measures of each exposure for all the individuals included on the analysis. It is a matrix-like file having a row per individual and a column per exposures. It must include a column with the subject's identifier.
- The `description` file contains a row for each exposure and, at last, defined the families of exposures. Usually, this file incorporates a description of the exposures, the matrix where it was obtained and the units of measurement among others.
- The `phenotypes` file contains the covariates to be included in the analysis as well as the health outcomes of interest. It contains a row per individual included in the analysis and a column for each covariate and outcome. Moreover, it must include a column with the individual's identifier.

Some remarks regarding this files:

- All three files have to share the same separator element, for `*.csv` files is typical to use a comma (,) but it could also be a semicolon (;).
- The exposure names have to start with a character [a-z/A-Z], leading special characters will cause the data entry to return errors.
- Exactly the same exposures have to be present on the description and exposures files.
- Exactly the same samples have to be present on the exposures and phenotypes files.
- The exposures and phenotypes files have an ID column, the description

file does not have an ID column nor row.

A visual representation of the three matrices and how they correlate is the following.



## Exposures data file example:

<b>id</b>	<b>bde100</b>	<b>bde138</b>	<b>bde209</b>	<b>PFOA</b>	...
sub01	2.4665	0.7702	1.6866	2.0075	...
sub02	0.7799	1.4147	1.2907	1.0153	...
sub03	-1.6583	-0.9851	-0.8902	-0.0806	...
sub04	-1.0812	-0.6639	-0.2988	-0.4268	...
sub05	-0.2842	-0.1518	-1.5291	-0.7365	...
...	...	...	...	...	...

## Description data file example:

exposure	family	matrix	description
bde100	PBDEs	colostrum	BDE 100 - log10
bde138	PBDEs	colostrum	BDE 138 - log10
bde209	PBDEs	colostrum	BDE 209 - log10
PFOA	PFAS	cord blood	PFOA - log10
PFNA	PFAS	cord blood	PFNA - log10
PFOA	PFAS	maternal serum	PFOA - log10
PFNA	PFAS	maternal serum	PFNA - log10
hg	Metals	cord blood	hg - log 10
Co	Metals	urine	Co (creatinine) - log10
Zn	Metals	urine	Zn (creatinine) - log10
Pb	Metals	urine	Pb (creatinine) - log10
THM	Water	---	Average total THM uptake - log10
CHCL3	Water	---	Average Chloroform uptake - log10
BROM	Water	---	Average Brominated THM uptake - log10
NO2	Air	---	NO2 levels whole pregnancy- log10
Ben	Air	---	Benzene levels whole pregnancy- log10

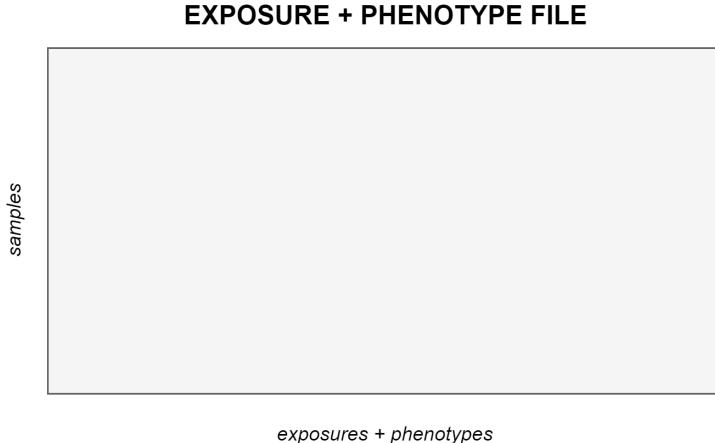
## Phenotypes data file example:

id	asthma	BMI	sex	age	...
sub01	control	23.2539	boy	4	...
sub02	asthma	24.4498	girl	5	...
sub03	asthma	15.2356	boy	4	...
sub04	control	25.1387	girl	4	...
sub05	control	22.0477	boy	5	...
...	...	...	...	...	...

### 3.2 Plain datasets

If the researcher has gathered all the data on a single file which contains both phenotype and exposure data, this file can be used too. The user interface has a selector for it, more information on the correspondent section.

A visual representation of a plain dataset is the following.



\* the exposures and phenotypes columns can be mixed

\* the Family infomation (Description file) can be manually set by the user when using the application

Plain dataset example (3 exposures + 2 phenotypes):

<b>id</b>	<b>bde100</b>	<b>bde138</b>	<b>bde209</b>	<b>asthma</b>	<b>BMI</b>	...
sub01	2.4665	0.7702	1.6866	control	23.2539	...
sub02	0.7799	1.4147	1.2907	asthma	24.4498	...
sub03	-1.6583	-0.9851	-0.8902	asthma	15.2356	...
sub04	-1.0812	-0.6639	-0.2988	control	25.1387	...
sub05	-0.2842	-0.1518	-1.5291	control	22.0477	...
...	...	...	...	...	...	...

### **3.3 Omics dataset**

The omics data inputed to the Shiny must be provided as an `*.RData`. This file has to contain an `ExpressionSet`, which is an S4 object. This object is a data container of the Bioconductor toolset.

For further information on `ExpressionSet` and how to create and manipulate them, please visit the official documentation and this selected vignette.

# Chapter 4

## Bioconductor packages

This Shiny application is a front end support for other Bioconductor packages in order to provide a comfortable environment on to conduct different analysis with those packages. In concrete the packages are rexposome, omicRexposome and CTDquerier.

### 4.1 rexposome

Rexposome is a package that allows to explore the exposome and to perform association analyses between exposures and health outcomes.

### 4.2 omicRexposome

OmicRexposome is a package that systematizes the association evaluation between exposures and omic data, taking advantage of MultiDataSet for coordinated data management, rexposome for exposome data definition and limma for association testing. Also to perform data integration mixing exposome and omic data using multi co-inherent analysis (omicade4) and multi-canonical correlation analysis (PMA).

### 4.3 CTDquerier

CTDquerier is a package to retrieve and visualize data from the Comparative Toxicogenomics Database. The downloaded data is formated as DataFrames for further downstream analyses.



# Chapter 5

## Analysis flowcharts

On this section, a detailed guide on how to perform different analyses using exposomeShiny will be provided. The guide contains screenshots of the analysis steps as well as some flowcharts.

### 5.1 Exposome health analysis

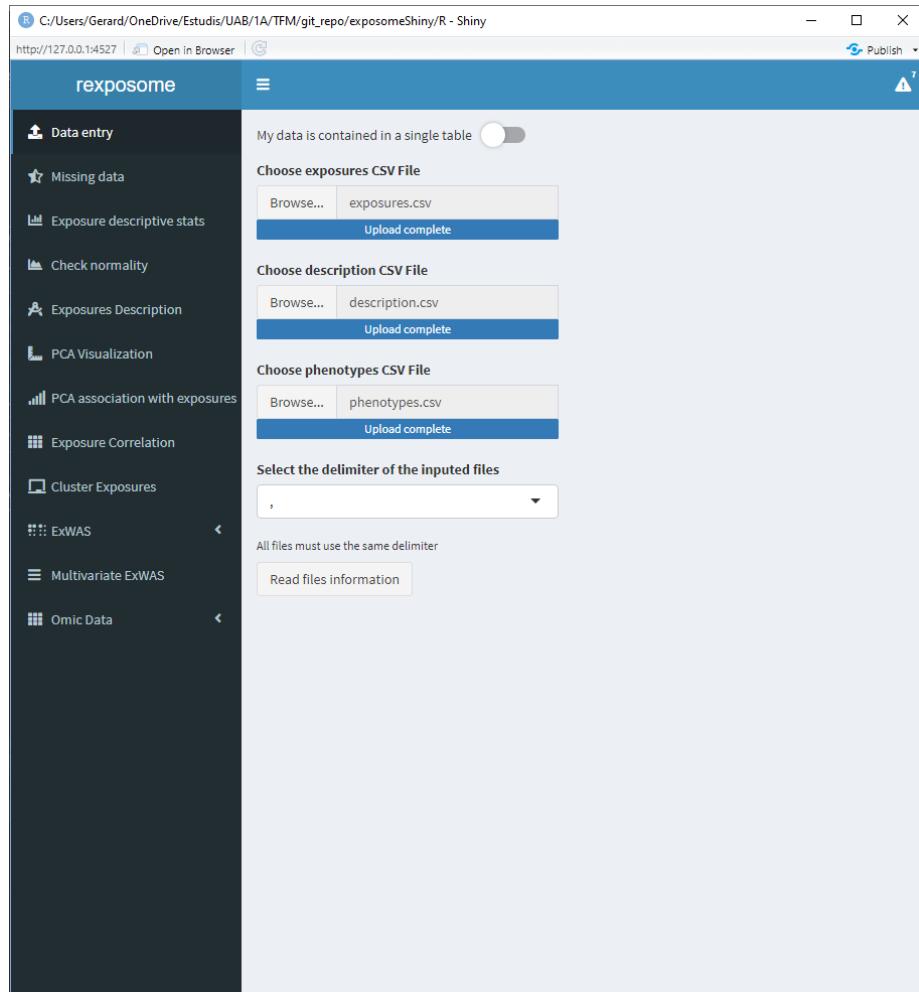
The exposome health analysis corresponds to the study of the relation between exposures (exposome) and health outcomes (phenotypes). Information about exposome data exploration and pre-processing can also be found on this subsection. Along this section, the data files used as examples are: `exposures.csv`, `exposures_lod.csv`, `description.csv`, `phenotypes.csv` and `exposome_plain.csv` which are available [here](#).

#### 5.1.1 Data entry

There are two different data entry methods. Exposome data which uses the three tables and plain data which only uses one.

##### 5.1.1.1 Exposome data

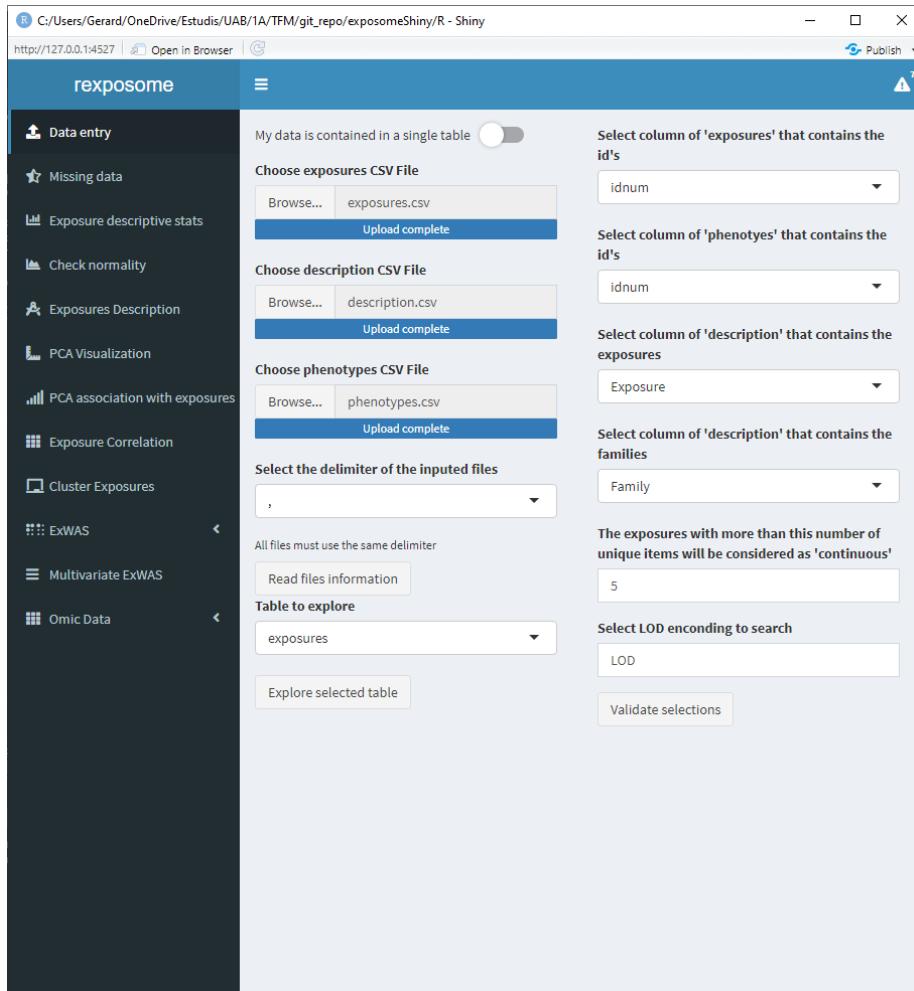
Three tables are needed to input an exposome dataset: the exposures, description and phenotypes. These files have to be provided as `csv` or `txt`. Different separator formats are supported (‘,’; ‘;’, tabs and spaces). Excel files or R objects are not supported as inputs. Be sure all three files share the same separator.



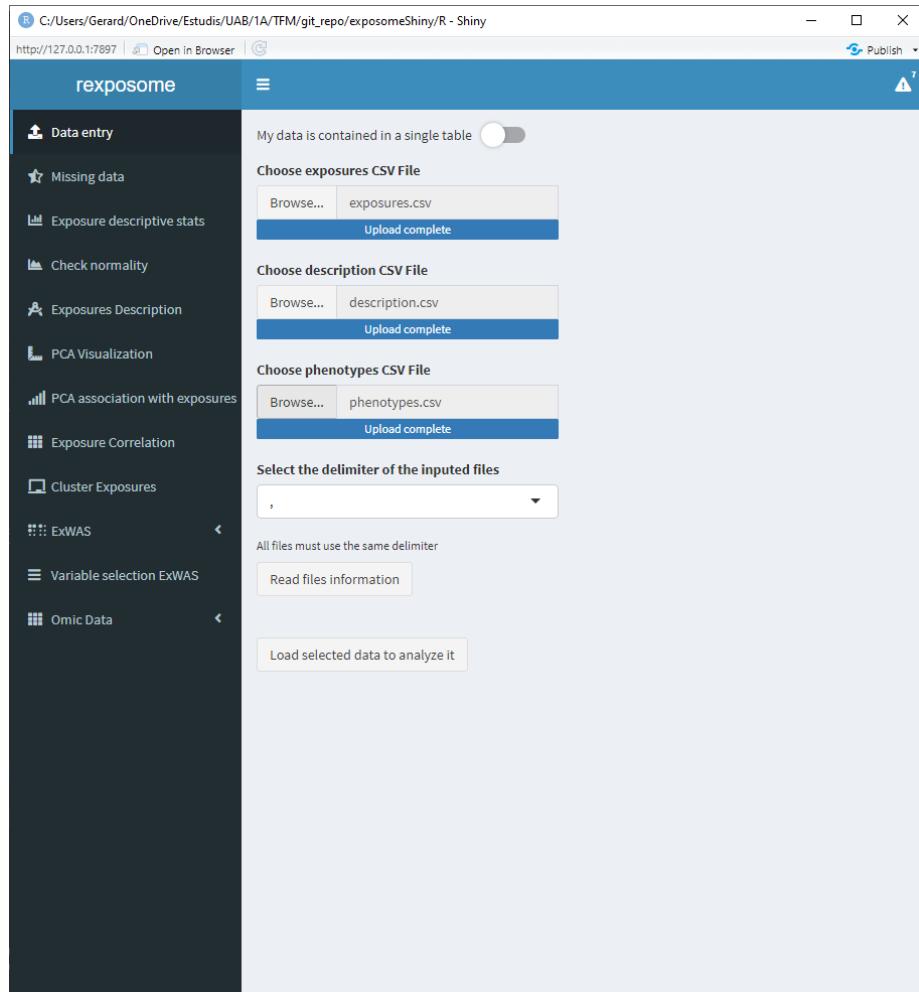
Once the tables are read two new main elements will appear, 1) the option to explore the inputted tables; and 2) six input fields which control the following file parameters:

- Column name in the *description* file that contains the exposures
- Column name in the *description* file that contains the families
- Column name in the *exposures* file that contains the identifier
- Column name in the *phenotypes* file that contains the identifier
- The threshold to select between continuous or factor exposures. More than this number of unique items on an exposure will be considered as 'continuous'
- The encoding to search for limit of detection (LOD) missings. It can be either a number (example: -1) or a string (example: LOD). All the cells that contain this encoder will be considered LOD missings.

This is illustrated on the following figure.

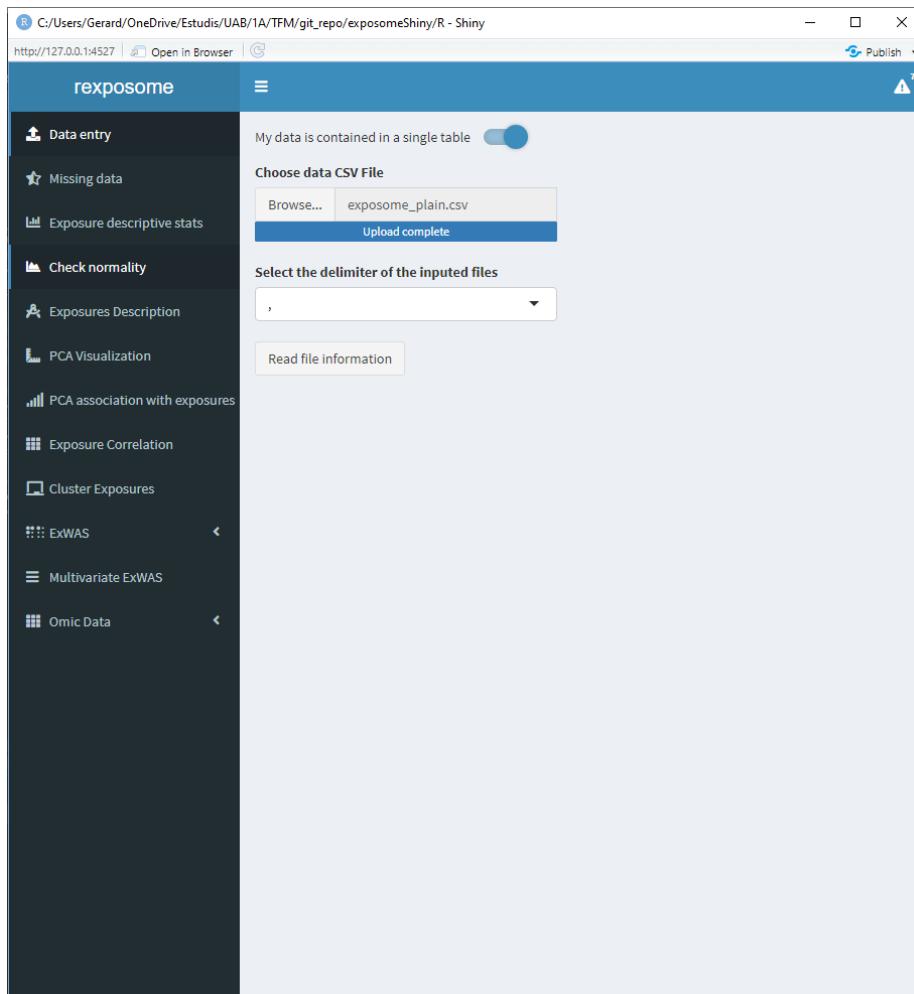


Once the fields are completed, press the *Validate selections* button in order to check if all the parameters allow for a successful load of the exposome dataset, if not, a pop-up will be prompted to the user with the R error message that stopped the execution. In the case that everything is correct, the interface will be updated and a button that reads *Load selected data to analyze it* will appear, by clicking this button the dataset will be loaded.

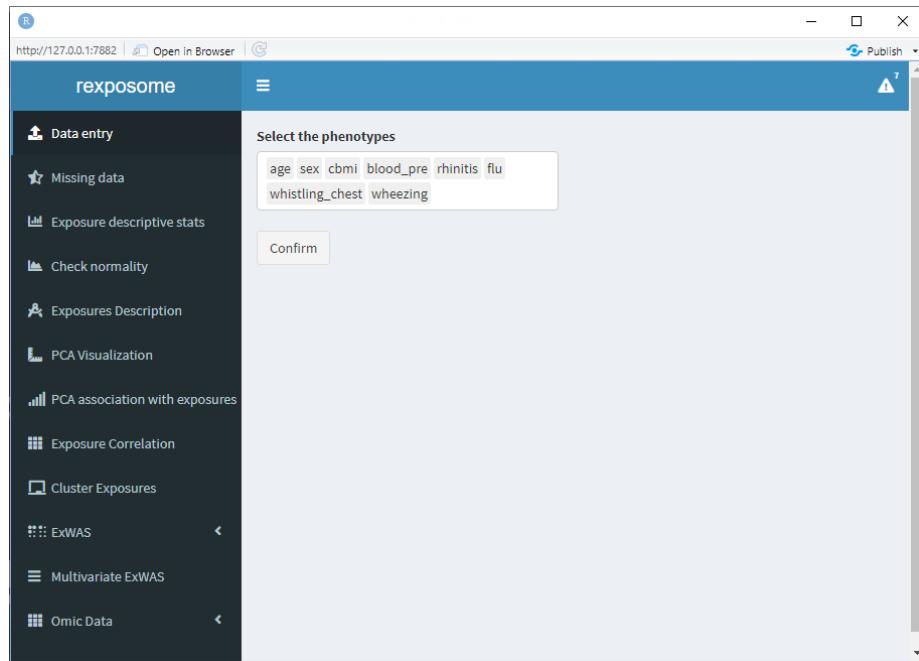


### 5.1.1.2 Plain data

When dealing with a single file that contains the exposures and phenotype data, press the “My data is contained in a single table” toggle. This will change the interface to only show one file selector.



Select a file and press “Read file information”. This will change the interface to show a multiple selector input, all the available columns will be listed, select the ones which correspond to phenotypes.



When all the phenotypes are selected, press “Confirm”. Now the exposures can be grouped into families, to do so:

- Select all the exposures from the same family
- Write the family name on the box “Family of selected exposures”
- Press the “Assign” button

The table will be updated to visualize the action performed.

Exposures left with an empty “Family” field will be treated as they are their own family.

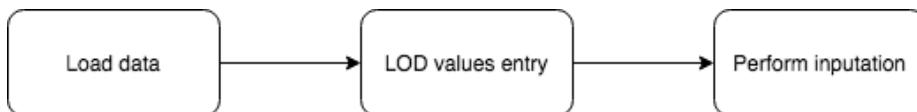
The screenshot shows the rexposome RStudio interface. The sidebar on the left lists various analysis options: Data entry, Missing data, Exposure descriptive stats, Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, and Omic Data. The main panel displays a table of exposures. The table has two columns: 'Exposures' and 'Family'. The 'Exposures' column lists PMcoarse, AbsPM25, Dens, Conn, Green, Noise\_d, Noise\_n, and Temp. The 'Family' column lists Noises for all entries except Conn. There are configuration fields on the right: 'Show 10 entries' (dropdown), 'The exposures with more than this number of unique items will be considered as 'continuous'' (input field with value 5), 'Select LOD encoding to search' (input field with value -1), 'Family of selected exposures' (input field with value Noises), and an 'Assign' button. Below the table, it says 'Showing 81 to 88 of 88 entries' with a page navigation bar (Previous, 1, ..., 5, 6, 7, 8, 9, Next) and a 'Load data' button.

Finally, there are two configuration fields:

- The threshold to select between continuous or factor exposures. More than this number of unique items on an exposure will be considered as ‘continuous’
- The encoding to search for limit of detection (LOD) missings (example: LOD). All the cells that contain this encoder will be considered LOD missings.

Be sure to revise them before pressing “Load data”.

### 5.1.2 LOD imputation



When loading the data, if LOD missings are detected a small table with the

exposures that have LOD missings will appear at the bottom of the “Data entry” page.

The screenshot shows the 'Data entry' page of the exposomeShiny R-Shiny application. On the left, there is a sidebar with various analysis options: Data entry (selected), Missing data, Exposure descriptive stats, Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Variable selection ExWAS, and Omic Data. The main area has several input fields and controls:

- Choose exposures CSV File:** A file input field containing "exposures\_lod.csv" with a blue "Upload complete" button.
- Choose description CSV File:** A file input field containing "description.csv" with a blue "Upload complete" button.
- Choose phenotypes CSV File:** A file input field containing "phenotypes.csv" with a blue "Upload complete" button.
- Select the delimiter of the inputted files:** A dropdown menu set to ",".
- All files must use the same delimiter:** A note below the dropdown.
- Read files information:** A button to view details about the uploaded files.
- Load selected data to analyze it:** A button to start analysis.
- Show 10 entries:** A dropdown to change the number of entries displayed.
- Search:** An input field to search the table.
- Exposure LOD table:**

Exposure	LOD
PFHxS	1
PFOS	2
- Showing 1 to 2 of 2 entries**
- Previous** and **Next** buttons.
- Help about the LOD substitution:** A link to provide more information.
- Choose imputation method:** A dropdown menu currently set to "LOD/sqrt(2)".
- Perform LOD imputation:** A button to execute the imputation.

This table has a column named “LOD”, which by default reads as 1, 2, 3, ... Those values are meant to be modified by the user and input the limit of detection values for the different exposures. Those values will be used if the LOD imputation method is LOD/sqrt(2). If the imputation method used is QRILC there is no need to modify those values, as they won’t be used.

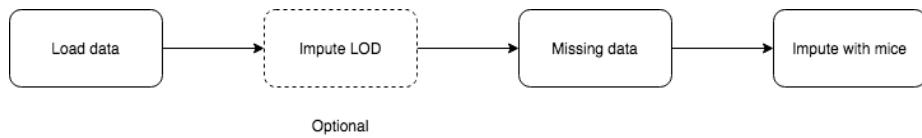
To modify the LOD values of the table, double click on the cell of interest and type the value (use points for decimal separation e.j. 1.156).

The screenshot shows the 'exposomeShiny' R-Shiny application interface. On the left, a sidebar lists various analysis options: Data entry, Missing data, Exposure descriptive stats, Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Variable selection ExWAS, and Omic Data. The main panel is titled 'Choose exposures CSV File' and shows 'exposures\_lod.csv' uploaded. Below it are sections for 'description.csv' and 'phenotypes.csv', both also showing 'Upload complete'. A section titled 'Select the delimiter of the inputed files' has a dropdown set to a comma. A note says 'All files must use the same delimiter'. Below this is a 'Read files information' button. A table titled 'Load selected data to analyze it' displays two entries: PFHxS with LOD 0.85 and PFOS with LOD 2.26. The table includes columns for 'Exposure' and 'LOD'. Navigation buttons 'Previous' and 'Next' are shown, along with a link 'Help about the LOD substitution'. A dropdown menu 'Choose imputation method:' is set to 'LOD/sqrt(2)'. At the bottom is a button 'Perform LOD imputation'.

