4.1-section-result

February 14, 2025

1 Section 4 - Avoiding data overinterpretation

- One common source of data overinterpretation is a failure to correct for multiple hypothesis testing
- When we use the same set of data to test a set of hypotheses we need to correct for **false discovery rate**
- **False discovery rate** (FDR) is a statistical measure used to estimate the proportion of false positives within a set of hypothesis tests
- When performing many hypothesis tests on the same data (*e.g.*, when creating many different contingency tables from the same set of data and running Fisher's Exact Test on each of them) it is best practice to correct p-values for FDR

1.1 Example 4.1

Application 4.1: We hypothesize that entangled proteins are not randomly distributed within biological pathways in humans

- Many biological pathways exist in humans (**Figure 4.1**), meaning that testing the hypothesis in **Application 4.1** actually requires many different hypothesis tests

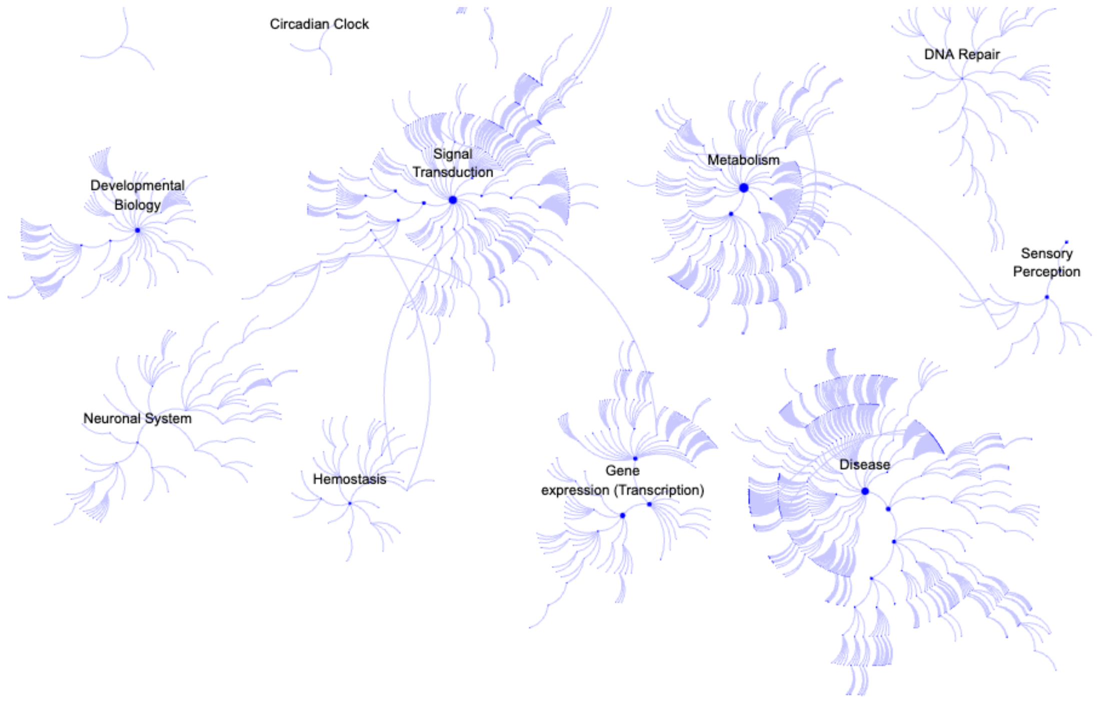


Figure 4.1 *A subset of the biological pathways identified for humans in the Reactome database. Nodes represent individual processes/reactions, e.g. "WNT5A-dependent internalization of FZD4", and edges represent interactions between processes. Image from https://reactome.org/PathwayBrowser/*

- For each pathway in humans, we need to construct a contingency table and compute the odds ratio as well as the *p*-value
- For the sake of simplicity, we will omit the initial calculation of {a, b, c, d}, odds ratios, and *p*-values to focus on correcting the *p*-values for **FDR**

1.1.1 Step 0 - Load libraries

[1]:
```
import pandas as pd
from statsmodels.stats.multitest import multipletests
import matplotlib.pyplot as plt
```

1.1.2 Step 1 - Load the data

[2]:
```
# list of columns to use from the dataset
column_list = ["unique_pathways",␣
 ↪"a_in_pathway_entangled",         "b_out_pathway_entangled",
                "c_in_pathway_not_entangled",␣
 ↪"d_out_pathway_not_entangled",        "odds_ratios", "two_sided_p_values"]

# load the specified columns; "data9" is a pandas DataFrame object
data_path = "/home/jovyan/data-store/data/iplant/home/shared/NCEMS/
 ↪BPS-training-2025/"
```

```
data9     = pd.read_csv(data_path + "pathways-and-entangled-proteins.csv",␣
 ↪usecols = column_list)
```

