`comoRbidity`: An R package to analyze disease comorbidities

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Contents

1 Introduction

The term comorbidity refers to the coexistence or presence of multiple diseases or disorders in relation to a primary disease or disorder in a patient [1, 2]. Clinical and epidemiological studies indicate that the comorbidities in patients have a great impact on the evolution of health status, selection of appropriate treatments and health system costs [3, 4]. Understanding comorbidities and their etiology is key to identify new preventive and therapeutic strategies.

The goal of `comoRbidity` R package is to provide a general overview about the disease comorbidities according to an index diseases, from both, clinical and molecular perspective. Analysing clinical data allows to extract significant comorbidities based on population, while exploring the gene-disease associations will allow to understand the mechanisms underlying comorbidities.

The `comoRbidity` R package is a user-friendly disease comorbidity prediction software. It provides different comorbidity measures as well as visualization of comorbidity results (Figure 1). To obtain statistically significant comorbidities, the `comoRbidity` R package uses clinical data, provided by the user, and gene-disease association data based on DisGeNET [12] database (www.disgenet.org)(Figure 1A, 1B). Special effort has been made in the results' visualization. Visualization of patients suffering the disorders of interest (clinical comorbidity) and visualization of gene-disease information (molecular comorbidity) is available in `comoRbidity` R package before doing the comorbidity analysis (Figure 1-3). After comorbidity analysis, results can also be visualized, as heatmaps and networks figures for an easily interpretation (Figure 1-5).

The tasks that can be performed with `comoRbidity` package are the following:

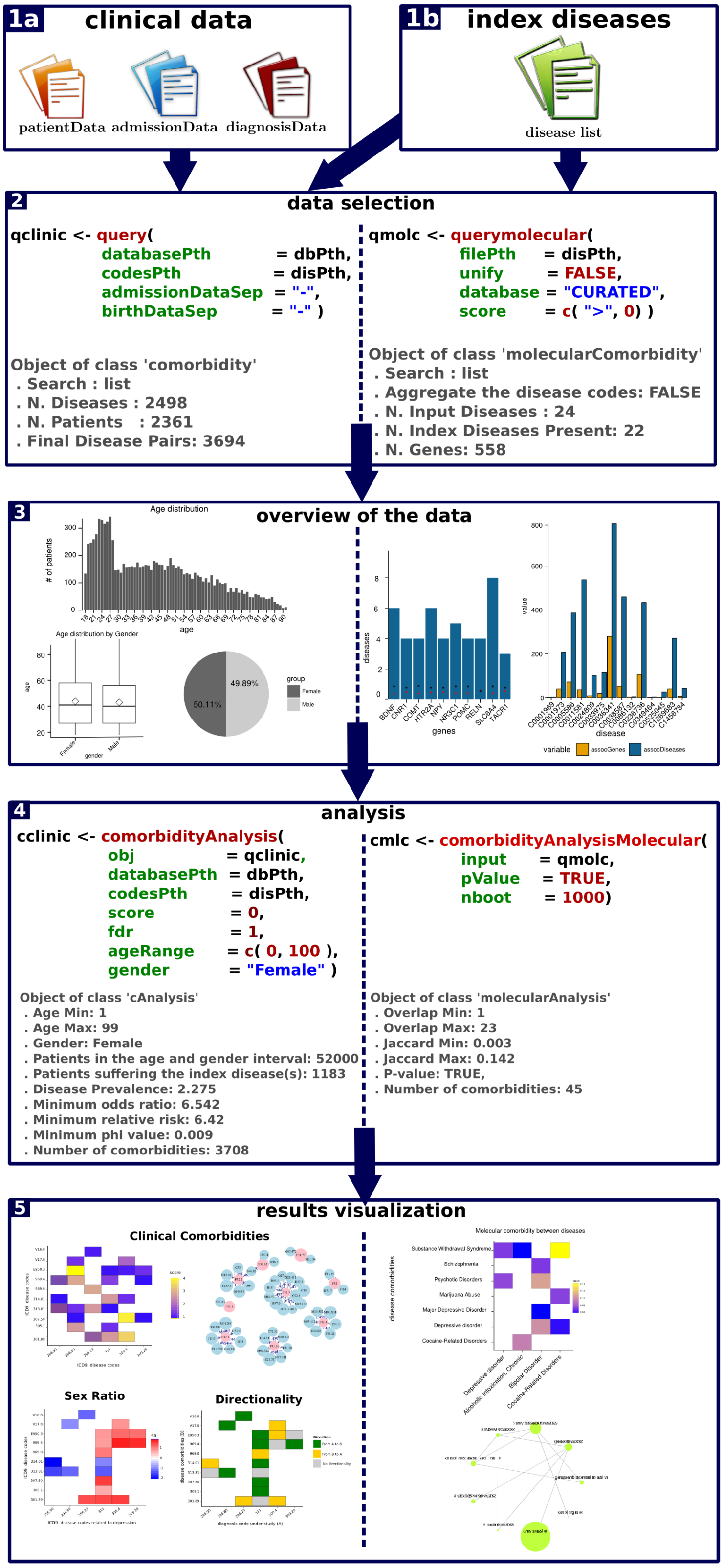
1. Age and gender analysis of the population suffering the disease of interest.
2. Clinical comorbidity analysis, based on diagnosis data, in an specific gender and age interval.
3. Analysis of the comorbidity temporal directionality.
4. Molecular comorbidity analysis, based on shared genes.
5. Visualization of the results in a clear way, easily interpretable.

The `comoRbidity` package also expedites the integration of comorbidity results with other R packages, and allows the development of complex bioinformatics workflow. In the following sections the specific functions that can be used to address each one of these tasks are presented.

1.1 Installation

The package `comoRbidity` is provided via Bitbucket. To install the `comoRbidity` R package the user must type the following commands in an R session:

```
library( "devtools" )
install_bitbucket( "ibi_group/disgenet2r" )
install_bitbucket( "ibi_group/comoRbidity" )
library( "comoRbidity" )
```



```
qclinic <- query(
              databasePth        = dbPth,
              codesPth           = disPth,
              admissionDataSep   = "-",
              birthDataSep       = "-" )

Object of class 'comorbidity'
 . Search : list
 . N. Diseases : 2498
 . N. Patients   : 2361
 . Final Disease Pairs: 3694
```

```
qmolc <- querymolecular(
            filePth     = disPth,
            unify       = FALSE,
            database = "CURATED",
            score       = c( ">", 0) )

Object of class 'molecularComorbidity'
 . Search : list
 . Aggregate the disease codes: FALSE
 . N. Input Diseases : 24
 . N. Index Diseases Present: 22
 . N. Genes: 558
```

```
cclinic <- comorbidityAnalysis(
              obj             = qclinic,
              databasePth     = dbPth,
              codesPth        = disPth,
              score           = 0,
              fdr             = 1,
              ageRange        = c( 0, 100 ),
              gender          = "Female" )

Object of class 'cAnalysis'
 . Age Min: 1
 . Age Max: 99
 . Gender: Female
 . Patients in the age and gender interval: 52000
 . Patients suffering the index disease(s): 1183
 . Disease Prevalence: 2.275
 . Minimum odds ratio: 6.542
 . Minimum relative risk: 6.42
 . Minimum phi value: 0.009
 . Number of comorbidities: 3708
```

```
cmlc <- comorbidityAnalysisMolecular(
           input      = qmolc,
           pValue     = TRUE,
           nboot      = 1000)

Object of class 'molecularAnalysis'
 . Overlap Min: 1
 . Overlap Max: 23
 . Jaccard Min: 0.003
 . Jaccard Max: 0.142
 . P-value: TRUE,
 . Number of comorbidities: 45
```

Figure 1: Overview framework of comoRbidity package

1.2 Implementation and Limitations

The comoRbidity R package works under:

- **Linux**
- **Mac**
- **Windows** Note that there are some limitations related to two comoRbidity R functions:
    - query function: set python argument to FALSE
    - comorbidityAnalysis function: set cores argument to 1

2 Clinical Comorbidity

The comoRbidity R package identifies disease comorbidities using different statistical tests and metrics. The disease comorbidity analysis can be performed stratifying the population by age and/or gender. More importantly, it allows the user to analyze his **own clinical record data**, and its own definition of comorbidity (e.g. regarding to the time interval considered). In addition, the comoRbidity package allows to compute other parameters like the sex ratio parameter and temporal directionality of the concomitant diseases.

2.1 Requirements

Four files are required for the comorbidity analysis with the comoRbidity package:

- patientData
- diagnosisData
- admissionData
- indexDiseaseCodes

An example of the data is shown in the next subsections. This data has been obtained from an artificial medical data set of 100000 patients with 361760 admissions (http://EMRbots.org).

2.1.1 Patient Data

The patientData file must contain at least three predefined columns named as stated below:

- **patient_id**: a patient identifier, that can be numeric, alphanumeric or a list of characters.
- **patient_gender**: patient gender is required to perform a comorbidity analysis stratified by gender and for the sex ratio analysis. It can be numeric (*e.g., 0 for one gender and 1 for the other*), character (*e.g., M for male and F for female*) or a list of characters (*e.g., male and female*).
- **patient_dateBirth**: patient birth date is required to calculate the age when the patient has been diagnosed with a particular disease. The structure of patient birth date follows format: year, month, day (XXXX/XX/XX), separated by any type of character.

If the patientData file does not contain the required columns the following notification message will appear:

```
## Check the patientData file structure.  Remember that this
## + file must contain at least three columns with the column
## + names as follows:
##  -> patient_id
##  -> patient_gender
##  -> patient_dateBirth
```

```
head( patientData )

##                             patient_id patient_gender
## 1 F7CF0FE9-AFCD-49EF-BFB3-E42302FFA0D3         Female
## 2 C3935FBC-DBBA-4844-BBE4-A175FA508454           Male
## 3 1CA33F6F-2E84-4C99-AF6A-D40F7B4DB27F           Male
## 4 81606388-2471-42A4-A6F1-1868AE25CFC3           Male
## 5 E3120DE9-3361-40CF-A618-265C769E75A2         Female
## 6 5C043111-3F94-44BC-A889-97D44ACCC7F6         Female
##         patient_dateBirth      PatientRace PatientMaritalStatus
## 1 1951-07-10 07:29:47.293            Asian               Single
## 2 1956-01-27 22:46:39.380 African American               Single
## 3 1972-12-22 10:11:01.867            White              Married
## 4 1984-01-17 00:49:06.903            Asian            Separated
## 5 1978-12-21 07:24:08.957            White              Married
## 6 1974-09-25 18:38:02.440 African American              Married
##   PatientLanguage PatientPopulationPercentageBelowPoverty
## 1         English                                   13.70
## 2         English                                   15.73
## 3         English                                    7.09
## 4         Spanish                                    2.17
## 5         English                                   18.67
## 6         English                                    2.57
```

2.1.2 Diagnosis Data

The diagnosisData file must contain at least three predefined columns named as stated below:

- **patient_id**: a patient identifier, that can be numeric, alphanumeric or a list of character. The patient identifier must be the same that is used in the patientData file.
- **admission_id**: an identifier related to the admission or visit, that allows to distinguish between different data entries of the same patient in the data base. It can be numeric, alphanumeric or a list of characters.
- **diagnosis_code**: the disease code or codes assigned to an admission or visit (using any vocabulary or standard to identify disease codes). **Note that the index diseases in the indexDiseaseCode file must use the same standard or vocabulary as the diagnoses in diagnosisData file.**

If the diagnosisData file does not contain the required columns the following notification message will appear:

```
## Check the diagnosisData file structure.  Remember that this
##                    file must contain at least three columns with the column
##                    names as follows:
##  -> patient_id
##  -> admission_id
##  -> diagnosis_code
```

```
head( diagnosisData )

##                             patient_id admission_id diagnosis_code
## 1 54C6E968-45B3-46B1-A64F-2CE3124F2A65            3          F80.1
## 2 54C6E968-45B3-46B1-A64F-2CE3124F2A65            4         R04.81
## 3 54C6E968-45B3-46B1-A64F-2CE3124F2A65            5            I36
## 4 9DD23357-9BEB-43E4-802D-1AB7ACDD4A3A            1         H16.43
## 5 9DD23357-9BEB-43E4-802D-1AB7ACDD4A3A            2        M05.161
## 6 9DD23357-9BEB-43E4-802D-1AB7ACDD4A3A            3        M06.011
##                                             diagnosis_description
## 1                                    Expressive language disorder
## 2                Acute idiopathic pulmonary hemorrhage in infants
## 3                          Nonrheumatic tricuspid valve disorders
## 4                            Localized vascularization of cornea
## 5 Rheumatoid lung disease with rheumatoid arthritis of right knee
## 6  Rheumatoid arthritis without rheumatoid factor, right shoulder
```

2.1.3 Admission Data

The admissionData file must contain at least three predefined columns named as stated below:

- **patient_id**: a patient identifier, that can be numeric, alphanumeric or a list of character. The patient identifier must be the same one that is used in the patientData file.
- **admission_id**: an identifier related to the admission or visit in which a disease diagnosis was made, that allows to distinguish between different entrances of the patient in the data base. It can be numeric, alphanumeric or a list of characters
- **admissionStartDate**: the date of the admission or visit in which the patient was diagnosed with the diseases under study. This information is needed for the directionality analysis. The data format followed must be: year, month, day (XXXX/XX/XX). Any separator symbol between them is allowed.