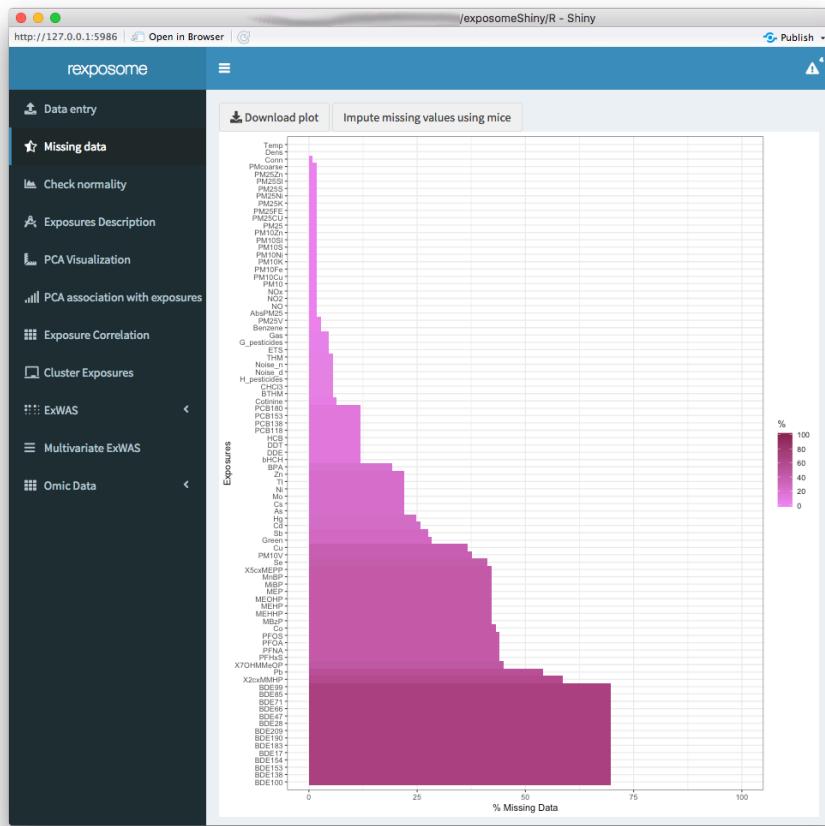
After clicking “Perform LOD imputation”, a new button to download the imputed dataset will appear, this will download the exposures data with the LOD missings imputed.

Once the LOD imputation is completed, the exposome dataset that will be used on the following steps is imputed.

### 5.1.3 Missing imputation



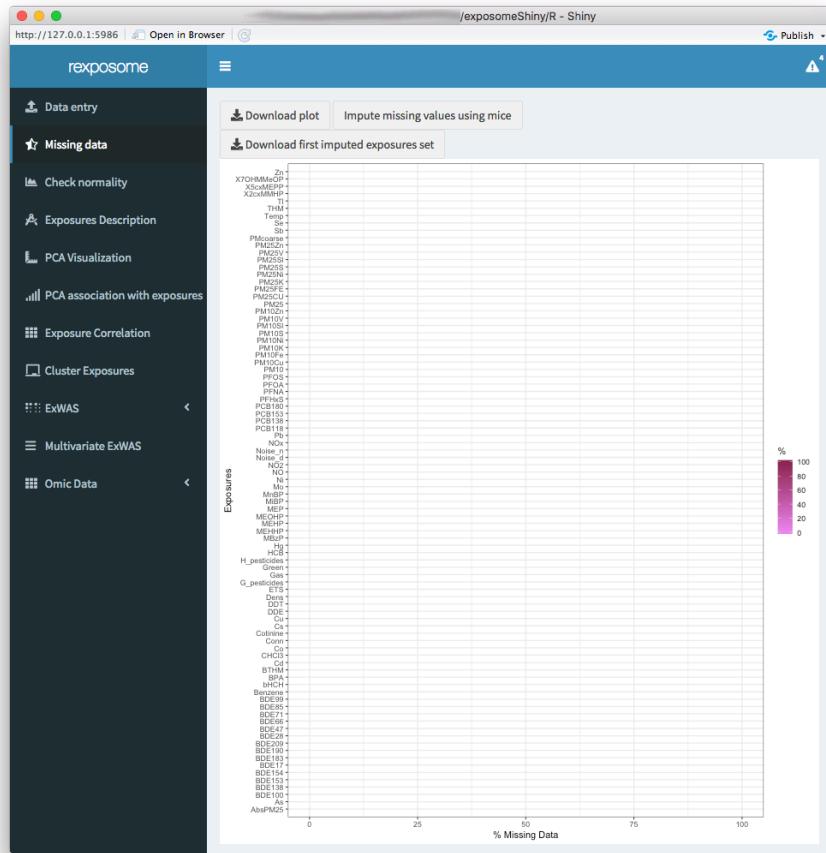
The “Missing data” tab displays a plot with the percentages of missing data for each exposure.



The missings can be imputed using Multiple Imputation by Chained Equations (MICE) by clicking the “Impute missing values using mice” button.

Please note that missings imputation might require other methodologies or more fine control. If that is the case, we encourage users to impute the data beforehand and input to our application data that is already imputed.

Once the missings imputation is completed, the exposome dataset that will be used on the following steps is imputed. This is reflected by refreshing the plot, which should read 0% missings for all exposures.



The imputed exposures set can be downloaded as a `*.csv` file.

#### 5.1.4 Exposures description

The exposure descriptive stats tab, provides a table with the main descriptive stats of the quantitative exposures. The descriptive stats (per exposure) included on the table are:

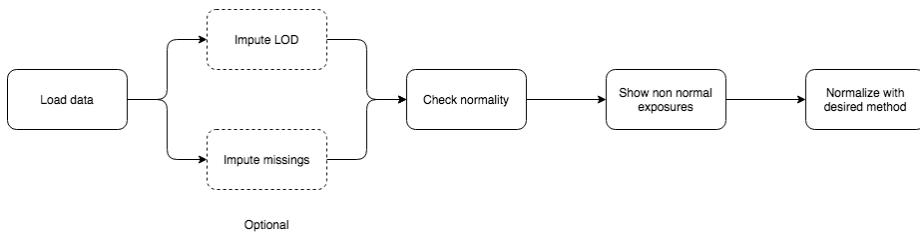
- Number of values
- Number of NULLs
- Number of NAs
- Minimum
- Maximum
- Range of values
- Sum of values
- Median

- Mean
- Standard Error of mean
- 0.95 confidence interval of the mean
- Variance
- Standard deviation
- Variance coefficient

Remember that after imputing the missings, the imputed dataset becomes active, this will be reflected showing 0 NAs for example.

Stat	AbsPM25	As	BDE100	BDE138	BDE153	BDE154	BDE17
nbr.val	107	85	33	33	33	33	3
nbr.null	0	0	0	0	0	0	0
nbr.na	2	24	76	76	76	76	7
min	0.2	0.775	-1.415	-2.119	-0.76	-1.153	-2.30
max	0.556	2.613	0.504	-0.909	0.428	0.203	-0.17
range	0.356	1.838	1.919	1.21	1.188	1.356	2.12
sum	37.951	129.504	-18.092	-55.046	-3.518	-14.069	-60.61
median	0.354	1.506	-0.554	-1.688	-0.087	-0.446	-1.87
mean	0.355	1.524	-0.548	-1.668	-0.107	-0.426	-1.83
SE.mean	0.007	0.042	0.07	0.04	0.058	0.044	0.06
CI.mean.0.95	0.014	0.083	0.142	0.081	0.118	0.089	0.12
var	0.005	0.148	0.161	0.053	0.111	0.063	0.12
std.dev	0.074	0.385	0.401	0.229	0.334	0.251	0.35
coef.var	0.209	0.253	-0.731	-0.137	-3.13	-0.589	-0.19

### 5.1.5 Normality correction

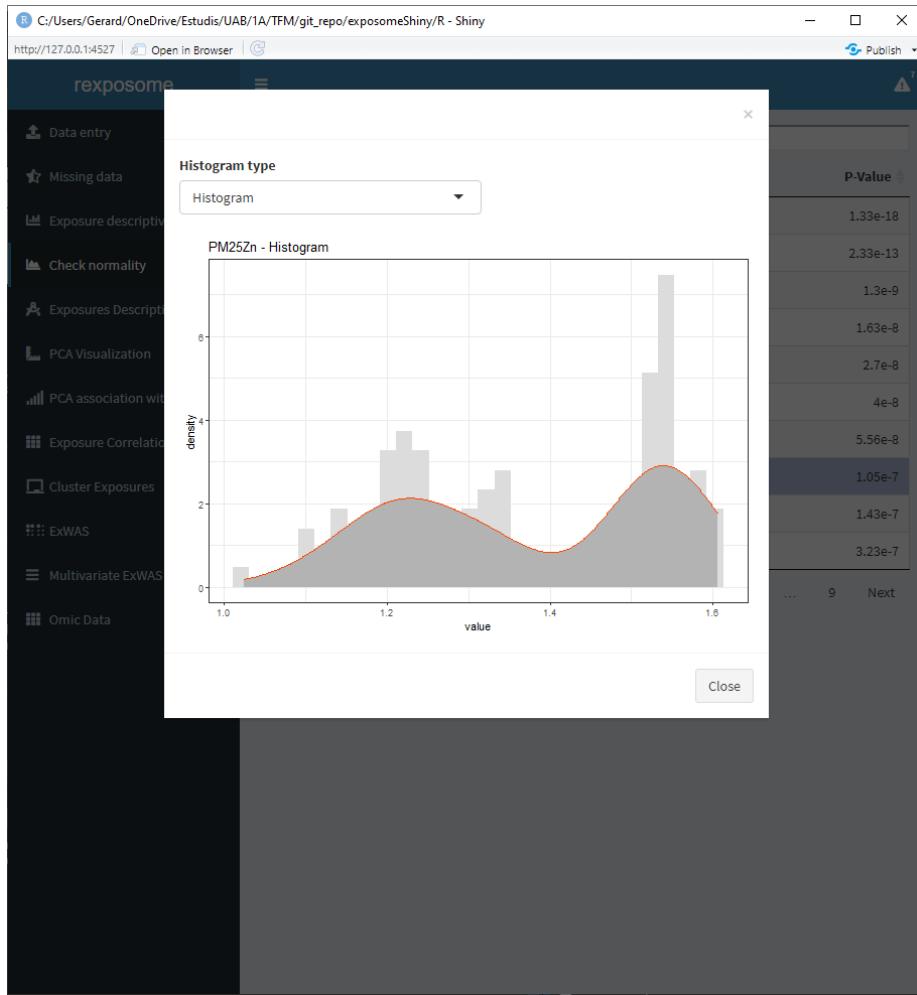


The table shown on the ‘Check normality’ tab contains all the exposures, if they can be considered normal distributed and the p-value of the normality test (Shapiro-Wilk).

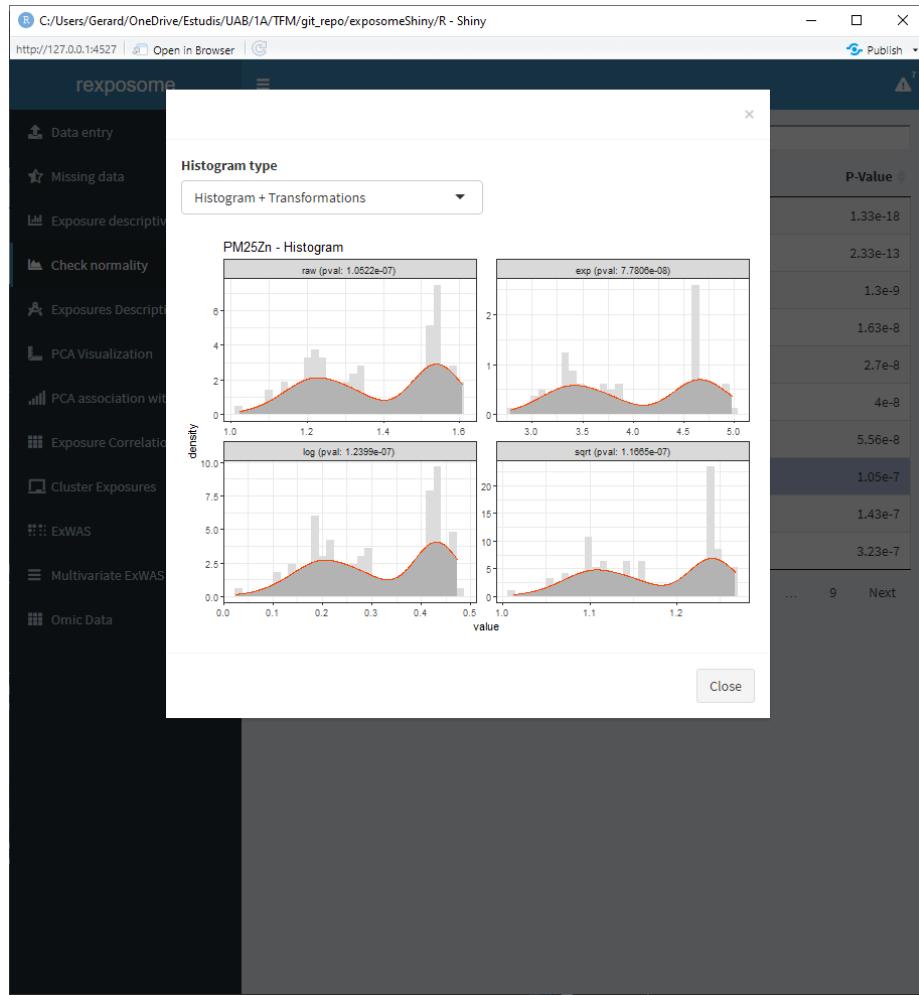
The screenshot shows a Shiny application window titled 'rexosome'. On the left is a sidebar with various menu items: Data entry, Missing data, Exposure descriptive stats, Check normality (which is selected), Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, and Omic Data. The main area displays a table with columns: Exposure, Normality, and P-Value. The table lists 10 entries out of 84 total, showing that all exposures listed are not normally distributed (Normality: false) and their corresponding p-values. At the bottom of the table, there are navigation links for 'Previous' and 'Next' pages, and two buttons: 'Plot histogram of selected exposure' and 'Show false'.

Exposure	Normality	P-Value
DDT	false	1.33e-18
PM10SI	false	2.33e-13
PM25K	false	1.3e-9
PM25SI	false	1.63e-8
PCB118	false	2.7e-8
Tl	false	4e-8
PM10V	false	5.56e-8
PM25Zn	false	1.05e-7
PM25FE	false	1.43e-7
PM10K	false	3.23e-7

Select an exposure from the table and click on ‘Plot histogram of selected exposure’ to see the histogram of that exposure.



There is the option to visualize the histograms with the available transformations applied, in order to see how an exposure will be affected by them. To see it select 'Histogram + transformations' on the Histogram type.



Aside from visualizing if exposures are normal, this tab can be used to apply transformations to the exposures. The available transformations are “log” (default, natural logarithm), “ $^{1/3}$ ” and “sqrt”. To do so, the procedure is the following:

- Press the ‘Show false’ button. A pop-up will appear with a table. This table contains all the exposures that did not pass the normality test, next to the exposures the transformation to be applied is shown.

The screenshot shows a shiny application window titled "rexposome". On the left, a sidebar lists various analysis options: Data entry, Missing data, Exposure description, Check normality, Exposures Description, PCA Visualization, PCA association with, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, and Omic Data. The main area displays two tables. The first table, titled "Exposure" and "Normalization method", lists ten entries: DDT (log), PM10SI (log), PM25K (log), PM25SI (log), PCB118 (log), Ti (log), PM10V (log), PM25Zn (log), PM25FE (log), and PM10K (log). The second table, titled "P-Value", lists values from 1.33e-18 down to 3.23e-7. A "Close" button is at the bottom right of the modal.

- Modify the transformation method by double clicking on the cell to be changed and typing the required method. Input 'none' if no transformation is desired for a certain exposure.

The screenshot shows a Shiny application window titled "reXposome". The left sidebar contains a menu with the following items:

- Data entry
- Missing data
- Exposure descriptive stats
- Check normality** (highlighted)
- Exposures Description
- PCA Visualization
- PCA association with exposures
- Exposure Correlation
- Cluster Exposures
- ExWAS
- Multivariate ExWAS
- Omic Data

The main content area displays a table titled "Show 10 entries" with the following columns: Exposure, Normality, and P-Value. The table lists 10 entries from a total of 84:

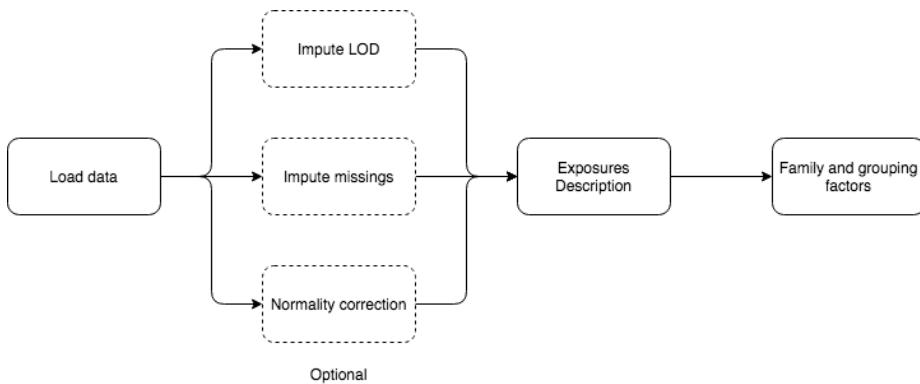
Exposure	Normality	P-Value
DDT	false	1.33e-18
PM10SI	false	2.33e-13
PM25K	false	1.3e-9
PM25SI	false	1.63e-8
PCB118	false	2.7e-8
TL	false	4e-8
PM10V	false	5.56e-8
PM25Zn	false	1.05e-7
PM25FE	false	1.43e-7
PM10K	false	3.23e-7

Below the table, there are navigation buttons for "Previous" and "Next", and two buttons: "Plot histogram of selected exposure" and "Show false".

- Press ‘Normalize’ to apply the transformations.

After the transformations are applied, the table of normality tests will be updated. The dataset used after transforming is updated with the new values.

### 5.1.6 Exposures description



On the ‘Exposures description’ tab, exploratory analysis of the exposures can be performed. Again, the data analyzed by this tab will be imputed or normalized if the user has performed it before.

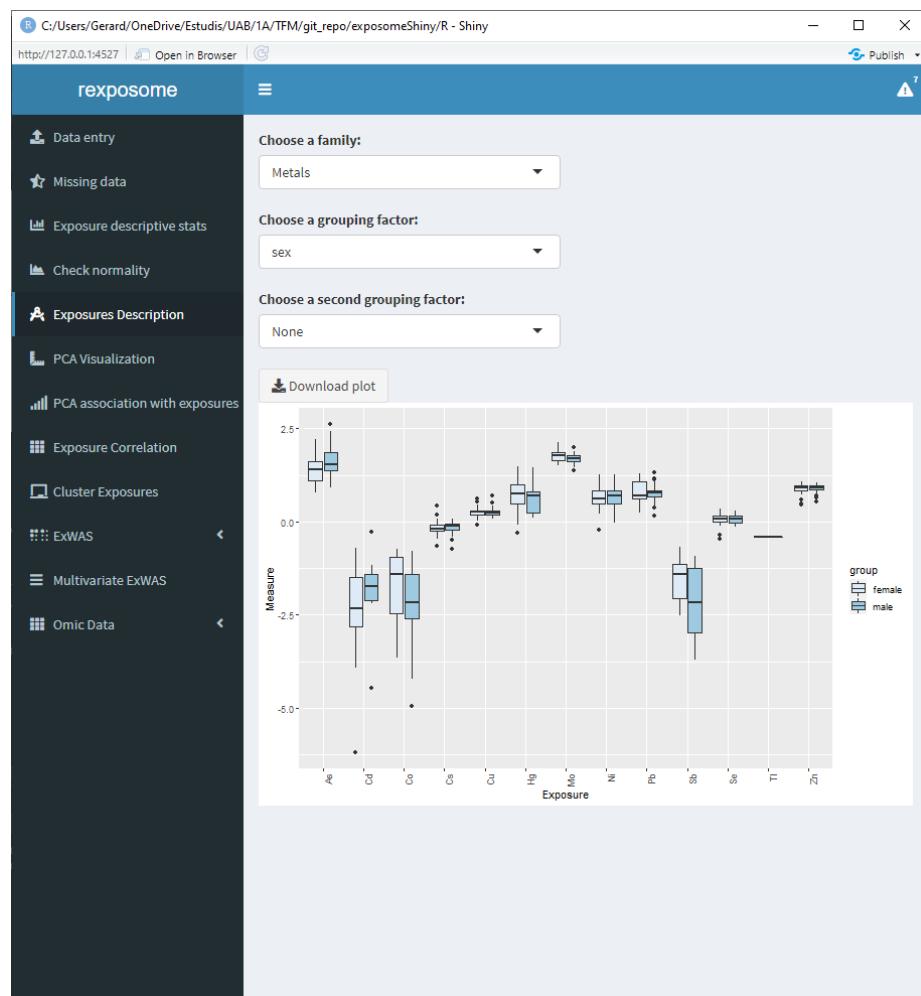
There are three input fields to obtain different plots:

- Family: To define which family of exposures will be plotted
- Grouping factor: Qualitative phenotype to group the exposures
- Second grouping factor: Second qualitative phenotype to group the exposures

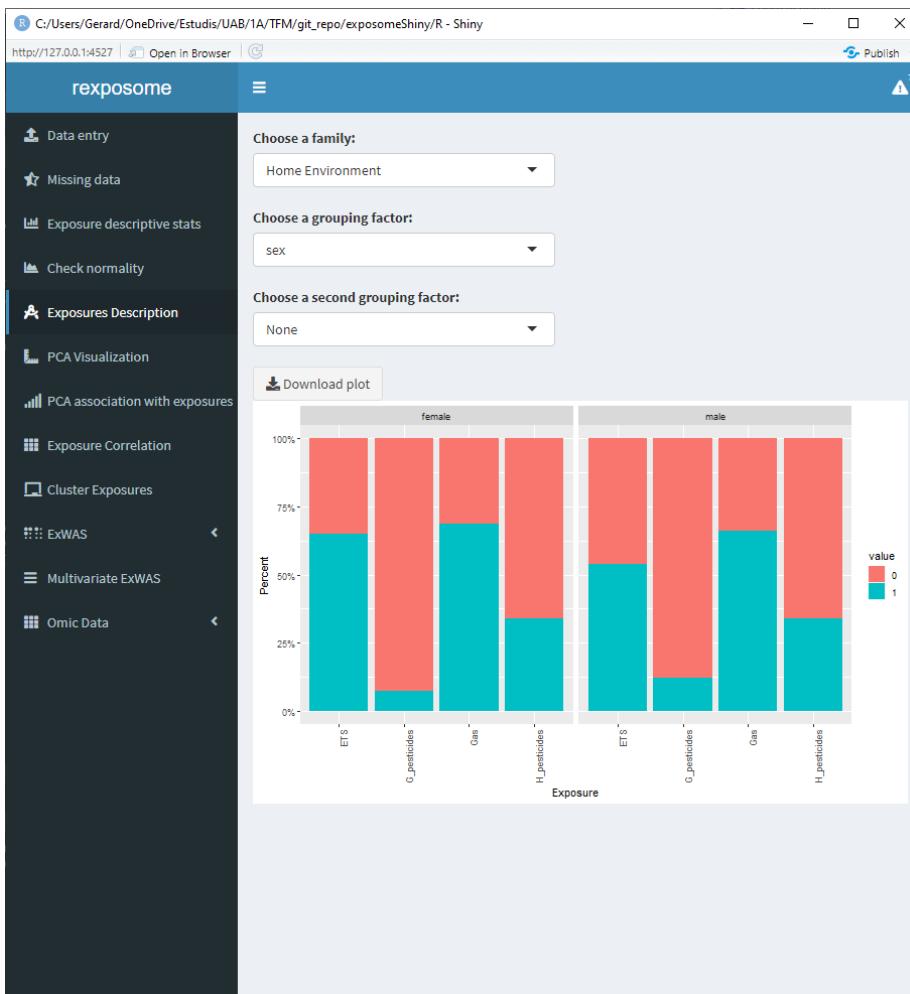
The visualization is different for categorical and quantitative exposures.

Some examples:

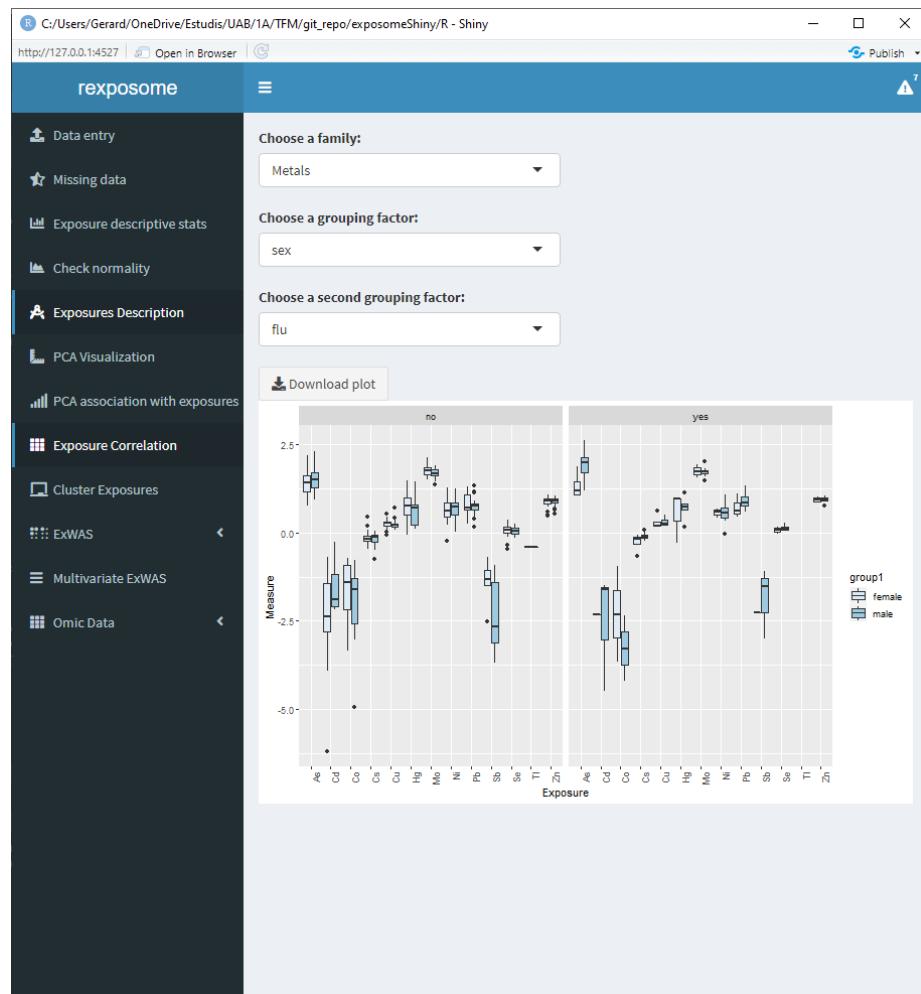
### 5.1.6.1 Quantitative family, grouped by sex



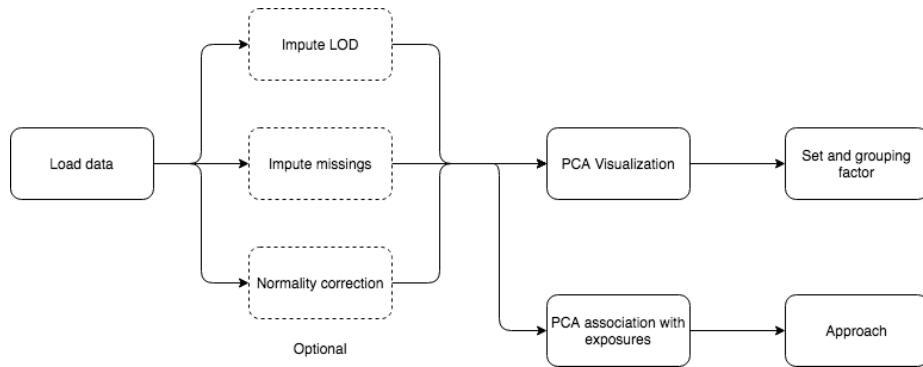
### 5.1.6.2 Qualitative family, grouped by sex



### 5.1.6.3 Quantitative family grouped by sex and flu diagnosis (binomial factor)



### 5.1.7 PCA Analysis



On the ‘PCA visualization’ tab, the results of a PCA analysis on the exposome data is displayed.

