Q4-Python

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1 NAFLD detection using RNA-Seq data

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In this part, we need to perform a regression task to predict the age of samples based on their gene expression profiles and clinical data.

We will then split the dataset based on sex, repeat the regression task, and compare the results between the groups.

2 Q4 - Python section

[24]:
```
# 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
import warnings
warnings.filterwarnings("ignore")
from sklearn.linear_model import LinearRegression, Ridge, ElasticNet
from sklearn.svm import SVR
from sklearn.metrics import mean_squared_error, r2_score
from sklearn.preprocessing import StandardScaler, OneHotEncoder
from sklearn.compose import ColumnTransformer
from sklearn.pipeline import Pipeline
from sklearn.feature_selection import VarianceThreshold
```

[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  ENSG00000000003   5.965571   5.741587   5.996891   5.551919   6.430237
     1  ENSG00000000005   1.612375   2.147793   0.418542   0.702492   1.215978
     2  ENSG00000000419   4.133821   4.120969   4.086129   4.116240   4.393797
     3  ENSG00000000457   4.111056   3.922234   3.964871   3.978350   4.018235
     4  ENSG00000000460   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)
```

[13]:
```
print("Number of Female samples: ", meta_data['SEX'].to_list().count("Female"))
print("Number of Male samples: ", meta_data['SEX'].to_list().count("Male"))
```

```
Number of Female samples:  154
Number of Male samples:  38
```

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.

[14]:
```
# 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)])
```

[15]:
```
clinical_data = preprocessor.fit_transform(meta_data)
```

[16]:
```
clinical_data = pd.DataFrame(clinical_data).iloc[:, [0, 2, 4]]
clinical_data.columns = ['BMI_surg', 'Sex', 'Diabet']
```

[17]:
```
clinical_data
```

[17]:
```
     BMI_surg  Sex  Diabet
0   -1.308155  1.0     0.0
1   -0.857325  1.0     1.0
2    0.143131  0.0     0.0
3   -0.600056  1.0     0.0
4    0.660065  1.0     0.0
..        ...  ...     ...
187 -0.772196  0.0     1.0
188  0.284524  1.0     0.0
189  6.161411  0.0     1.0
190  0.007788  1.0     0.0
191 -0.214761  1.0     0.0
```

```
[192 rows x 3 columns]
```

[18]:
```
gene_names = normal_counts.iloc[:,0]
gene_names.at[17396] = 'BMI_surg'
gene_names.at[17397] = 'SEX'
gene_names.at[17398] = 'Diabet'
```

[19]:
```
gene_names
```

[19]:
```
0        ENSG00000000003
1        ENSG00000000005
2        ENSG00000000419
3        ENSG00000000457
4        ENSG00000000460
              …
17394    ENSG00000273274
17395    ENSG00000273294
17396           BMI_surg
17397                SEX
17398             Diabet
Name: gene, Length: 17399, dtype: object
```

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

[20]:
```
combined_data = np.hstack((normal_counts.iloc[0:,1:].T, clinical_data))
df_deg = pd.DataFrame(combined_data)
df_deg.columns = gene_names
```

[21]:
```
df_deg
```

[21]:
```
gene  ENSG00000000003  ENSG00000000005  ENSG00000000419  ENSG00000000457  \
0            5.965571         1.612375         4.133821         4.111056
1            5.741587         2.147793         4.120969         3.922234
2            5.996891         0.418542         4.086129         3.964871
3            5.551919         0.702492         4.116240         3.978350
4            6.430237         1.215978         4.393797         4.018235
..                …                …                …                …
187          6.480745        -1.083676         4.361634         4.313540
188          6.360397         0.886550         4.093280         4.205119
189          6.367705        -0.902201         4.148010         4.506058
190          6.604050        -0.865036         4.351489         4.072137
191          6.514539        -1.588749         3.859711         4.341988

gene  ENSG00000000460  ENSG00000000938  ENSG00000000971  ENSG00000001036  \
0            4.150662         2.975845        11.005488         4.405768
1            3.732756         3.199989        10.860700         3.895350
2            3.634637         2.949733        10.934025         4.282577
3            3.853979         2.991061        10.760445         4.297722
```