If the admissionData file does not contain the required columns the following notification message will appear:

```
## Check the admissionData file structure.  Remember that this
##                    file must contain at least three columns with the column
##                    names as follows:
##  -> patient_id
##  -> admission_id
##  -> admissionStartDate
```

```
head( admissionData )

##                              patient_id admission_id admissionStartDate
## 1 9380F9E3-1927-42F3-9731-03A74D4E4C6B            5         2011-03-23
## 2 0A89658C-C739-45CA-9BF1-CBDDDFB922C0            1         1974-02-10
## 3 0A89658C-C739-45CA-9BF1-CBDDDFB922C0            2         1991-05-22
## 4 0A89658C-C739-45CA-9BF1-CBDDDFB922C0            3         1995-02-26
## 5 0A89658C-C739-45CA-9BF1-CBDDDFB922C0            4         2005-03-17
## 6 0A89658C-C739-45CA-9BF1-CBDDDFB922C0            5         2008-04-12
##   admissionEndDate
## 1       2011-03-28
## 2       1974-02-16
## 3       1991-05-29
## 4       1995-02-28
## 5       2005-04-04
## 6       2008-04-16
```

2.1.4 Index Disease Codes

The indexDiseaseCode file contains the diseases in which you are interested for the comorbidity analysis. As explained before, the index diseases in the indexDiseaseCode file must be in the same format as the diagnoses in `diagnosisData` file.

`indexDiseaseCode` file must contain at least one predefined columns named as stated below:

- **Code**: the disease code (in any format).
- **Agg**: this column is not compulsory for performing the comorbidity analysis. It must be included if the user wants to collapse the index disease codes in a higher category (e.g., the example below shows all index diseases being collapsed in two disease categories, depression (Dep) and bipolar disorder (BD)).

```
head( indexDiseaseCode )

##    Code
## 1   F32
## 2 F30.9
## 3 F32.0
## 4 F32.1
## 5 F32.2
## 6 F32.3
##                                                                       Description
## 1                                    Major depressive disorder, single episode
## 2                                                   Manic episode, unspecified
## 3                              Major depressive disorder, single episode, mild
## 4                          Major depressive disorder, single episode, moderate
## 5 Major depressive disorder, single episode, severe without psychotic features
## 6    Major depressive disorder, single episode, severe with psychotic features
##   Agg
## 1 Dep
## 2 Dep
## 3 Dep
## 4 Dep
## 5 Dep
## 6 Dep
```

While the afore mentioned columns are required, the files may contain other additional information, as shown in the previous examples. The extra information will no be used by the `comoRbidity` R package.

2.2 comoRbidity objects

2.2.1 comorbidity object

The `comorbidity` object is obtained when the `query` function is applied. This object is used as input for other functions in the package that enable the user to have an overview of the population under study, including age, gender and diagnosis. `comorbidity` object is also used as input in the function that performs the comorbidity analysis (`comorbidityAnalysis`).

In summary, `comorbidity` object is the input for the following functions:

- `comorbidityAnalysis`
- `summaryDB`
- `populationAge`
- `diagnosisUse`

The `comorbidity` object contains the query information as well as a summary about the results. It shows:

- the type of search that has been done (`Search`)
- if the comorbidities will be estimated only between the index diseases (`Only comorbidities between index diseases: TRUE`), or if they will be estimated with among the index disease and all the disorders (Only comorbidities between index diseases: FALSE)
- if the individual index disease codes are used for the comorbidity study (`Aggregate the disease codes: FALSE`) or if they are grouped into a higher category (`Aggregate the disease codes: TRUE`)
- the number of index disorders used as input (`N. Input Index Diseases`)
- the total number of disorders present in this subset (`N. Index Diseases Present`)
- the number of concomitant disorders present in the results (`N. Concomitant Disorders`)
- the patients that suffer at least one of the input disorders (`N. Patients`)

All the `comoRbidity` **R package objects come with a function called** `extract`**. The** `extract` **function allows the user to retrieve data stored in the object. The** `extract` **function returns a formatted** `data.frame` **with the complete set of information obtained from the required data.**

```
comor_obj

## Object of class 'comorbidity'
##  . Search:                    list
##  . Only comorbidities between index diseases: FALSE
##  . Aggregate the disease codes: FALSE
##  . N. Input Index Diseases:   18
##  . N. Index Diseases Present: 17
##  . N. Concomintant Diseases:  2498
##  . N. Patients:               2361
```

2.2.2 cAnalysis object

The `cAnalysis` object is obtained when `comorbidityAnalysis` function is applied. This object is used as input for other functions in the package that enable the user to visualize the results in different graphical ways. Moreover, `cAnalysis` object is used as input for further analysis in the comorbidity results, like the sex ratio analysis and the directionality analysis.

`cAnalysis` object is the input for the following functions:

- `network`
- `heatmapPlot`
- `sexRatio`
- `directionality`

The `cAnalysis` object contains the results of the comorbidity analysis and other relevant information for the user. `cAnalysis` object shows the age interval that has been applied for the analysis (`Age Min` and `Age Max`), the gender, the number of patients that belong to this group from the total data (`Patients in the age and gender interval`) as well as the number of them that suffer the disease of interest (`Patients diagnosed with the index disease(s)`). Other data such as the disease prevalence and the range values obtained for each parameter estimated to measure the comorbidity are also contained in the `cAnalysis` object. Finally the number of comorbidities that pass the `cutOff` determined by the user are also shown (`Number of comorbidities`).

All the `comoRbidity` **R package objects come with a function called** `extract`**. The** `extract` **function allows the user to retrieve data stored in the object. The** `extract` **function**

returns a formatted `data.frame` with the complete set of information obtained from the required data.

```
comorMale

## Object of class 'cAnalysis'
##  . Age Min : 0
##  . Age Max : 100
##  . Gender  : Male
##  . Patients in the age and gender interval: 48000
##  . Patients suffering the index disease(s): 1178
##  . Disease Prevalence: 2.454
##  . Odds ratio range: [6.182 , 34.497]
##  . Relative risk range: [6.067 , 32.107]
##  . Phi value range: [0.009 , 0.043]
##  . Number of comorbidities: 3694

class(comorMale)

## [1] "cAnalysis"
## attr(,"package")
## [1] "comoRbidity"
```

```
comorbidityData <- extract ( comorMale )
head( comorbidityData )

##      disAcode disBcode disA disB AB AnotB BnotA notAnotB fisher oddsRatio
## 817    F31.11   I25.41   59   78  3    56    75    47866  0.000    34.162
## 108    F31.62      D11   64   75  3    61    72    47864  0.000    32.657
## 1639    F32.4  M05.561   77   64  3    74    61    47862  0.000    31.790
## 1643    F32.1   O10.11   65   46  2    63    44    47891  0.002    34.497
## 620     F32.5    B57.3   53   57  2    51    55    47892  0.002    34.103
## 1608    F32.3   E10.63   51   62  2    49    60    47889  0.002    32.545
##      relativeRisk   phi expect score   fdr sumRank
## 817        31.291 0.043  0.096 1.868 0.127       1
## 108        30.000 0.042  0.100 1.862 0.127       2
## 1639       29.221 0.041  0.103 1.859 0.127       3
## 1643       32.107 0.035  0.062 1.498 0.127       4
## 620        31.778 0.035  0.063 1.497 0.127       5
## 1608       30.361 0.034  0.066 1.493 0.127       6
```

2.3 Data extraction

The first step in order to perform the comorbidity analysis is extracting the data related to the patients diagnosed with the index diseases. These index diseases are determined by the `indexDiseaseCode` file.

The `query` function allows the user to extract the data and store it in a `comorbidity` class object. As input the `query` function requires:

- `databasePth`: determines the path where the three required input files (`patientData, diagnosisData, admissionData`) are located.
- `codesPth`: determines the path where the file with the index diseases is located (`indexDiseaseCode`).
- `admissionDateSep`: determines what separator symbol is used in the admission date.
- `birthDateSep`: determines separator symbol is used in the birth date.

Table 1: Optional arguments for data extraction

aggregatedDis argument	intraCodes argument	Description
FALSE	FALSE	Data extraction is done using the Codes column from index disease file. The comorbidities will be estimated between the index diseases and the rest of diseases that the patient had suffered (*e.g.*, ***ff***).
TRUE	FALSE	Data extraction is done using the Agg column from the index disease file, that collapse the diseases in a supperior class. The comorbidities will be estimated between the index diseases and the rest of diseases that the patient has suffered (*e.g.*, ***aggQuery***).
FALSE	TRUE	Data extraction is done using the Codes column from index disease file. Comorbidities will be estimated only between the index diseases (*e.g.*, ***queryIntra***).
TRUE	TRUE	Data extraction is done using the Agg column from the index disease file, that collapse the diseases in a supperior class. Comorbidities will be estimated only between the index diseases (*e.g.*, ***aggQueryIntra***).

As a result, a `comorbidity` object is obtained. This object will contain those patients that have been diagnosed with at least one of the index diseases presented in the `indexDiseaseCode` file, and the data related to them, according to the options selected in the `query` function.

> **Note that the `query` function has an optional argument, `python`, that by default is `FALSE`, but that can be changed to `TRUE` to run the `query` function faster by using python script. In order to use this option it is necesary to have python installed in your computer.**

In the following we illustrate the different options for data extraction shown in Table 1.