1.1.3 Step 2 - Explore the data

[3]:
```
# print a quick summary of "data9"
data9.info()

# print the first 10 rows of "data9"
data9.head(10)
```

```
<class 'pandas.core.frame.DataFrame'>
RangeIndex: 2150 entries, 0 to 2149
Data columns (total 7 columns):
 #   Column                       Non-Null Count  Dtype
---  ------                       --------------  -----
 0   unique_pathways              2150 non-null   object
 1   a_in_pathway_entangled       2150 non-null   int64
 2   b_out_pathway_entangled      2150 non-null   int64
 3   c_in_pathway_not_entangled   2150 non-null   int64
 4   d_out_pathway_not_entangled  2150 non-null   int64
 5   odds_ratios                  2150 non-null   float64
 6   two_sided_p_values           2150 non-null   float64
dtypes: float64(2), int64(4), object(1)
memory usage: 117.7+ KB
```

[3]:
```
  unique_pathways  a_in_pathway_entangled  b_out_pathway_entangled  \
0    R-HSA-166663                      38                    11517
1    R-HSA-173623                      33                    11522
2    R-HSA-198933                     100                    11455
3    R-HSA-202733                      66                    11489
4   R-HSA-2029481                      34                    11521
5   R-HSA-2029482                      77                    11478
6   R-HSA-2029485                      45                    11510
7   R-HSA-2168880                      35                    11520
8   R-HSA-2454202                      29                    11526
9   R-HSA-2730905                      37                    11518

   c_in_pathway_not_entangled  d_out_pathway_not_entangled  odds_ratios  \
0                          41                         8908     0.716891
1                          37                         8912     0.689880
2                          97                         8852     0.796677
3                          85                         8864     0.599089
4                          40                         8909     0.657315
5                          46                         8903     1.298366
6                          43                         8906     0.809763
7                          39                         8910     0.694132
```

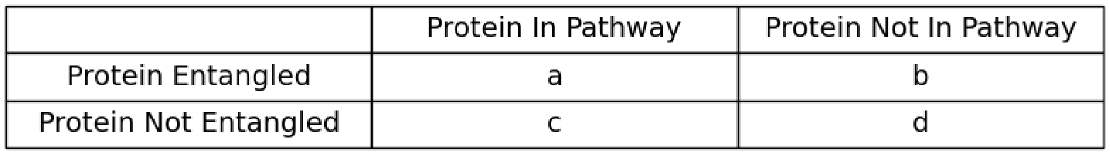
```
8                          37                          8912     0.606054
9                          38                          8911     0.753317

   two_sided_p_values
0            0.141226
1            0.146931
2            0.112900
3            0.002205
4            0.078217
5            0.171969
6            0.333862
7            0.127166
8            0.046602
9            0.243788
```

- Each row of this DataFrame corresponds to a unique Reactome pathway
- The columns correspond to:
  - `unique_pathways`: The pathway identifier
  - `a_in_pathway_entangled`: Number of proteins in the pathway that are entangled (a in contingency table)
  - `b_out_pathway_entangled`: Number of proteins *not* in the pathway that are entangled (b in contingency table)
  - `c_in_pathway_not_entangled`: Number of proteins in the pathway that are *not* entangled (c in contingency table)
  - `d_out_pathway_not_entangled`: Number of proteins *not* in the pathway that are *not* entangled (d in contingency table)
  - `odds_ratios`: The odds ratio calculated from a 2x2 contingency table (see below)
  - `two_sided_p_values`: Initial uncorrected p-value from Fisher's Exact Test
- The contingency tables used in this analysis had the following form:

[4]:
```
# put values into a new format to enable a nice print statement & analysis
contingency_table = pd.DataFrame({"Protein In Pathway"    : ['a', 'c'],
                                  "Protein Not In Pathway": ['b', 'd']},
                                 index = ["Protein Entangled", "Protein Not␣
 ↪Entangled"])

# print the output
print ("This is our contingency table:\n")

# create a table from our contingency_table using matplotlib
plt.clf()
fig, ax    = plt.subplots(figsize = (5, 2))
ax.axis("tight")
ax.axis("off")
cell_text  = contingency_table.reset_index().values.tolist()
col_labels = [""] + contingency_table.columns.tolist()
```

```
table      = ax.table(cellText=cell_text, colLabels=col_labels, loc="center",␣
 ↪cellLoc="center")
table.auto_set_font_size(False)
table.set_fontsize(14)
table.scale(2, 2)
plt.show()
```

```
This is our contingency table:


<Figure size 640x480 with 0 Axes>
```



	Protein In Pathway	Protein Not In Pathway
Protein Entangled	a	b
Protein Not Entangled	c	d

