

# Q2-Python

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## 1 NAFLD detection using RNA-Seq data

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In this part, we need to assess whether clinical data such as BMI, age, sex, and diabetes are important enough to be included in our feature selection. Additionally, we will evaluate whether their inclusion improves upon our previous results. Let's proceed and find out.

## 2 Q2 - Python section

```
[39]: # Import needed libraries
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
import seaborn as sns
from sklearn.metrics import precision_score, recall_score
from sklearn.model_selection import train_test_split
from sklearn.preprocessing import LabelEncoder
from sklearn.linear_model import LassoCV
from sklearn.decomposition import PCA
from sklearn.manifold import TSNE
import warnings
warnings.filterwarnings("ignore")
from sklearn.linear_model import LogisticRegression
from sklearn.svm import SVC
from sklearn.ensemble import RandomForestClassifier
from sklearn.neural_network import MLPClassifier
```

```
[2]: # Load Data
normal_counts = pd.read_csv('Normal.counts.voom.csv')
meta_data = pd.read_csv('meta_data.csv')
```

```
[3]: normal_counts.head()
```

```
[3]:
```

	gene	DLDR_0001	DLDR_0002	DLDR_0003	DLDR_0004	DLDR_0005	\
0	ENSG000000000003	5.965571	5.741587	5.996891	5.551919	6.430237	

```

1 ENSG00000000005    1.612375    2.147793    0.418542    0.702492    1.215978
2 ENSG000000000419    4.133821    4.120969    4.086129    4.116240    4.393797
3 ENSG000000000457    4.111056    3.922234    3.964871    3.978350    4.018235
4 ENSG000000000460    4.150662    3.732756    3.634637    3.853979    3.614220

```

```

      DLDR_0006 DLDR_0007 DLDR_0008 DLDR_0009 ... DLDR_0183 DLDR_0184 \
0    6.234619    6.071503    6.441882    5.752712 ...    6.304802    6.576246
1    0.920810    0.458163    0.927224    1.089389 ...   -0.031596   -1.091275
2    4.390909    4.148242    4.554655    4.203819 ...    4.176599    4.244459
3    3.864521    4.263119    3.896271    4.139546 ...    4.378342    4.453838
4    3.500857    4.000565    4.016287    3.904500 ...    2.974209    3.720038

```

```

      DLDR_0185 DLDR_0186 DLDR_0187 DLDR_0188 DLDR_0189 DLDR_0190 \
0    6.735760    6.344234    6.608924    6.480745    6.360397    6.367705
1   -0.942637   -0.026585   -0.757399   -1.083676    0.886550   -0.902201
2    4.342765    4.179319    4.274450    4.361634    4.093280    4.148010
3    4.685598    4.438796    4.042577    4.313540    4.205119    4.506058
4    4.640011    3.814717    2.126408    3.120196    3.336802    3.982071

```

```

      DLDR_0191 DLDR_0192
0    6.604050    6.514539
1   -0.865036   -1.588749
2    4.351489    3.859711
3    4.072137    4.341988
4    3.143138    2.741172

```

[5 rows x 193 columns]

```
[4]: normal_counts.shape
```

```
[4]: (17396, 193)
```

```
[5]: meta_data.head()
```

```

[5]: Patient_ID    SEX    BMI_surg    Age    Run    Diabet \
0 DLDR_0001  Female  35.214555    55  SRR8378590  Non Diabetic
1 DLDR_0002  Female  39.421748    47  SRR8378589    Diabetic
2 DLDR_0003   Male  48.758108    46  SRR8378432  Non Diabetic
3 DLDR_0004  Female  41.822607    36  SRR8378431  Non Diabetic
4 DLDR_0005  Female  53.582192    54  SRR8378434  Non Diabetic

```

```

Simplified_class
0           Normal
1           Normal
2           Normal
3           Normal
4           Normal

```

```
[6]: meta_data.shape
```

```
[6]: (192, 7)
```

```
[7]: print("Number of Normal samples: ", meta_data['Simplified_class'].to_list().
      ↪count("Normal"))
      print("Number of Non_advanced_Fibrosis samples: ",
      ↪meta_data['Simplified_class'].to_list().count("Non_advanced_Fibrosis"))
      print("Number of Advanced_fibrosis: ", meta_data['Simplified_class'].to_list().
      ↪count("Advanced_fibrosis"))
```

Number of Normal samples: 74

Number of Non\_advanced\_Fibrosis samples: 53

Number of Advanced\_fibrosis: 65

To include our clinical data, we need to perform some preprocessing steps. For categorical data, we will use one-hot encoding, and for continuous data, we will apply normalization.

```
[8]: # One-Hot Encoding for categorical features
      categorical_features = ['SEX', 'Diabet']
      ohe = OneHotEncoder()
      # Standardize continuous variables
      numerical_features = ['BMI_surg', 'Age']
      scaler = StandardScaler()
      # Preprocess clinical data
      preprocessor = ColumnTransformer(
          transformers=[
              ('num', scaler, numerical_features),
              ('cat', ohe, categorical_features)])
```

```
[9]: clinical_data = preprocessor.fit_transform(meta_data)
```

```
[10]: clinical_data = pd.DataFrame(clinical_data).iloc[:, [0, 1, 2, 4]]
      clinical_data.columns = ['BMI_surg', 'Age', 'Sex', 'Diabet']
```

```
[11]: clinical_data
```

```
[11]:
```

	BMI_surg	Age	Sex	Diabet
0	-1.308155	0.855160	1.0	0.0
1	-0.857325	0.145913	1.0	1.0
2	0.143131	0.057257	0.0	0.0
3	-0.600056	-0.829302	1.0	0.0
4	0.660065	0.766504	1.0	0.0
..	...	...	...	...
187	-0.772196	1.209783	0.0	1.0
188	0.284524	0.589192	1.0	0.0
189	6.161411	-1.538548	0.0	1.0
190	0.007788	-0.829302	1.0	0.0

```
191 -0.214761  0.677848  1.0      0.0
```

```
[192 rows x 4 columns]
```

```
[12]: print(clinical_data.shape)
      print(normal_counts.shape)
```

```
(192, 4)
(17396, 193)
```

```
[13]: gene_names = normal_counts.iloc[:,0]
      gene_names.at[17396] = 'BMI_surg'
      gene_names.at[17397] = 'Age'
      gene_names.at[17398] = 'SEX'
      gene_names.at[17399] = 'Diabet'
```

```
[14]: gene_names
```

```
[14]: 0      ENSG000000000003
      1      ENSG000000000005
      2      ENSG000000000419
      3      ENSG000000000457
      4      ENSG000000000460
      ...
      17395  ENSG000000273294
      17396      BMI_surg
      17397      Age
      17398      SEX
      17399      Diabet
      Name: gene, Length: 17400, dtype: object
```