A. aggregatedDis = FALSE intraCodes = FALSE (Default option)

The `databasePth` argument should contain the ubication of the folder that contains the input files. As an example, it will be used the path where example data is located.

```
databasePth <- system.file("extdata", package="comoRbidity")
diagnosticCodes <- system.file("extdata", package="comoRbidity")
```

The user should indicate his own path following the next structure:

```
databasePthEx <- "/home/user/.../..."
diagnosticCodesEx <- "/home/user/.../..."
```

```
ff <- query( databasePth       = databasePth,
             codesPth          = diagnosticCodes,
             admissionDataSep = "-",
             birthDataSep      = "-"
             )

## Starting querying the index diseases in the dataset
## Loading the input datasets
## Checking the patientData file structure
## Checking the diagnosisData file structure
## Checking the admissionData file structure
## Checking the patients
## Starting querying for your index diseases
## Generating the resulting objects

ff

## Object of class 'comorbidity'
##   . Search:                    list
##   . Only comorbidities between index diseases: FALSE
##   . Aggregate the disease codes: FALSE
##   . N. Input Index Diseases:    18
##   . N. Index Diseases Present: 17
##   . N. Concomitant Diseases:   2498
##   . N. Patients:               2361
```

B. aggregatedDis = TRUE intraCodes = FALSE

```
aggQuery <- query( databasePth      = databasePth,
             codesPth         = diagnosticCodes,
             admissionDataSep = "-",
             birthDataSep     = "-",
             aggregatedDis    = TRUE
             )
aggQuery
```

```
## Object of class 'comorbidity'
##   . Search:                    list
##   . Only comorbidities between index diseases: FALSE
##   . Aggregate the disease codes: TRUE
##   . N. Input Index Diseases:    2
##   . N. Index Diseases Present: 2
##   . N. Concomitant Diseases:   2483
##   . N. Patients:               2361
```

C. aggregatedDis = FALSE intraCodes = TRUE

```
queryIntra <- query( databasePth      = databasePth,
             codesPth         = diagnosticCodes,
             admissionDataSep = "-",
             birthDataSep     = "-",
             intraCodes       = TRUE,
             aggregatedDis    = FALSE
             )
queryIntra
```

```
## Object of class 'comorbidity'
##   . Search:                    list
##   . Only comorbidities between index diseases: TRUE
##   . Aggregate the disease codes: FALSE
##   . N. Input Index Diseases:    18
##   . N. Index Diseases Present: 17
##   . N. Concomitant Diseases:   17
##   . N. Patients:               2361
```

D. aggregatedDis = TRUE intraCodes = TRUE

```
aggQueryIntra <- query( databasePth      = databasePth,
             codesPth         = diagnosticCodes,
             admissionDataSep = "-",
             birthDataSep     = "-",
             intraCodes       = TRUE,
             aggregatedDis    = TRUE
             )
aggQueryIntra
```

```
## Object of class 'comorbidity'
##   . Search:                      list
##   . Only comorbidities between index diseases: TRUE
##   . Aggregate the disease codes: TRUE
##   . N. Input Index Diseases:   2
##   . N. Index Diseases Present: 2
##   . N. Concomintant Diseases:  2
##   . N. Patients:               2361
```

2.4 Overview of the clinical data

2.4.1 Summary DB

The `comoRbidity` R package allows the user to analyze and characterize the population that suffer the index diseases. To have a general idea about the population main characteristics, the user can apply the `summaryDB` function.

As a input, the `summaryDB` function requires:

- `input`: a `comorbidity` object, obtained after applying the `query` function.
- `maleCode`: the symbol which denotes males in users' database (e.g., 0, M, Male...etc)
- `femaleCode`: the symbol which denotes females in users' database (*e.g., 1, F, Female...etc*)

The output of the `summaryDB` function is a plot with three different graphics (Figure 2):

- A barplot with the age distribution of the patients suffering the disease of interest.
- A boxplot showing the age distribution by gender.
- A pie chart representing the gender distribution.

```
summaryDB(input = ff,
          maleCode = "Male",
          femaleCode ="Female")
                             ## Checking the input object
```

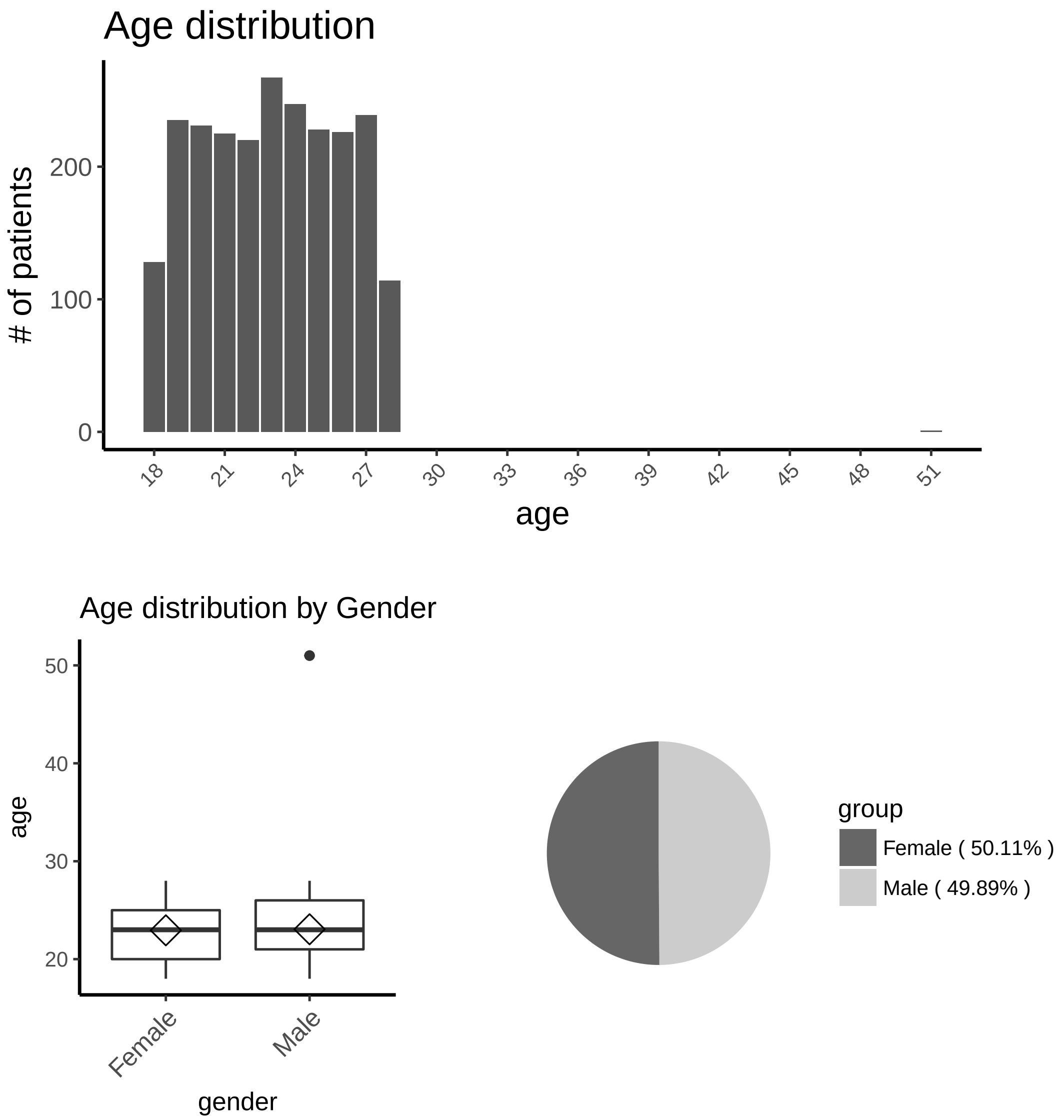


Figure 2: Summary plot containing: age-distribution, age distribution by gender and gender distribution.

2.4.2 Population analysis based on the disease under study

Following with the age analysis, the `comoRbidity` R package also allows to analyze the age distribution of patients suffering the index disease(s) compared with all the patients of the database. The age of the patients suffering the disorder of interest is estimated taking into account the first time in which they have been diagnosed with the index disease(s). For the rest of patients, the age is estimated taking into account the first time the patient has an entrace in the database.

As an input, `populationAge` function requires:

- `input`: a `comorbidity` object, obtained with the `query` function.
- `codesPth`: determines the path where the file with the index diseases is located (`indexDiseaseCode`).

- `databasePth`: determines the path where the three required input files (`patientData, diagnosisData, admissionData`) are located.

The `populationAge` function has two additional arguments, that are optional for the user:

- `type` argument allows the user to select the output barplot. By default the type is `"together"` (Figure 3), but it can be set to `"separate"` (Figure 4).

- `interactive` argument allows to create an interactive barplot, that show you the specific information of each bar in the barplot in an interactive way.

The results of the population analysis can be visualized together (Figure 3) or separately. **Note that the patient age in all population is estimated taking into account the first admission date of the patient to the database while the disorder age is estimated taking into account the first admission data in which the patient has been diagnosed with the index disease.**

```
populationAge ( input       = ff,
                codesPth    = diagnosticCodes,
                databasePth = databasePth,
                type        = "together",
                interactive = FALSE)
                 ## Checking the input object
```

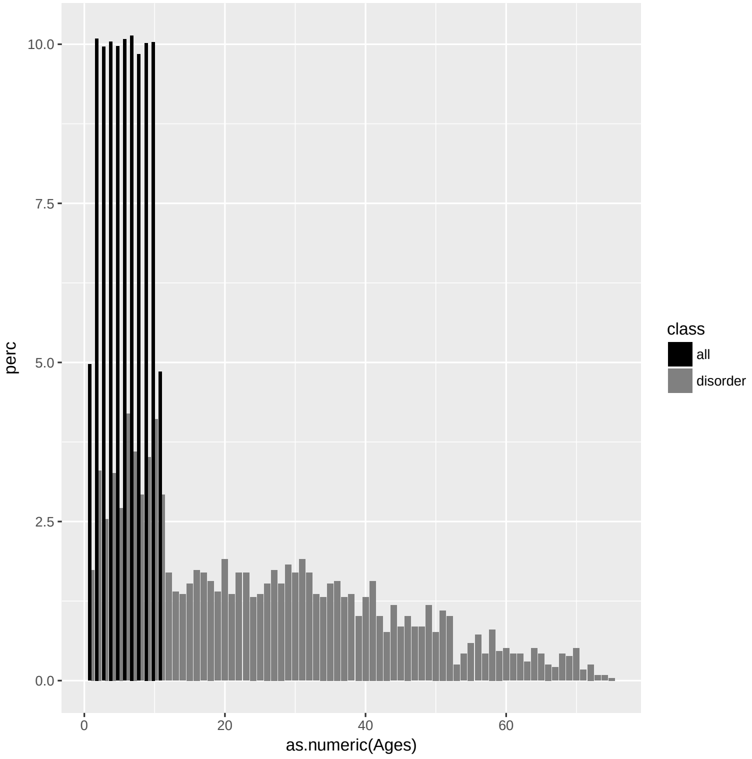


Figure 3: Barplot representing the age-distribution of the patients suffering the disorder of interest vs all population age.

```
populationAge ( input       = ff,
                codesPth    = diagnosticCodes,
                databasePth = databasePth,
                type        = "separate",
                interactive = FALSE)
                            ## Checking the input object
```

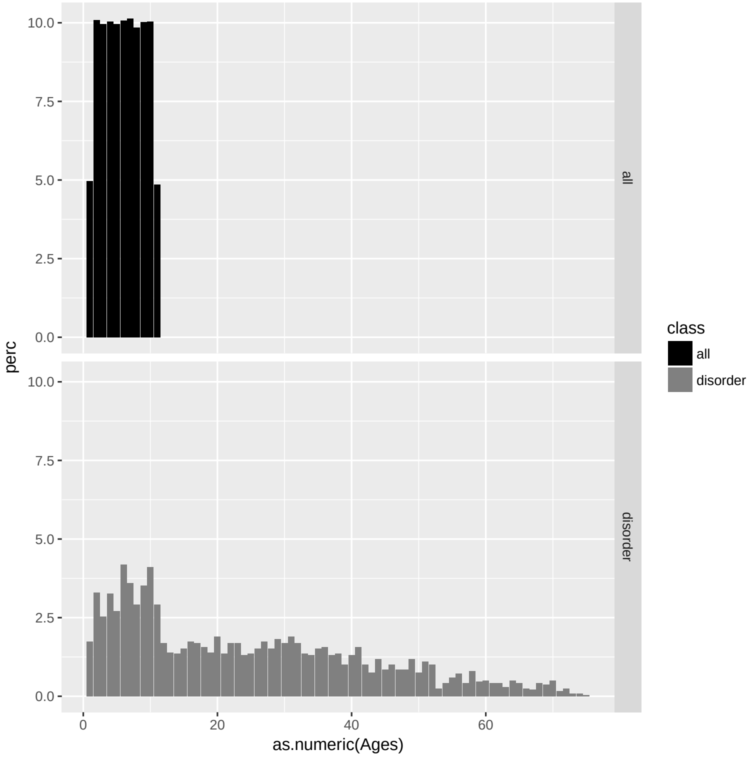


Figure 4: Separate barplot comparing the age-distribution of the patients suffering the disorder of interest vs all population age.

2.4.3 Disease Prevalence

The `comoRbidity` R package allows the user to extract the disease prevalence. In order to obtain this information, the user can apply the `diseasePrevalence` function.

As a input, the `diseasePrevalence` function requires:

- `input`: a `comorbidity` object, obtained after applying the `query` function.
- `maleCode`: the symbol which denotes males in users' database (e.g., 0, M, Male...etc)
- `femaleCode`: the symbol which denotes females in users' database (*e.g., 1, F, Female...etc*)
- `databasePth`: determines the path where the three required input files (`patientData, diagnosisData, admissionData`) are located.