```
4           3.614220         2.836130        11.491427         4.405558
..               …                …               …                 …
187         3.120196         1.941859        11.451981         4.556052
188         3.336802         2.911496        11.658711         4.315153
189         3.982071         2.202136        11.864397         4.348255
190         3.143138         4.037476        11.782524         4.358527
191         2.741172         2.803568        11.474777         3.895962

gene  ENSG00000001084  ENSG00000001167  …  ENSG00000272658  ENSG00000272869  \
0            6.825329         4.221450  …         1.365460         0.691230
1            6.453687         4.218183  …         0.935490         1.004202
2            6.437658         3.736947  …         0.675699         0.943633
3            6.710840         4.003661  …         0.702492         0.431190
4            7.437655         4.377965  …         0.898496         0.085581
..               …                …   …            …                 …
187          7.183779         5.066071  …         0.632531        -0.431600
188          7.384726         4.627703  …        -0.147398        -1.080284
189          7.326297         4.528530  …         0.048889        -1.108652
190          8.468526         5.013154  …        -1.154543        -0.624028
191          7.617180         4.778544  …        -0.114818        -0.510747

gene  ENSG00000273079  ENSG00000273173  ENSG00000273259  ENSG00000273274  \
0            5.637483        -0.005377         1.675800         2.683536
1            5.975612         0.532134         1.555218         2.926666
2            5.531648        -0.184123         2.391906         2.260662
3            5.571799        -0.034474         1.639298         2.341393
4            5.636848        -1.216981         1.974160         1.351861
..               …                …               …                 …
187          2.616763         1.063165         1.941859        -2.306069
188          2.975985         0.589568         2.754906        -0.839275
189          3.218814        -0.416774         2.246191        -1.639167
190          2.050571        -1.517113         2.084923        -4.324468
191          2.572243         0.667591         2.160189        -3.173712

gene  ENSG00000273294  BMI_surg  SEX  Diabet
0           -0.339797 -1.308155  1.0     0.0
1            0.435919 -0.857325  1.0     1.0
2           -0.691083  0.143131  0.0     0.0
3            0.096771 -0.600056  1.0     0.0
4           -0.079478  0.660065  1.0     0.0
..               …         …   …      …
187          0.632531 -0.772196  0.0     1.0
188         -0.016153  0.284524  1.0     0.0
189          0.145105  6.161411  0.0     1.0
190          0.067849  0.007788  1.0     0.0
191          1.218606 -0.214761  1.0     0.0
```

```
[192 rows x 17399 columns]
```

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

```
[22]: X_train, X_test, y_train, y_test = train_test_split(df_deg, meta_data['Age'],␣
       ↪test_size=0.2, random_state = 10101)
```

First, we applied a variance threshold to filter out features that do not have sufficient variation.

```
[25]: threshold = 0.8
      selector = VarianceThreshold(threshold)
      filtered_data = selector.fit_transform(X_train)
      selected_features = X_train.columns[selector.get_support()]
      X_train_filtered = pd.DataFrame(filtered_data, columns=selected_features)
```

```
[26]: X_train_filtered.shape
```

```
[26]: (153, 5229)
```

Next, we performed LASSO to extract the most relevant features for this task.

```
[21]: lasso = LassoCV(cv=5).fit(X_train_filtered, y_train)
      # Extract the non-zero coefficients
      important_genes = X_train_filtered.columns[(lasso.coef_ != 0)]
```

```
[22]: important_genes
```

```
[22]: Index(['ENSG00000001630', 'ENSG00000007001', 'ENSG00000011083',
             'ENSG00000024526', 'ENSG00000064205', 'ENSG00000071909',
             'ENSG00000086548', 'ENSG00000091129', 'ENSG00000103154',
             'ENSG00000104369', 'ENSG00000109182', 'ENSG00000115009',
             'ENSG00000115457', 'ENSG00000129824', 'ENSG00000131080',
             'ENSG00000134184', 'ENSG00000141668', 'ENSG00000144229',
             'ENSG00000151025', 'ENSG00000152726', 'ENSG00000152779',
             'ENSG00000162391', 'ENSG00000164266', 'ENSG00000165246',
             'ENSG00000166923', 'ENSG00000170160', 'ENSG00000171496',
             'ENSG00000171747', 'ENSG00000181333', 'ENSG00000182771',
             'ENSG00000182870', 'ENSG00000184674', 'ENSG00000184698',
             'ENSG00000189266', 'ENSG00000221890', 'ENSG00000236882',
             'ENSG00000244694', 'ENSG00000255798', 'ENSG00000256618'],
            dtype='object', name='gene')
```