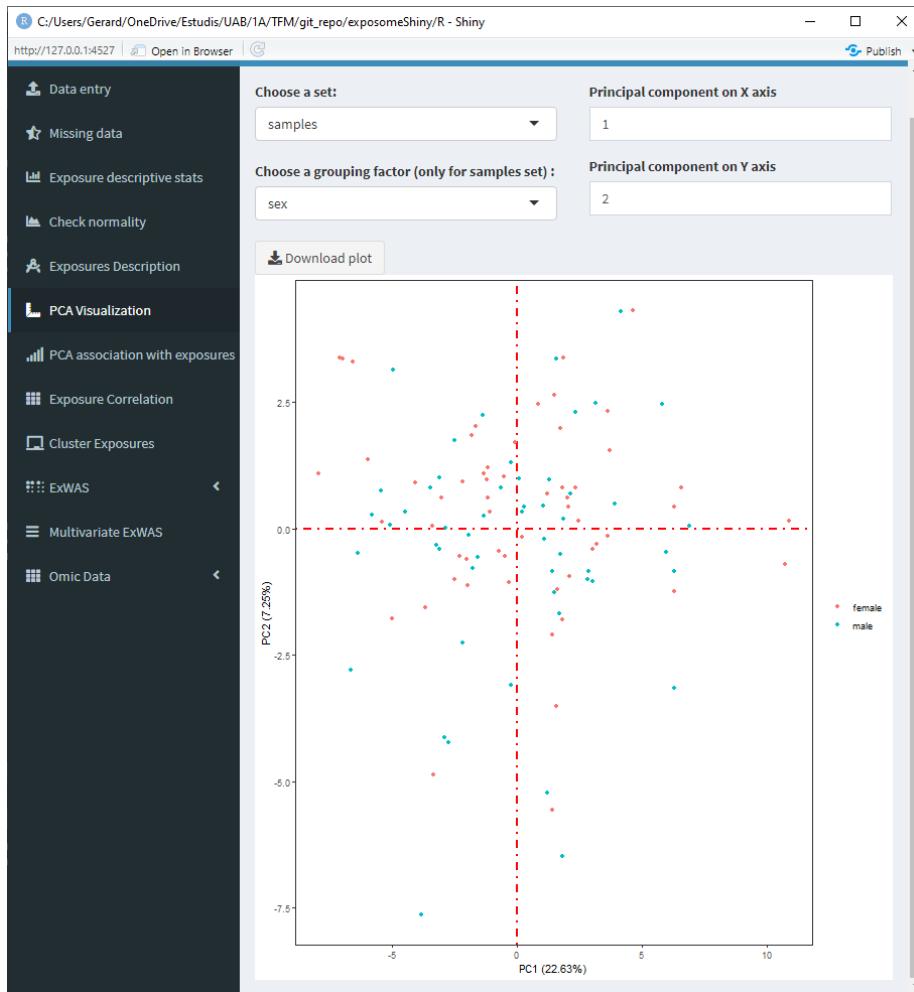
There are some input fields to modify the plot:

- Set: Select ‘all’ to see an array of four plots with different PCA related information. There is also the option of selecting ‘samples’ and ‘exposures’ to only display those two plots on a bigger scale.
- Grouping factor: To group the ‘samples’ set using a qualitative phenotype.
- Principal components: By default the first and second principal components are used for the plots. They can be changed using those inputs, which affect all the plots.

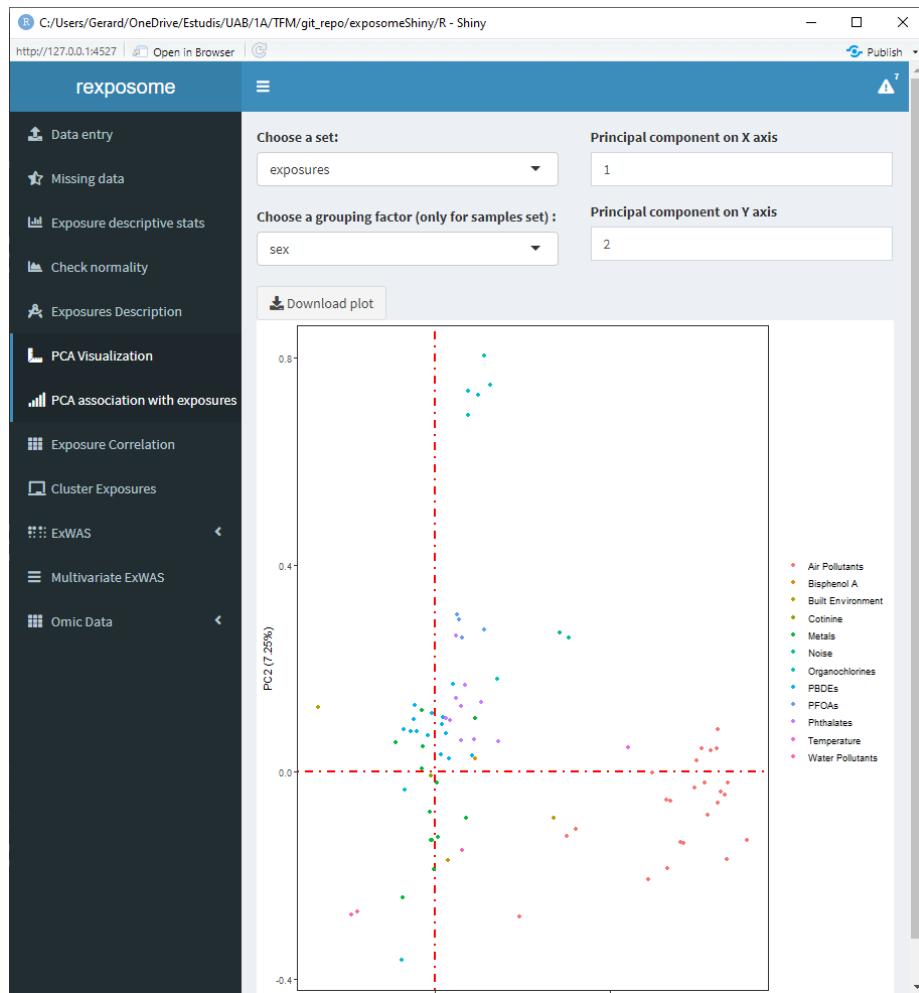
### 5.1.7.1 Set: 'all'



## 5.1.7.2 Set: ‘samples’ grouped by sex

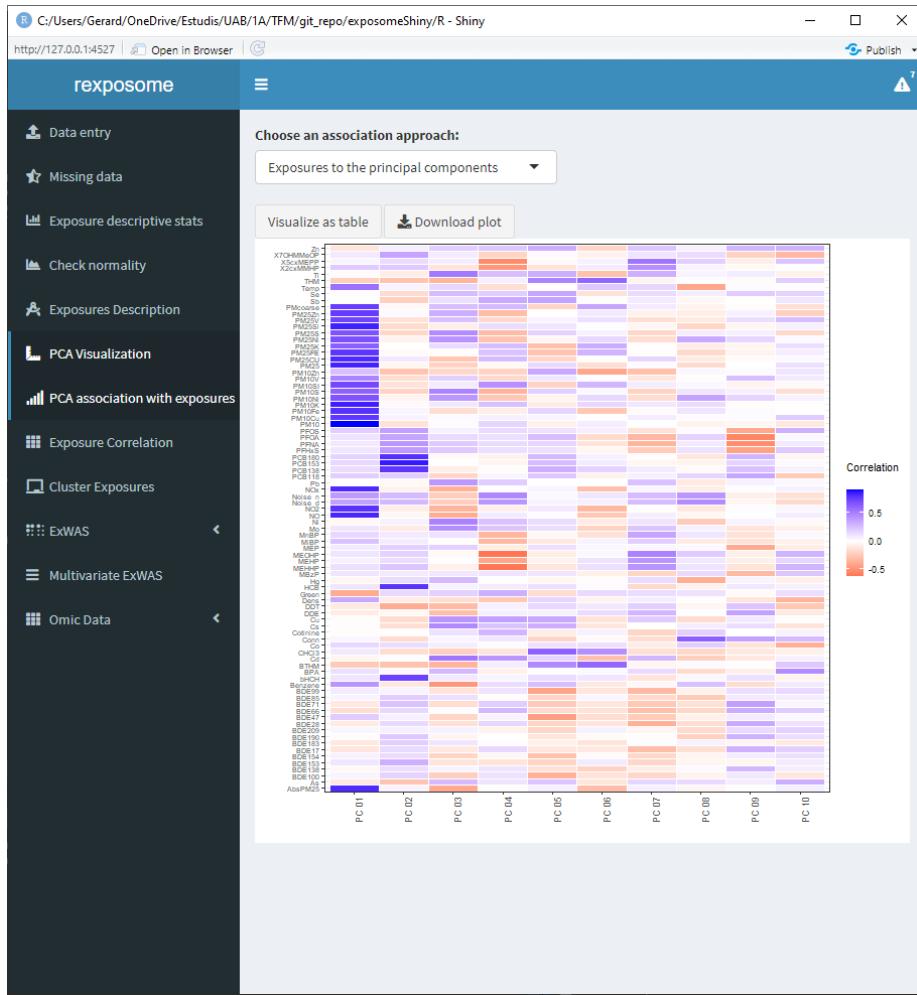


### 5.1.7.3 Set: ‘exposures’

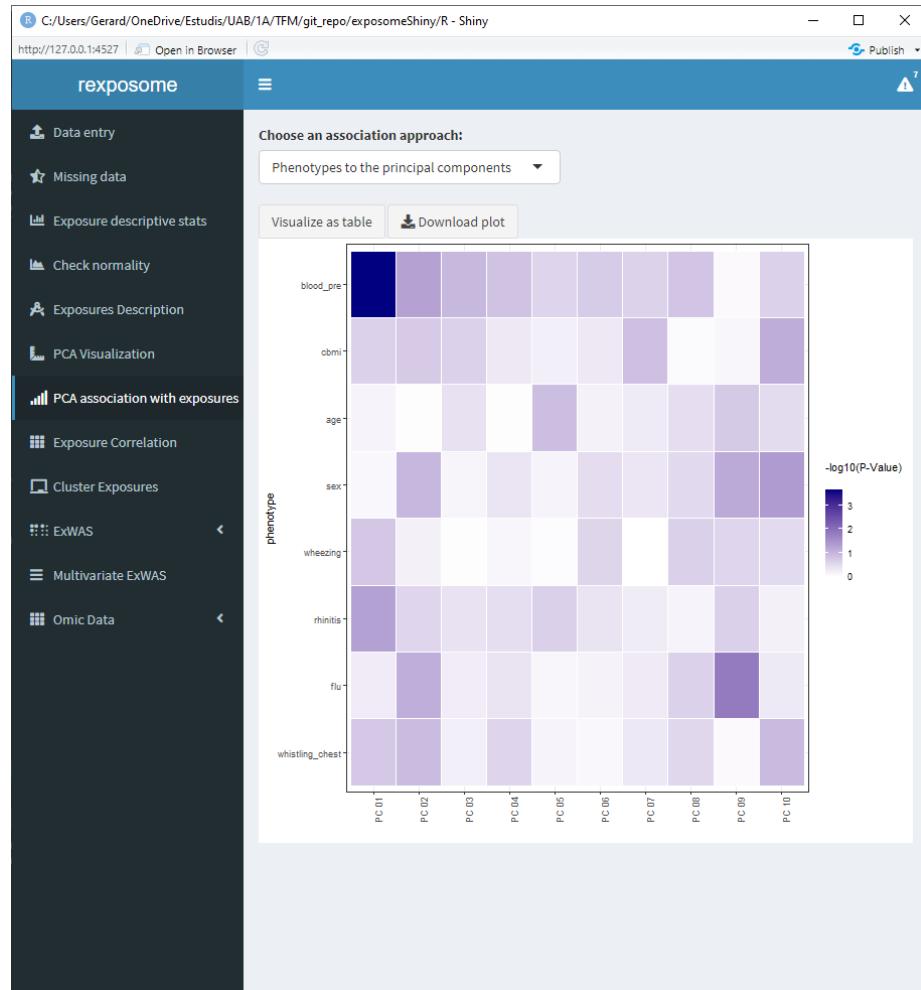


Moreover, there is an additional PCA visualization tab named ‘PCA association with exposures’. On this tab there are two heatmap plots available.

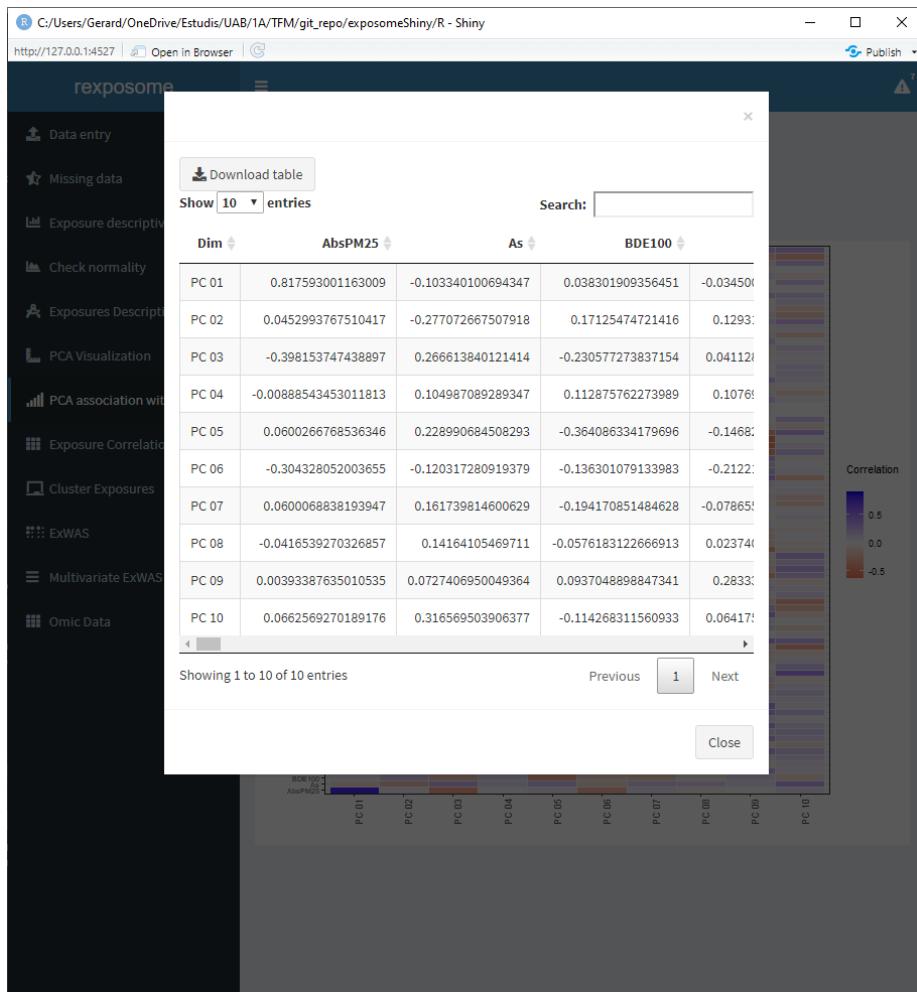
- Association of the exposures to the principal components



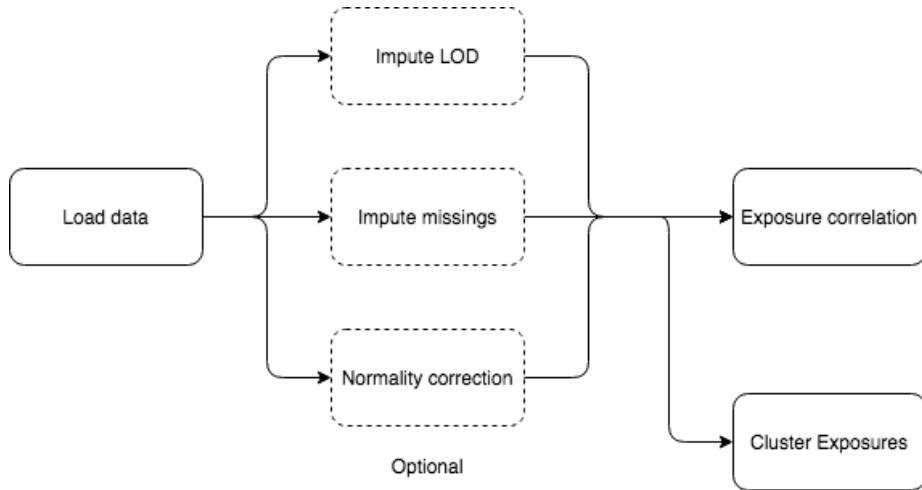
- Association of the phenotypes to the principal components



The values of this heatmaps can be visualized as tables by clicking on 'Visualize as table' and be downloaded as \*.csv.



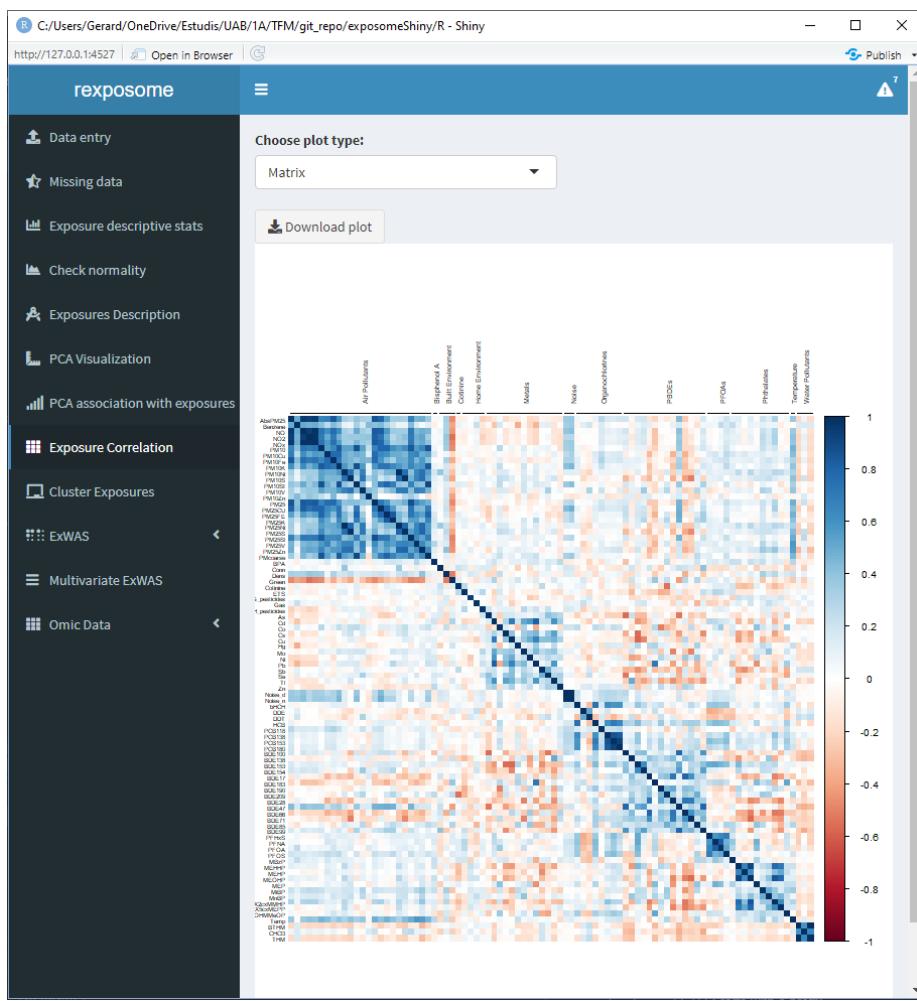
### 5.1.8 Correlation of exposures



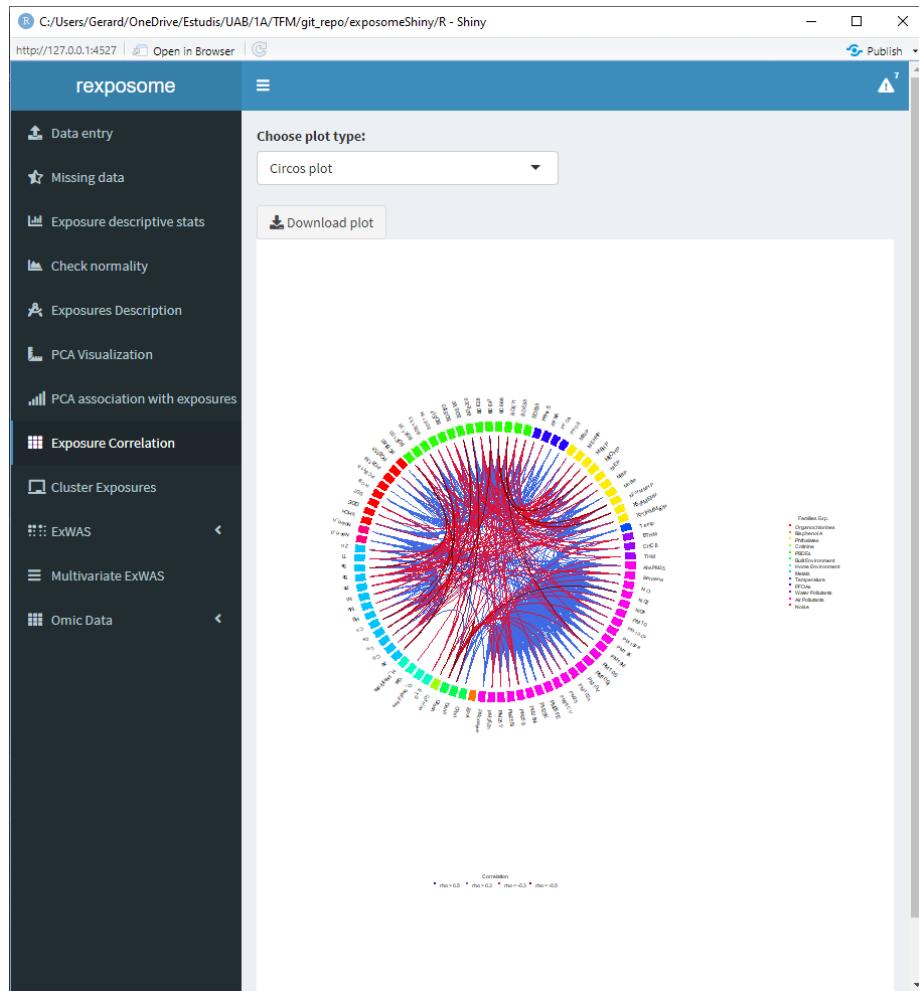
Displaying the correlation of the exposures can help to visualize intra and inter family relations between the exposures, for that reason there are two different visualization options, the circos and the matrix.

The correlation is computed using Pearson method for numerical-to-numerical correlation, Cramer's V for categorical-to-categorical correlation and linear models for categorical-to-numerical.

### 5.1.8.1 Matrix plot

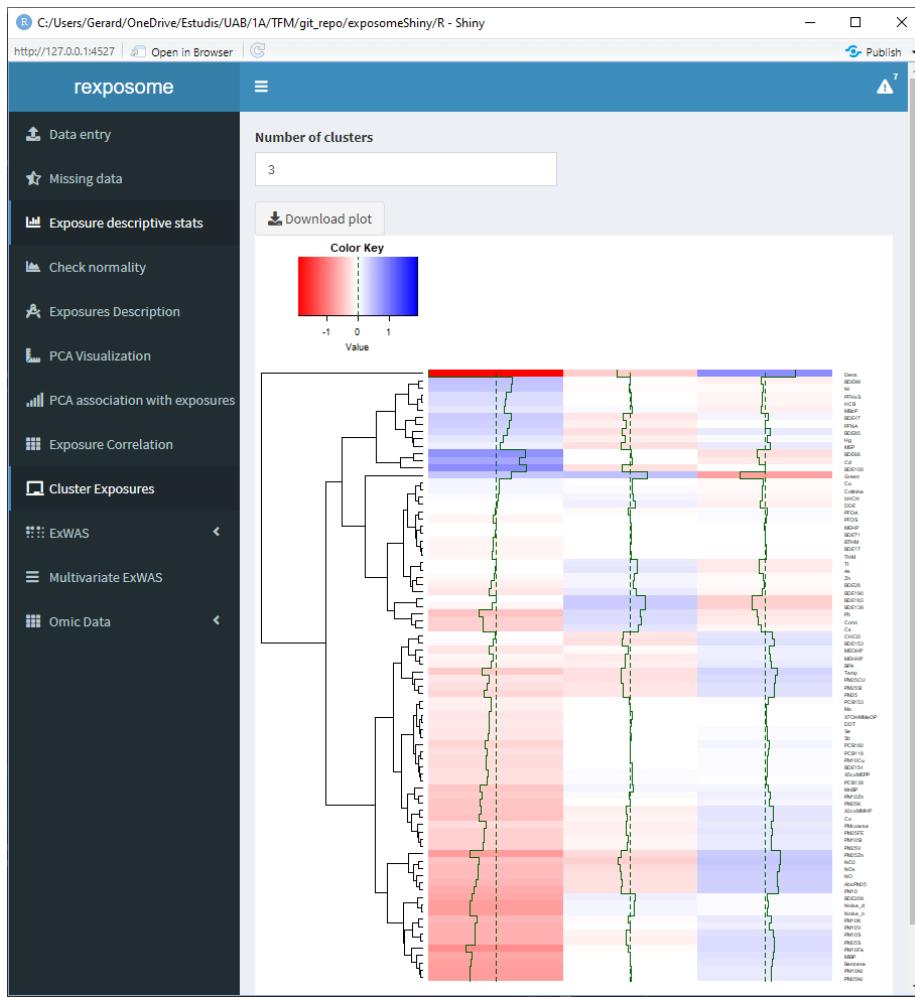


### 5.1.8.2 Circos plot

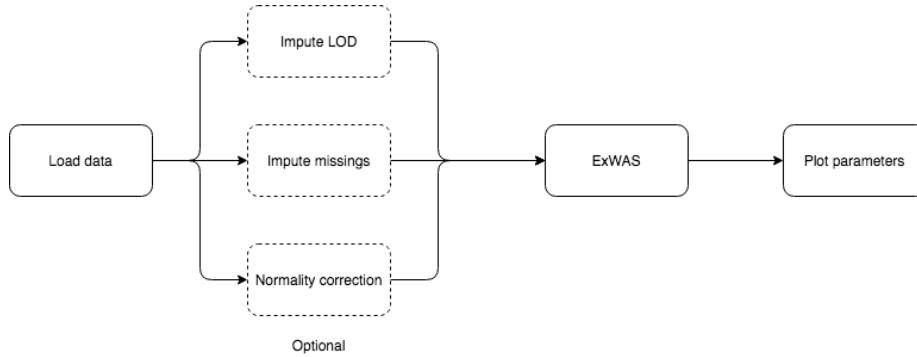


### 5.1.9 Clusterization of exposures

The clusterization of exposures uses a hierarchical clustering algorithm to classify the individuals profiles of exposures in k groups, where k can be selected by the user. The plot shows the profile for each group of individuals.