The output of the `diseasePrevalence` function is a barplot showing the disease prevalence in the entire population and the disease prevalence according to the gender (Figure 5):

```
diseasePrevalence(input = ff,
          maleCode = "Male",
          femaleCode ="Female",
          databasePth = databasePth)
                             ## Checking the input object
```

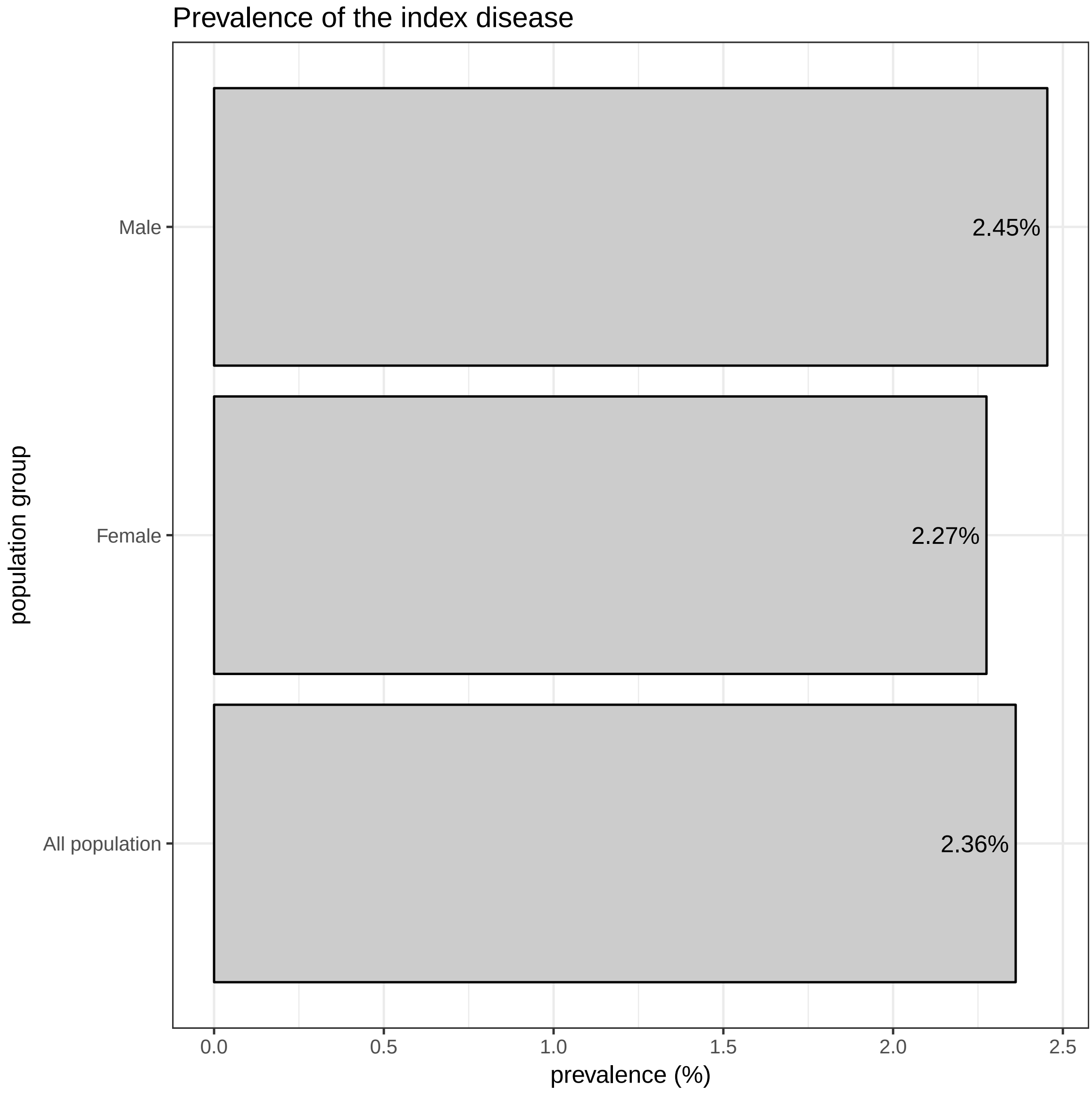


Figure 5: Barplot showing the disease prevalence in all the population and also, according to the gender.

2.4.4 Code Use

When studying a disorder that is defined by more than one diagnosed code, `comoRbidity` R package allows to analyze the percentage of use of each one of the index disease codes (Figure 6).
As an input, `diagnosticUse` function requires:

- `input`: a `comorbidity` object, obtained with the `query` function.
- `codesPth`: determines the path where the file with the index diseases is located (`indexDiseaseCode`).

The `diagnosticUse` function has two more arguments that are optional for the user:

- `cutOff` argument allows the user to select those index disease codes that will be represented in the output barplot. By default the `cutOff` is set as 0, but it can be set to any other percentage value.
- `interactive` argument allows to create an interactive barplot, that show you the specific information of each bar in the barplot in an interactive way. By default the `interactive` argument is set as `FALSE`.

```
diagnosticUse( codesPth     = diagnosticCodes,
               input        = ff,
               cutOff       = 0,
               interactive  = FALSE
               )
                            ## Checking the input object
```

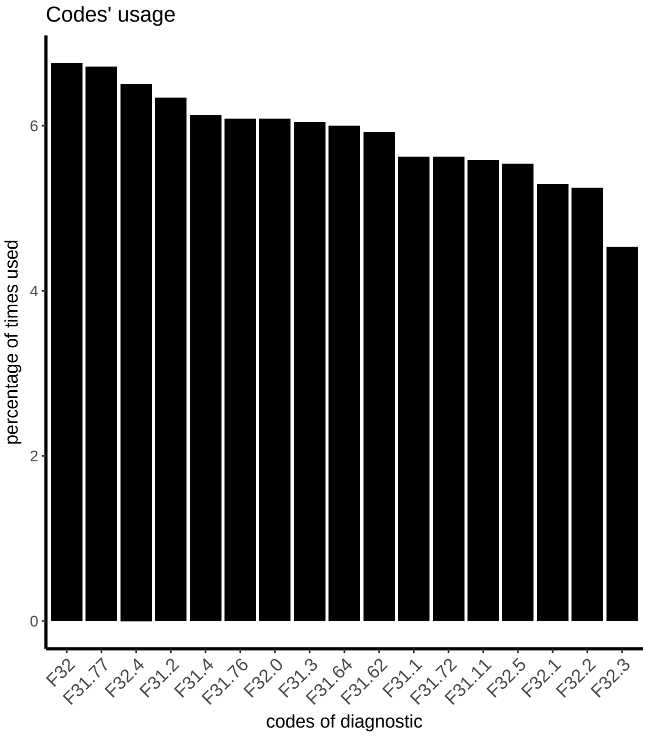


Figure 6: Index disease codes usage in percentage

If `aggregatedDis` argument has been set to `TRUE`, the graphic will show the percentage of use of each disease category, in this case bipolar disorder (BD) and depression (Dep) (Figure 7).

```
diagnosticUse( codesPth     = diagnosticCodes,
               input          = aggQueryIntra,
               cutOff       = 0,
               interactive  = FALSE
               )
                            ## Checking the input object
```

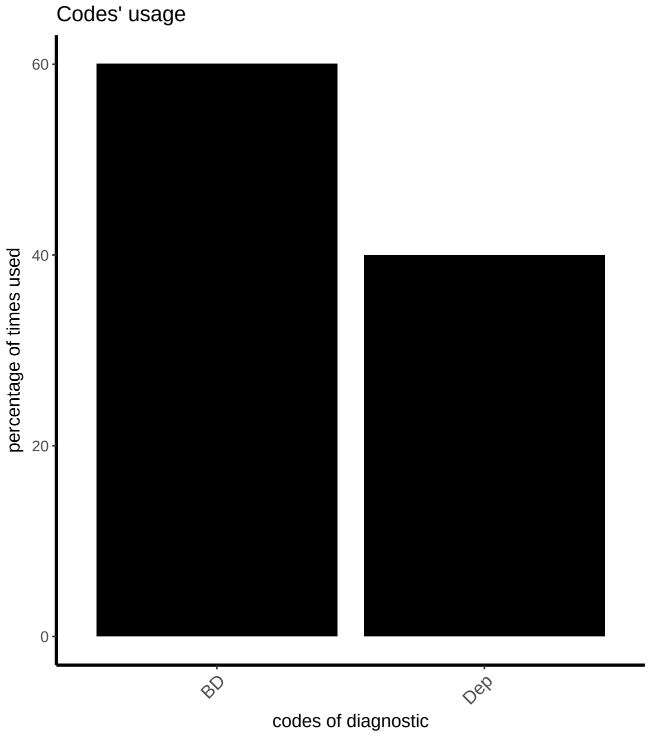


Figure 7: Index disease codes usage in percentage when aggregation has been performed

2.5 Clinical Comorbidity Analysis

Having a general overview about the patients that suffer the index diseases in the database and the main characteristics of this population, the next step is to perform the comorbidity analysis.

The user can estimate the statistically significant comorbidities by applying the `comorbidityAnalysis` function to the `comorbidity` object previously generated with the `query` function.

2.5.1 Comorbidity Measurements

The `comoRbidity` R package estimates several measures to determine if two diseases are comorbid in the population under study:

Fisher test A Fisher exact test for each pair of diseases is performed to assess the null hypothesis of independence between the two diseases. Four groups of patients are defined in order to perform the statistical testing: patients suffering disease A and disease B, patients diagnosed with disease A but not disease B, patients suffering disease B but not disease A and patients not suffering disease A nor disease B. The Fisher exact test is then applied to estimate the p-value for each pair of diseases. The Benjamini-Hochberg false discovery rate method [13] is applied on the ranked list to correct for multiple testing.

Comorbidity score This score is defined in Roque et al. as follows [14]:

$$comorbidityscore = log_2\left(\frac{observed + 1}{expected + 1}\right), expected = \frac{P_A P_B}{N} \tag{1}$$

where *observed* stands for the number of patients diagnosed with both diseases (disease A and disease B), and *expected* is estimated based on the prevalence of each disease (prevalence of disease A, *PA*, multiplied by the prevalence of disease B, *PB* , and divided by the total number of patients, *N*). A pseudocount of 1 is added to correct bias of the Comorbidity score towards low prevalent diseases. Since logarithm is applied, a comorbidity score of 1.0 means that the observed comorbidities are higher than two fold (approximately) than expected.

Relative risk or Risk Ratio (RR) The Relative Risk or Risk Ratio (RR) expresses the relationship between disease A and disease B as a ratio measure of effect (or risk) on the disease prevalence. If we consider for instance that disease A is the outcome variable and disease B the exposure variable, and we ask the question: does a diagnosis of disease B increase the risk of having a diagnosis of disease A? In other words, does disease A co-occur with disease B in patients?
The RR is estimated as the fraction between the number of patients diagnosed with both diseases and random expectation based on disease prevalence, as described in [2]:

$$RR_{AB} = \frac{C_{AB}N}{P_A P_B} \tag{2}$$

where *CAB* is the number of patients diagnosed with both diseases, *N* is the total number of patients in the population and *PA* and *PB* are the prevalences of diseases A and B.
The the RR can take the following values:

- RR = 1: Disease A and disease B are independent regarding prevalence.
- RR > 1: Disease A and disease B co-occur more often than expected by chance.
- RR < 1: Disease A and disease B co-occur less often than expected by chance.