Now, let's combine our gene expression data with clinical data

```
[15]: combined_data = np.hstack((normal_counts.iloc[0:,1:].T, clinical_data))
      combined_data = pd.DataFrame(combined_data)
```

```
[16]: combined_data
```

```
[16]:
```

	0	1	2	3	4	5	6	\
0	5.965571	1.612375	4.133821	4.111056	4.150662	2.975845	11.005488	
1	5.741587	2.147793	4.120969	3.922234	3.732756	3.199989	10.860700	
2	5.996891	0.418542	4.086129	3.964871	3.634637	2.949733	10.934025	
3	5.551919	0.702492	4.116240	3.978350	3.853979	2.991061	10.760445	
4	6.430237	1.215978	4.393797	4.018235	3.614220	2.836130	11.491427	
..	...	...	...	...	...	...	...	
187	6.480745	-1.083676	4.361634	4.313540	3.120196	1.941859	11.451981	
188	6.360397	0.886550	4.093280	4.205119	3.336802	2.911496	11.658711	
189	6.367705	-0.902201	4.148010	4.506058	3.982071	2.202136	11.864397	

```

190  6.604050 -0.865036  4.351489  4.072137  3.143138  4.037476  11.782524
191  6.514539 -1.588749  3.859711  4.341988  2.741172  2.803568  11.474777

```

```

      7      8      9      ...      17390      17391      17392  \
0  4.405768  6.825329  4.221450  ...  0.691230  5.637483 -0.005377
1  3.895350  6.453687  4.218183  ...  1.004202  5.975612  0.532134
2  4.282577  6.437658  3.736947  ...  0.943633  5.531648 -0.184123
3  4.297722  6.710840  4.003661  ...  0.431190  5.571799 -0.034474
4  4.405558  7.437655  4.377965  ...  0.085581  5.636848 -1.216981
..      ...      ...      ...      ...      ...      ...
187  4.556052  7.183779  5.066071  ... -0.431600  2.616763  1.063165
188  4.315153  7.384726  4.627703  ... -1.080284  2.975985  0.589568
189  4.348255  7.326297  4.528530  ... -1.108652  3.218814 -0.416774
190  4.358527  8.468526  5.013154  ... -0.624028  2.050571 -1.517113
191  3.895962  7.617180  4.778544  ... -0.510747  2.572243  0.667591

```

```

      17393      17394      17395      17396      17397  17398  17399
0  1.675800  2.683536 -0.339797 -1.308155  0.855160    1.0    0.0
1  1.555218  2.926666  0.435919 -0.857325  0.145913    1.0    1.0
2  2.391906  2.260662 -0.691083  0.143131  0.057257    0.0    0.0
3  1.639298  2.341393  0.096771 -0.600056 -0.829302    1.0    0.0
4  1.974160  1.351861 -0.079478  0.660065  0.766504    1.0    0.0
..      ...      ...      ...      ...      ...
187  1.941859 -2.306069  0.632531 -0.772196  1.209783    0.0    1.0
188  2.754906 -0.839275 -0.016153  0.284524  0.589192    1.0    0.0
189  2.246191 -1.639167  0.145105  6.161411 -1.538548    0.0    1.0
190  2.084923 -4.324468  0.067849  0.007788 -0.829302    1.0    0.0
191  2.160189 -3.173712  1.218606 -0.214761  0.677848    1.0    0.0

```

[192 rows x 17400 columns]

Let's split our data and labels to train and test sets

```
[17]: X_train, X_test, y_train, y_test = train_test_split(combined_data,
↳ meta_data['Simplified_class'], test_size=0.3, random_state = 10101)
```

```
[18]: X_train.head()
```

```

[18]:      0      1      2      3      4      5      6      \
35  5.820135 -1.060061  4.388400  4.080172  2.564430  3.552685  11.011379
80  6.546299  0.582165  3.752090  4.645175  3.840899  3.201075  11.433579
190  6.604050 -0.865036  4.351489  4.072137  3.143138  4.037476  11.782524
187  6.480745 -1.083676  4.361634  4.313540  3.120196  1.941859  11.451981
129  6.550016 -1.222374  4.534941  4.370763  3.512952  2.517867  12.041229

      7      8      9      ...      17390      17391      17392  \
35  4.682305  6.951539  4.589555  ... -0.323095  3.157170  0.459313
80  3.705547  7.143316  5.482169  ...  0.582165  4.041596  0.096738

```

```

190  4.358527  8.468526  5.013154  ... -0.624028  2.050571 -1.517113
187  4.556052  7.183779  5.066071  ... -0.431600  2.616763  1.063165
129  4.315374  7.485927  4.655817  ... -0.981365  2.594319  0.447478

      17393      17394      17395      17396      17397  17398  17399
35   3.115198 -2.645023  0.760969 -0.001936 -0.120055    1.0    0.0
80   2.418666 -2.225190  0.582165 -0.661021 -1.361237    1.0    1.0
190  2.084923 -4.324468  0.067849  0.007788 -0.829302    1.0    0.0
187  1.941859 -2.306069  0.632531 -0.772196  1.209783    0.0    1.0
129  2.556600 -2.359877  0.932905  0.932361 -1.627204    1.0    0.0

```

[5 rows x 17400 columns]

```
[19]: X_train.shape
```

```
[19]: (134, 17400)
```

```
[20]: y_train.head()
```

```

[20]: 35          Normal
      80    Advanced_fibrosis
      190          Normal
      187          Normal
      129  Non_advanced_Fibrosis
      Name: Simplified_class, dtype: object

```

```
[21]: y_train.shape
```

```
[21]: (134,)
```

```

[22]: print("Number of Normal samples: ", y_train.to_list().count("Normal"))
      print("Number of Non_advanced_Fibrosis samples: ", y_train.to_list().
      ↪count("Non_advanced_Fibrosis"))
      print("Number of Advanced_fibrosis samples: ", y_train.to_list().
      ↪count("Advanced_fibrosis"))

```

```

Number of Normal samples:  44
Number of Non_advanced_Fibrosis samples:  38
Number of Advanced_fibrosis samples:  52

```

Now, let's save the training set data to use in the R Jupyter Notebook for feature selection

```

[23]: X_train.T.to_csv('train_normal_counts.csv', index=False)
      y_train.T.to_csv('train_meta_data.csv', index=False)

```

**Run R Jupyter Notebook** Let's load the output of R to continue the task and check if any clinical data have been selected or not

```
[24]: subset_data = pd.read_csv('subset_data.csv')
```

```
[25]: subset_data
```

```
[25]:
```