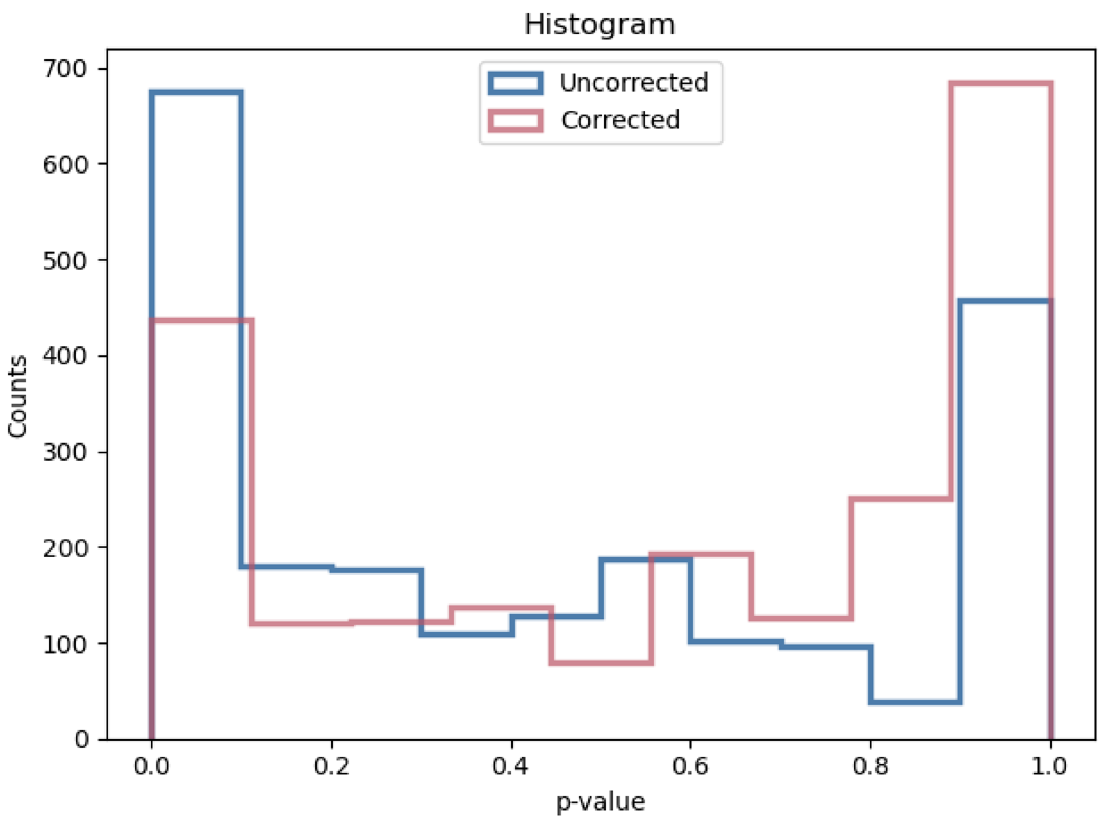
1.1.4 Step 3 - Run the analysis

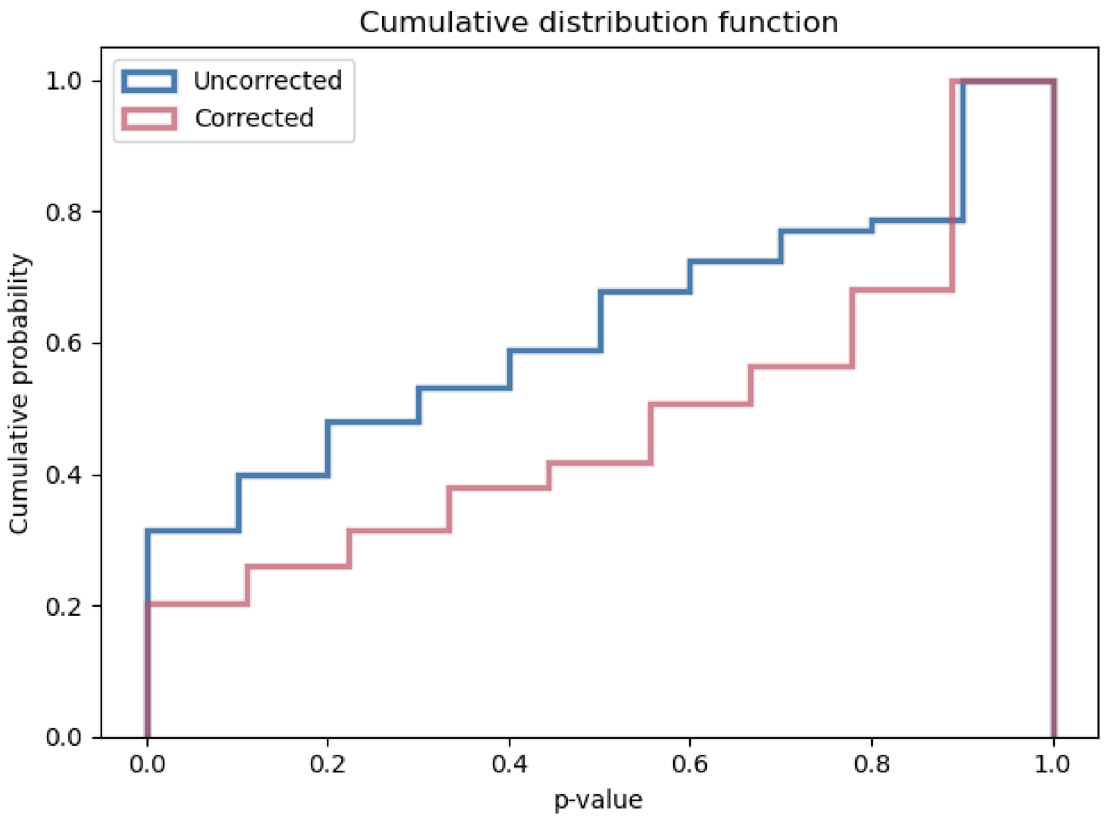
- With the data loaded, we are ready to correct our *p*-values and interpret the results