Phi value (Pearsons correlation for binary variables) measures the robustness of the comorbidity association. It can be expressed mathematically as:

$$\phi_{AB} = \frac{C_{AB}N - P_A P_B}{\sqrt{P_A P_B (N - P_A)(N - P_A)}} \tag{3}$$

where *N* is the total number of patients in the population, *PA* i and *PB* are prevalences of diseases A and B respectively. *CAB* is the number of patients that have been diagnosed with both diseases A and B, and *PA PB* is the random expectation based on disease prevalence. The Pearson correlation coefficient, can take a range of values from +1 to -1:

- $\phi_{AB} = 0 : indicatesthatthereisnocorrelationbetweenthetwodiseases; \phi_{AB} > 0 : indicatesapositivecorrelationbe$

- $\phi_{AB} < 0 : indicatesanegativecorrelation.$

Odds ratio The odds ratio represents the increased chance that someone suffering disease A will have the comorbid disorder B. It shows the extent to which suffering a disorder increases the risk of developing another illness or disorder. The odds ratio is derived from a comparison of rates of the illness among individuals who do and do not exhibit the factor of interest. A statistically significant odds ratio (significantly different from 1.00 at the .05 level) indicates an appreciable risk associated with a particular factor. For example, an odds ratio of 2.00 indicates a doubled risk of the appearance of the disorder.

These measures allow the user to quantify the co-occurrence of disease pairs compared with the random expectation. The user can select the measure and the cut-off value in order to asses disease comorbidity.

`comorbidityAnalysis` function allows the user to perform the comorbidity analysis and store it in a `cAnalysis` class object. As input the function requires:

- `input`: a `comorbidity` object obtained after applying the `query` function.

- `codesPth`: determines the path where the file with the index diseases is located (`indexDiseaseCode`).
- `databasePth`: determines the path where the three required input files (`patientData, diagnosisData, admissionData`) are located.
- `ageRange`: determines what is the age range of interest for performing the comorbidity analysis. By default it is set from 0 to 100 years old.
- `gender`: determine what is the gender of interest for performing the comorbidity analysis.

Moreover, the `comorbidityAnalysis` function allows to restrict the results according to the comorbidity measurements values:

- score
- fdr
- oddsRatio
- rr
- phi

The user can filter the results by applying all the comorbidity measurements that are considered necesary. The cut-off value for these measurements must be numeric. The example below shows a query in which two of the five comorbidity measurements are applied, the `score` and the `fdr`.

```
comorFemale <- comorbidityAnalysis( input = ff,
                              codesPth    = diagnosticCodes,
                              databasePth = databasePth,
                              score       = 0,
                              fdr         = 1,
                              ageRange    = c( 0, 100 ),
                              gender      = "Female",
                              verbose     = FALSE
                              )
save(comorFemale, file=paste0(databasePth, "comorFemale.RData"))
```

As a result, a `cAnalysis` object is obtained. This object contains a summary of the comorbidities that have been found.

```
load(system.file("extdata", "comorFemale.RData", package="comoRbidity"))
comorFemale

## Object of class 'cAnalysis'
##   . Age Min : 0
##   . Age Max : 100
##   . Gender  : Female
##   . Patients in the age and gender interval: 52000
##   . Patients suffering the index disease(s): 1183
##   . Disease Prevalence: 2.275
##   . Odds ratio range: [6.542 , 35.037]
##   . Relative risk range: [6.42 , 32.369]
##   . Phi value range: [0.009 , 0.042]
##   . Number of comorbidities: 3716
```

All the `comoRbidity` R package objects come with a function called `extract`. The `extract` function allows the user to retrieve data stored in the object. The `extract` function returns a formatted `data.frame` with the complete set of information obtained from the comorbidity analysis results.

```
comorbidityDataFem <- extract ( comorFemale )
head( comorbidityDataFem )

##      disAcode disBcode disA disB AB AnotB BnotA notAnotB fisher oddsRatio
## 328    F31.62    D37.4   77   63  3    74    60    51863  0.000    35.037
## 1017   F31.72      Z11   63   51  2    61    49    51888  0.002    34.713
## 1521    F32.4   C40.81   78   72  3    75    69    51853  0.000    30.045
## 913     F32.3    C40.3   57   59  2    55    57    51886  0.002    33.041
## 1191   F31.11   E10.52   74   78  3    71    75    51851  0.000    29.200
## 490     F32.1   D31.01   61   56  2    59    54    51885  0.002    32.543
##      relativeRisk   phi expect score   fdr sumRank
## 328        32.158 0.042  0.093 1.871 0.124     1.0
## 1017       32.369 0.034  0.062 1.498 0.124     2.0
## 1521       27.778 0.039  0.108 1.852 0.124     3.0
## 913        30.925 0.033  0.065 1.495 0.124     4.0
## 1191       27.027 0.038  0.111 1.848 0.124     5.5
## 490        30.445 0.033  0.066 1.493 0.124     5.5
```

2.5.2 Clinical Comorbidity Visualization

In order to visualize the comorbidity analysis results, `comoRbidity` package provides two different options:

- Network: obtained by applying `network` function.
- Heatmap: obtained by applying `heatmapPlot` function.

Comorbidity Network

`network` function allows the user to visualize the data contained in the `cAnalysis` object obtained after applying the `comorbidityAnalysis` function.

As input the `network` function requires:

- `input`: a `cAnalysis` object obtained after applying the `comorbidityAnalysis` function.
- `databasePth`: determines the path where the three required input files (`patientData, diagnosisData, admissionData`) are located.
- `layout`: by deafult `"layout.fruchterman.reingold"`. It can be set to other of the possible igraph layouts.
- `selectValue`: By default `"score"` variable will be selected. It can be set to any of the other possible variables ('fdr', 'odds ratio', 'phi', 'rr').
- `cutOff`: By default '0.05'. The value of the argument can be changed to any other numeric variable, according to the range of the selected value.
- `npairs`: By default '0'. The value of the argument can be changed to any other numeric variable to show in the network only those comorbidities suffered by at least `npairs` of patients.
- `prop`: Determines the node size proportionality. By default it is set to `1`. The value of the argument can be changed to any other numeric variable.
- `title`: Determines the title of the network figure. By default `'Comorbidity network'`.
- `interactive` Determines if the output network is interactive or not. By default the `interactive` argument is set to `FALSE`. The value of the argument can be changed to `TRUE`, as a result an interactive network will be obtained.

```
load(system.file("extdata", "comorFemale.RData", package="comoRbidity"))
network ( input = comorFemale,
          databasePth = databasePth,
          layout = "layout.fruchterman.reingold",
          selectValue = "score",
          cutOff = 1.45,
          npairs = 2,
          prop  = 1,
          title = "Female comorbidity network",
          interactive = FALSE
        )
```

As a result, a network is obtained (Figure 8). The nodes in pink belong to the disorder of interest, while the blue nodes correspond to the comorbidity disorders. Note that the color of the nodes can be changed by adding the following arguments to the `network` function:

- `diseaseColor`: determines the node color for the disorder of interest. By default it is set to `"pink"`.
- `comorColor`: determines the node color for the comorbid disorders. By default it is set to `"lightblue"`.

```
## Checking the input object
```

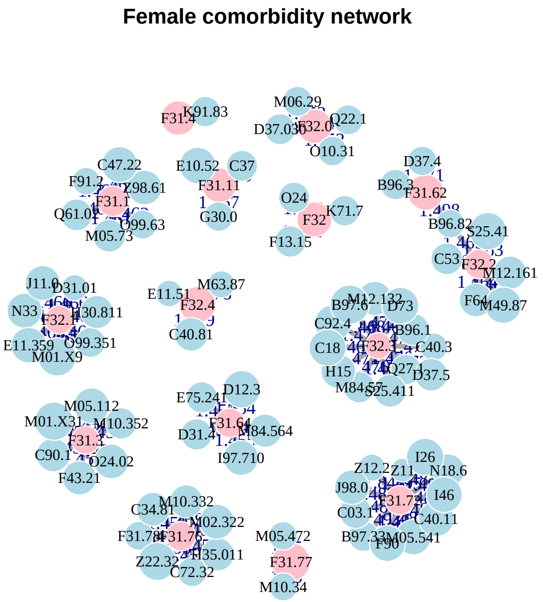


Figure 8: Comorbidity network in female population

The `comoRbidity` package also allows to visualize `cAnalysis` object in a heatmap (Figure 9). The required input is the same as for the `network` function.

```
heatmapPlot( input       = comorFemale,
             selectValue = "score",
             npairs      = 2,
             cutOff      = 1.45,
             verbose     = FALSE,
             interactive = FALSE)
```

Note that the color of the heatmap can be changed adding the next arguments to the `heatmaPlot` function:

- `lowColor`: By default `"0000FF"`. It defines the heatmap color for the lowest value.
- `highColor`: By default `"yellow"`. It defines the heatmap color for the highest value.

```
## Checking the input object
```

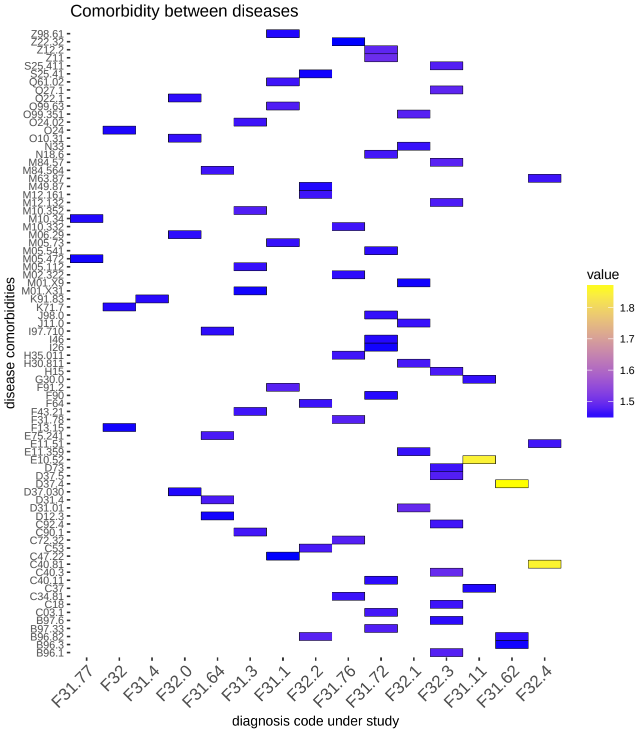


Figure 9: Comorbidity heatmap in female population

2.6 Sex ratio analysis

The `comoRbidity` package also estimates the sex ratio (SR) parameter. The sex ratio (SR) parameter allows to see if a comorbidity suffered in both, men and women, is equally likely in both genders or if it is more likely in one gender than in another. For a comorbidity A and age group t, SR (2.6) is defined by Klimek et al. [15] as follows:

$$SR(A,B) = log\left(\frac{1+\dfrac{D_f(B)}{D_f(A,B)}}{1+\dfrac{D_m(B)}{D_m(A,B)}}\right)$$

where Dm(f)(B) stands for the number of patients (men or women) suffering disease B in age group t, and Dm(f)(A,B) denotes those patients suffering disease B who also have been diagnosed with a disease A. SR values close to 0 mean that the comorbidity is equally likely for men and women. Positive SR values indicate that the comorbidity is more likely for women, while negative SR values indicate that the comorbidity is more likely in men.

To obtain the sex ratio heatmap, two steps should be followed:

1. Apply the `sexRatio` function using as input the `cAnalysis` object obtained for both genders.
2. Apply the `heatmapSexRatio` to the previous results. `interactive` argument can be set to `TRUE` if an interactive heatmap is required.