	Unnamed: 0	X35	X80	X190	X187	X129	X12	\
0	10	4.589555	5.482169	5.013154	5.066071	4.655817	4.299078	
1	57	-0.719024	0.582165	-1.154543	-1.083676	-0.289488	-0.006469	
2	265	0.587637	-2.225190	-0.076540	0.196432	0.272391	2.523150	
3	275	1.662405	2.529697	1.508422	1.991612	1.467942	1.934152	
4	278	5.529902	5.846272	5.553583	5.359267	5.774549	4.962662	
..	...	...	...	...	...	...	...	
522	16863	3.811126	3.329399	2.183327	2.412750	3.323819	2.690386	
523	16887	3.576080	2.418666	3.355012	3.288878	3.231084	3.757011	
524	16892	4.007463	3.060212	2.951656	3.366357	2.649112	2.774044	
525	17075	6.925971	7.323632	7.411511	7.189120	7.519400	6.661844	
526	17187	-2.645023	-2.225190	-4.324468	-3.891031	-4.681805	0.820694	
		X78	X130	X134	...	X174	X51	X86 \
0	4.752957	5.409514	4.993777	...	4.853565	4.567173	4.985158	
1	-1.138550	-4.060128	-2.104255	...	-2.285986	-0.110595	-0.385647	
2	1.424386	0.332190	0.217673	...	1.083248	0.352377	-0.309698	
3	2.031375	2.048397	0.528013	...	1.083248	2.556469	2.055951	
4	5.201300	5.500205	5.782051	...	5.862067	5.038603	5.665231	
..	...	...	...	...	...	...	...	
522	3.515760	3.197260	2.112975	...	1.555316	3.901539	3.344479	
523	2.435441	3.079423	2.393996	...	2.974541	3.737944	2.476362	
524	3.123117	2.951099	2.974696	...	2.604785	3.934981	3.001089	
525	7.087205	7.366661	7.586092	...	7.491543	6.721953	7.511299	
526	-3.013019	-4.060128	-4.426183	...	-3.870949	-3.735085	-4.010138	
		X154	X91	X186	X185	X178	X181	X0
0	5.234776	4.900754	4.923087	5.004500	5.058062	5.002454	4.221450	
1	-3.580073	0.317259	-0.757399	-3.486017	-1.346471	-1.103280	-0.005377	
2	0.120366	0.890444	-0.291735	0.811664	-0.246936	0.416094	2.729099	
3	1.374123	1.902221	1.838969	1.643266	1.272439	1.932344	2.467558	
4	5.781140	5.745159	5.438062	5.570169	5.558701	5.733822	5.220121	
..	...	...	...	...	...	...	...	
522	1.911780	3.763838	3.667268	2.742802	2.446087	3.857108	2.844001	
523	2.817958	2.596331	3.203431	3.162640	2.353968	3.445156	3.271068	
524	2.596515	3.945549	4.002569	3.359473	3.497226	3.563476	2.830128	
525	7.684565	7.620817	7.419428	7.417614	7.354464	7.633460	6.752731	
526	-5.165036	-2.718365	-5.149716	-5.070979	-2.568864	-3.425208	1.202731	

```
[527 rows x 135 columns]
```

```
[26]: subset_data.shape
```

```
[26]: (527, 135)
```

We need to extract the same features for the X test dataset as well. To do this, we must identify the indices of our selected DEGs and apply the same subsetting to the test data.

```
[27]: selected_genes_R = subset_data.T.iloc[0,:].to_list()
```

```
[28]: selected_genes_Python = [int(i-1) for i in selected_genes_R ]
```

```
[29]: selected_genes_Python[:10]
```

```
[29]: [9, 56, 264, 274, 277, 296, 309, 340, 351, 389]
```

```
[30]: len(selected_genes_Python)
```

```
[30]: 527
```

```
[31]: clinical_indices =[17396,17397,17398,17399]
common_members = set(selected_genes_Python).intersection(set(clinical_indices))
if common_members:
    print("Some clinical data is identified among DEGs")
else:
    print("No clinical data is identified among DEGs")
```

No clinical data is identified among DEGs

So in this case, no clinical data have been selected

### 2.0.1 Classification

Now, we need to determine whether adding clinical data improves the performance of our classification models compared to the previous results. To evaluate this, we will perform classification under three conditions: 1- using only DEGs, 2- combining DEGs and clinical data, and 3- using only clinical data. This process will help us assess the impact of clinical data on classification, both with and without the presence of DEGs.

#### 1- Using only DEGs

```
[32]: df_deg = subset_data.iloc[0:,1:].T
df_deg_test = X_test[selected_genes_Python]
```

```
[33]: df_deg.head()
```

```
[33]:
```

	0	1	2	3	4	5	6	\
X35	4.589555	-0.719024	0.587637	1.662405	5.529902	7.549487	0.318451	
X80	5.482169	0.582165	-2.225190	2.529697	5.846272	7.759228	0.096738	
X190	5.013154	-1.154543	-0.076540	1.508422	5.553583	7.612538	1.033084	
X187	5.066071	-1.083676	0.196432	1.991612	5.359267	8.070057	0.356896	
X129	4.655817	-0.289488	0.272391	1.467942	5.774549	7.439405	0.362589	



	7	8	9	...	517	518	519	\
X35	6.089009	5.898008	9.044188	...	3.104511	1.602904	0.915692	
X80	6.539682	6.101239	8.633568	...	2.819204	1.234242	-0.640228	
X190	6.434588	6.098648	8.244201	...	2.275445	0.884985	0.067849	
X187	6.476384	6.118797	7.951712	...	2.708882	0.356896	-0.431600	
X129	6.486240	6.239292	8.248378	...	2.969247	1.248932	-1.222374	

	520	521	522	523	524	525	526
X35	7.886943	5.319606	3.811126	3.576080	4.007463	6.925971	-2.645023
X80	6.310085	3.797178	3.329399	2.418666	3.060212	7.323632	-2.225190
X190	7.139567	3.909152	2.183327	3.355012	2.951656	7.411511	-4.324468
X187	7.167637	4.021858	2.412750	3.288878	3.366357	7.189120	-3.891031
X129	6.106913	3.570860	3.323819	3.231084	2.649112	7.519400	-4.681805

[5 rows x 527 columns]

```
[34]: df_deg_test.head()
```

```
[34]:
```

	9	56	264	274	277	296	309	\
21	3.967031	0.177378	2.880522	2.254454	5.101756	7.696151	2.077214	
15	4.603126	0.002788	2.608214	1.992446	5.079298	7.453528	2.274384	
3	4.003661	-0.178864	2.418699	2.287454	5.125878	6.943351	2.538993	
164	5.286333	0.143691	0.332137	1.587791	5.827910	7.489948	1.225221	
126	5.118729	-0.818750	0.897457	1.530400	5.446251	7.622416	0.897457	

	340	351	389	...	16641	16686	16715	\
21	5.845657	5.444621	8.076772	...	2.825450	0.246091	0.928350	
15	5.735367	5.394124	9.027927	...	2.934201	0.299770	0.488215	
3	6.049955	5.583037	8.243201	...	2.418699	0.217065	-0.519901	
164	6.994423	6.261908	8.733452	...	2.368478	0.143691	0.389852	
126	6.472366	5.832644	8.246698	...	2.137661	0.811301	1.128783	