### 5.1.10 ExWAS

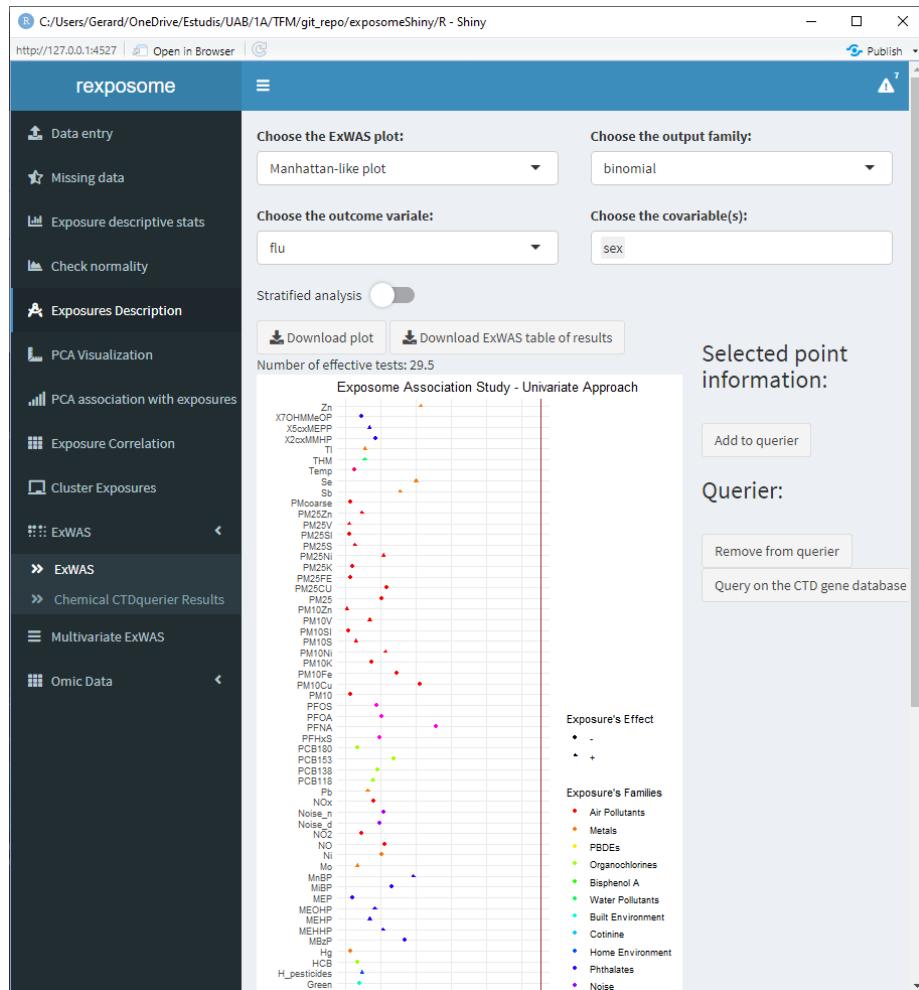


There are two ExWAS methods implemented, the regular and the stratified. The difference is that the stratified performs multiple ExWAS subsetting the data with a quantitative phenotype (example: ExWAS with male data and ExWAS with female data).

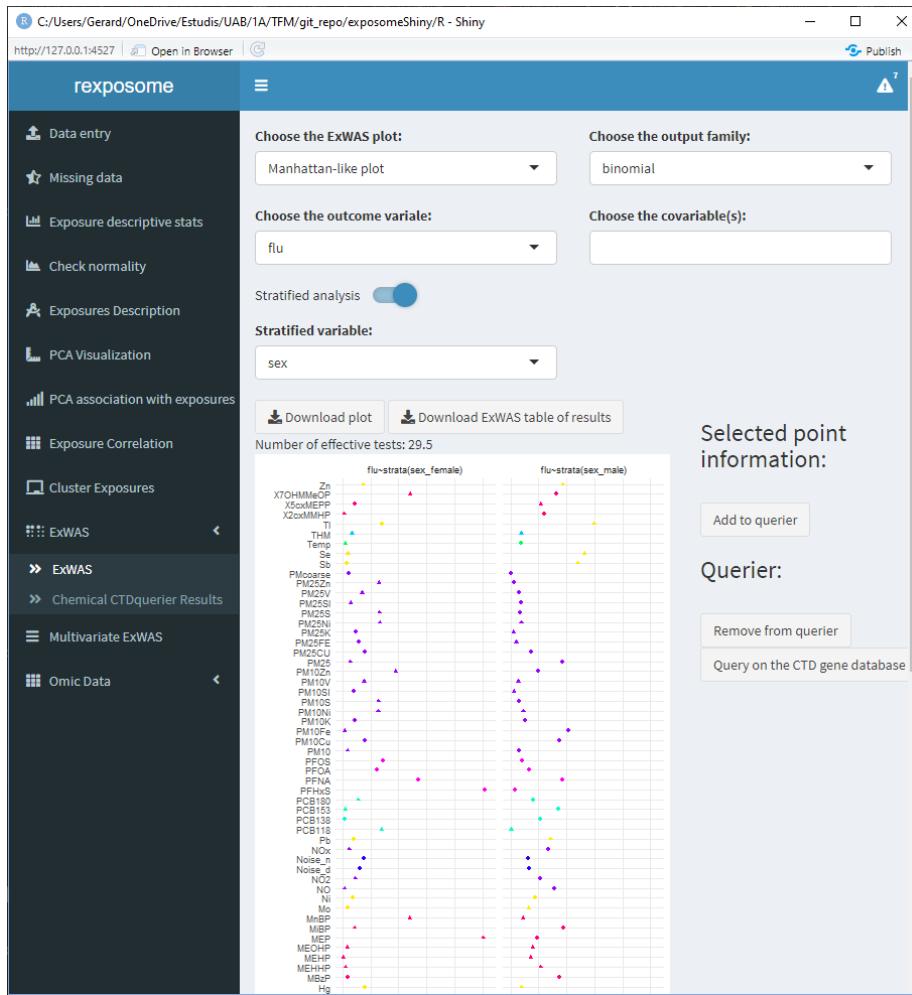
To perform an ExWAS enter the required fields:

- Outcome variable: Output phenotype
- Covariate(s): Adjusting phenotype(s)
- Output family: Family of the outcome variable (Gaussian/Binomial/Poisson)
- Type of plot: Manhattan / Effects

#### 5.1.10.1 Regular ExWAS. Outcome: flu, Covariate: Sex, Family: Binomial

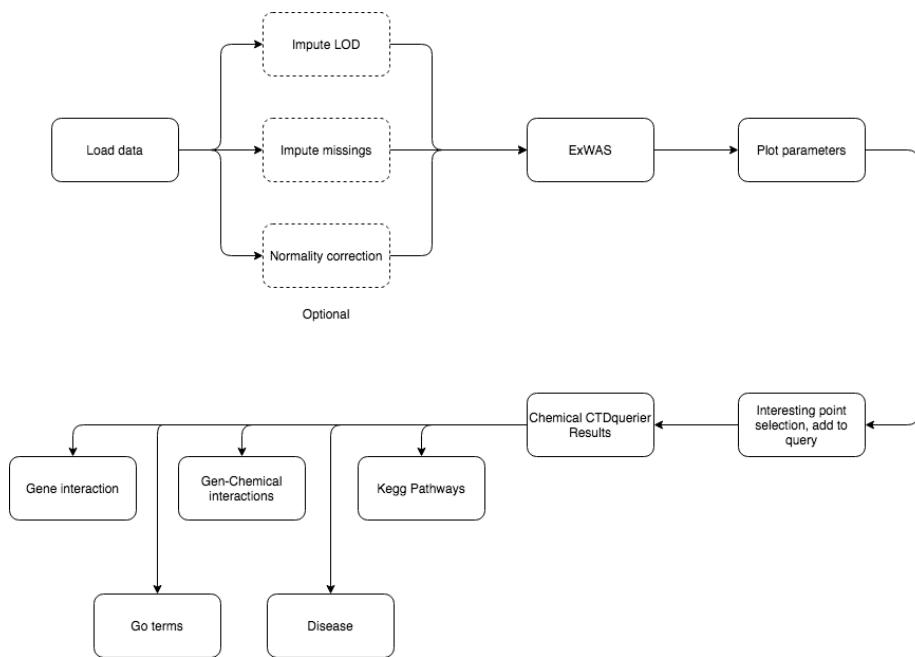


### 5.1.10.2 Stratified ExWAS. Outcome: flu, Cobariable: None, Family: Binomial, Stratifying variable: Sex



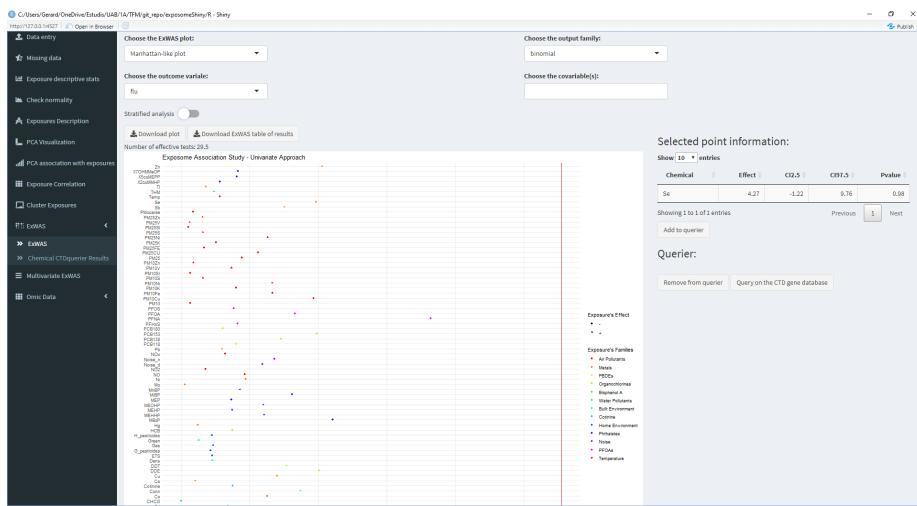
The plots and results table can be downloaded. The results table contains the exposure names, association pvalue and effect (with CI 95% for the effect). For the stratified ExWAS, the results table is actually a \*.zip file with a file for each subset.

### 5.1.11 ExWAS - CTDquerier

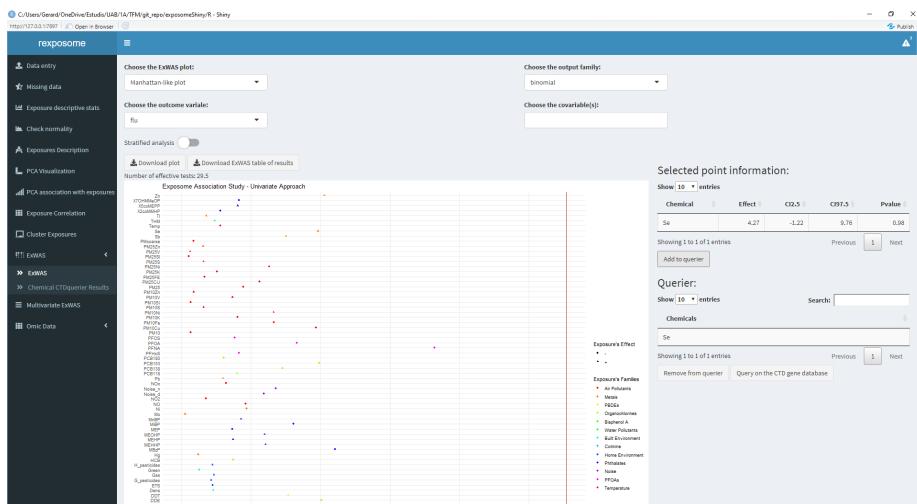


The ExWAS analysis can reveal high association between health outcomes and exposures. To obtain further information (from the CTD database) on exposures of interest:

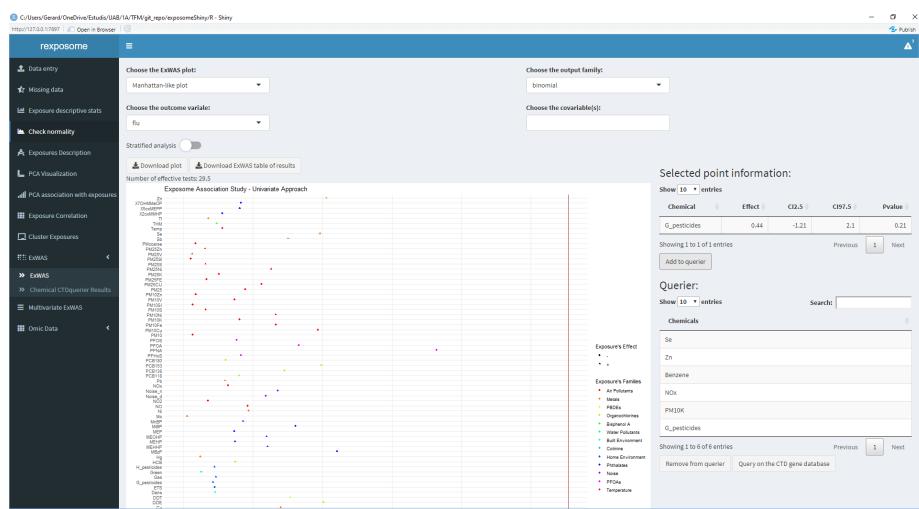
1. Perform the ExWAS
2. Click on the exposure of interest on the plot
3. A table with information about the selected exposure will appear on the right



4. If the exposure shown on the right table is of interest, click on ‘Add to querier’



5. Repeat 2/3/4 for all the exposures of interest

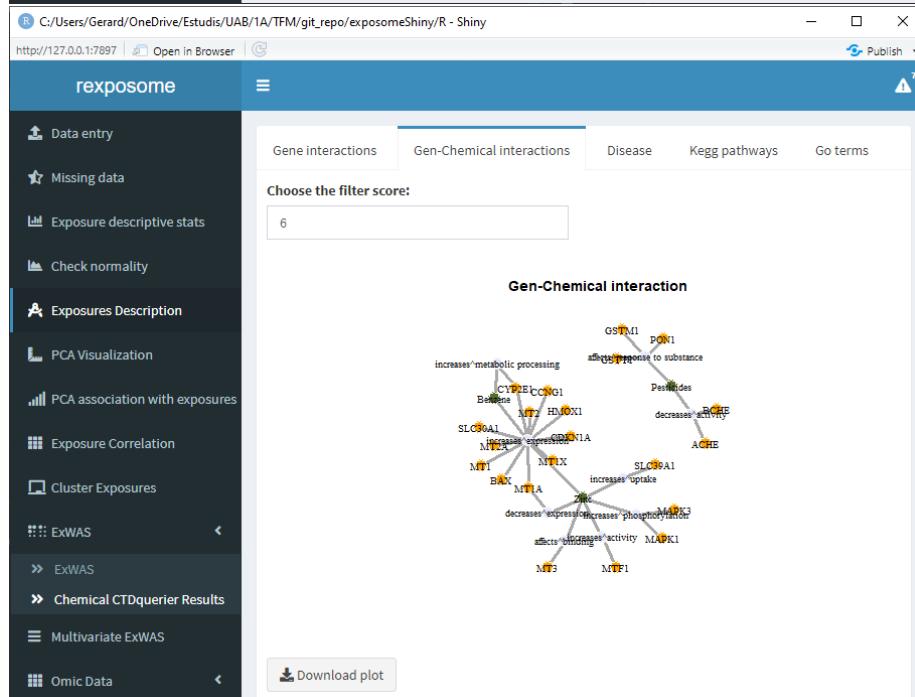
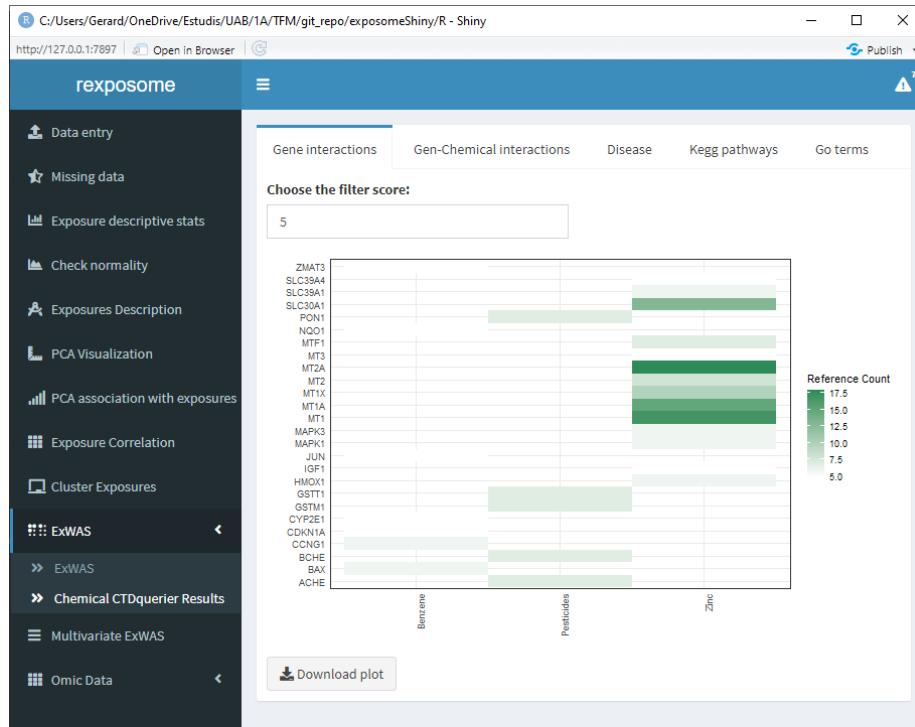


- Once all the exposures of interest are on the ‘Querier’ table, click ‘Query on the CTD gene database’

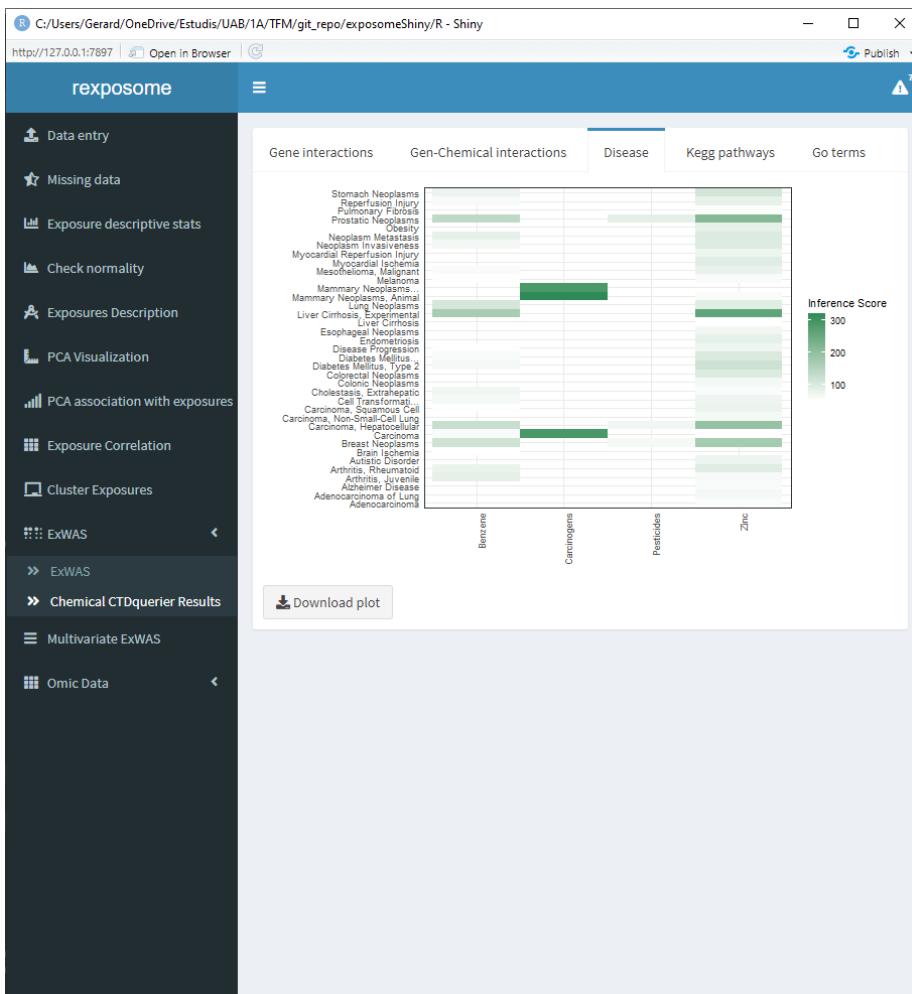
The results of this query can be visualized on the ‘Chemical CTDquerier Results’. There are five visualization options:



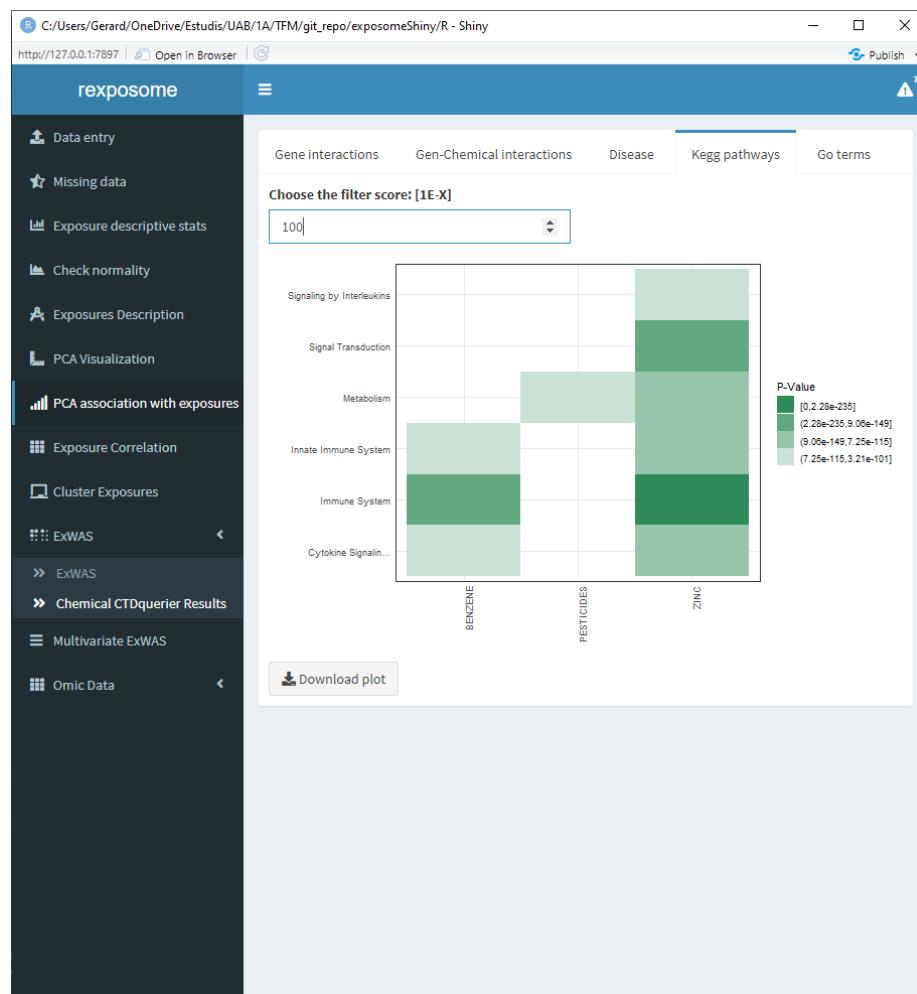
### 5.1.11.1 Gene - chemical interactions



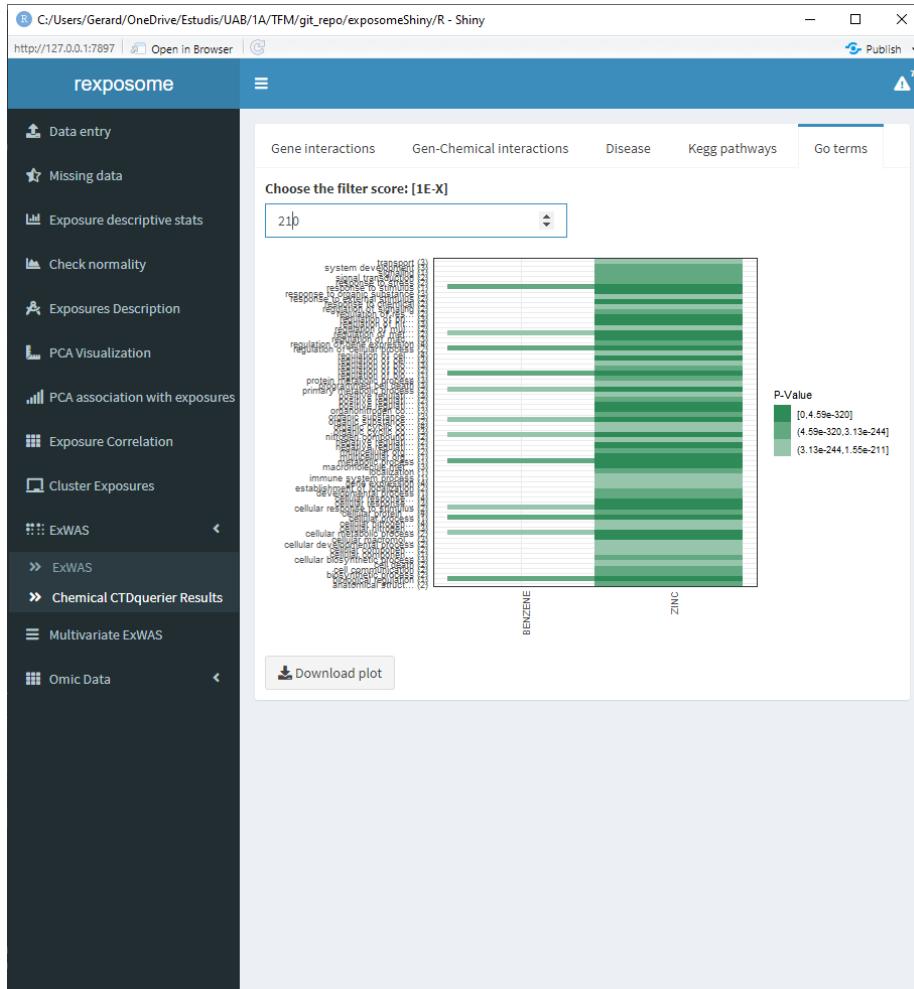
### 5.1.11.2 Disease relation



### 5.1.11.3 Kegg pathways

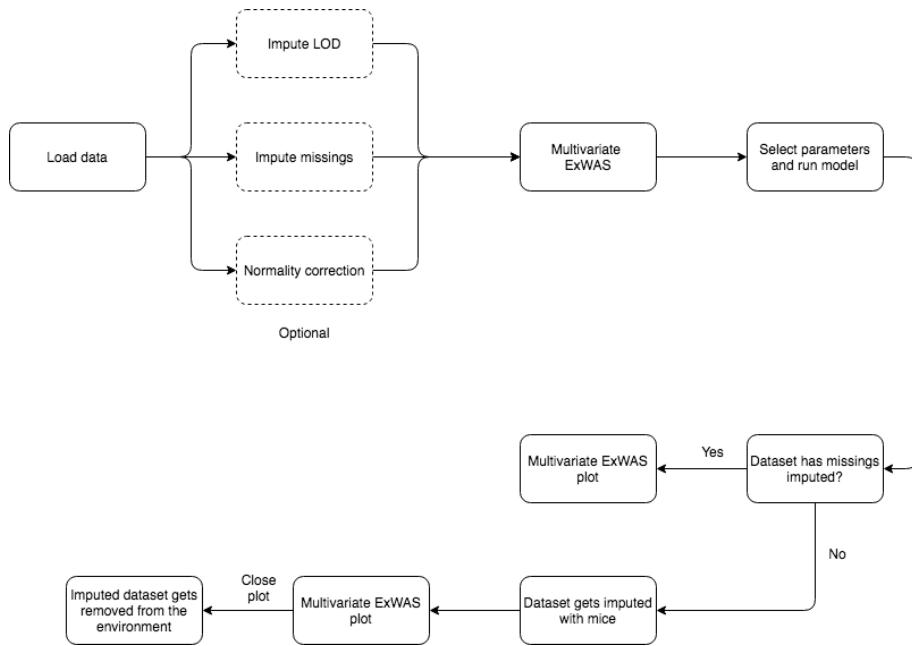


### 5.1.11.4 Go terms



### 5.1.12 Variable selection ExWAS

Variable selection ExWAS applies elastic net (LASSO regression) to the exposures given a health outcome of interest. The resulting heat map is coloured with the coefficient of each exposure in relation to the health outcome, so the ones in white are not associated. The two columns of the heat map correspond to the minimum lambda (Min) and to the lambda which gives the most regularized model such that error is within one standard error of the minimum (1SE).