In the next example, a filter to the SR results has been applied to show only extreme SR values.

```
srAnalysis <- sexRatio(female    = comorFemale,
                       male      = comorMale,
                       fisherTest = 0
                       )

## Checking the input objects
## Warning in matrix(value, n, p):  la longitud de los datos [4] no es un submúltiplo
o múltiplo del número de columnas [3] en la matriz
## Warning in matrix(value, n, p):  la longitud de los datos [4] no es un submúltiplo
o múltiplo del número de columnas [3] en la matriz

srAnalysis <- srAnalysis[as.numeric(srAnalysis$SR) <= -0.5
                         | as.numeric(srAnalysis$SR) >= 0.5,]
```

```
heatmapSexRatio(srAnalysis,
                interactive = FALSE
                )
```

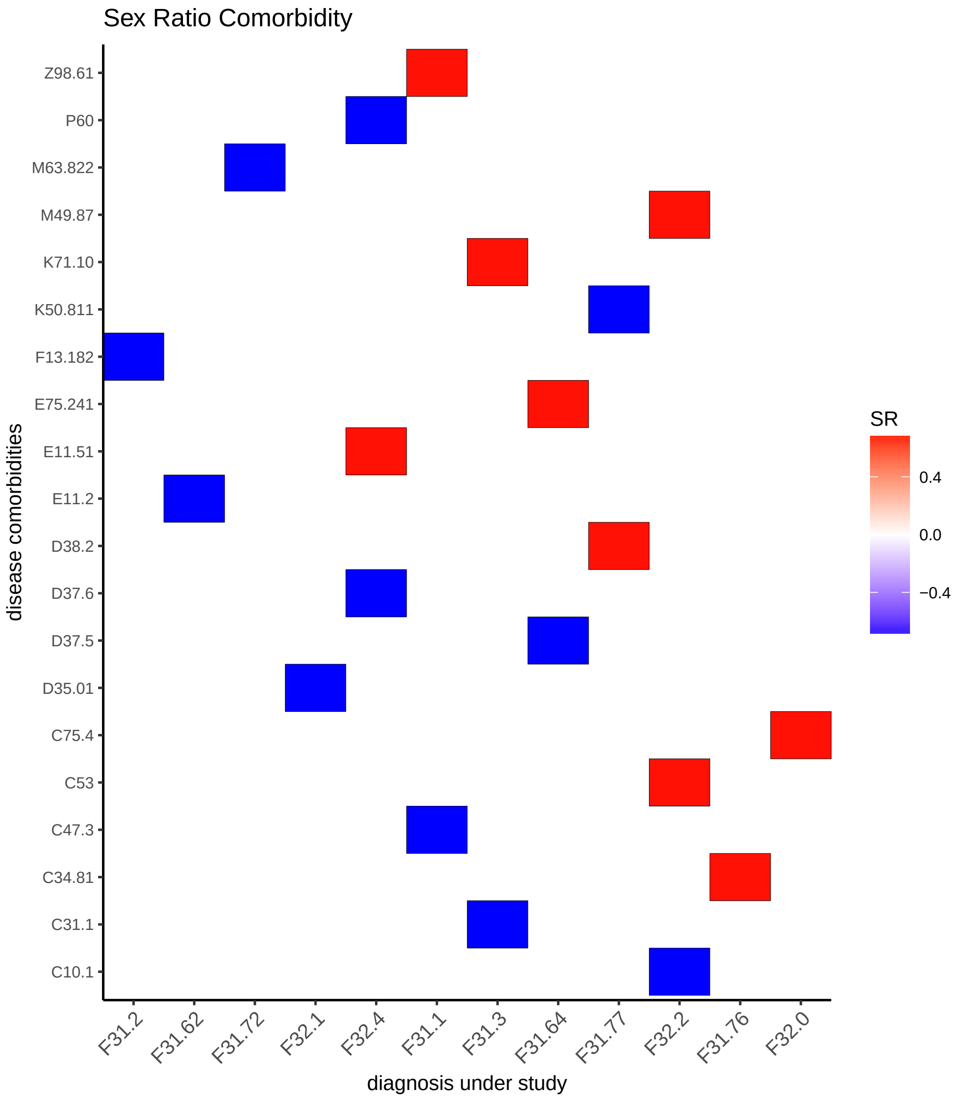


Figure 10: Sex ratio (SR) comorbidities heatmap

As a result a heatmap (Figure 10) is obtained. Red color (positive values) in the heatmap belong to those comorbidities that are more likely for women, while blue color (negative values) belong to those that are more likely for men. These colors can be changed adding the following arguments:

- `maleColor` Determines the heatmap color for those comorbidities that ' are more likely in men than women. By default `"blue"`.
- `femaleColor` Determines the heatmap color for those comorbidities that ' are more likely in women than men. By default `"red"`.

2.7 Directionality analysis

Additionally, the `comoRbidity` package allows to analyze of the temporal directionality of the co-occurring diseases, which allows the inference of temporal disease trajectories (Figure 11).

The temporal direction of disease association (dA ??? dB and dB ??? dA) is assessed for the diagnosis pairs with a significant Bonferroni-corrected p-value. Specifically, the number of patients for whom diagnosis dB follows diagnosis dA or vice versa, is calculated and an exact binomial test is, subsequently, used with a probability of success equal to 0.5. A preferred (significant) direction is determined for those diagnosis pairs that result in binomial tests with p-values < 0.05 and according to the pair that appears more often.

The `directionality` function allows the user to perform the directionality analysis. As input the function requires:

- `input`: an object of class `cAnalysis`.
- `databasePth`: determines the path where the three required input files (`patientData, diagnosisData, admissionData`) are located.
- `minPairs`: determines the minimum number of patients that must suffer the comorbidity to take them into account for the directionality analysis. By default the minPairs value is set to `1`.
- `gender`: determine what is the gender of interest for performing the directionality analysis.
- `ageRange`: determines what is the age range of interest for performing the directionality analysis. By default it is set from 0 to 100 years old.
- `days`: determines the number of days of difference needed for considering two diseases as comorbid for the directionality analysis.
- `dataSep`: determines the separator symbol used in the admission date.

```
comorbidityDirection <- directionality( input       = comorFemale,
                                        databasePth = databasePth,
                                        gender      = "Female",
                                        ageRange    = c(0,100),
                                        days        = 0,
                                        minPairs    = 1,
                                        dataSep     = "-")

## Checking the input objects

summary(as.factor(comorbidityDirection$result))

## No directionality
##             3716
```

As a result a `data.frame` is obtained. This `data.frame` contains 6 columns, with the comorbidity disorders and the directionality results in numeric and character format.

```
##      disAcode disBcode AtoB BtoA test           result
## 328    F31.62    D37.4    2    1  1.0 No directionality
## 1017   F31.72      Z11    1    1  1.0 No directionality
## 1521    F32.4   C40.81    1    2  1.0 No directionality
## 913     F32.3    C40.3    2    0  0.5 No directionality
## 1191   F31.11   E10.52    2    1  1.0 No directionality
## 490     F32.1   D31.01    1    1  1.0 No directionality
```

These results can be visualized in a heatmap by applying the `heatmapDirection` function. As input this function requires the `data.frame` obtained after applying the `directionality` function. The `interactive` argument is also available. By default it is set to `FALSE`.

```
heatmapDirection(test,
                 interactive = FALSE)
                             ## Checking the input object
```

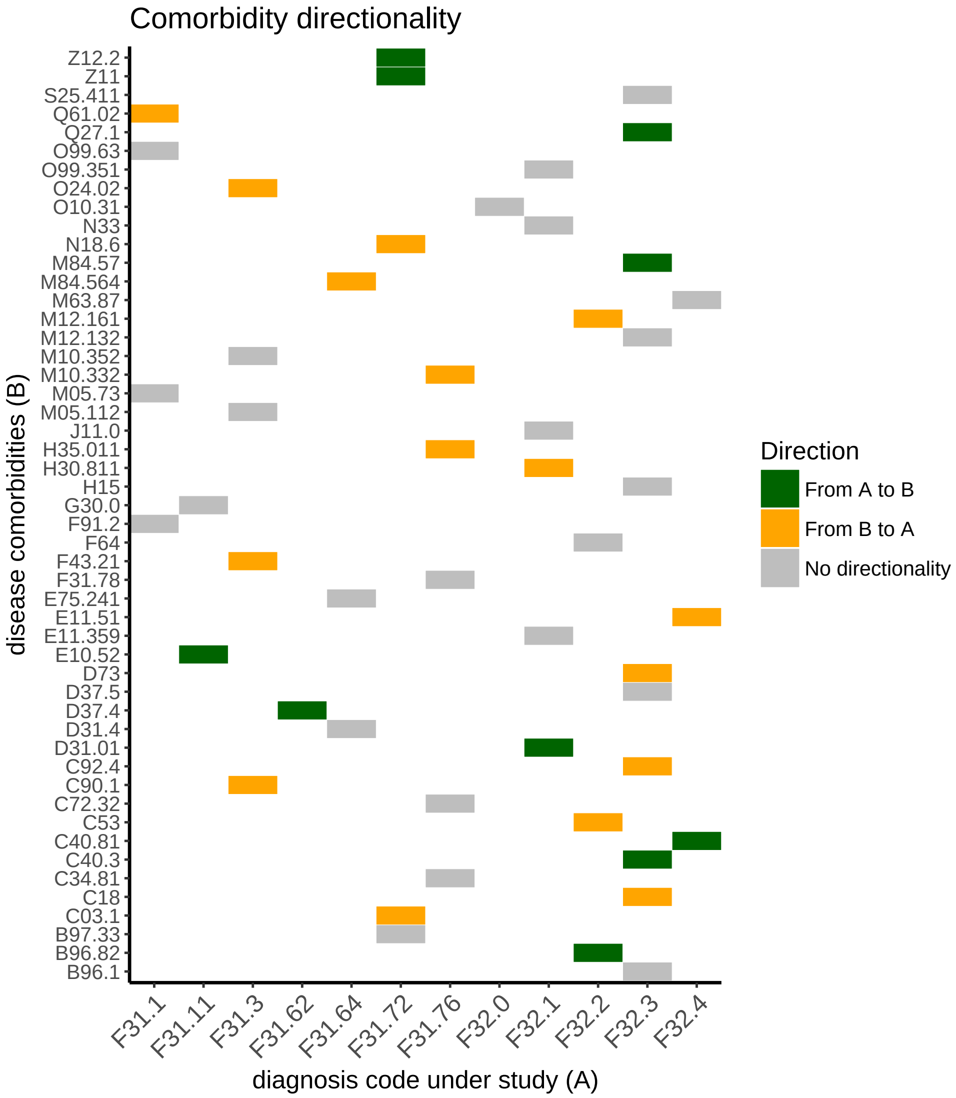


Figure 11: Comorbidities directionality heatmap

As a result, a heatmap is obtained (Figure 11). Note that three possible results can be shown in the heatmap in three different colors:

- From A to B in green.
- From B to A in yellow.
- No directionality in grey.

3 Molecular Comorbidity

3.1 Requirements

The `cuiDiseaseList` file is required in order to perform the molecular comorbidity analysis with `comoRbidity` package. An example of the data is shown below.

3.1.1 Index Disease Codes

The `cuiDiseaseList` file must contain at least two predefined column name, as stated below:

- **identifier_id**: the UMLS identifier of the disease/s of interest.
- **name**: this column is not compulsory for performing the comorbidity analysis. It must be included if the user want to collapse the index disease codes in a higher category. The example below shows all index diseases collapsed in 7 different categories.

If the cuiDiseaseList file does not contain the required columns the next message will appear:

```
## Check the input file structure.  Remember that this
## + file must contain at least two columns with the column
## + names as follows:
##   -> identifier
##   -> name
```

```
filePth <- system.file("extdata", package="comoRbidity")
cuiDiseaseList <- read.delim( paste0(filePth, "/cuiDiseaseList.txt"), header = TRUE, sep = "\t" )
head(cuiDiseaseList)

##   identifier                                     name
## 1   C0001969                    Alcohol use disorders
## 2   C0001973                    Alcohol use disorders
## 3   C0005586 Bipolar disorders and related disorders
## 4   C0006870                   Cannabis use disorders
## 5   C0011570                     Depressive disorders
## 6   C0011581                     Depressive disorders
```

While the afore mentioned columns are required, the files may contain other additional information. The extra information will no be used by the `comoRbidity` R package for the molecular comorbidity analysis.

3.2 molecular comoRbidity objects

3.2.1 molecularComorbidity object

`molecularComorbidity` object is obtained when `querymolecular` function is applied. This object is used as input for other functions in the package that enable the user to have an overview about their index diseases as well as the genes associated with them. `molecularComorbidity` object is used as input in the function that performs the molecular comorbidity analysis (`comorbidityAnalysisMolecular`).