	16731	16762	16862	16886	16891	17074	17186
21	7.735892	4.644785	2.335526	3.460071	3.723708	6.646771	0.246091
15	7.956485	5.527246	2.274384	3.722128	3.226652	6.739625	0.545930
3	7.122512	3.978350	2.974088	3.280568	2.418699	6.827647	0.999473
164	6.695064	3.291074	1.485611	2.862085	2.892352	7.434889	-3.697611
126	6.668899	3.631283	3.308569	2.881690	2.101816	7.391864	0.215197

[5 rows x 527 columns]

## Logistic Regression

```
[40]: # Logistic Regression
LR_model = LogisticRegression(solver='saga')
LR_model.fit(df_deg, y_train)
y_pred_LR = LR_model.predict(df_deg_test)
LR_precision = precision_score(y_test, y_pred_LR, average='macro')
```

```
LR_recall = recall_score(y_test, y_pred_LR, average='macro')
print(LR_precision)
print(LR_recall)
```

0.7842803030303029

0.752991452991453

## Support Vector Machine

```
[41]: # Support Vector Machine
SVM_model = SVC(kernel='linear', C=1)
SVM_model.fit(df_deg, y_train)
y_pred_SVM = SVM_model.predict(df_deg_test)
SVM_precision = precision_score(y_test, y_pred_SVM, average='macro')
SVM_recall = recall_score(y_test, y_pred_SVM, average='macro')
print(SVM_precision)
print(SVM_recall)
```

0.8441558441558442

0.8008547008547008

## Random Forest

```
[42]: # Random Forest
RF_model = RandomForestClassifier(random_state = 10101)
RF_model.fit(df_deg, y_train)
y_pred_RF = RF_model.predict(df_deg_test)
RF_precision = precision_score(y_test, y_pred_RF, average='macro')
RF_recall = recall_score(y_test, y_pred_RF, average='macro')
print(RF_precision)
print(RF_recall)
```

0.7743589743589744

0.7606837606837606

## Multi Layer Perceptron

```
[43]: # Multi Layer Perceptron
MLP_model = MLPClassifier(hidden_layer_sizes=(100,), max_iter=300,
    ↪activation='relu', solver='adam', random_state = 10101)
MLP_model.fit(df_deg, y_train)
y_pred_MLP = MLP_model.predict(df_deg_test)
MLP_precision = precision_score(y_test, y_pred_MLP, average='macro')
MLP_recall = recall_score(y_test, y_pred_MLP, average='macro')
print(MLP_precision)
print(MLP_recall)
```

0.7317550505050505

0.7051282051282052

## 2- Combining DEGs and clinical data

```
[44]: clinical_df_deg = np.hstack((df_deg,X_train[clinical_indices]))
```

```
[45]: clinical_df_deg = pd.DataFrame(clinical_df_deg)
clinical_df_deg.head()
```

```
[45]:
```

	0	1	2	3	4	5	6	\
0	4.589555	-0.719024	0.587637	1.662405	5.529902	7.549487	0.318451	
1	5.482169	0.582165	-2.225190	2.529697	5.846272	7.759228	0.096738	
2	5.013154	-1.154543	-0.076540	1.508422	5.553583	7.612538	1.033084	
3	5.066071	-1.083676	0.196432	1.991612	5.359267	8.070057	0.356896	
4	4.655817	-0.289488	0.272391	1.467942	5.774549	7.439405	0.362589	

	7	8	9	...	521	522	523	524	\
0	6.089009	5.898008	9.044188	...	5.319606	3.811126	3.576080	4.007463	
1	6.539682	6.101239	8.633568	...	3.797178	3.329399	2.418666	3.060212	
2	6.434588	6.098648	8.244201	...	3.909152	2.183327	3.355012	2.951656	
3	6.476384	6.118797	7.951712	...	4.021858	2.412750	3.288878	3.366357	
4	6.486240	6.239292	8.248378	...	3.570860	3.323819	3.231084	2.649112	

	525	526	527	528	529	530
0	6.925971	-2.645023	-0.001936	-0.120055	1.0	0.0
1	7.323632	-2.225190	-0.661021	-1.361237	1.0	1.0
2	7.411511	-4.324468	0.007788	-0.829302	1.0	0.0
3	7.189120	-3.891031	-0.772196	1.209783	0.0	1.0
4	7.519400	-4.681805	0.932361	-1.627204	1.0	0.0

[5 rows x 531 columns]

```
[46]: clinical_df_deg_test = np.hstack((df_deg_test,X_test[clinical_indices]))
```

```
[47]: clinical_df_deg_test = pd.DataFrame(clinical_df_deg_test)
clinical_df_deg_test.head()
```

```
[47]:
```

	0	1	2	3	4	5	6	\
0	3.967031	0.177378	2.880522	2.254454	5.101756	7.696151	2.077214	
1	4.603126	0.002788	2.608214	1.992446	5.079298	7.453528	2.274384	
2	4.003661	-0.178864	2.418699	2.287454	5.125878	6.943351	2.538993	
3	5.286333	0.143691	0.332137	1.587791	5.827910	7.489948	1.225221	
4	5.118729	-0.818750	0.897457	1.530400	5.446251	7.622416	0.897457	

	7	8	9	...	521	522	523	524	\
0	5.845657	5.444621	8.076772	...	4.644785	2.335526	3.460071	3.723708	
1	5.735367	5.394124	9.027927	...	5.527246	2.274384	3.722128	3.226652	
2	6.049955	5.583037	8.243201	...	3.978350	2.974088	3.280568	2.418699	
3	6.994423	6.261908	8.733452	...	3.291074	1.485611	2.862085	2.892352	
4	6.472366	5.832644	8.246698	...	3.631283	3.308569	2.881690	2.101816	

	525	526	527	528	529	530
0	6.646771	0.246091	-0.082413	1.298439	1.0	0.0
1	6.739625	0.545930	-0.446496	-0.474678	1.0	0.0
2	6.827647	0.999473	-0.600056	-0.829302	1.0	0.0
3	7.434889	-3.697611	0.875886	-1.183925	1.0	0.0
4	7.391864	0.215197	-0.699417	-0.208711	1.0	1.0

[5 rows x 531 columns]

### Logistic Regression

```
[48]: # Logistic Regression
LR_model = LogisticRegression(solver='saga')
LR_model.fit(clinical_df_deg, y_train)
y_pred_LR = LR_model.predict(clinical_df_deg_test)
LR_precision = precision_score(y_test, y_pred_LR, average='macro')
LR_recall = recall_score(y_test, y_pred_LR, average='macro')
print(LR_precision)
print(LR_recall)
```

0.8010912698412698

0.7752136752136751

### Support Vector Machine

```
[49]: # Support Vector Machine
SVM_model = SVC(kernel='linear', C=1)
SVM_model.fit(clinical_df_deg, y_train)
y_pred_SVM = SVM_model.predict(clinical_df_deg_test)
SVM_precision = precision_score(y_test, y_pred_SVM, average='macro')
SVM_recall = recall_score(y_test, y_pred_SVM, average='macro')
print(SVM_precision)
print(SVM_recall)
```