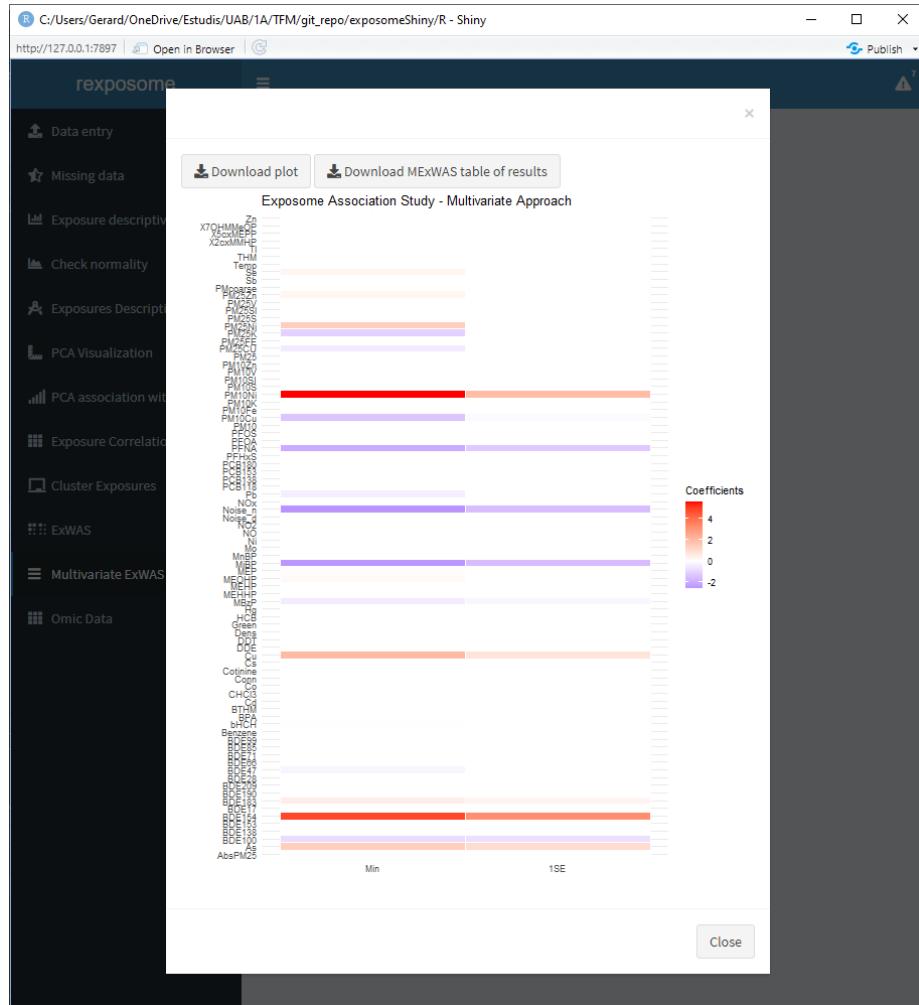
To perform a variable selection ExWAS study, check the Variable selection ExWAS tab and select the desired output parameter, click on run model to generate the plot.

This analysis requires data without missings, so if there are missings MICE imputation is applied beforehand. After the model is fitted, the imputed dataset will be removed.

The screenshot shows the user interface of the rexposome R-Shiny application. The left sidebar contains a vertical list of analysis modules:

- Data entry
- Missing data
- Exposure descriptive stats
- Check normality
- Exposures Description
- PCA Visualization
- PCA association with exposures
- Exposure Correlation
- Cluster Exposures
- ExWAS
- Multivariate ExWAS
- Omic Data

The main panel is titled "Choose the outcome variable:" and contains a dropdown menu set to "flu". Below it is another dropdown menu titled "Choose the output family:" set to "binomial". At the bottom of this panel is a "Run model" button.



The table of results and plot can be downloaded.

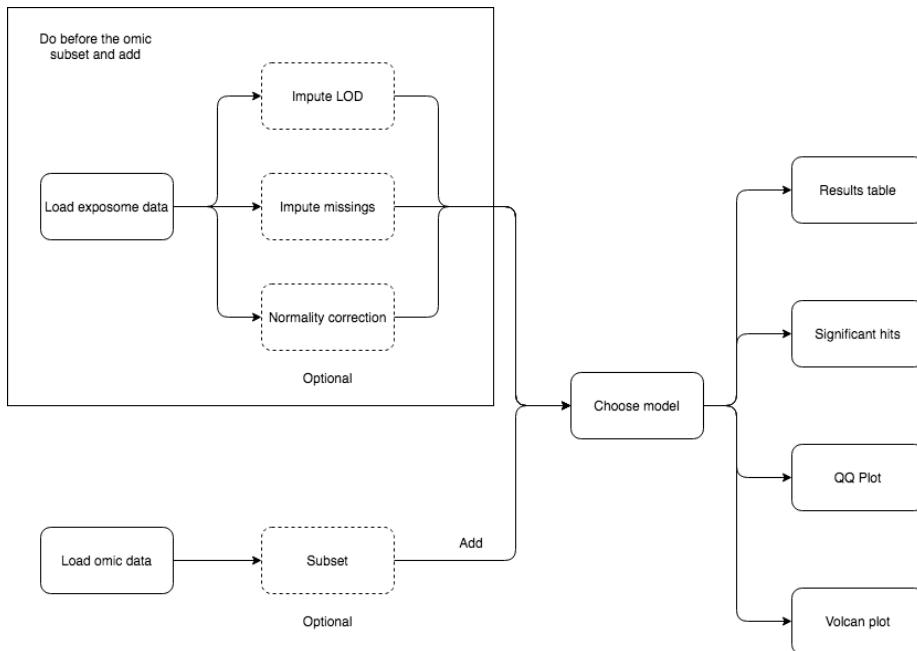
## 5.2 Exposome-Omic analysis

The aim of this analysis is to perform an association test between the gene expression levels and the exposures. The datasets used in this section are `exposures.csv`, `description.csv`, `phenotypes.csv` and `gene_exp.Rdata` which are available here.

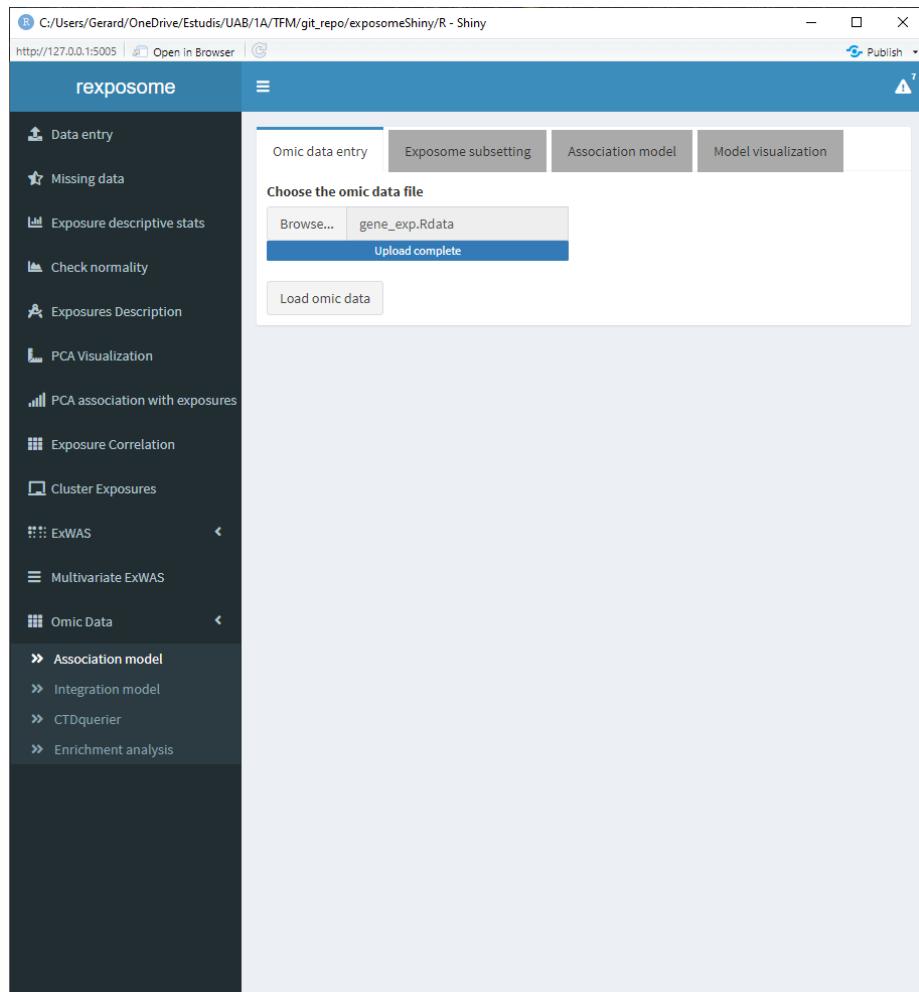
It's important noting that (by default) the maximum size of the omics data is 30 MB, if the omics file to be analyzed is bigger, change the line number 2 of the `server.R` file.

```
# the "30" refers to 30MB, change as needed
options(shiny.maxRequestSize=30*1024^2)
```

### 5.2.1 Association analysis



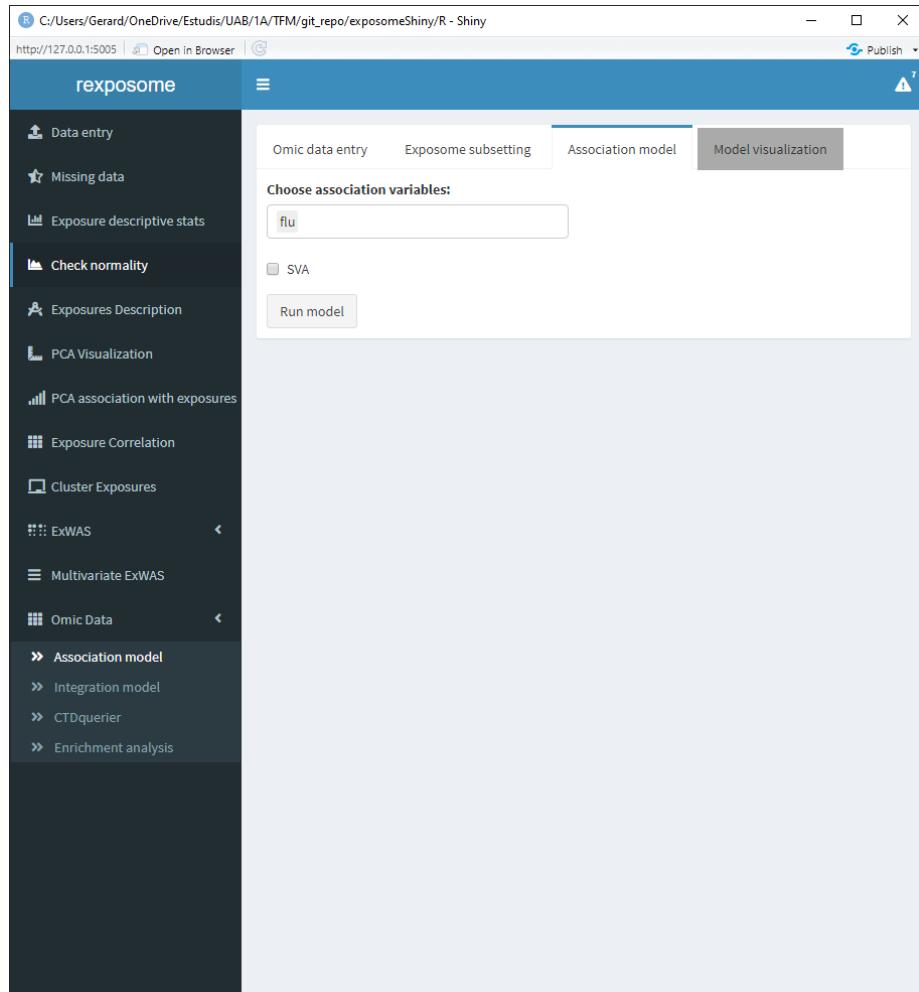
First, make sure that the exposome dataset is loaded. Then, proceed to loading the omics data, which should be provided as a **\*.RData**.



This type of analysis is usually very computationally intensive, so it's useful to be able to limit the scope. The exposome data can be subsetted by families for that reason. If all the families are desired don't input any and proceed to click the "Subset and add".

The screenshot shows the rexposome shiny application interface. The left sidebar contains a vertical list of analysis tools: Data entry, Missing data, Exposure descriptive stats, Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, Omic Data, Association model, Integration model, CTDquerier, and Enrichment analysis. The main panel has a header with tabs: Omic data entry, Exposome subsetting (which is active), Association model, and Model visualization. Below the tabs, there is a section titled "Choose an exposure family:" with a text input field containing "Metals". A button labeled "Subset and add" is present. A note at the bottom of this section says: "If no subsetting is required, do not input any family and click 'Subset and add'".

Select the variables for the association analysis (linear models) and if SVA (surrogate variable analysis) is wanted on the “Association model” subtab.



Once complete, there are various tabs to visualize the results.

The “Results table” shows the gene, log of the fold change, p-value and adjusted p-value.

The screenshot shows the rexposome shiny application interface. The left sidebar contains a navigation menu with various options: Data entry, Missing data, Exposure descriptive stats, Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, Omic Data, Association model (which is selected), Integration model, CTDquerier, and Enrichment analysis. The main panel has tabs for Omic data entry, Exposome subsetting, Association model (selected), and Model visualization. Below these tabs is a sub-tab bar with Results table (selected), Significant hits, QQ Plot, and Volcano plot. A search bar allows users to show 10 entries and search for specific terms. The main content area displays a table with columns for logFC, PValue, and adj.PVal. The table lists 10 entries from a total of 20,000, with the first few rows being TC02001791.hg.1, TC02003390.hg.1, TC01004554.hg.1, TC01004476.hg.1, TC05001945.hg.1, TC03001844.hg.1, TC03003225.hg.1, TC05001428.hg.1, TC02000545.hg.1, and TC02000374.hg.1. The table includes a footer indicating it is showing 1 to 10 of 20,000 entries and provides navigation links for Previous, Next, and page numbers 1 through 2000.

	logFC	PValue	adj.PVal
TC02001791.hg.1	-0.18	0	1
TC02003390.hg.1	-0.21	0	1
TC01004554.hg.1	-0.16	0	1
TC01004476.hg.1	-0.13	0	1
TC05001945.hg.1	-0.12	0	1
TC03001844.hg.1	-0.23	0	1
TC03003225.hg.1	0.15	0	1
TC05001428.hg.1	-0.21	0	1
TC02000545.hg.1	-0.2	0	1
TC02000374.hg.1	0.1	0	1

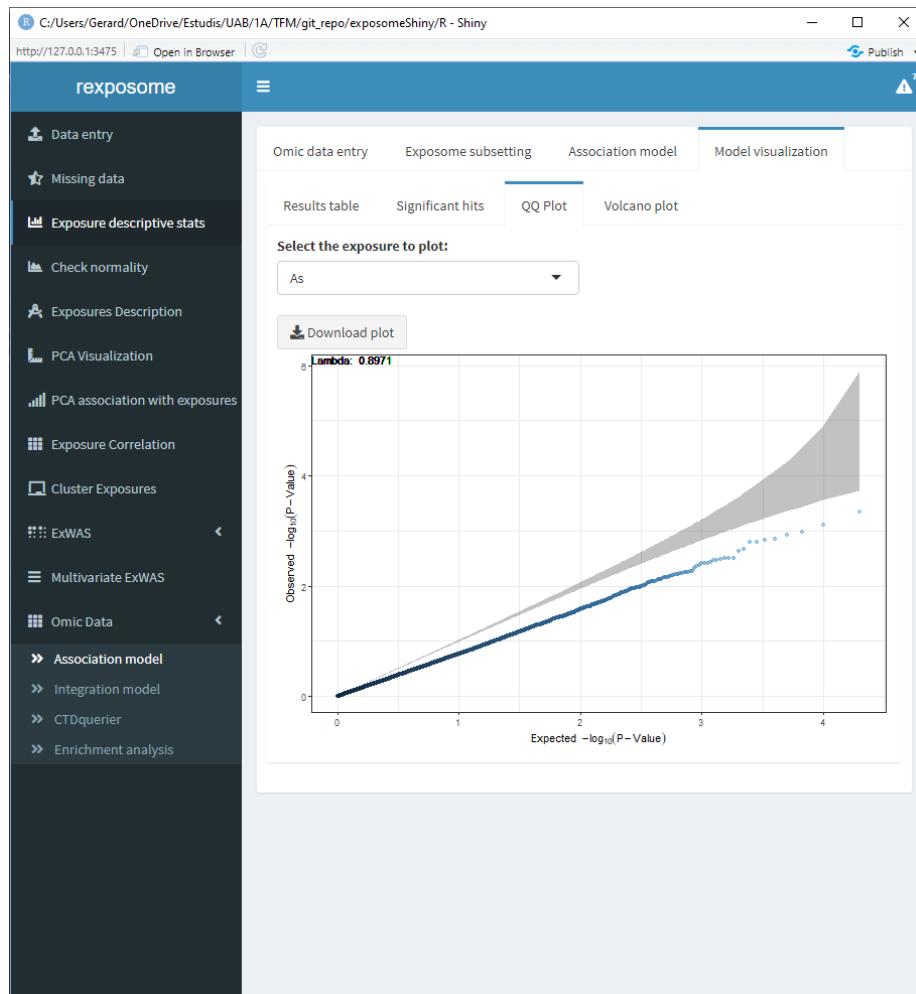
The “Significant hits” shows the exposure, hits and lambda.

The screenshot shows a Shiny application window titled "reXposome". The left sidebar contains a menu with various options: Data entry, Missing data, Exposure descriptive stats, Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, Omic Data, Association model, Integration model, CTDquerier, and Enrichment analysis. The main panel has tabs for Omic data entry, Exposome subsetting, Association model, and Model visualization. The Model visualization tab is active, showing sub-tabs for Results table, Significant hits, QQ Plot, and Volcano plot. The Results table tab is selected, displaying a table with columns: Exposure, Hits, and Lambda. The table lists 13 entries:

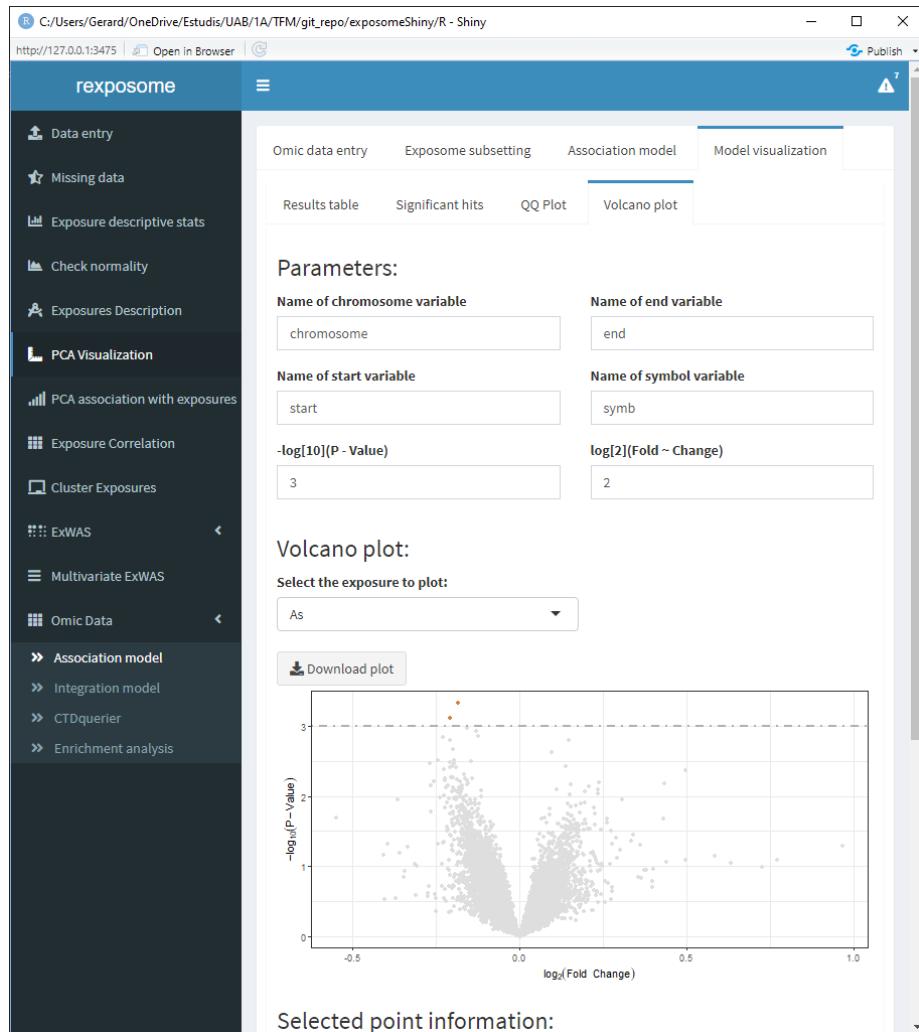
Exposure	Hits	Lambda
As	2	0.9
Cd	28	1.01
Co	5	0.84
Cs	17	0.99
Cu	18	0.94
Hg	4	0.9
Mo	5	0.93
Ni	56	1.82
Pb	9	0.95
Sb	5	0.83

Below the table, it says "Showing 1 to 10 of 13 entries" and has navigation buttons for Previous, Next, and page numbers 1 and 2.

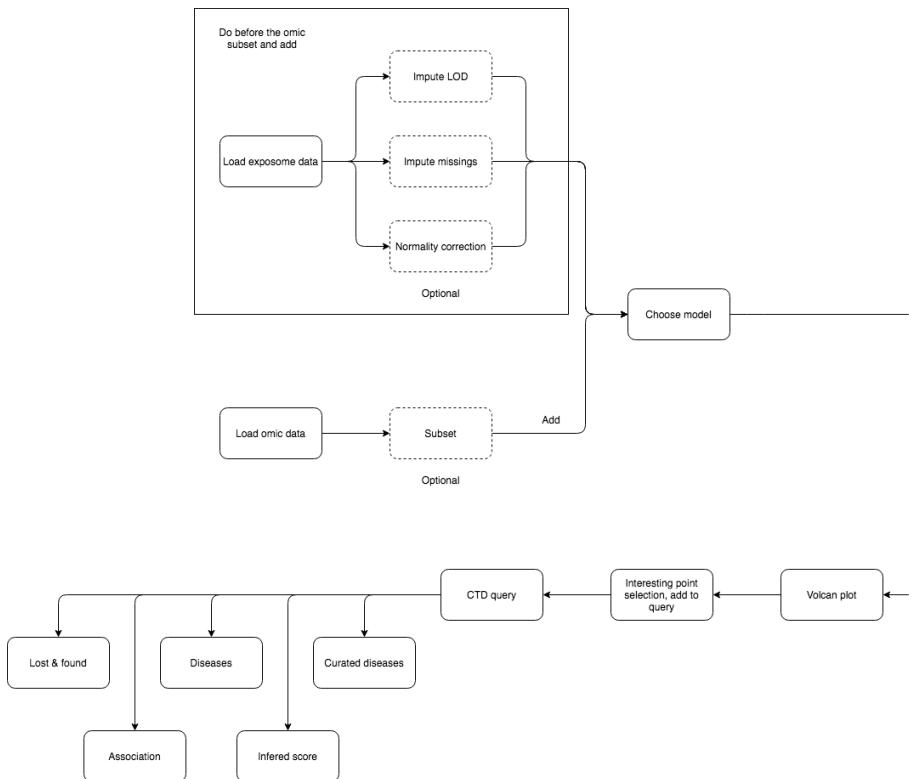
The QQ Plot shows a QQ plot (expected vs. observed -lo10(p-value)) for the selected exposure.