In summary `molecularComorbidity` object is the input for the functions:

- `summaryDiseases`
- `comorbidityAnalysisMolecular`

The `molecularComorbidity` object contains the query information as well as the summary of the results. It shows:

- The type of search that has been done (Search).
- If the identifier column is used for the comorbidity study (Aggregate the disease codes: FALSE), or if they are collapsed into a higher category and the name column is used for the comorbidity study (Aggregate the disease codes: TRUE).
- N. Input Diseases: number of diseases that the user gives as input.
- N. Index Diseases Present: number of diseases that present associated genes in DisGeNET.
- N. Genes: number of genes associated to the diseases.

```
class(mc)

## [1] "molecularComorbidity"
## attr(,"package")
## [1] "comoRbidity"

mc

## Object of class 'molecularComorbidity'
##   . Search:                         list
##   . Aggregate the disease codes: FALSE
##   . N. Input Diseases:            24
##   . N. Index Diseases Present:    22
##   . N. Genes   :                  558
```

All the comoRbidity R package objects come with a function called extract. The extract function allows the user to retrieve data stored in the object. The extract function returns a formatted data.frame with the complete set of information obtained from the required data.

```
head(extract(mc))

##   geneId geneSymbol     diseaseId                     diseaseName
## 1   8864       PER2 umls:C0001969          Alcoholic Intoxication
## 2    217      ALDH2 umls:C0001973 Alcoholic Intoxication, Chronic
## 3    125     ADH1B umls:C0001973 Alcoholic Intoxication, Chronic
## 4   4852        NPY umls:C0001973 Alcoholic Intoxication, Chronic
## 5   6532     SLC6A4 umls:C0001973 Alcoholic Intoxication, Chronic
## 6    126      ADH1C umls:C0001973 Alcoholic Intoxication, Chronic
```

3.2.2 molecularcAnalysis object

molecularcAnalysis object is obtained when comorbidityAnalysisMolecular function is applied. This object is used as input for other functions in the package that enable the user to visualize the results in different graphical ways.

molecularcAnalysis object is the input for the following functions:

- network
- heatmapPlot

molecularcAnalysis object contains the results of the comorbidity analysis and other relevant information for the user. molecularcAnalysis object shows the gene overlap interval (minimum and maximum value of the gene overlap for the disease comorbidities, Overlap Min and Overlap Max) as well as the Jaccard Index interval (Jaccard Min and Jaccard Max). Other data such as if the p-value has been estimated or not is also shown (P-value). Finally, the number of comorbidities found based on the genes shared between diseases are also shown (Number of comorbidities).

```
class(mcAnalysis)

## [1] "molecularcAnalysis"
## attr(,"package")
## [1] "comoRbidity"

mcAnalysis

## Object of class 'molecularcAnalysis'
##   . Overlap Min : 1
##   . Overlap Max : 23
##   . Jaccard Min : 0.003
##   . Jaccard Max : 0.142
##   . P-value     : TRUE
##   . Number of comorbidities: 45
```

All the `comoRbidity` R package objects come with a function called `extract`. The `extract` function allows the user to retrieve data stored in the object. The `extract` function returns a formatted `data.frame` with the complete set of information obtained from the required data.

```
head(extract(mcAnalysis))

##                                  V1                            V2 geneV1
## 59           Alcoholic Intoxication Substance Withdrawal Syndrome      1
## 61 Alcoholic Intoxication, Chronic              Bipolar Disorder     41
## 64 Alcoholic Intoxication, Chronic     Cocaine-Related Disorders     41
## 65 Alcoholic Intoxication, Chronic           Depressive disorder     41
## 67 Alcoholic Intoxication, Chronic     Major Depressive Disorder     41
## 70 Alcoholic Intoxication, Chronic                Mood Disorders     41
##    geneV2 overlap jaccard  pval
## 59     53       1   0.019 0.000
## 61     72       5   0.046 0.000
## 64    108      13   0.096 0.000
## 65     37       2   0.026 0.011
## 67     41       2   0.025 0.000
## 70      3       1   0.023 0.013
```

3.3 Data extraction

The first step to perform the molecular comorbidity analysis is extracting the gene-disease association data for the index diseases. This information is extracted from DisGeNET database (http://disgenet.org).

`querymolecular` function allows the user to extract the genes associated to the index diseases, based on DisGeNET data, and store it in a `molecularComorbidity` class object. As input the function requires:

- `filePth`: determines the file name with the complete path where the file with disorders of interest is located.
- `unify`: the default value is set to `FALSE`. If the argument is set to `TRUE`, the `name` colum from the cui disease file will be selected for doing the comorbidity analysis.
- `database`: the default value is set to `'CURATED'`. User can select any of the databases available in DisGeNET (Table 2).
- `score`: by default it is set to `(">", 0)`. It means that all the data available in DisGeNET will be used for the comorbidity analysis. For detailed information about DisGeNET score: *http://disgenet.org/web/DisGeNET/menu/dbinfoscore*.

Table 2: Source databases included in DisGeNET

Name	Description
CTD_human	The Comparative Toxicogenomics Database, human data
UNIPROT	The Universal Protein Resource
CLINVAR	ClinVar, public archive of relationships among sequence variation and human phenotype
ORPHANET	The portal for rare diseases and orphan drugs
GWASCAT	The NHGRI-EBI GWAS Catalog
PSYGENET	Psychiatric disorders Gene association NETwork
HPO	Human Phenotype Ontology
GAD	The Genetic Association Database
CTD_mouse	The Comparative Toxicogenomics Database, *Mus musculus* data
CTD_rat	The Comparative Toxicogenomics Database, *Rattus norvergicus* data
MGD	The Mouse Genome Database
RGD	The Rat Genome Database
LHGDN	Literature-derived human gene-disease network generated by text mining Entrez's GeneRIFs
BEFREE	Data from text mining medline abstracts using the BeFree System
CURATED	Human curated sources:(CTD_human), ClinVar, GWASCAT, UniProt, Orphanet, PsyGeNET, and HPO
PREDICTED	All data from animal models: CTD_rat, RGD, CTD_mouse, MGD
ALL	All previous data sources

As a result, a `molecularComorbidity` object is obtained. This object contains all the gene-disease associations for the index diseases available in DisGeNET according to the user database and score selection.

```r
diseaseCodes <- paste0(filePth, "/cuiDiseaseList.txt")
mc <- querymolecular ( filePth = diseaseCodes,
                       unify = FALSE,
                       database = "CURATED",
                       score = c(">", 0))
mc

## Object of class 'molecularComorbidity'
##   . Search:                      list
##   . Aggregate the disease codes: FALSE
##   . N. Input Diseases:           24
##   . N. Index Diseases Present:   22
##   . N. Genes   :                 1759
```

3.4 Overview of the gene-disease data

3.4.1 Genes summary

The `comoRbidity` R package allows the user to analyze and characterize the genes associated to the index diseases. In order to have a general overview about the genes, the `summaryDiseases` function can be applied, by setting the `type` argument to `gene_barplot`.

As input, the `summaryDiseases` function requires:

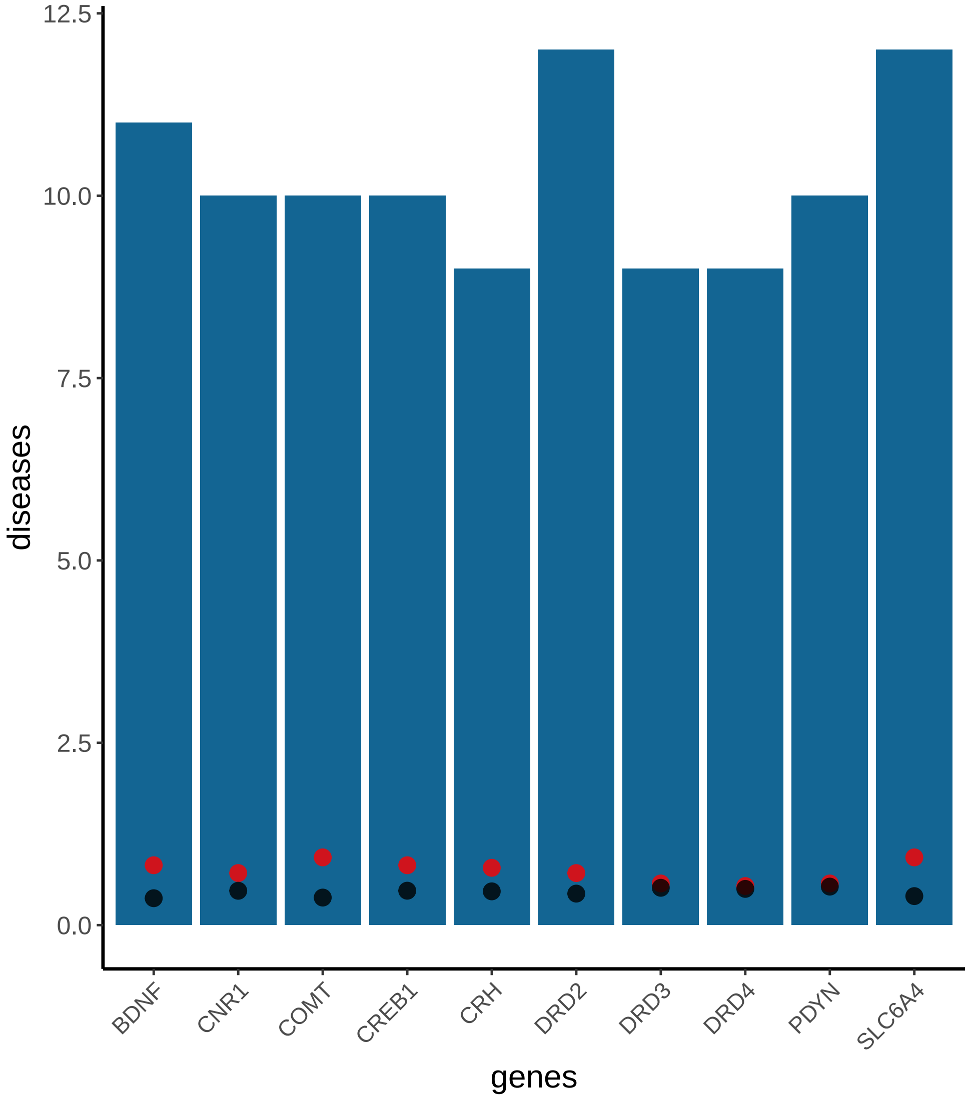
- `input`: a `molecularComorbidity` object, obtained after applying the `querymolecular` function.
- `type`: `'gene_barplot'` is selected to perform the gene analysis.
- `database`: the DisGeNET database from where the gene information will be retrieved (by default: 'CURATED').
- `interactive`: Determines if the output barplot is interactive or not. By default the `interactive` argument is set to `FALSE`. The value of the argument can be changed to `TRUE`, as a result an interactive barplot generated with Shiny will be obtained.