0.8288084464555053

0.7752136752136751

### Random Forest

```
[50]: # Random Forest
RF_model = RandomForestClassifier(random_state = 10101)
RF_model.fit(clinical_df_deg, y_train)
y_pred_RF = RF_model.predict(clinical_df_deg_test)
RF_precision = precision_score(y_test, y_pred_RF, average='macro')
RF_recall = recall_score(y_test, y_pred_RF, average='macro')
print(RF_precision)
print(RF_recall)
```

0.753968253968254

0.7461538461538462

## Multi Layer Perceptron

```
[51]: # Multi Layer Perceptron
MLP_model = MLPClassifier(hidden_layer_sizes=(100,), max_iter=300,
    ↪activation='relu', solver='adam', random_state = 10101)
MLP_model.fit(clinical_df_deg, y_train)
y_pred_MLP = MLP_model.predict(clinical_df_deg_test)
MLP_precision = precision_score(y_test, y_pred_MLP, average='macro')
MLP_recall = recall_score(y_test, y_pred_MLP, average='macro')
print(MLP_precision)
print(MLP_recall)
```

0.7662337662337663

0.7273504273504273

## 3- using only clinical data

### Logistic Regression

```
[53]: # Logistic Regression
LR_model = LogisticRegression(solver='saga')
LR_model.fit(X_train[clinical_indices], y_train)
y_pred_LR = LR_model.predict(X_test[clinical_indices])
LR_precision = precision_score(y_test, y_pred_LR, average='macro')
LR_recall = recall_score(y_test, y_pred_LR, average='macro')
print(LR_precision)
print(LR_recall)
```

0.49887766554433216

0.511965811965812

### Support Vector Machine

```
[158]: # Support Vector Machine
SVM_model = SVC(kernel='linear', C=1)
SVM_model.fit(X_train[clinical_indices], y_train)

y_pred_SVM = SVM_model.predict(X_test[clinical_indices])
SVM_precision = precision_score(y_test, y_pred_SVM, average='macro')
SVM_recall = recall_score(y_test, y_pred_SVM, average='macro')
print(SVM_precision)
print(SVM_recall)
```

0.47980407944176057

0.511965811965812

### Random Forest

```
[54]: # Random Forest
RF_model = RandomForestClassifier(random_state = 10101)
RF_model.fit(X_train[clinical_indices], y_train)
y_pred_RF = RF_model.predict(X_test[clinical_indices])
```

```

RF_precision = precision_score(y_test, y_pred_RF, average='macro')
RF_recall = recall_score(y_test, y_pred_RF, average='macro')
print(RF_precision)
print(RF_recall)

```

0.5344751866490997

0.5418803418803418

## Multi Layer Perceptron

```

[55]: # Multi Layer Perceptron
MLP_model = MLPClassifier(hidden_layer_sizes=(100,), max_iter=300,
    ↪activation='relu', solver='adam', random_state = 10101)
MLP_model.fit(X_train[clinical_indices], y_train)
y_pred_MLP = MLP_model.predict(X_test[clinical_indices])
MLP_precision = precision_score(y_test, y_pred_MLP, average='macro')
MLP_recall = recall_score(y_test, y_pred_MLP, average='macro')
print(MLP_precision)
print(MLP_recall)

```

0.5412962962962963

0.5307692307692308

So far, we have obtained only a single result for each classifier, but these results are not statistically valid. To test for reproducibility, we have put the entire process into a loop to run it 100 times. Additionally, we created an R script to perform DE analysis automatically using subprocess. Let's run this cell and save the results for statistical analysis.

For each iteration, in addition to saving precision and recall for statistical validation, we need to check whether the clinical data have been included in our feature selection.

```

[57]: import subprocess

# Load Data
normal_counts = pd.read_csv('Normal.counts.voom.csv')
meta_data = pd.read_csv('meta_data.csv')

categorical_features = ['SEX', 'Diabet']
ohe = OneHotEncoder()
numerical_features = ['BMI_surg', 'Age']
scaler = StandardScaler()
preprocessor = ColumnTransformer(
    transformers=[
        ('num', scaler, numerical_features),
        ('cat', ohe, categorical_features)])
clinical_data = preprocessor.fit_transform(meta_data)
clinical_data = pd.DataFrame(clinical_data).iloc[:, [0, 1, 2, 4]]
clinical_data.columns = ['BMI_surg', 'Age', 'Sex', 'Diabet']
gene_names = normal_counts.iloc[:,0]
gene_names.at[17396] = 'BMI_surg'

```

```

gene_names.at[17397] = 'Age'
gene_names.at[17398] = 'SEX'
gene_names.at[17399] = 'Diabet'

combined_data = np.hstack((normal_counts.iloc[0:,1:].T, clinical_data))
combined_data = pd.DataFrame(combined_data)

n_iterations = 300
test_size = 0.3

LR_precisions = []
LR_recalls = []
LR_precisions2 = []
LR_recalls2 = []
LR_precisions3 = []
LR_recalls3 = []

SVM_precisions = []
SVM_recalls = []
SVM_precisions2 = []
SVM_recalls2 = []
SVM_precisions3 = []
SVM_recalls3 = []

RF_precisions = []
RF_recalls = []
RF_precisions2 = []
RF_recalls2 = []
RF_precisions3 = []
RF_recalls3 = []

MLP_precisions = []
MLP_recalls = []
MLP_precisions2 = []
MLP_recalls2 = []
MLP_precisions3 = []
MLP_recalls3 = []

for i in range(100):
    print('iteration',i)
    X_train, X_test, y_train, y_test = train_test_split(combined_data,
↳ meta_data['Simplified_class'], test_size=0.3, random_state = i)
    X_train.T.to_csv('train_normal_counts.csv', index=False)
    y_train.T.to_csv('train_meta_data.csv', index=False)

    r_script_path = r"q2r.R"
    rscript_path = r"C:\Program Files\R\R-4.2.1\bin\Rscript.exe"