The Volcan plot shows a volcano plot ( $\log_2(\text{fold change})$  vs  $-\log_{10}(\text{p-value})$ ). For this plot there are two input cells to adjust the horizontal and vertical limit lines to filter out the results.



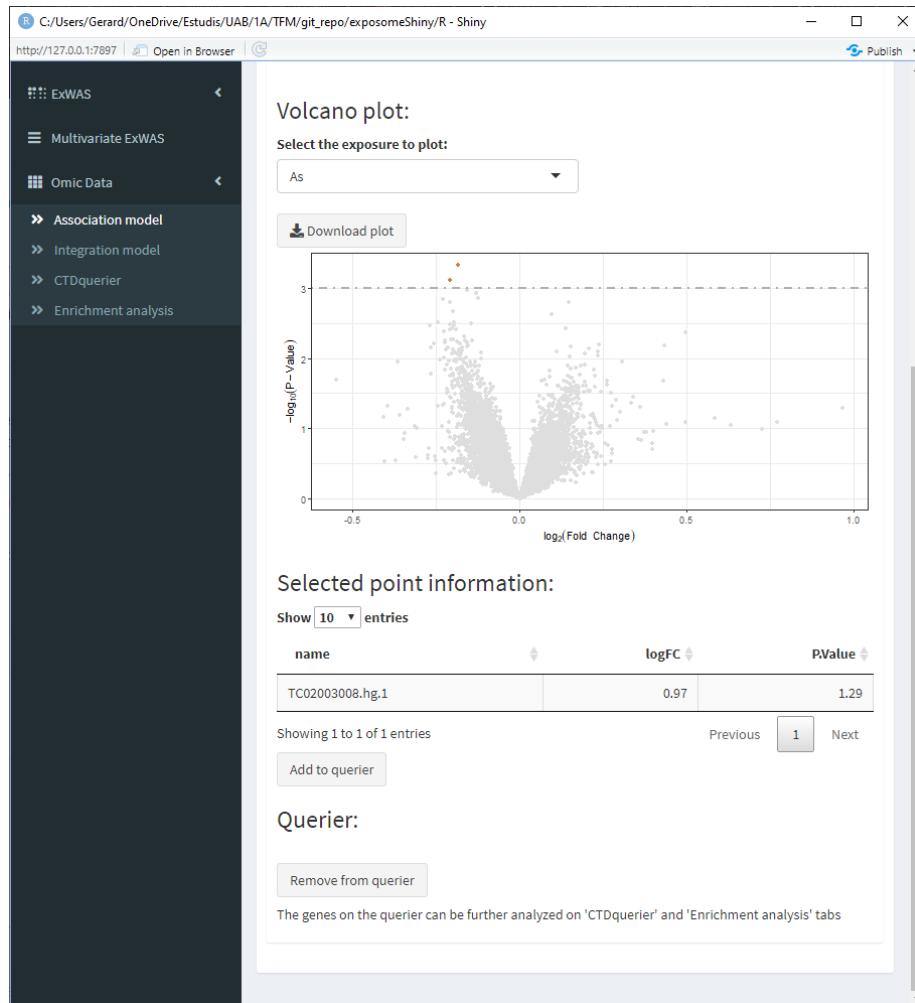
### 5.2.2 CTD querier



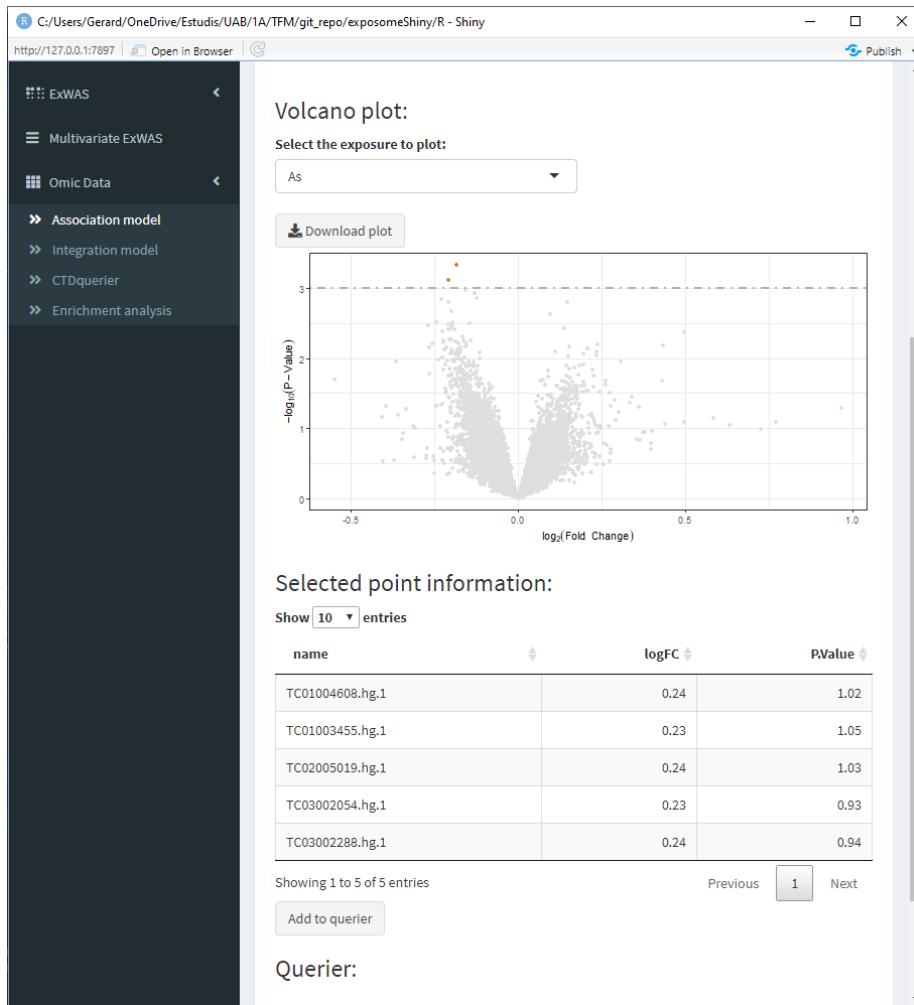
The association analysis of exposome data and omic data may point some genes of interest. Information about these genes can be queried on the CTD database.

To do so:

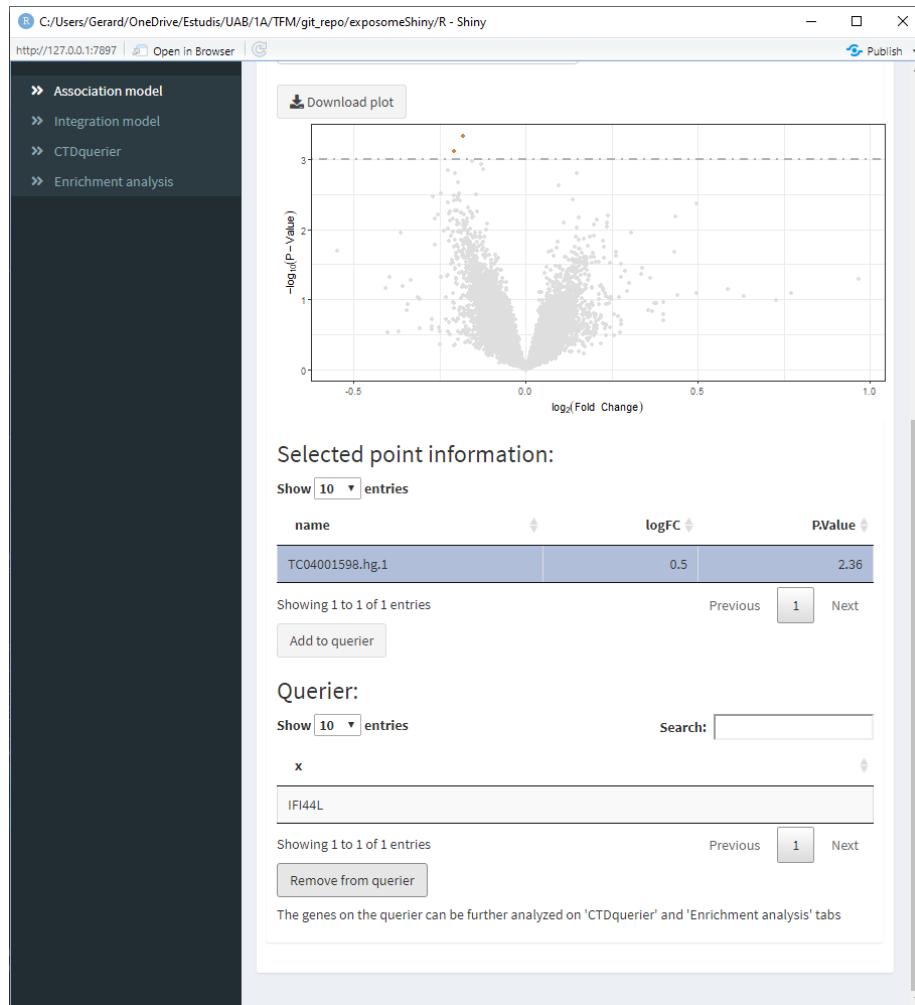
1. Perform association analysis
2. Go to the volcano plot visualization
3. Select one point. This will update the 'Selected point information' table at the bottom



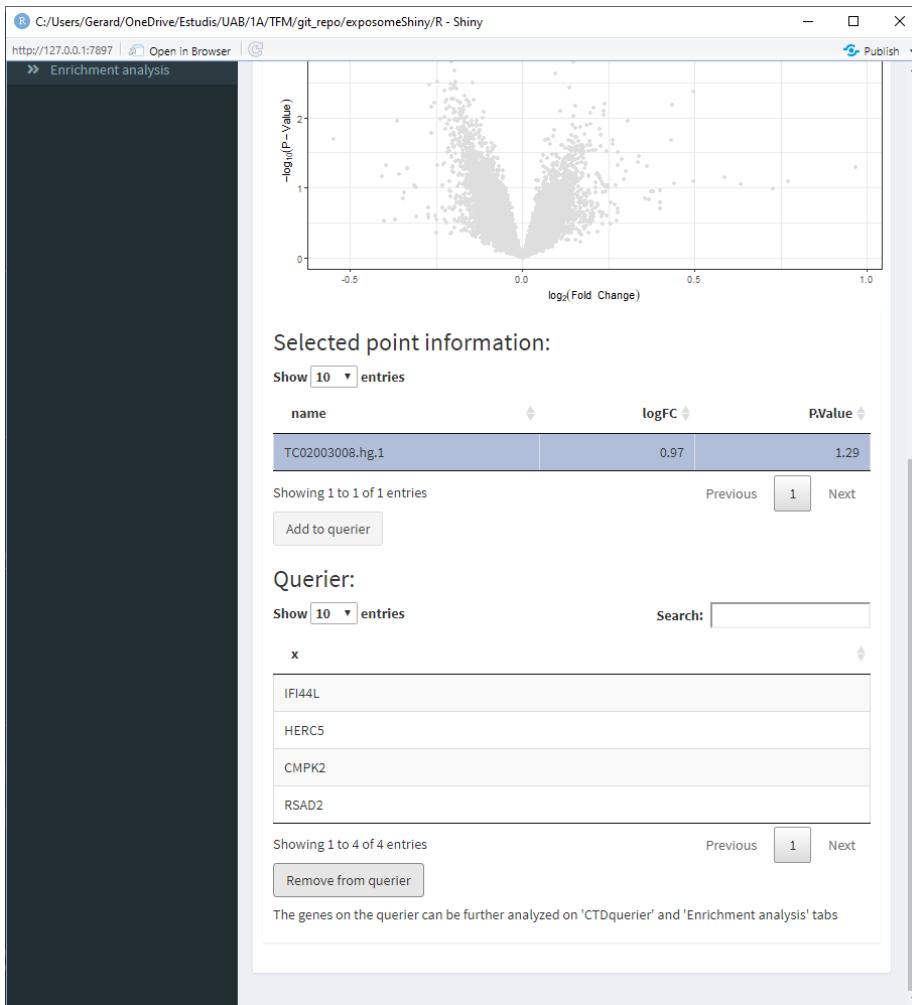
Sometimes multiple points may be very close, so this table will show information about them



4. Select the point of interest from the 'Selected point information' table and click on 'Add to querier'. This will initiate the search of the gene related to the selected SNP. Sometimes the search may not yield a resulting gene.



5. Repeat 3/4 for all the points of interest



When all the genes of interest are on the querier, move to the 'CTDquerier' tab. On it, the genes to be queried can be visualized before performing the query. Once the query is performed, the results can be visualized on the different tabs.

The screenshot shows the 'reXosome' Shiny application interface. The left sidebar contains a list of analysis tools:

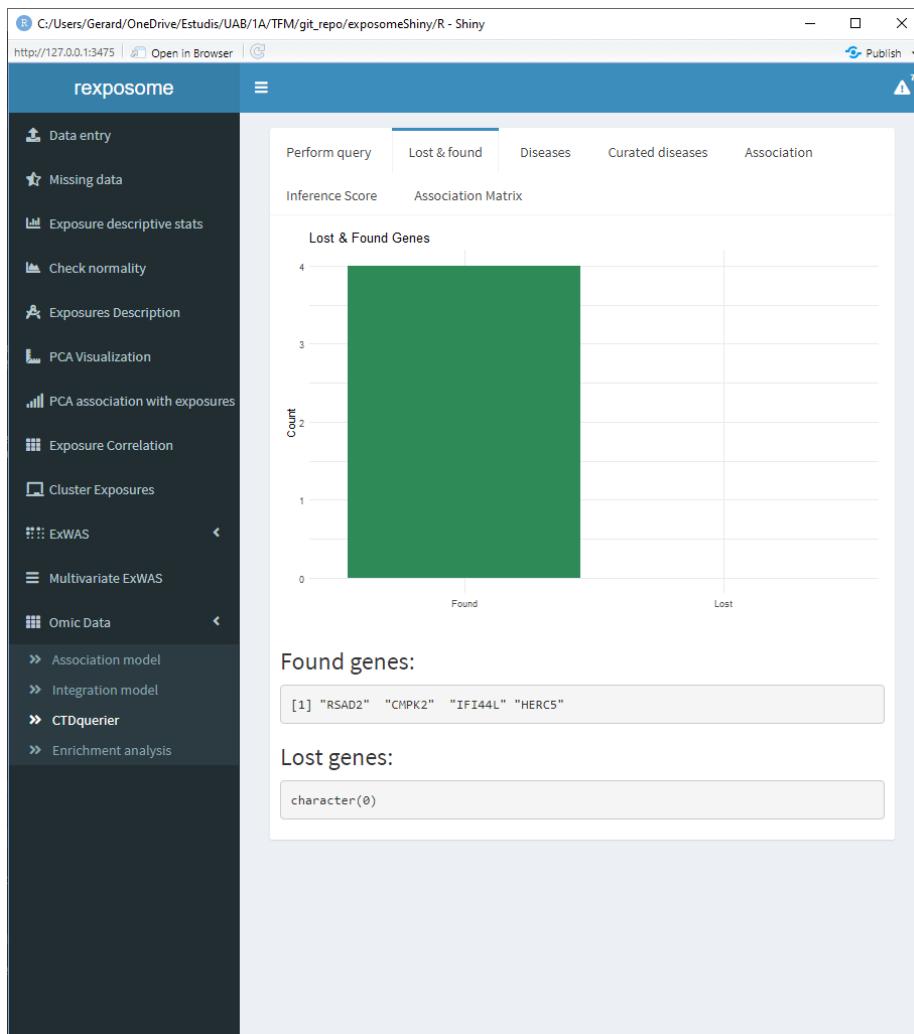
- Data entry
- Missing data
- Exposure descriptive stats
- Check normality
- Exposures Description
- PCA Visualization
- PCA association with exposures
- Exposure Correlation
- Cluster Exposures
- ExWAS
- Multivariate ExWAS
- Omic Data
  - Association model
  - Integration model
  - CTDquerier
  - Enrichment analysis

The main panel features a navigation bar with tabs: Perform query, Lost & found, Diseases, Curated diseases, and Association. The 'Perform query' tab is selected, displaying a table titled 'Querier' with the following data:

RSAD2
CMPK2
IFI44L
HERC5

Below the table, there are buttons for 'Previous' (disabled), '1', and 'Next'. A button labeled 'Query genes on the CTD gene database' is also visible.

There are six tabs showing different results interpretations. First there's the "Lost & found" tab which a plot to see the amount of genes found on the CTD database and the ones that were not found , there's also two lists stating the names of them.



The diseases tab shows a table of all the associated diseases found on the CTD database.

The screenshot shows the 'reXposome' Shiny application interface. The left sidebar contains a navigation menu with various options: Data entry, Missing data, Exposure descriptive stats, Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, Omic Data (with sub-options: Association model, Integration model, CTDquerier, Enrichment analysis), and a 'lost & found' section.

The main panel has tabs at the top: Perform query, Lost & found, Diseases (which is selected), Curated diseases, and Association. Below these are Inference Score and Association Matrix buttons. A search bar and a dropdown for 'Show 10 entries' are also present.

The central part of the screen displays a table of associated diseases:

	Disease.Name	Disease.ID	Direct.Evidence	Inference.Score	Reference
1	Myocardial Ischemia	MESH:D017202	marker/mechanism	16.99	
2	Influenza, Human	MESH:D007251	marker/mechanism	4.75	
3	Necrosis	MESH:D009336		228.75	
4	Chemical and Drug Induced Liver Injury	MESH:D056486		221.51	
5	Prenatal Exposure Delayed Effects	MESH:D011297		200.96	
6	Weight Loss	MESH:D015431		200.8	
7	Hyperplasia	MESH:D006965		192.75	
8	Inflammation	MESH:D007249		187.53	
9	Hepatomegaly	MESH:D006529		181.99	
10	Poisoning	MESH:D011041		164.68	

Below the table, it says 'Showing 1 to 10 of 6,168 entries' and includes a page navigation bar with buttons for Previous, 1, 2, 3, 4, 5, ..., 617, and Next.

The curated diseases tab shows the table of associated diseases but only shows the ones with direct evidence.

The screenshot shows the rexposome shiny application interface. The left sidebar contains a navigation menu with various options like Data entry, Missing data, Exposure descriptive stats, Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, Omic Data, Association model, Integration model, CTDquerier, and Enrichment analysis. The main panel has tabs at the top: Perform query, Lost & found, Diseases, Curated diseases (which is selected), and Association. Below these are two buttons: Inference Score and Association Matrix. A search bar with 'Search:' and a dropdown 'Show 10 entries' are also present. The main content area displays a table with the following data:

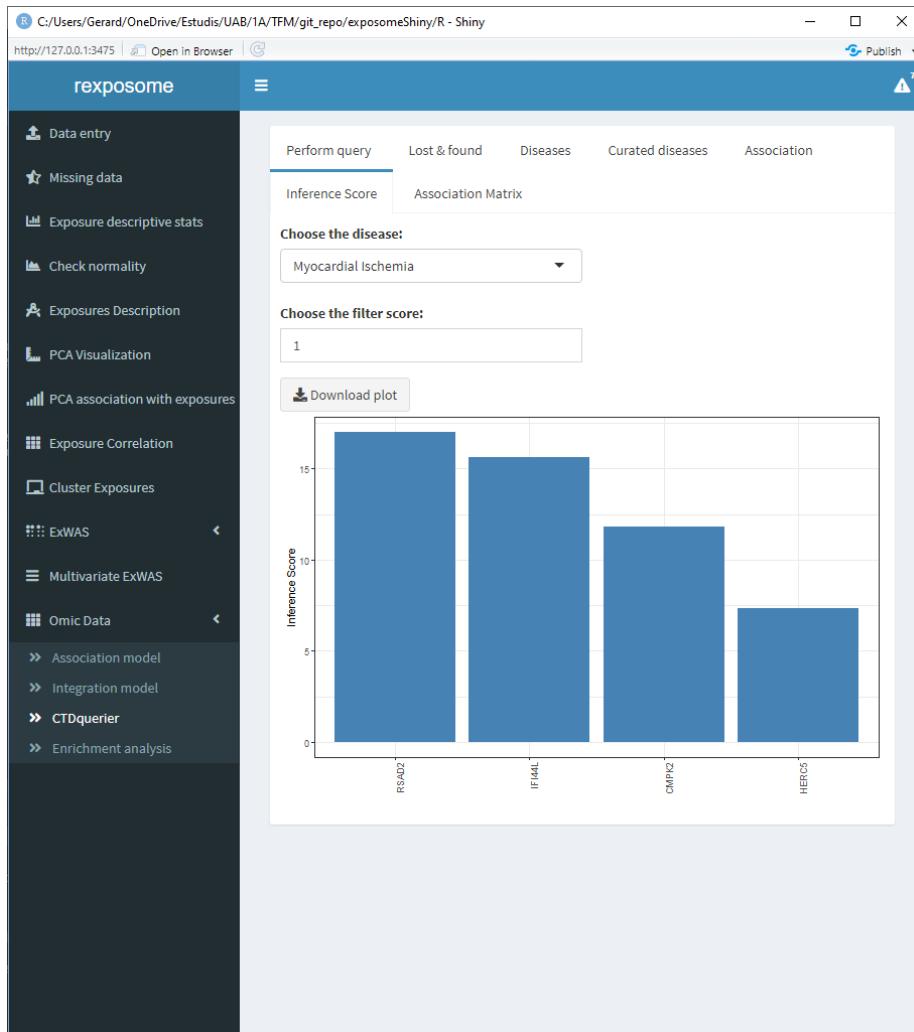
	Disease.Name	Disease.ID	Inference.Score	Reference.Count	Gene
1	Myocardial Ischemia	MESH:D017202	16.99	20	RSAD2
2	Influenza, Human	MESH:D007251	4.75	2	RSAD2
3	Depressive Disorder, Major	MESH:D003865	11.64	7	IFI44L
4	Influenza, Human	MESH:D007251		1	IFI44L
5	Carcinoma, Hepatocellular	MESH:D006528	40.21	90	HERC5
6	Endometriosis	MESH:D004715	4.08	8	HERC5
7	Influenza, Human	MESH:D007251		1	HERC5

At the bottom, it says 'Showing 1 to 7 of 7 entries' with buttons for Previous, Next, and page number 1.

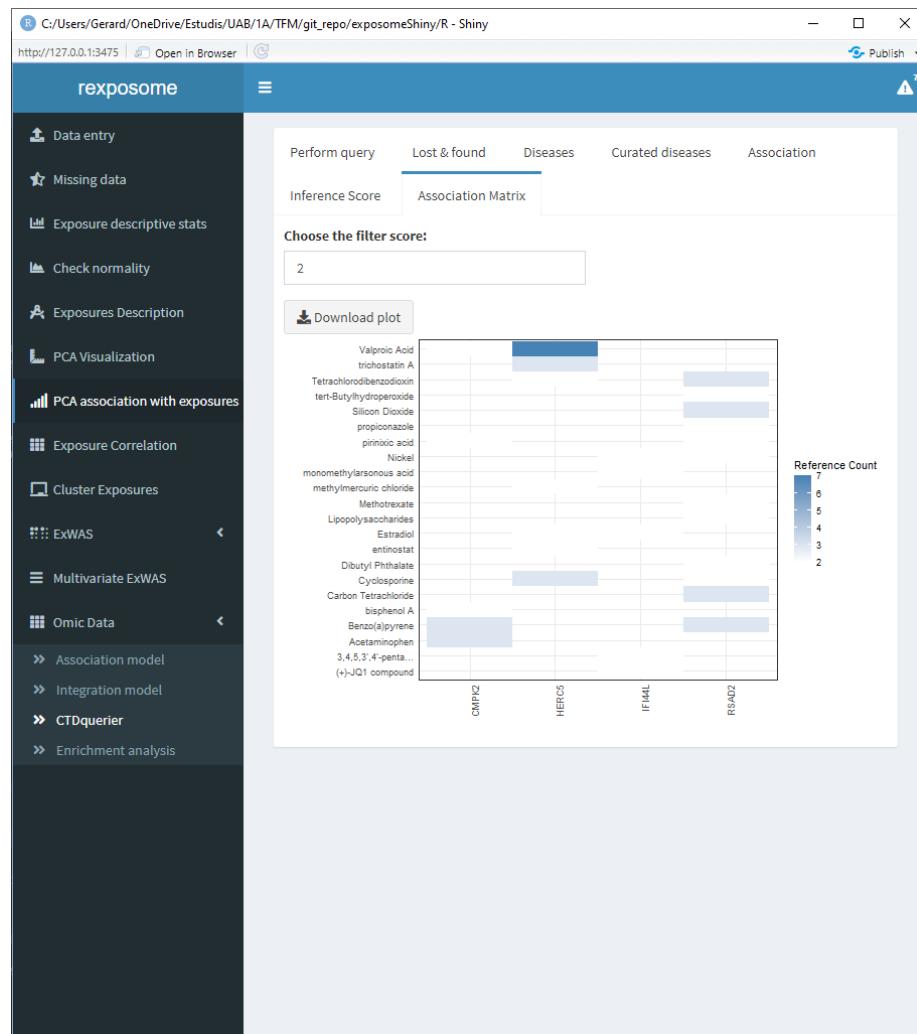
The association tab shows information about all the direct evidence associated diseases. Select the disease of interest to see the score and reference count of it.

The screenshot shows the 'reXosome' Shiny application interface. The left sidebar contains a list of analysis tools: Data entry, Missing data, Exposure descriptive stats, Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, and Omic Data. The Omic Data section is expanded, showing sub-options: Association model, Integration model, CTDquerier, and Enrichment analysis. The main panel has tabs at the top: Perform query, Lost & found, Diseases, Curated diseases, and Association. The Association tab is selected. Below the tabs are two input fields: 'Inference Score' and 'Association Matrix'. A dropdown menu labeled 'Choose the disease:' is set to 'Myocardial Ischemia'. Below it, the 'Disease score:' field contains '[1] 14.09158' and the 'Reference count:' field contains '[1] 10929'.