As a result, a barplot showing the number of diseases associated to each gene will be displayed. Moreover, some gene attributes like the Disease Pleiotropy Index (DPI) and Disease Specificity Index

(DSI) can be displayed. For more information about these indexes: *http://disgenet.org/web/DisGeNET/menu/dbinfospecificity.*

```
summaryDiseases( input  = mc,
                  type    = "gene_barplot",
                  database = "CURATED",
                 interactive = FALSE
           )

## Checking the input object
## The top 10 genes with more diseases associated are shown
```

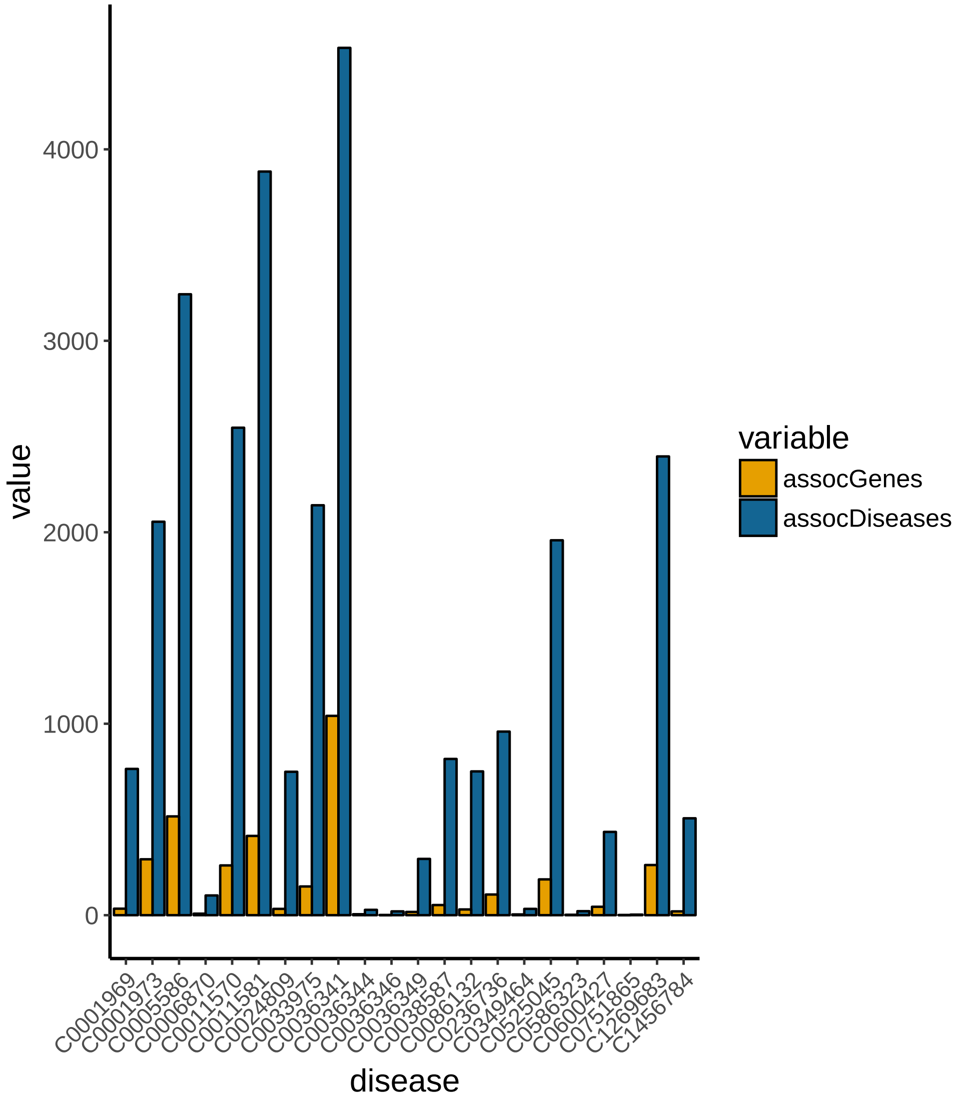


3.4.2 Diseases summary

The `comoRbidity` R package allows the user to analyze and characterize the diseases. In order to have a general overview about the number of genes associated to each one of the index diseases as well as the number of disorders that share some genes with them, the `summaryDiseases` function can be applied, setting the `type` argument to `dis_barplot`.

```
summaryDiseases( input      = mc,
                 type       = "dis_barplot",
                 database   = "CURATED")

## Checking the input object
```



3.5 Molecular comorbidity analysis

Having a general overview about the index diseases and the genes associated with them, the next step is to perform the molecular comorbidity analysis.

The user can estimate the molecular comorbidities by applying the `comorbidityAnalysisMolecular` function to the `molecularComorbidity` object previously generated with the `queryMolecular` function.

3.5.1 Molecular Comorbidity Measurements

The comorbidity is estimated from the molecular perspective taking into account the number of genes shared between the diseases according to DisGeNET data. The `comoRbidity` R package estimates the Jaccard estimation index and optionally a P-value can also be estimated.

Jaccard Index The Jaccard Index, also known as the Jaccard similarity coefficient, is a statistic measurement used for comparing the similarity of two sets, and is defined as the size of the intersection divided by the size of the union of the sample sets:

$$J(A,B) = \frac{\mid A \cap B \mid}{\mid A \cup B \mid}$$

`comorbidityAnalysisMolecular` function computes the Jaccard Index as an estimation of the similarity of two diseases based on the number of genes shared between the diseases according to DisGeNET data.

P-Value To determine if the association between two diseases as estimated by the Jaccard Index is statistically significant, a bootstrap procedure can be applied. `comorbidityAnalysisMolecular` function has two optional arguments, `pValue` and `nboot`. By applying this function, random gene sets of size n and p (being n, p the number of genes associated to disease 1 and 2, respectively) are sampled from a population of human disease genes obtained from DisGeNET. These random gene sets (n and p) are then used to compute the Jaccard Index for diseases 1 and 2. This procedure is repeated `nboot` times. Then the number of times that it has been obtained a Jaccard Index for the random gene sets larger than the observed value of the Jaccard Index is estimated.

The `comorbidityAnalysisMolecular` function allows the user to perform the molecular comorbidity analysis and store it in a `molecularcAnalysis` object. As input this function requires:

- `input`: a `molecularComorbidity` object obtained after applying the `querymolecular` function.
- `pValue`: determines if the p-value is estimated or not. By default it is set to `'FALSE'`. The `pValue` argument can be set to `'TRUE'` in order to estimate the P-value associated to each Jaccard Index.
- `nboot`: determines the number of random times that the Jaccard Index is computed using random sets. By default it is set to `100`. The value of the argument can be changed to any other numeric variable.

```
mcAnalysis <- comorbidityAnalysisMolecular(input  = mc,
                                           pValue = TRUE,
                                           nboot  = 1000
                                           )

## Checking the input object
## Estimating the overlap and jaccard
## Estimating the p-value
## A total of 8948 genes obtained from DisGeNET CURATED database are
##                   being used for the bootstrap process
## Pvalue estimation for each comorbidity
```

As a result, a `molecularcAnalysis` object is obtained. The `molecularcAnalysis` object contains the results of the molecular comorbidity analysis and other relevant information for the user. `molecularcAnalysis` object shows the gene overlap interval for the disease comorbidities (`Overlap Min` and `Overlap Max`) as well as the Jaccard Index interval for the disease comorbidities (`Jaccard Min` and `Jaccard Max`). Other data such as if the p-value has been estimated or not is shown (`P-value`). Finally the number of comorbidities that have gene overlap are also shown (`Number of comorbidities`).

```
## Object of class 'molecularcAnalysis'
##   . Overlap Min : 1
##   . Overlap Max : 302
##   . Jaccard Min : 0.001
##   . Jaccard Max : 0.601
##   . P-value     : TRUE
##   . Number of comorbidities: 130
##                              V1                              V2 geneV1
## 23 Alcohol Withdrawal Seizures Alcoholic Intoxication, Chronic      2
## 42      Alcoholic Intoxication Alcoholic Intoxication, Chronic     34
## 43      Alcoholic Intoxication                Bipolar Disorder     34
## 45      Alcoholic Intoxication      Cocaine-Related Disorders     34
## 46      Alcoholic Intoxication              Cocaine Dependence     34
## 47      Alcoholic Intoxication             Depressive disorder     34
##    geneV2 overlap jaccard pval
## 23    292       2   0.007    0
## 42    292      17   0.055    0
## 43    516      19   0.036    0
## 45    108       5   0.036    0
## 46     44       3   0.040    0
## 47    414      16   0.037    0
```

3.5.2 Molecular comorbidity visualization

In order to visualized the molecular comorbidity analysis results, the `comoRbidity` R package provides two different options:

- Network: obtained by applying the `network` function.
- Heatmap: obtained by applying the `heatmapPlot` function.

Comorbidity Network

`network` function allows the user to visualize the data contained in the `molecularcAnalysis` object obtained after applying the `comorbidityAnalysisMolecular` function.

As input the `network` function requires:

- `input`: a `molecularcAnalysis` object obtained after applying the `comorbidityAnalysisMolecular` function.
- `layout`: by deafult `"layout.circle"`. It can be set to other of the possible igraph layouts.
- `selectValue`: By default `"jaccard"` variable will be selected. It can be set to to the p-value variable ('pval').
- `cutOff`: By default '0.05'. The value of the argument can be changed to any other numeric variable, according to the range of the selected value.
- `npairs`: By default '0'. The value of the argument can be changed to any other numeric variable to show in the network only those comorbidities in which the gene overlap is equal or greather than a the `npairs` value.
- `prop`: Determines the node size proportionality. By default it is set to `1`. The value of the argument can be changed to any other numeric variable.
- `title`: Determines the title of the network figure. By default `'Comorbidity network'`.
- `interactive` Determines if the output network is interactive or not. By default the `interactive` argument is set to `FALSE`. The value of the argument can be changed to `TRUE`, as a result an interactive network will be obtained.

```
mcNetwork <- network( input = mcAnalysis ,
                      layout = "layout.circle",
                      selectValue = "jaccard",
                      title = "Molecular comorbidity network",
                      cutOff = 0.05,
                      prop   = 0.2,
                      diseaseColor = "olivedrab1",
                      interactive = FALSE,
)
                                 ## Checking the input object
```

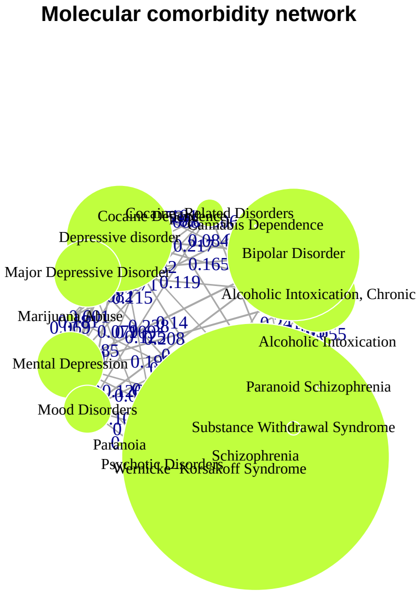


Figure 12: Molecular comorbidity network

As a result, a network is obtained (Figure 12). Note that the color of the nodes can be changed by adding the `diseaseColor` argument to the `network` function. The `diseaseColor` argument is set to `"pink"` color by default.

Comorbidity heatmap The `comoRbidity` package also allows to visualize `molecularcAnalysis` object in a heatmap (Figure 13). The required input is the same as for the `network` function.

```
mcHeatmap <- heatmapPlot( input = mcAnalysis ,
                          selectValue = "jaccard",
                          cutOff = 0.05,
                          interactive = FALSE,
                          lowColor = "#0000FF",
                          highColor = "yellow" )
                                 ## Checking the input object

mcHeatmap
```

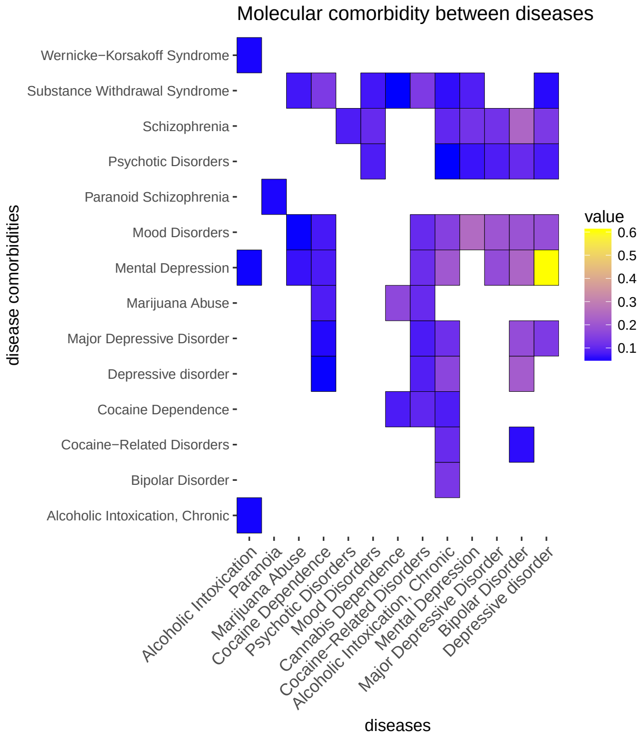


Figure 13: Molecular Comorbidity heatmap

Note that the color of the heatmap can be changed adding the next arguments to the `heatmaPlot` function:

- `lowColor`: By default `"0000FF"`. It defines the heatmap color for the lowest value.
- `highColor`: By default `"yellow"`. It defines the heatmap color for the highest value.

4 Warnings

All the functions in the `comoRbidity` R package first check that the input object belongs to the correct class. If the input is not correct the following message will be shown:

```
## Check the input object.  Remember that this
##                      object must be obtained after applying the query
##                      function to your input file.  The input object class must
##                      be:"cAnalysis" or "molecularcAnalysis"
```

5 Bibliography

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