```
[23]: len(important_genes)
```

```
[23]: 39
```

```
[24]: X_train[important_genes]
```

```
[24]: gene   ENSG00000001630  ENSG00000007001  ENSG00000011083  ENSG00000024526  \
      9            -0.579836         5.686951         2.307689         0.467470
      16            0.467219         3.764056         3.043866         2.238594
      149          -1.395729         6.789909        -5.096169        -1.008706
      63           -0.910103         2.403314         2.927247         1.445378
      41            0.522448         6.611324        -4.969405        -4.969405
      ..                 …                …                …                …
      186          -0.105322         7.730441        -5.149716        -5.149716
      185          -0.823052         7.831961        -3.486017        -1.370540
      178          -0.983901         7.563422        -2.568864        -2.568864
      181          -0.255283         4.830607        -5.010171        -5.010171
      0            -0.956468         3.061454         2.537521         1.019285

      gene   ENSG00000064205  ENSG00000071909  ENSG00000086548  ENSG00000091129  \
      9             1.067862         1.780060         0.036835         3.584736
      16            2.238594         3.534050         2.575022         3.603591
      149          -2.288814        -0.703852        -0.238188         0.685191
      63            1.770618         2.803334         1.991119         2.777221
      41           -1.268966        -4.969405        -1.509974         0.456859
      ..                 …                …                …                …
      186          -3.564753        -0.901788        -5.149716        -1.242825
      185          -5.070979        -3.486017        -2.749051         0.601446
      178          -1.831898        -4.153826        -4.153826        -0.246936
      181          -2.688243        -2.688243        -2.688243        -1.550739
      0             1.823142         2.924544         2.234674         2.937541

      gene   ENSG00000103154  ENSG00000104369  …  ENSG00000182771  ENSG00000182870  \
      9             3.075516        -1.027295  …         2.052432         2.254740
      16            3.027090         1.223948  …         2.957966         2.575022
      149           2.985980        -1.636737  …         2.355042        -5.096169
      63            3.635429         0.933171  …         2.533816         2.549329
      41            3.235166        -4.969405  …         1.096684        -3.384443
      ..                 …                …  …              …                …
      186           1.536785        -2.827788  …        -2.827788        -3.564753
      185           1.774511        -1.901054  …         2.047962        -5.070979
      178           1.868542        -1.831898  …         0.800370        -1.831898
      181           2.407682        -2.202816  …        -1.550739        -3.425208
      0             2.911429         0.691230  …         2.975845         2.055505

      gene   ENSG00000184674  ENSG00000184698  ENSG00000189266  ENSG00000221890  \
      9             4.527294        -0.399264         0.467470         0.722727
      16            3.659086         0.894640        -0.142835         2.267449
      149           3.889673        -2.288814        -2.774241         1.503744
      63            5.584576         0.674859        -4.997566         1.822613
      41           -4.969405        -2.647477         1.659951        -2.647477
      ..                 …                …                …                …
      186           4.975697        -5.149716         0.827564         0.059737
```

```
185       -5.070979      -1.611548       2.005836      -0.316092
178        3.782812      -2.568864       0.800370      -0.246936
181        3.644465      -5.010171       1.619186      -1.840246
0          5.048033       0.751351       1.644436       1.706497

gene  ENSG00000236882  ENSG00000244694  ENSG00000255798  ENSG00000256618
9            6.884506         0.798676         2.432137         6.505504
16           5.158060         2.209151        -0.690322         7.458252
149          4.096124        -1.395729        -5.096169         4.302575
63           5.936862         1.411825         1.541593         6.323799
41           8.626668        -2.647477        -2.162050         6.275553
..                …                …                …                …
186          7.257286        -1.242825        -1.062253         9.595696
185          4.631193        -2.263624         2.148189         8.637566
178          7.818077        -1.831898        -2.568864         7.130998
181          4.988419         0.481682        -1.840246         9.887060
0            3.960009         1.245166         2.007006         6.124616