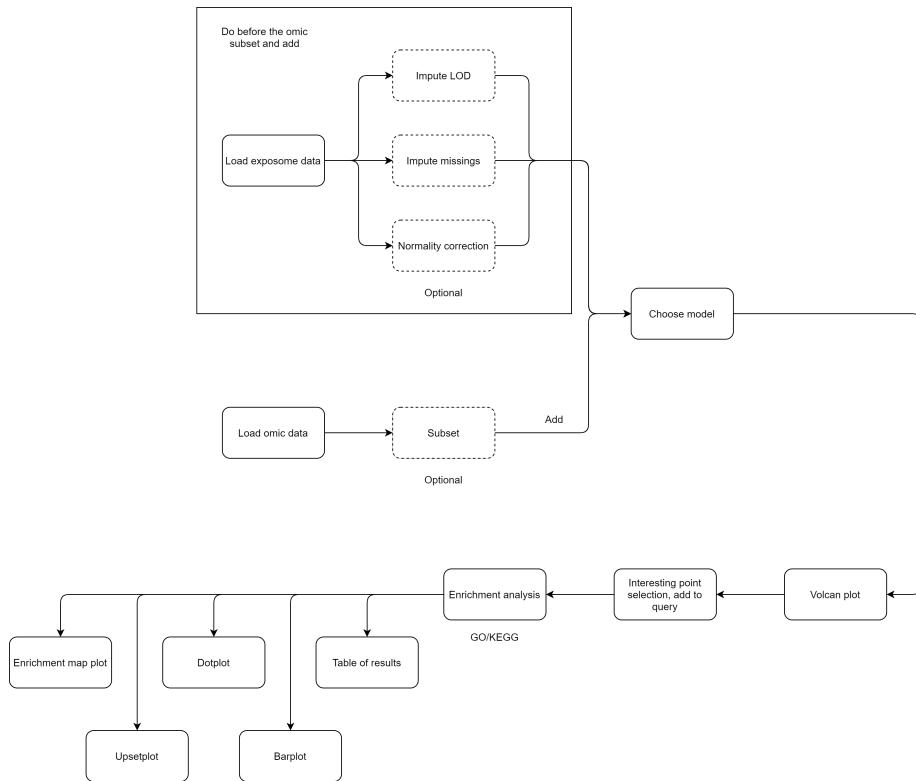
The inference score tab shows the inference score for each gene for a selected disease, the filter parameters puts out the genes with an inference score lower than the selected filter.



The association matrix tab shows a matrix of genes vs. chemicals with a heatmap representing the existing papers (references) providing evidence about the association between chemicals and genes.



### 5.2.3 Enrichment analysis



To perform an enrichment analysis, select the genes of interest by following the same procedure as with the CTDquerier.

After selecting them, go to the “Enrichment analysis” tab, on it there is a selector to choose between GO and KEGG databases and a threshold selector for the cutoff pvalue of the enrichment analysis.

The screenshot shows the 'reXposome' Shiny application interface. On the left, a sidebar menu lists various analysis options: Data entry, Missing data, Exposure descriptive stats (selected), Check normality, Exposures Description, PCA Visualization, PCA association with exposures, Exposure Correlation, Cluster Exposures, ExWAS, Multivariate ExWAS, Omic Data (selected), Association model, Integration model, CTDquerier, and Enrichment analysis. The main panel has tabs at the top: Database selection, Table of results (selected), Barplot, Dotplot, and Upsetplot. Under 'Database selection', 'GO' is selected. The 'Enrichment cutoff pvalue' is set to '0,05'. A table titled 'Querier:' lists four entries: RSAD2, CMPK2, IFI44L, and HERC5. Below the table, it says 'Showing 1 to 4 of 4 entries'. A 'Perform enrichment analysis' button is at the bottom of the table area.

When the enrichment analysis is performed, there are multiple visualization tabs for the results, the first one is a table with the plain results, which can be downloaded.

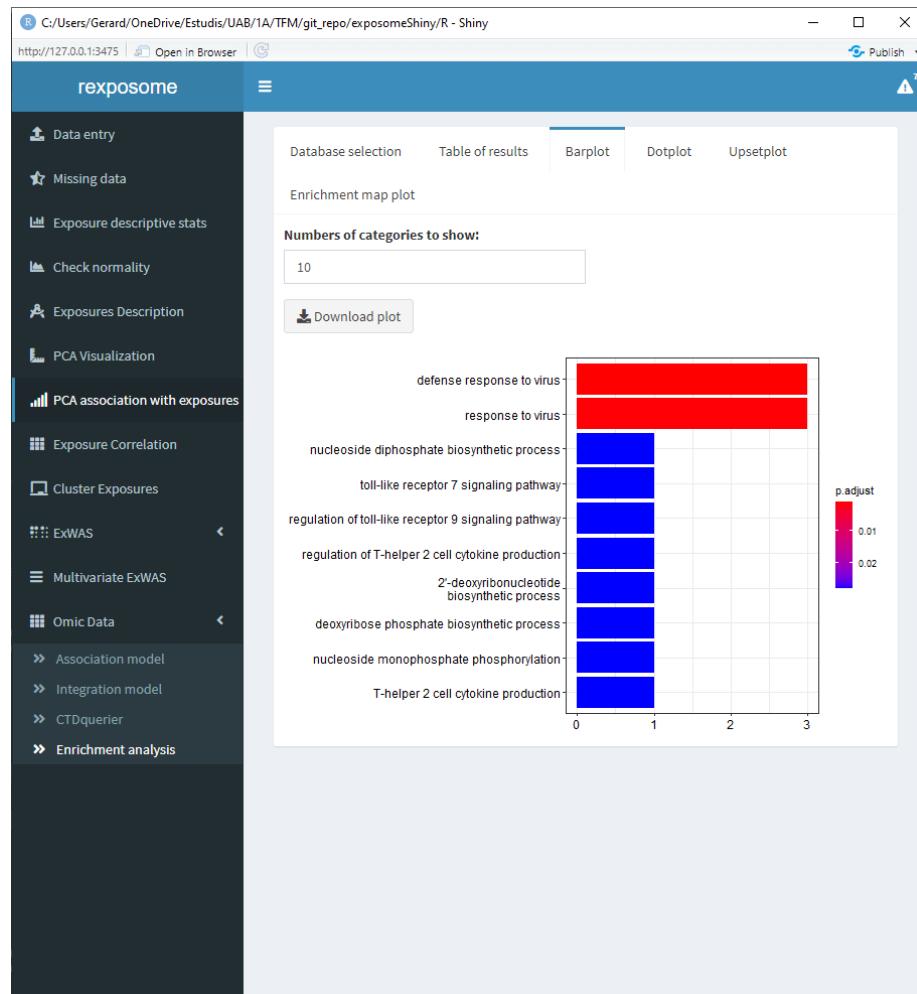
The screenshot shows the rexposome Shiny application interface. The left sidebar contains a navigation menu with the following items:

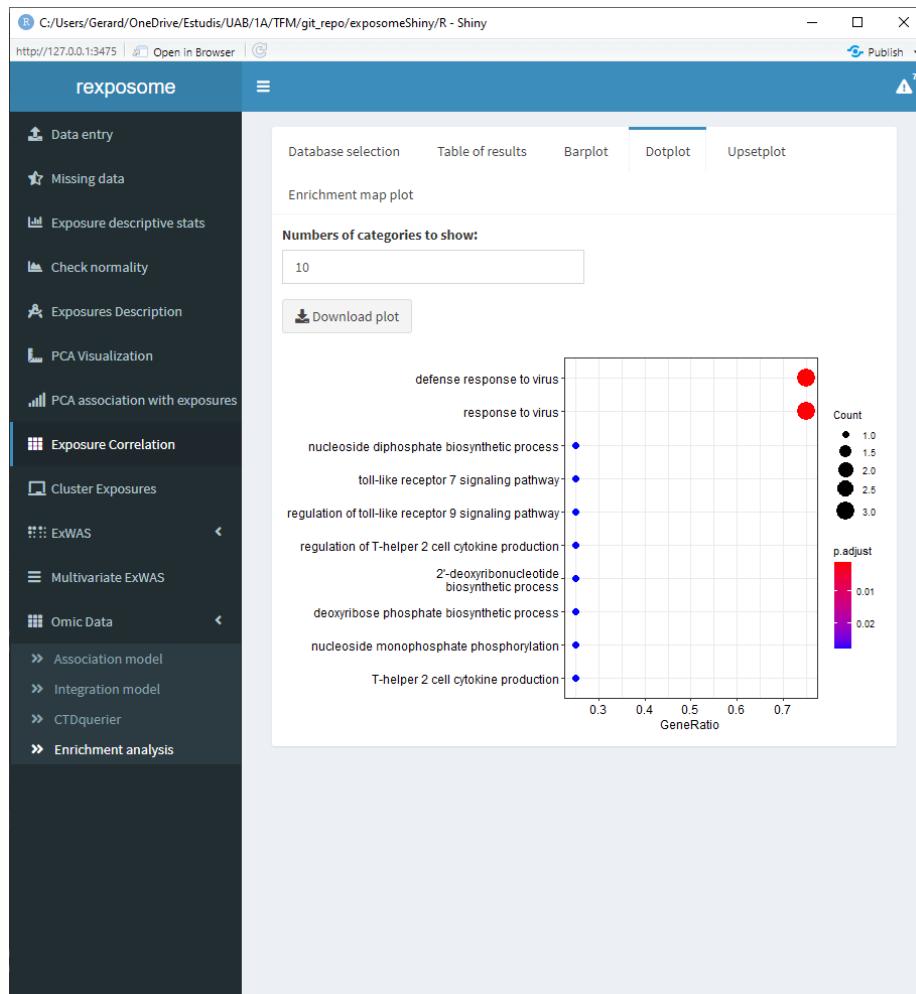
- Data entry
- Missing data
- Exposure descriptive stats
- Check normality
- Exposures Description
- PCA Visualization
- PCA association with exposures
- Exposure Correlation
- Cluster Exposures
- ExWAS
- Multivariate ExWAS
- Omic Data
- Association model
- Integration model
- CTDquerier
- Enrichment analysis

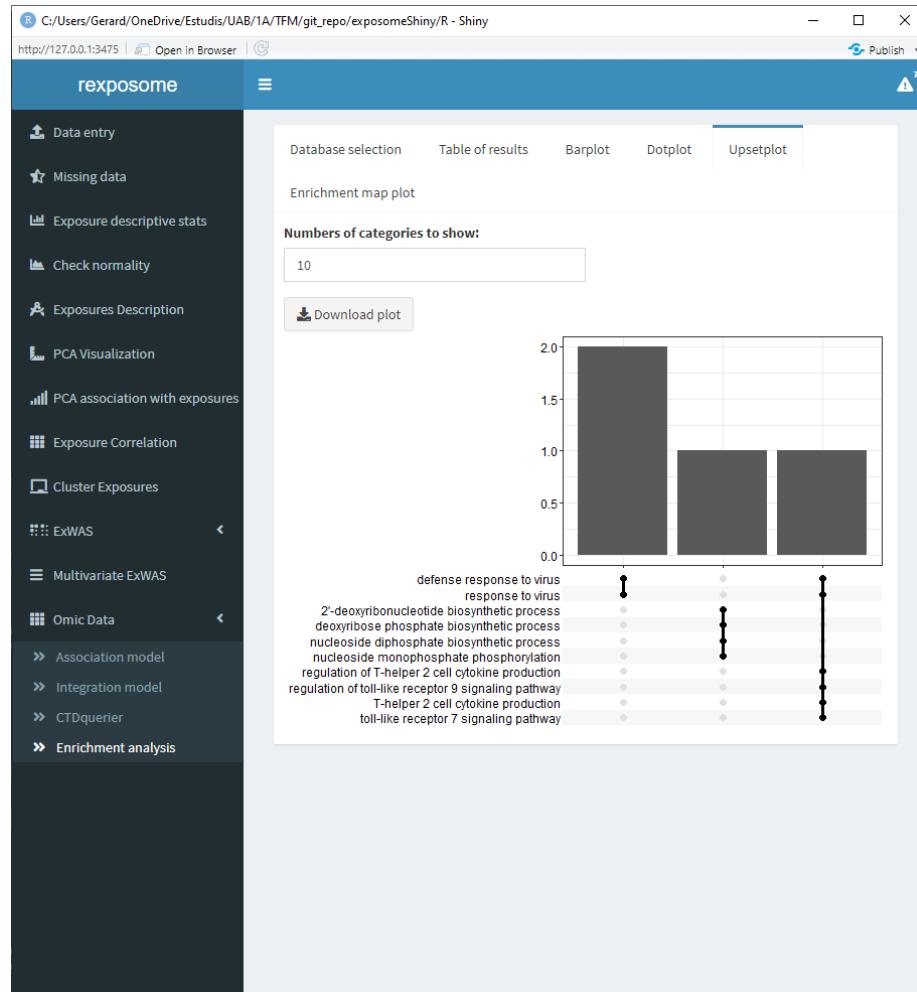
The main area has a blue header bar with tabs: Database selection, Table of results, Barplot, Dotplot, and Upsetplot. The "Table of results" tab is selected. Below it is an "Enrichment map plot" section with a "Download table" button and a search input field. A table is displayed with the following columns: ID, Description, GeneRatio, and BgRatio.

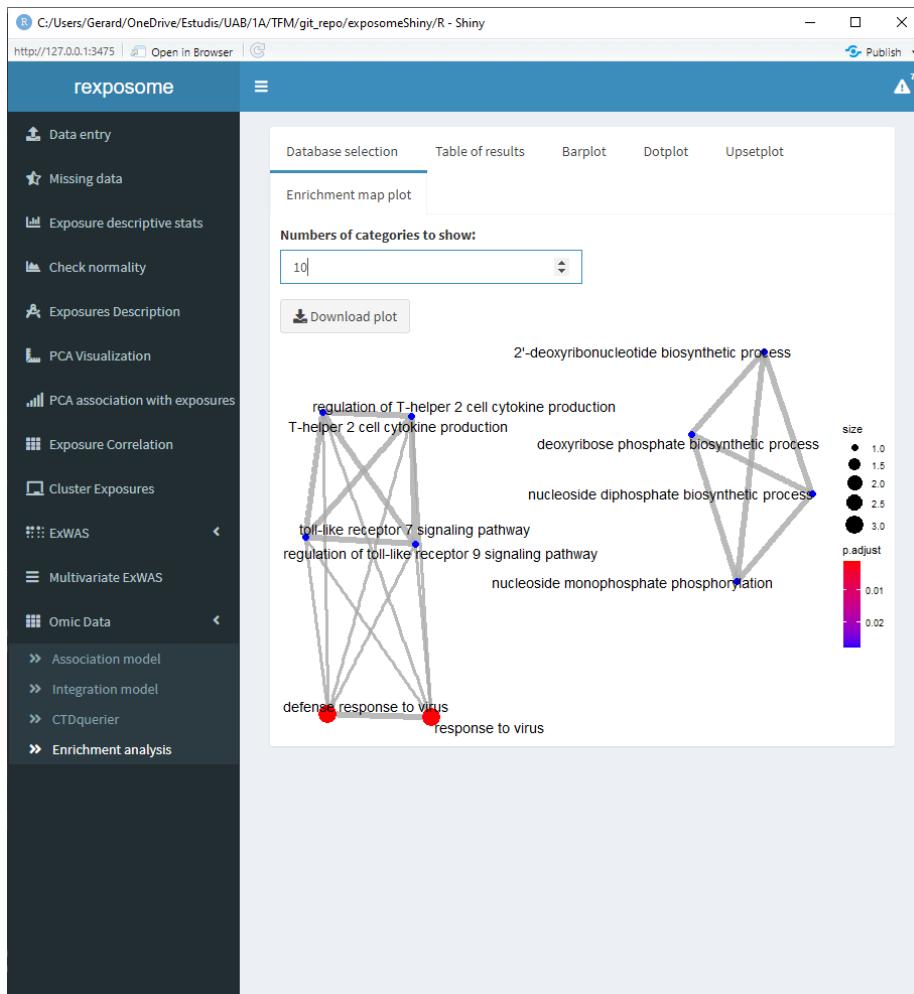
ID	Description	GeneRatio	BgRatio
GO:0051607	GO:0051607 defense response to virus	3/4	258/18866 0.00001001
GO:0009615	GO:0009615 response to virus	3/4	349/18866 0.00002476
GO:0009133	GO:0009133 nucleoside diphosphate biosynthetic process	1/4	10/18866 0.00211
GO:0034154	GO:0034154 toll-like receptor 7 signalling pathway	1/4	10/18866 0.00211
GO:0034163	GO:0034163 regulation of toll-like receptor 9 signalling pathway	1/4	10/18866 0.00211
GO:2000551	GO:2000551 regulation of T-helper 2 cell cytokine production	1/4	11/18866 0.002330
GO:0009265	GO:0009265 2'-deoxyribonucleotide biosynthetic process	1/4	13/18866 0.002753
GO:0046385	GO:0046385 deoxyribose phosphate biosynthetic process	1/4	13/18866 0.002753
GO:0046940	GO:0046940 nucleoside monophosphate phosphorylation	1/4	14/18866 0.002965
GO:0035745	GO:0035745 T-helper 2 cell cytokine production	1/4	15/18866 0.003176

The following four tabs correspond to different visualization options for the results. All of them can be downloaded.



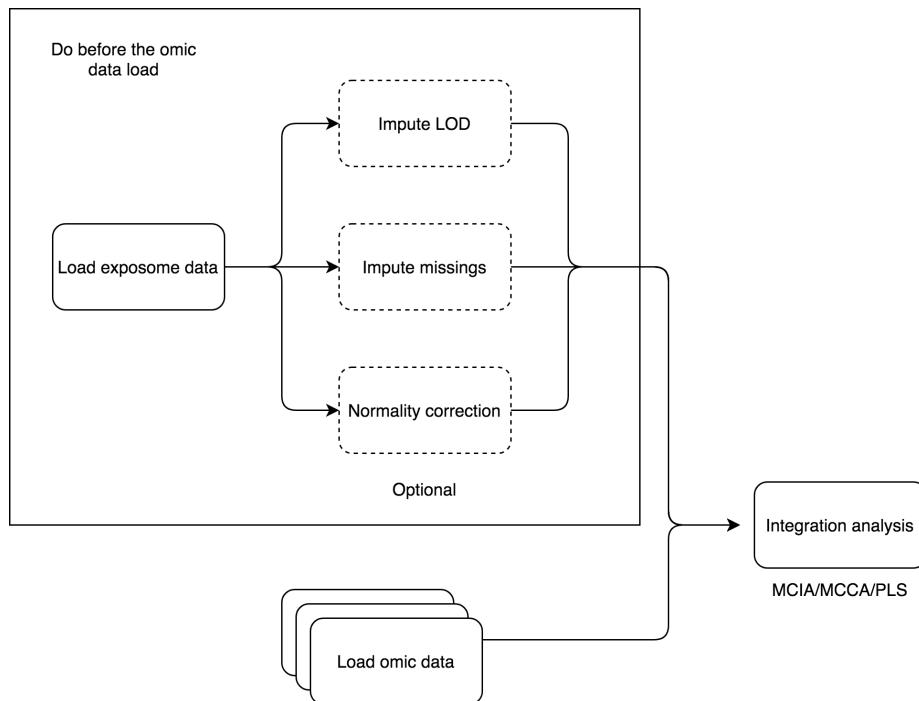






### 5.3 Exposome-Omic integration (e.g. crossomics)

The datasets used in this section are `exposures_2.csv`, `description_2.csv`, `phenotypes_2.csv`, `brge_gexp.rda` and `brge_prot.rda` which are available here.



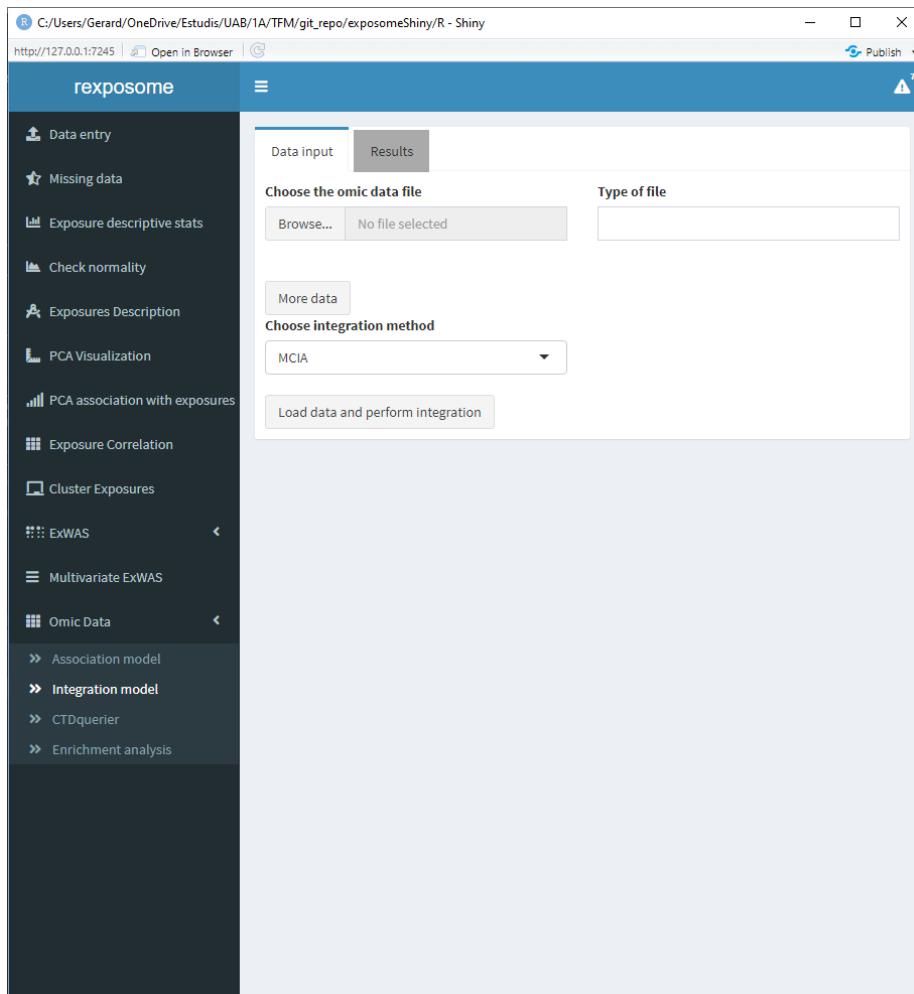
The relation between exposures and omic-features can be studied from another perspective, different from the association analyses. The integration analysis can be done, using multi canonical correlation analysis, multiple co-inertia analysis or partial least squares (this method only is supported with one omics dataset). The first method is implemented in R package PMA (CRAN) and the second in **omicade4** R package (Bioconductor). The PLS uses the **mixOmics** R package (Bioconductor).

Before conducting an integration model, an exposome dataset has to be loaded into the Shiny application.

For the MCIA and MCCA options, the exposome dataset can't contain missings, so it needs to be imputed beforehand. For the PLS there can be missings. Different visualizations are provided for each method, as the used R packages provide different visualization tools, all three methods generate different plots. The Rdata object that contains the results can be downloaded to be explored by the user on a separate R session.

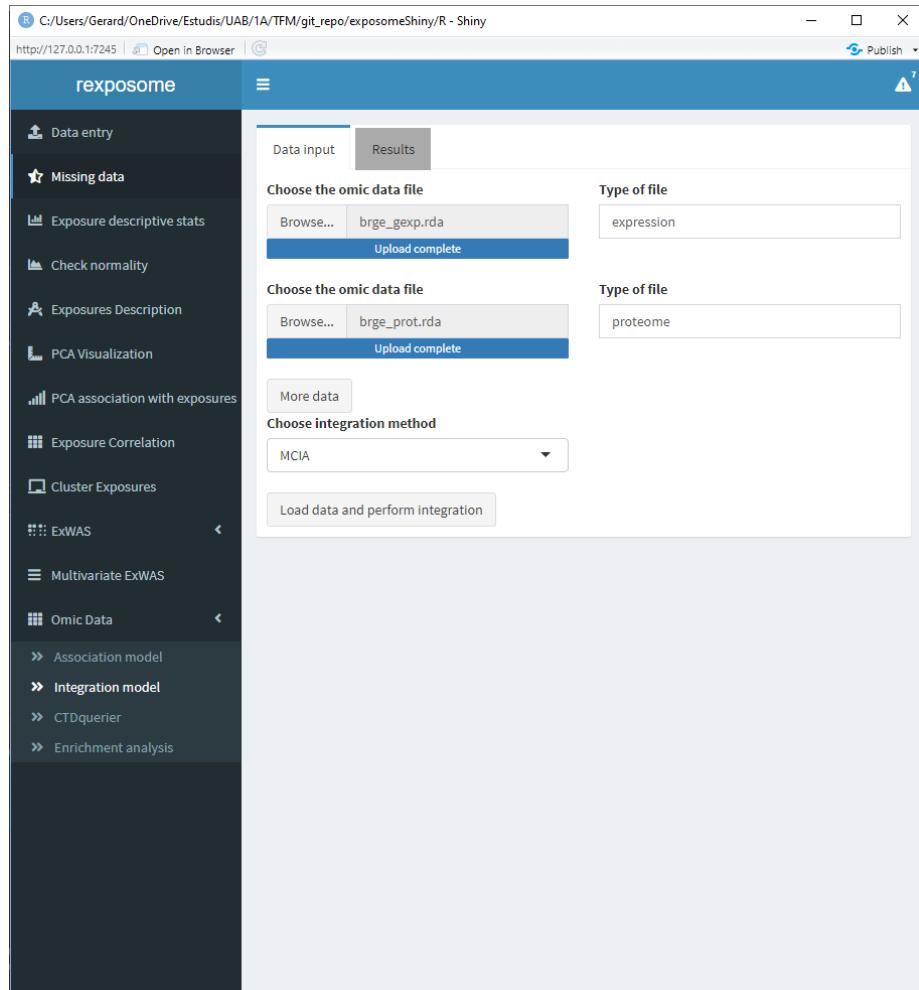
The differences between association and crossomics are that the first method test association between two complete data-sets, by removing the samples having missing values in any of the involved data-sets, and the second try to find latent relationships between two or more sets.

The initial page of the integration is the following.



The user has a field to select a omics file and a ‘Type of file’ field. This text field is to input whether the selected data is expression/proteome/... this field will be used for informative purposes when displaying the results.