[5]:
```
# define the significance level for our tests
alpha = 0.05

# apply the Benjamini-Hochberg procedure for FDR correction
_, pvals_corrected, _, _ = multipletests(data9['two_sided_p_values'], alpha =␣
 ↪alpha, method = 'fdr_bh')

# add corrected p-values as a new column
data9['two_sided_p_values-adjusted'] = pvals_corrected

# compute the proportions of uncorrected & corrected p-values < alpha
N_uncorr_acc = ((data9["two_sided_p_values"]          < alpha) &␣
 ↪(data9["odds_ratios"] != 1.0)).sum()
N_corr_acc   = ((data9["two_sided_p_values-adjusted"] < alpha) &␣
 ↪(data9["odds_ratios"] != 1.0)).sum()

print ("Using the uncorrected p-values, we would conclude", N_uncorr_acc,␣
 ↪"pathways have a non-random number of entangled proteins\n")
print ("Using the corrected p-values, we conclude", N_corr_acc, "pathways have␣
 ↪a non-random number of entangled proteins\n")

# make a plot of the distributions of p-values before & after the FDR correction
```

```
plt.clf()
plt.title("Histogram")
plt.hist(data9["two_sided_p_values"], color = "#004488", alpha = 0.7, label =␣
 ↪"Uncorrected", histtype = "step", bins = "fd", linewidth=2.5) # here, alpha !
 ↪= significance level
plt.hist(data9["two_sided_p_values-adjusted"], color = "#BB5566", alpha = 0.7,␣
 ↪label = "Corrected", histtype = "step", bins = "fd", linewidth=2.5)
plt.xlabel("p-value")
plt.ylabel("Counts")
plt.legend(loc = "best")
plt.tight_layout()
plt.show()

# it can be easier to see differences when considering the cumulative␣
 ↪distribution function
plt.clf()
plt.title("Cumulative distribution function")
plt.hist(data9["two_sided_p_values"], color = "#004488", alpha = 0.7, label =␣
 ↪"Uncorrected", histtype = "step", bins = "fd", density = True, cumulative =␣
 ↪True, linewidth=2.5) # here, alpha != significance level
plt.hist(data9["two_sided_p_values-adjusted"], color = "#BB5566", alpha = 0.7,␣
 ↪label = "Corrected", histtype = "step", bins = "fd", density = True,␣
 ↪cumulative = True, linewidth=2.5)
plt.xlabel("p-value")
plt.ylabel("Cumulative probability")
plt.legend(loc = "best")
plt.tight_layout()
plt.show()
```

```
Using the uncorrected p-values, we would conclude 539 pathways have a non-random
number of entangled proteins

Using the corrected p-values, we conclude 318 pathways have a non-random number
of entangled proteins
```


Histogram
Uncorrected
Corrected
Counts
p-value
0
100
200
300
400
500
600
700
0.0
0.2
0.4
0.6
0.8
1.0



1.1.5 Step 4 - Interpret the results

- We conclude that 318 pathways contain a non-random number of entangled proteins
  - We have eliminated 539 - 318 = 221 false positives
- The Benjamini-Hochberg procedure does not eliminate the presence of false positives; with our threshold of 0.05, we have reduced the false discovery rate to 5% or less
- Associations that are significant before the Benjamini-Hochberg correction and not significant afterwards likely arise from random chance