```

```

# Execute the R script
try:
    subprocess.run([rscript_path, r_script_path], capture_output=True,
↳text=True)
except subprocess.CalledProcessError as e:
    print(f"Error executing R script: {e}")

#
subset_data = pd.read_csv('subset_data.csv')
selected_genes_R = subset_data.T.iloc[0,:].to_list()
selected_genes_Python = [int(i-1) for i in selected_genes_R ]

clinical_indices =[17396,17397,17398,17399]
common_members = set(selected_genes_Python).
↳intersection(set(clinical_indices))
if common_members:
    print("Some clinical data is identified among DEGs")
else:
    print("No clinical data is identified among DEGs")

df_deg = subset_data.iloc[0:,1:].T
df_deg_test = X_test[selected_genes_Python]

# Logistic Regression
LR_model = LogisticRegression(solver='saga')
LR_model.fit(df_deg, y_train)
y_pred_LR = LR_model.predict(df_deg_test)
LR_precision = precision_score(y_test, y_pred_LR, average='macro')
LR_recall = recall_score(y_test, y_pred_LR, average='macro')
LR_precisions.append(LR_precision)
LR_recalls.append(LR_recall)

# Support Vector Machine
SVM_model = SVC(kernel='linear', C=1)
SVM_model.fit(df_deg, y_train)
y_pred_SVM = SVM_model.predict(df_deg_test)
SVM_precision = precision_score(y_test, y_pred_SVM, average='macro')
SVM_recall = recall_score(y_test, y_pred_SVM, average='macro')
SVM_precisions.append(SVM_precision)
SVM_recalls.append(SVM_recall)

# Random Forest
RF_model = RandomForestClassifier(random_state = i)
RF_model.fit(df_deg, y_train)
y_pred_RF = RF_model.predict(df_deg_test)
RF_precision = precision_score(y_test, y_pred_RF, average='macro')
RF_recall = recall_score(y_test, y_pred_RF, average='macro')

```



```

RF_precisions.append(RF_precision)
RF_recalls.append(RF_recall)

# Multi Layer Perceptron
MLP_model = MLPClassifier(hidden_layer_sizes=(100,), max_iter=300,
↪activation='relu', solver='adam', random_state = i)
MLP_model.fit(df_deg, y_train)
y_pred_MLP = MLP_model.predict(df_deg_test)
MLP_precision = precision_score(y_test, y_pred_MLP, average='macro')
MLP_recall = recall_score(y_test, y_pred_MLP, average='macro')
MLP_precisions.append(MLP_precision)
MLP_recalls.append(MLP_recall)

clinical_df_deg = np.hstack((df_deg,X_train[clinical_indices]))
clinical_df_deg = pd.DataFrame(clinical_df_deg)
clinical_df_deg_test = np.hstack((df_deg_test,X_test[clinical_indices]))
clinical_df_deg_test = pd.DataFrame(clinical_df_deg_test)

# Logistic Regression
LR_model = LogisticRegression(solver='saga')
LR_model.fit(clinical_df_deg, y_train)
y_pred_LR = LR_model.predict(clinical_df_deg_test)
LR_precision = precision_score(y_test, y_pred_LR, average='macro')
LR_recall = recall_score(y_test, y_pred_LR, average='macro')
LR_precisions2.append(LR_precision)
LR_recalls2.append(LR_recall)

# Support Vector Machine
SVM_model = SVC(kernel='linear', C=1)
SVM_model.fit(clinical_df_deg, y_train)
y_pred_SVM = SVM_model.predict(clinical_df_deg_test)
SVM_precision = precision_score(y_test, y_pred_SVM, average='macro')
SVM_recall = recall_score(y_test, y_pred_SVM, average='macro')
SVM_precisions2.append(SVM_precision)
SVM_recalls2.append(SVM_recall)

# Random Forest
RF_model = RandomForestClassifier(random_state = i)
RF_model.fit(clinical_df_deg, y_train)
y_pred_RF = RF_model.predict(clinical_df_deg_test)
RF_precision = precision_score(y_test, y_pred_RF, average='macro')
RF_recall = recall_score(y_test, y_pred_RF, average='macro')
RF_precisions2.append(RF_precision)
RF_recalls2.append(RF_recall)

# Multi Layer Perceptron

```

```

MLP_model = MLPClassifier(hidden_layer_sizes=(100,), max_iter=300,
↪activation='relu', solver='adam', random_state = i)
MLP_model.fit(clinical_df_deg, y_train)
y_pred_MLP = MLP_model.predict(clinical_df_deg_test)
MLP_precision = precision_score(y_test, y_pred_MLP, average='macro')
MLP_recall = recall_score(y_test, y_pred_MLP, average='macro')
MLP_precisions2.append(MLP_precision)
MLP_recalls2.append(MLP_recall)

```

#### *# Logistic Regression*

```

LR_model = LogisticRegression(solver='saga')
LR_model.fit(X_train[clinical_indices], y_train)
y_pred_LR = LR_model.predict(X_test[clinical_indices])
LR_precision = precision_score(y_test, y_pred_LR, average='macro')
LR_recall = recall_score(y_test, y_pred_LR, average='macro')
LR_precisions3.append(LR_precision)
LR_recalls3.append(LR_recall)

```

#### *# Support Vector Machine*

```

SVM_model = SVC(kernel='linear', C=1)
SVM_model.fit(X_train[clinical_indices], y_train)
y_pred_SVM = SVM_model.predict(X_test[clinical_indices])
SVM_precision = precision_score(y_test, y_pred_SVM, average='macro')
SVM_recall = recall_score(y_test, y_pred_SVM, average='macro')
SVM_precisions3.append(SVM_precision)
SVM_recalls3.append(SVM_recall)

```

#### *# Random Forest*

```

RF_model = RandomForestClassifier(random_state = i)
RF_model.fit(X_train[clinical_indices], y_train)
y_pred_RF = RF_model.predict(X_test[clinical_indices])
RF_precision = precision_score(y_test, y_pred_RF, average='macro')
RF_recall = recall_score(y_test, y_pred_RF, average='macro')
RF_precisions3.append(RF_precision)
RF_recalls3.append(RF_recall)

```

#### *# Multi Layer Perceptron*

```

MLP_model = MLPClassifier(hidden_layer_sizes=(100,), max_iter=300,
↪activation='relu', solver='adam', random_state = i)
MLP_model.fit(X_train[clinical_indices], y_train)
y_pred_MLP = MLP_model.predict(X_test[clinical_indices])
MLP_precision = precision_score(y_test, y_pred_MLP, average='macro')
MLP_recall = recall_score(y_test, y_pred_MLP, average='macro')
MLP_precisions3.append(MLP_precision)
MLP_recalls3.append(MLP_recall)

```

iteration 0  
No clinical data is identified among DEGs  
iteration 1  
No clinical data is identified among DEGs  
iteration 2  
No clinical data is identified among DEGs  
iteration 3  
No clinical data is identified among DEGs  
iteration 4  
No clinical data is identified among DEGs  
iteration 5  
No clinical data is identified among DEGs  
iteration 6  
No clinical data is identified among DEGs  
iteration 7  
No clinical data is identified among DEGs  
iteration 8  
No clinical data is identified among DEGs  
iteration 9  
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iteration 10  
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iteration 51  
No clinical data is identified among DEGs  
iteration 52  
No clinical data is identified among DEGs  
iteration 53  
No clinical data is identified among DEGs  
iteration 54  
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No clinical data is identified among DEGs

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iteration 96
No clinical data is identified among DEGs
iteration 97
No clinical data is identified among DEGs
iteration 98
No clinical data is identified among DEGs
iteration 99
No clinical data is identified among DEGs

```

As you can see, clinical data were not selected in any of the iterations.

Last but not least, we need to report the average and confidence interval for the results of each classifier. We will also create a bar plot to visually compare the results of the classifiers. We also need to compare the results of the three conditions to determine whether adding clinical data has a significant effect on performance or not.