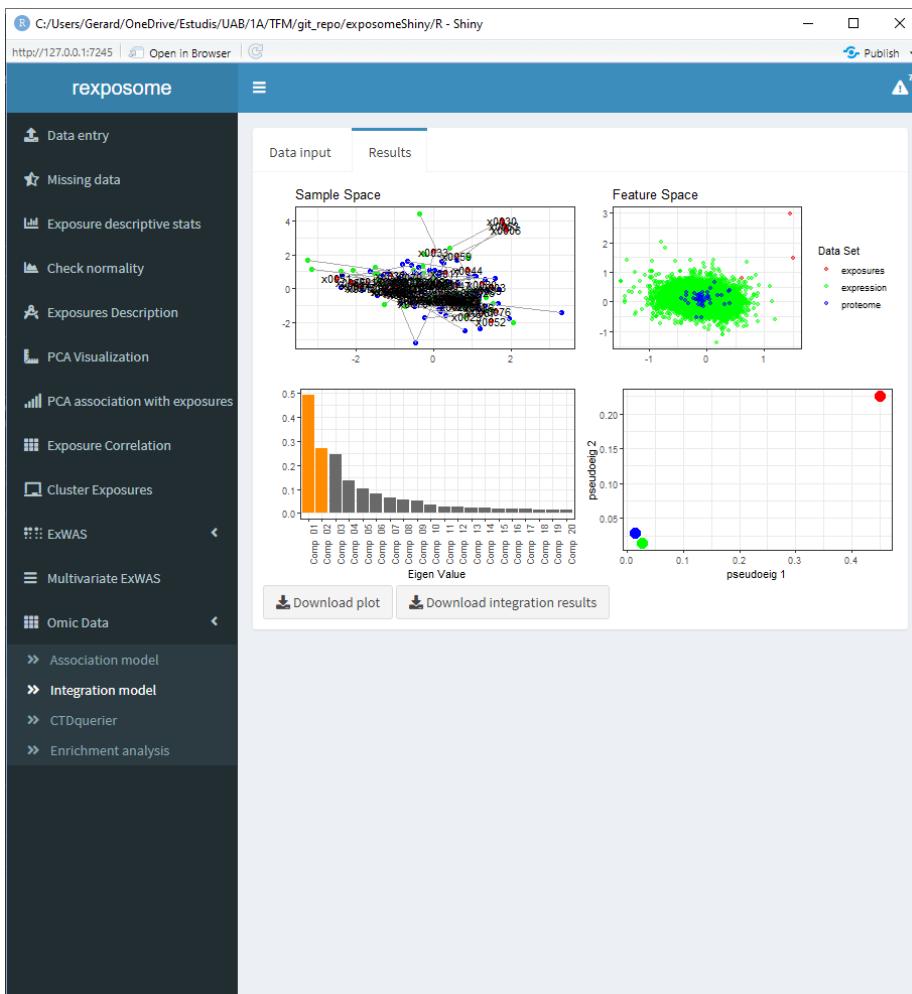
If more than one omics dataset is going to be used on the integration, click on ‘More data’ to get additional input fields, remember that only MCIA and MCCA accept more than one omics dataset.



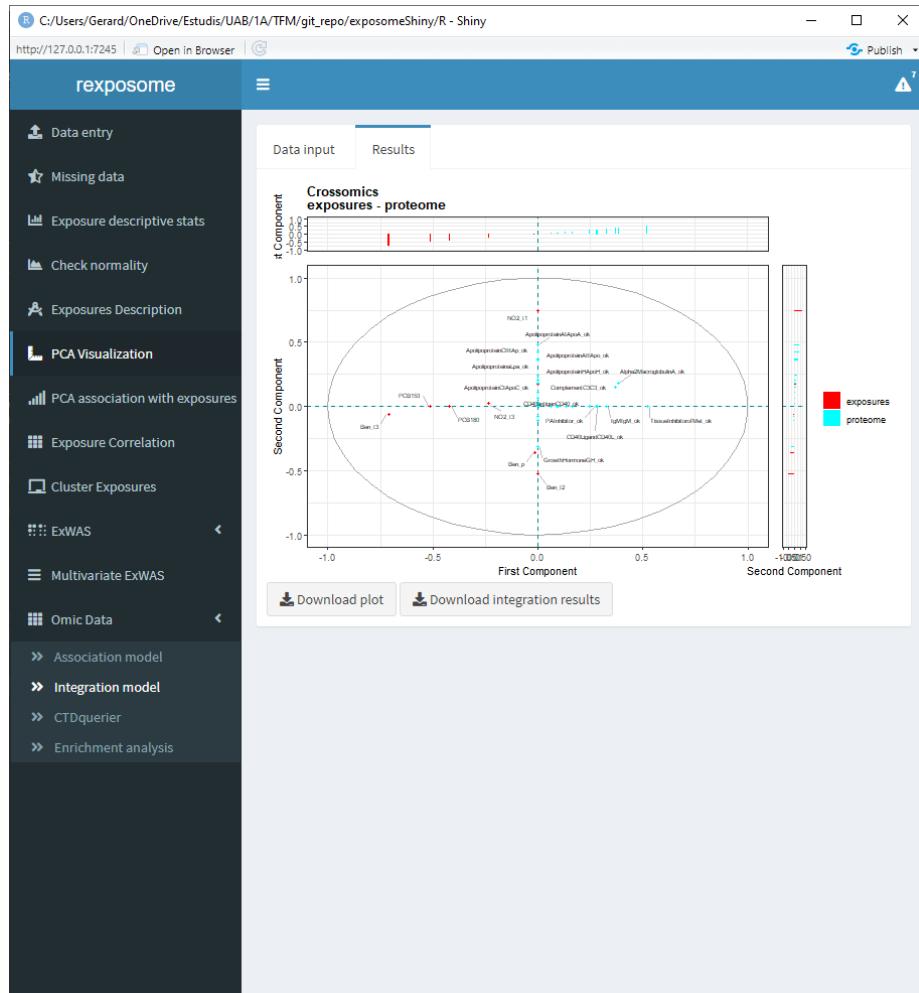
Once the data entry is completed, click on ‘Load data and perform integration’. Go to the results page to see the plots of the integration.

The following screenshots are the results of an integration analysis using the exosome dataset (`exposures_2.csv`, `description_2.csv`, `phenotypes_2.csv`) with the missings imputed (using the ‘Missing data’ tab of the Shiny) expression omics (`brge_gexp.rda`) and proteome omics (`brge_prot.rda`). All this mentioned datasets can be found here.

### 5.3.1 Integration using MCIA



### 5.3.2 Integration using MCCA (using only the proteome omics)



### 5.3.3 Integration using PLS (using only the proteome omics)

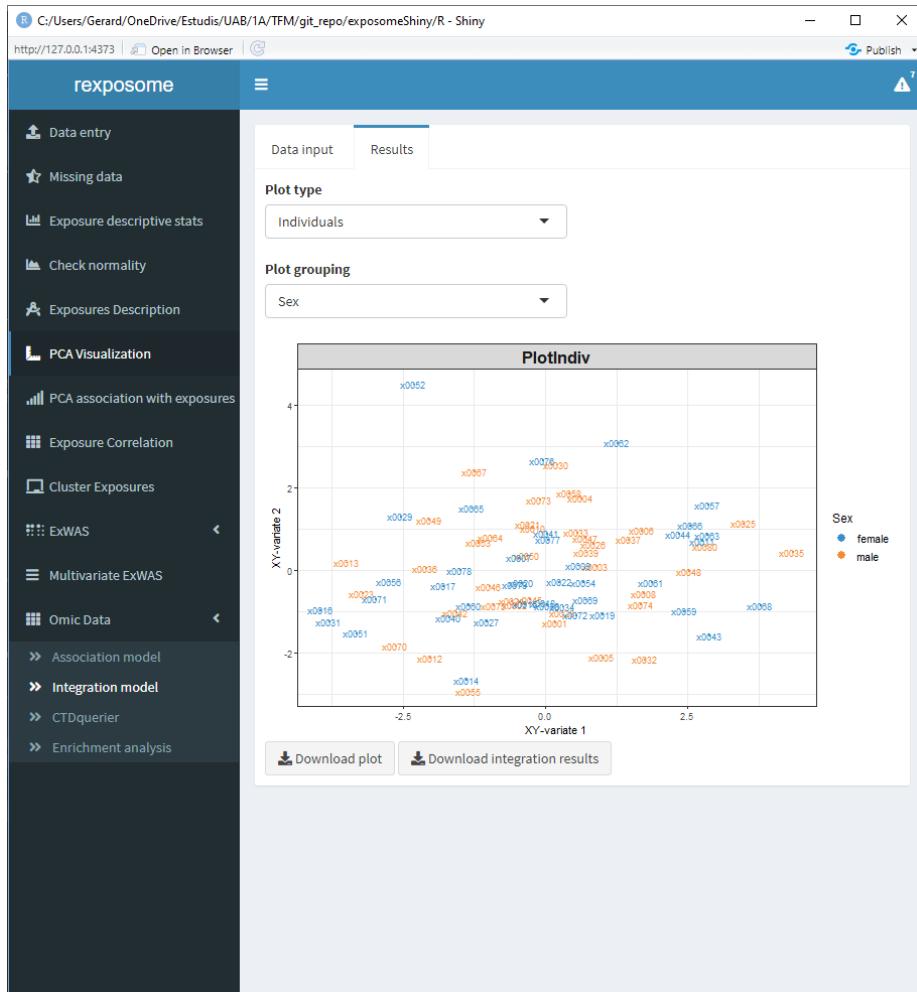
There are multiple visualization options for the PLS integration.

### 5.3.3.1 The exposures space

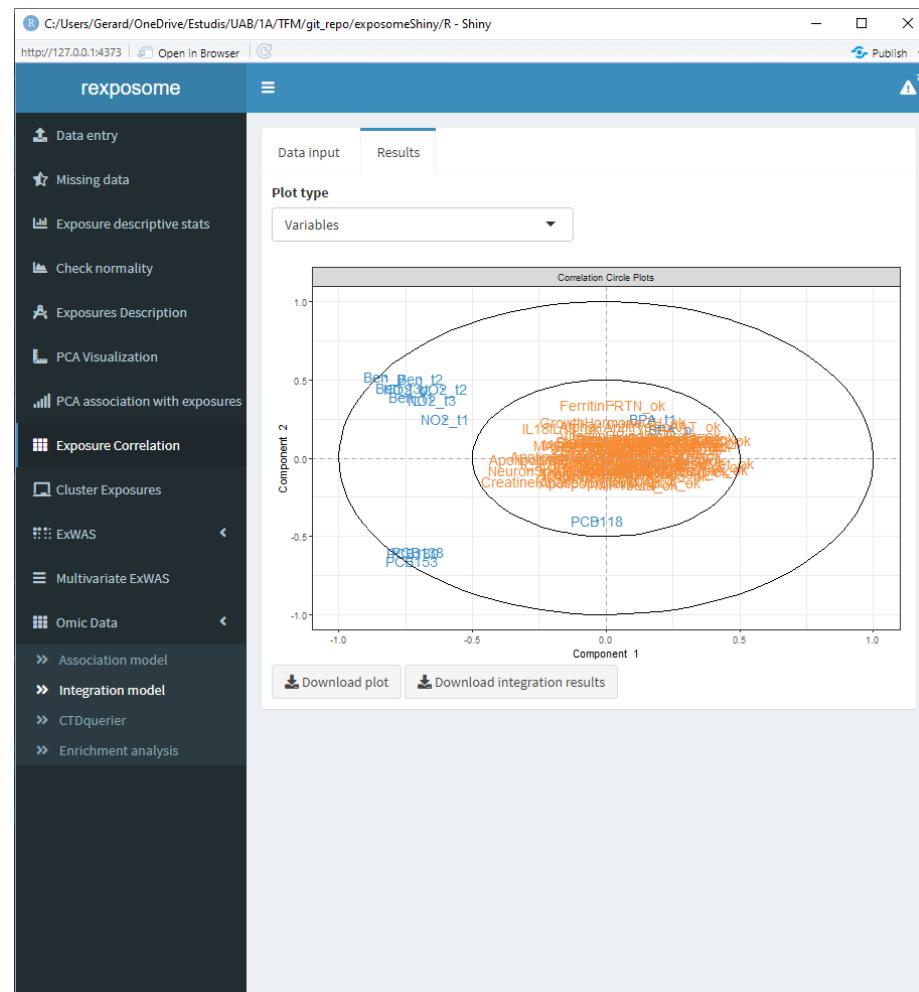
Can be grouped using the categorical phenotypes of the exposome data

### 5.3. EXPOSOME-OMIC INTEGRATION (E.G. CROSSOMICS)

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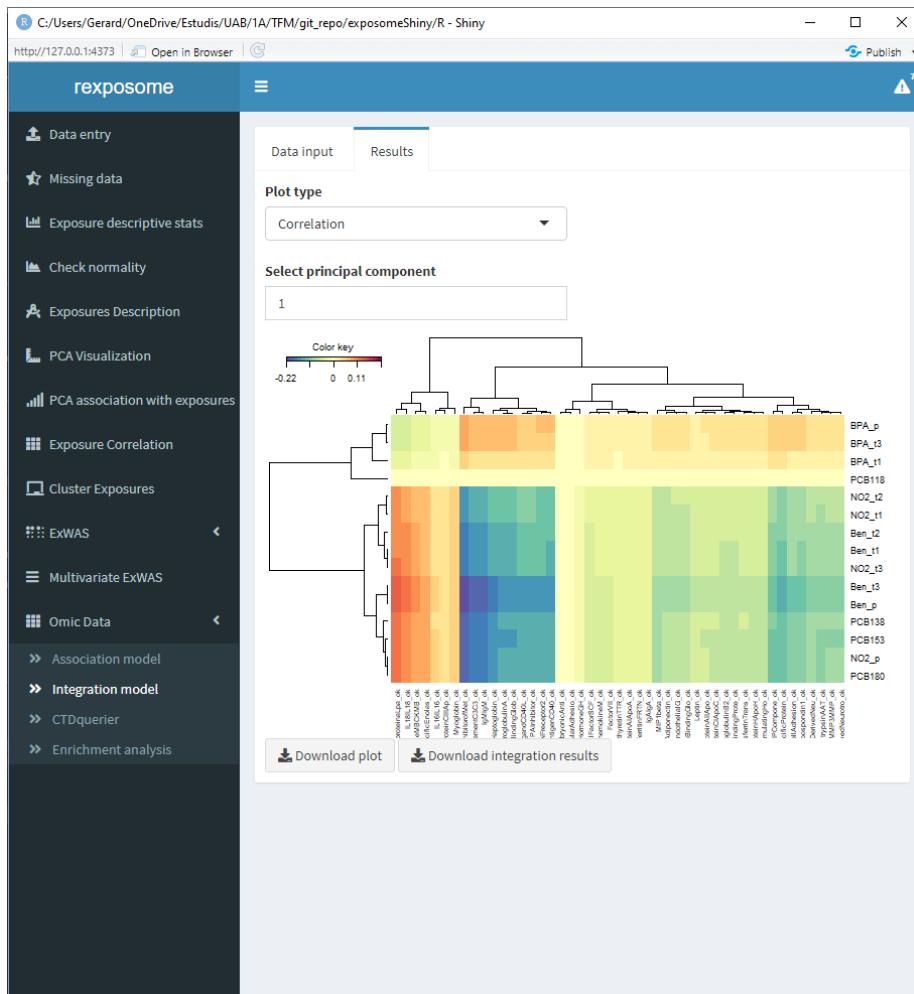


### 5.3.3.2 The variables space



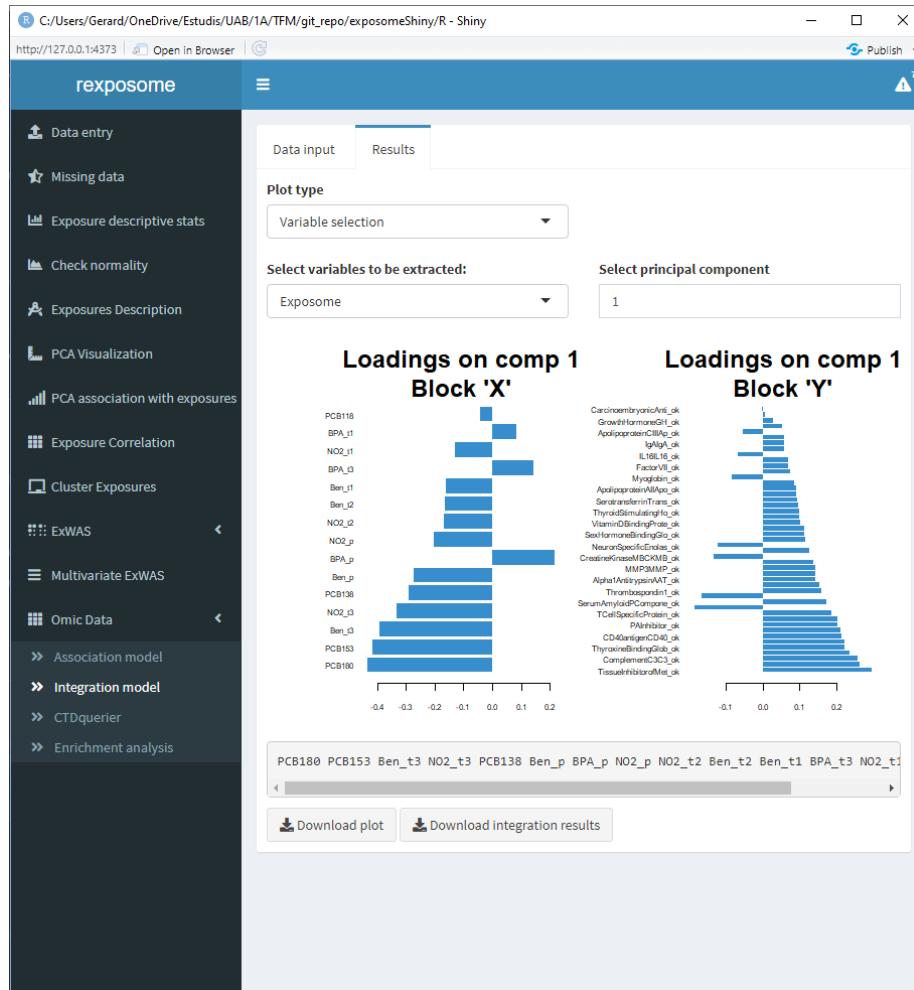
### 5.3.3.3 A correlation heatmap

Available for the first three principal components



#### 5.3.3.4 Loading plots (coefficients of each variable)

Available for the first three principal components

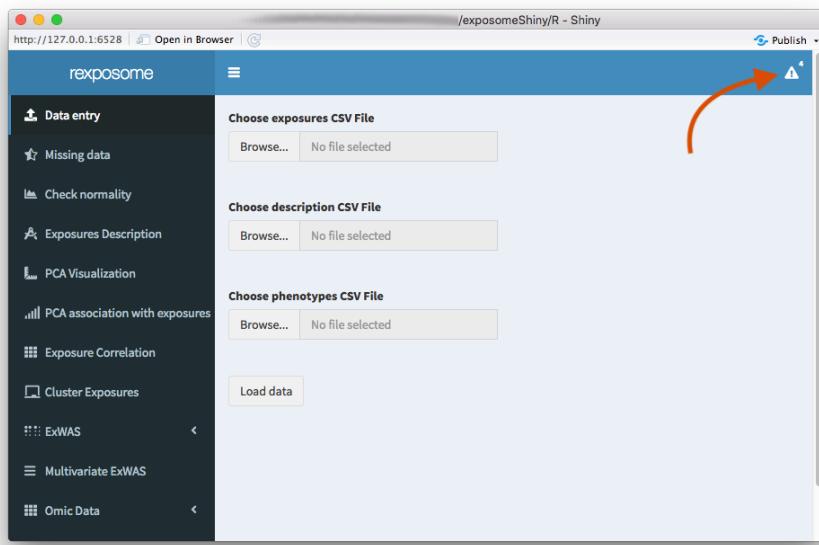


All plots can be downloaded individually, also, the Rdata file that contains the results of the integration can be downloaded.

## **Chapter 6**

# **General application functionalities**

This whole Shiny application serves the purpose to perform a various number of exposome and omic analysis with an input set of data. To perform this analysis some operations can be performed on the inputted dataset prior to the analysis and so in order to follow track of what exactly has been done or what is loaded on the current session, there's implemented some sort of state tracker inside the Shiny application. In order to access it, press the icon on the top right of the application



When clicking it a dropdown menu appears, inside there are seven different notifications:

- Exposome dataset: Turns to 100% when an exposome dataset is loaded in the environment. Here's a graphical example.

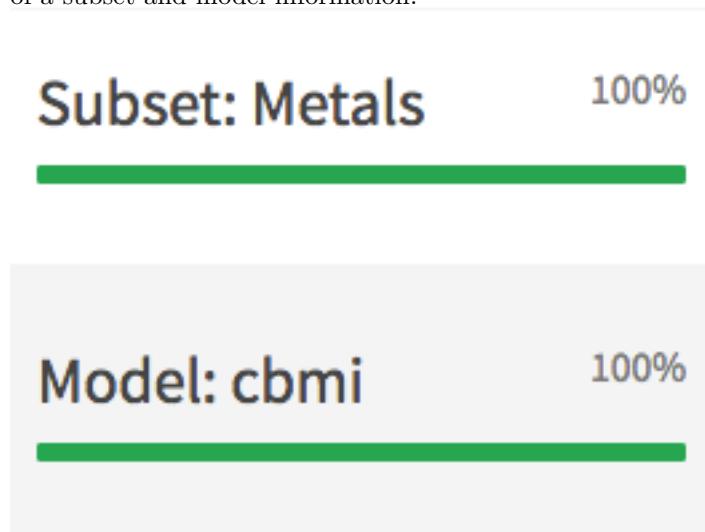
**Exposome dataset** 0%

**Exposome dataset** 100%

- LOD imputed: Turns to 100% if the exposome dataset is LOD imputed.
- Missing imputed: Turns to 100% if the exposome dataset has the missings imputed.
- Normality corrected: Turns to 100% if the exposome dataset is normality

corredted.

- Omics dataset: Turns to 100% when an omics dataset is loaded in the environment.
- Subset: Turns to 100% (and displays the subset family(ies)) when the exposome dataset is subseted.
- Model: Turns to 100% (and displays the association variable(s)) when a model is performed for an omics association analysis. Here's an example of a subset and model information.





# Chapter 7

## Methods

### 7.1 Missing data imputation

LOD missings are discovered through a encoding provided by the user, there is no method implemented to separate missing values between missing at random at LOD, meaning that all NA values are considered missing at random.

#### 7.1.1 Limit of detection (LOD) missing

LOD missings can be imputed using two methodologies:

- LOD value /  $\sqrt{2}$  : Use a LOD value provided by the user (one value per exposures) divided by the square root of two. Richardson and Ciampi (2003)
- QRILC: a quantile regression approach for the imputation of left-censored missing data Lazar (2015).

#### 7.1.2 Missing at random

Multiple imputation chained equations (MICE) is used to impute missing at random data. The *mice* package is used to do so. A brief explanation on the algorithm:

1. Imputation of the variable (exposure)  $x_n$  with the mean of all it's values.
2. Perform 1 for all the variables.
3. Set the mean imputed values from one variable back to missing.
4. Perform a regression model and fill those missings.
5. Repeat 3 and 4 for all the variables.
6. Repeat 3, 4 and 5 until the imputed values obtained are stabilized.

## 7.2 Normality

### 7.2.1 Normality testing

To test the normality of a variable, a Shapiro-Wilks test is used. The Shapiro-Wilks test, tests the null hypothesis of a sample (variable of the dataset) is normally distributed, to perform the test it calculates the W statistic.

$$W = \frac{(\sum_{i=1}^n a_i x_{(i)})^2}{\sum_{i=1}^n (x_i - \bar{x})^2}$$

To perform this test exposome uses the *shapiro.test* function from the *base* package of R.

### 7.2.2 Normalization

A user selected function can be applied to exposures (selected by the user) to normalize them. The available functions are: *log*, *sqrt* and  $\sqrt[3]{\cdot}$ .

## 7.3 Principal component analysis (PCA)

Reposome contains two PCA methodologies

- Regular PCA Jolliffe and Cadima (2016) (only numerical exposures)
- FAMD Chavent et al. (2014) (numerical and categorical)

exposomeShiny uses regular PCA from the *FactoMineR* package. A toggle to select between the two may be added in future releases.

## 7.4 Exposures correlation

The correlation method takes into account the nature of each pair of exposures: continuous vs. continuous uses *cor* function from R *base*, categorical vs. categorical uses *cramerV* function from *lsr* R package and categorical vs. continuous exposures correlation is calculated as the square root of the adjusted r-square obtained from fitting a lineal model with the categorical exposures as dependent variable and the continuous exposure as independent variable.

## 7.5 Exposures clustering

Clustering analysis on samples can be performed to cluster individuals having similar exposure profiles. This is done using hierarchical clustering using the function *hclust* from the *stats* R package. The results this analysis yields are the exposure profiles of a selected number of groups.

## 7.6 Exposome Association Analysis

### 7.6.1 Single Association Analysis

Exposome-Wide Association Study (ExWAS) is equivalent to a Genome-Wide Association Study (GWAS) in genomics or to Epigenetic-Wide Association Study (EWAS) in epigenomics. The ExWAS was first described by Patel et al. Patel et al. (2010) . ExWAS are based on generalized linear models using any formula describing the model that should be adjusted for (following standard formula options in R). That is, continuous or factor variables can be incorporated in the design, as well as interaction or splines using standard R functions and formulas. Multiple comparisons in the ExWAS analysis is addressed by computing the number of effective (Neff) tests as described by Li and Ju Li and Ji (2005) . The method estimates Neff by using the exposure correlation matrix that is corrected when it is not positive definite by using *nearPD* R function. The significant threshold is computed as  $1-(1-0.05)M_{eff}$ . This threshold is added to the Manhattan plots. When using imputed data, analysis is done for each imputed set and P-Values are pooled to obtain a global association score.

### 7.6.2 Stratified Single Association Analysis

The stratified analysis option for the ExWAS corresponds to applying the same method as regular ExWAS to subsetted datasets. As example, a stratified analysis with the `sex` variable stratified corresponds to performing two ExWAS, one to the `male` and one for the `female` group.

### 7.6.3 Variable selection ExWAS

There are some authors that proposed to perform association analysis in a multivariate fashion, just to take into account the correlation across exposures Agier et al. (2016) . A Lasso regression is implemented using Elastic-Net regularized generalized linear models implemented in *glmnet* R package.

## 7.7 Exposome-Omic Association Analysis

Perform association analyses between exposures and omic data by fitting linear models as described in the *limma* R package Ritchie et al. (2015) . The pipeline implemented in association allows performing surrogate variable analysis in order to correct for unwanted variability. This adjustment is provided by *SVA* R package Leek et al. (2020) .

## 7.8 Integration analysis

There are three different methodologies to perform the integration analysis:

- Multiset canonical correlation analysis (MCCA). Implemented using the `Multicca` function of `PMA` R package Witten et al. (2020) .
- Multiple co-inertia analysis (MCIA). Implemented using the `mcia` function of `omicae4` R package Meng et al. (2013) , Min and Long (2020) .
- Partial least squares (PLS). Implemented using the `pls` function of `pls` R package Mevik and Wehrens (2015) .

## 7.9 Enrichment analysis

Functional profiles of selected genes are obtained using the Bioconductor package `clusterProfiler` Yu et al. (2012) . The available enrichment databases are GO and KEGG.

## **Chapter 8**

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