```

[58]: def
    Evaluation(LR_precisions,LR_recalls,SVM_precisions,SVM_recalls,RF_precisions,RF_recalls,MLP

    LR_mean_precision = np.mean(LR_precisions)
    LR_mean_recall = np.mean(LR_recalls)
    LR_precision_conf_interval = np.percentile(LR_precisions, [2.5, 97.5])
    LR_recall_conf_interval = np.percentile(LR_recalls, [2.5, 97.5])
    print(f'Mean Precision for Logistic Regression: {LR_mean_precision}, 95% CI:
    {LR_precision_conf_interval}')
    print(f'Mean Recall for Logistic Regression: {LR_mean_recall}, 95% CI:
    {LR_recall_conf_interval}')

    SVM_mean_precision = np.mean(SVM_precisions)
    SVM_mean_recall = np.mean(SVM_recalls)
    SVM_precision_conf_interval = np.percentile(SVM_precisions, [2.5, 97.5])
    SVM_recall_conf_interval = np.percentile(SVM_recalls, [2.5, 97.5])
    print(f'Mean Precision for Support Vector Machine: {SVM_mean_precision}, 95%
    CI: {SVM_precision_conf_interval}')
    print(f'Mean Recall for Support Vector Machine: {SVM_mean_recall}, 95% CI:
    {SVM_recall_conf_interval}')

    RF_mean_precision = np.mean(RF_precisions)
    RF_mean_recall = np.mean(RF_recalls)
    RF_precision_conf_interval = np.percentile(RF_precisions, [2.5, 97.5])
    RF_recall_conf_interval = np.percentile(RF_recalls, [2.5, 97.5])
    print(f'Mean Precision for Random Forest: {RF_mean_precision}, 95% CI:
    {RF_precision_conf_interval}')

```

```

print(f'Mean Recall for Random Forest: {RF_mean_recall}, 95% CI:␣
↪{RF_recall_conf_interval}')
␣
↪print("-----

MLP_mean_precision = np.mean(MLP_precisions)
MLP_mean_recall = np.mean(MLP_recalls)
MLP_precision_conf_interval = np.percentile(MLP_precisions, [2.5, 97.5])
MLP_recall_conf_interval = np.percentile(MLP_recalls, [2.5, 97.5])
print(f'Mean Precision for Multi Layer Perceptron: {MLP_mean_precision}, 95%␣
↪CI: {MLP_precision_conf_interval}')
print(f'Mean Recall for Multi Layer Perceptron: {MLP_mean_recall}, 95% CI:␣
↪{MLP_recall_conf_interval}')

model_results = {
    'RandomForest': {
        'mean_precision': RF_mean_precision,
        'precision_ci': RF_precision_conf_interval,
        'mean_recall': RF_mean_recall,
        'recall_ci': RF_precision_conf_interval
    },
    'SVM': {
        'mean_precision': SVM_mean_precision,
        'precision_ci': SVM_precision_conf_interval,
        'mean_recall': SVM_mean_recall,
        'recall_ci': SVM_precision_conf_interval
    },
    'LogisticRegression': {
        'mean_precision': LR_mean_precision,
        'precision_ci': LR_precision_conf_interval,
        'mean_recall': LR_mean_recall,
        'recall_ci': LR_precision_conf_interval
    },
    'MLP': {
        'mean_precision': MLP_mean_precision,
        'precision_ci': MLP_precision_conf_interval,
        'mean_recall': MLP_mean_recall,
        'recall_ci': MLP_precision_conf_interval
    }
}

models = list(model_results.keys())
mean_precisions = [model_results[model]['mean_precision'] for model in models]
precision_cis = [model_results[model]['precision_ci'] for model in models]
mean_recalls = [model_results[model]['mean_recall'] for model in models]
recall_cis = [model_results[model]['recall_ci'] for model in models]

```



```

precision_errors = np.array([[mean - ci[0], ci[1] - mean] for mean, ci in
zip(mean_precisions, precision_cis)]).T
recall_errors = np.array([[mean - ci[0], ci[1] - mean] for mean, ci in
zip(mean_recalls, recall_cis)]).T

fig, ax = plt.subplots(1, 2, figsize=(14, 6))

# Plot precision
ax[0].bar(models, mean_precisions, yerr=precision_errors, capsize=5,
color='skyblue')
ax[0].set_title('Mean Precision with 95% Confidence Interval')
ax[0].set_ylabel('Precision')
ax[0].set_ylim([0.4, 1])
ax[0].set_xticklabels(models, rotation=45, ha="right")

# Plot recall
ax[1].bar(models, mean_recalls, yerr=recall_errors, capsize=5,
color='lightgreen')
ax[1].set_title('Mean Recall with 95% Confidence Interval')
ax[1].set_ylabel('Recall')
ax[1].set_ylim([0.4, 1])
ax[1].set_xticklabels(models, rotation=45, ha="right")

plt.tight_layout()
plt.show()

```

## 1- Using only DEGs

[59]: Evaluation(LR\_precisions,LR\_recalls,SVM\_precisions,SVM\_recalls,RF\_precisions,RF\_recalls,MLP\_pr

Mean Precision for Logistic Regression: 0.7640169927513462, 95% CI: [0.64210043  
0.85890152]

Mean Recall for Logistic Regression: 0.7651338428612129, 95% CI: [0.63696044  
0.87401786]

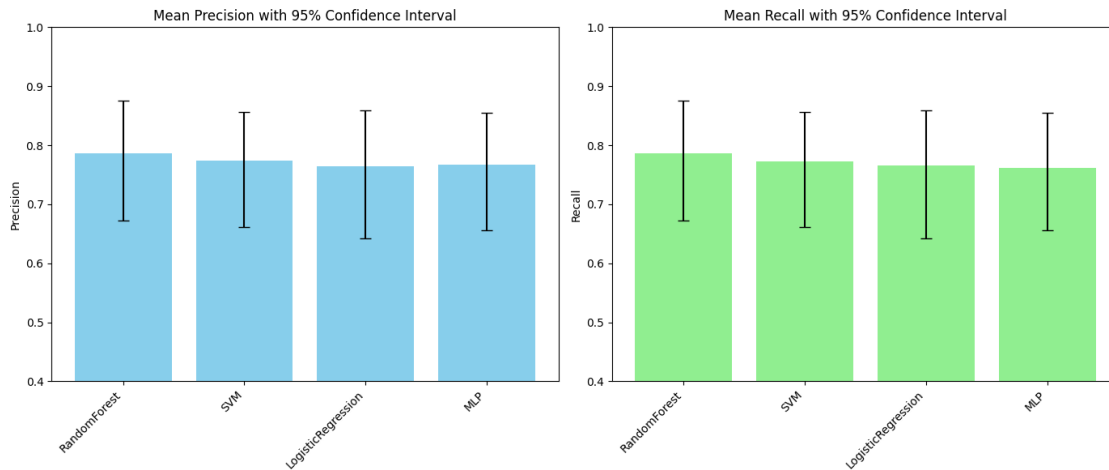
-----  
-----  
Mean Precision for Support Vector Machine: 0.7743040927880918, 95% CI:  
[0.66116453 0.85674319]

Mean Recall for Support Vector Machine: 0.7723320280185707, 95% CI: [0.65753472  
0.86513971]

-----  
-----  
Mean Precision for Random Forest: 0.7860810773240244, 95% CI: [0.6725455  
0.87525208]

Mean Recall for Random Forest: 0.7867412414621653, 95% CI: [0.65607372  
0.88233974]

-----  
Mean Precision for Multi Layer Perceptron: 0.7677514303520464, 95% CI: [0.65656233 0.85438582]  
Mean Recall for Multi Layer Perceptron: 0.7618658834643532, 95% CI: [0.6144042 0.8572071]



## 2- Combining DEGs and clinical data

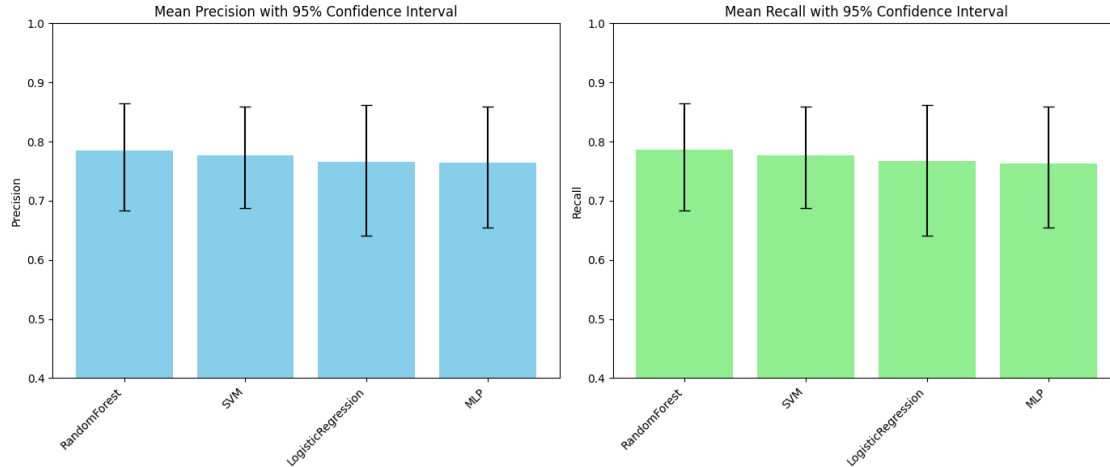
[60]: Evaluation(LR\_precisions2,LR\_recalls2,SVM\_precisions2,SVM\_recalls2,RF\_precisions2,RF\_recalls2,

Mean Precision for Logistic Regression: 0.7657572934644726, 95% CI: [0.64076229 0.86249775]  
Mean Recall for Logistic Regression: 0.766437798402862, 95% CI: [0.63156272 0.87401786]

-----  
Mean Precision for Support Vector Machine: 0.7773571309688684, 95% CI: [0.68729898 0.85893579]  
Mean Recall for Support Vector Machine: 0.7760440256206106, 95% CI: [0.67974551 0.85704685]

-----  
Mean Precision for Random Forest: 0.7853592442842011, 95% CI: [0.6831345 0.86472959]  
Mean Recall for Random Forest: 0.7858822709176627, 95% CI: [0.68036487 0.86493412]

-----  
Mean Precision for Multi Layer Perceptron: 0.7649651583043456, 95% CI: [0.65414083 0.85956706]  
Mean Recall for Multi Layer Perceptron: 0.7626787599565183, 95% CI: [0.65620388 0.86896226]



### 3- using only clinical data

[61]: Evaluation(LR\_precisions3,LR\_recalls3,SVM\_precisions3,SVM\_recalls3,RF\_precisions3,RF\_recalls3,

Mean Precision for Logistic Regression: 0.515218903229282, 95% CI: [0.31170101 0.66907334]

Mean Recall for Logistic Regression: 0.47621360370841015, 95% CI: [0.36085483 0.59306812]

Mean Precision for Support Vector Machine: 0.4216381464824728, 95% CI: [0.25569444 0.65331303]

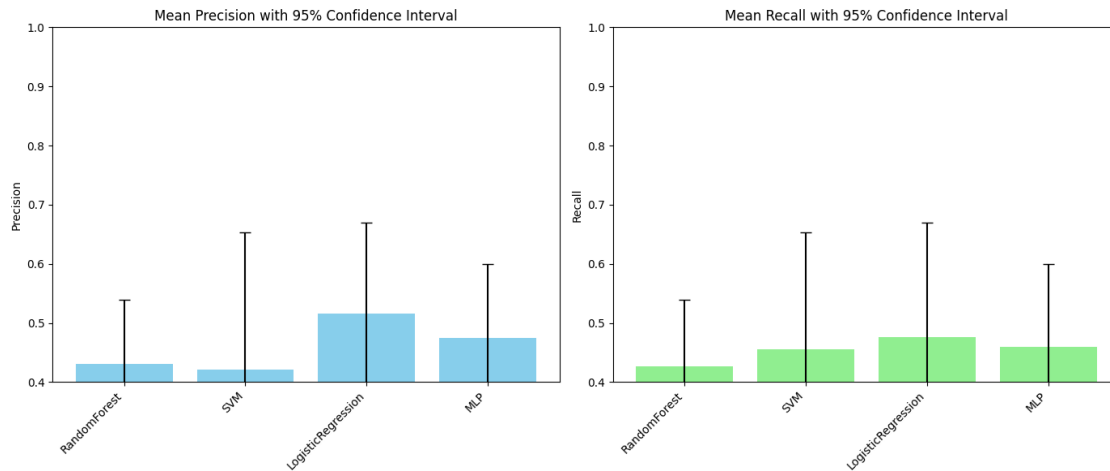
Mean Recall for Support Vector Machine: 0.45489894310748175, 95% CI: [0.36710967 0.55240385]

Mean Precision for Random Forest: 0.4311592063399727, 95% CI: [0.33722599 0.53896528]

Mean Recall for Random Forest: 0.4260025261322037, 95% CI: [0.33441138 0.52781951]

Mean Precision for Multi Layer Perceptron: 0.4748118320079058, 95% CI: [0.33603222 0.59929803]

Mean Recall for Multi Layer Perceptron: 0.4600780548510615, 95% CI: [0.33597553 0.57160134]



As you can see, adding clinical data to DEGs does not have a significant effect on model performance, and the clinical data alone are not strong features for this classification task.