[153 rows x 39 columns]
```

[25]:
```
X_test[important_genes].shape
```

[25]:
```
(39, 39)
```

It's time for regression. I have used three types of regressors in this part to ensure consistency. These regressors are well-established in machine learning and are well-suited for this task. The regressors used are: Linear Regression, Ridge Regression, and ElasticNet Regression.

[52]:
```
models = {
    "Linear Regression": LinearRegression(),
    "Ridge Regression": Ridge(alpha=0.5),
    "ElasticNet Regression": ElasticNet(alpha=1.0, l1_ratio=0.5),
}

results = {}

for name, model in models.items():
    model.fit(X_train_filtered[important_genes], y_train)
    y_pred = model.predict(X_test[important_genes])

    mse = mean_squared_error(y_test, y_pred)
    r2 = r2_score(y_test, y_pred)
    results[name] = {
        "MSE": mse,
        "R²": r2
    }

for model_name, metrics in results.items():
    print(f"{model_name}:\n\tMSE: {metrics['MSE']}\n\tR²: {metrics['R²']}\n")
```

```
Linear Regression:
        MSE: 109.75763313259237
        R²: 0.14127465204431466

Ridge Regression:
        MSE: 109.66346309043713
        R²: 0.14201142268986122

ElasticNet Regression:
        MSE: 98.05173665922788
        R²: 0.23285962645861957
```

Let's split the dataset based on sex, repeat the regression task, and compare the results between the groups.

2.0.1 Female

```
[27]: female_df_deg = df_deg[df_deg['SEX'] == 1]
```

```
[28]: female_df_deg.shape
```

```
[28]: (154, 17399)
```

```
[29]: female_meta_data = meta_data[meta_data['SEX'] == 'Female']
```

```
[30]: female_meta_data.shape
```

```
[30]: (154, 7)
```

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

```
[31]: X_train, X_test, y_train, y_test = train_test_split(female_df_deg,␣
       ↪female_meta_data, test_size=0.2, random_state = 10101)
```

First, we applied a variance threshold to filter out features that do not have sufficient variation.

```
[32]: threshold = 0.8
      selector = VarianceThreshold(threshold)
      filtered_data = selector.fit_transform(X_train)
      selected_features = X_train.columns[selector.get_support()]
      X_train_filtered = pd.DataFrame(filtered_data, columns=selected_features)
```

```
[33]: X_train_filtered.shape
```

```
[33]: (123, 5223)
```

Next, we performed LASSO to extract the most relevant features for this task.

```
[33]: lasso = LassoCV(cv=5).fit(X_train_filtered, y_train['Age'])
      # Extract the non-zero coefficients
      female_important_genes = X_train_filtered.columns[(lasso.coef_ != 0)]
```

```
[34]: female_important_genes
```

```
[34]: Index(['ENSG00000007001', 'ENSG00000070193', 'ENSG00000086548',
              'ENSG00000100867', 'ENSG00000104044', 'ENSG00000104435',
              'ENSG00000112852', 'ENSG00000128313', 'ENSG00000129824',
              'ENSG00000131080', 'ENSG00000132872', 'ENSG00000134121',
              'ENSG00000134640', 'ENSG00000134668', 'ENSG00000139515',
              'ENSG00000151962', 'ENSG00000161973', 'ENSG00000162391',
              'ENSG00000162426', 'ENSG00000164266', 'ENSG00000165246',
              'ENSG00000166206', 'ENSG00000169876', 'ENSG00000171747',
              'ENSG00000174226', 'ENSG00000179674', 'ENSG00000180440',
              'ENSG00000182256', 'ENSG00000182771', 'ENSG00000196844',
              'ENSG00000198885', 'ENSG00000205358', 'ENSG00000206077',
              'ENSG00000213088', 'ENSG00000221890', 'ENSG00000223609',
              'ENSG00000236882', 'ENSG00000237693', 'ENSG00000239839',
              'ENSG00000250361', 'ENSG00000251209', 'ENSG00000255798',
              'ENSG00000256618', 'ENSG00000258484'],
             dtype='object', name='gene')
```

```
[35]: len(female_important_genes)
```

```
[35]: 44
```

```
[34]: female_df_deg[female_important_genes]
```

```
[34]: gene  ENSG00000007001  ENSG00000070193  ENSG00000086548  ENSG00000100867  \
      0            3.061454         0.346095         2.234674         2.830128
      1            8.213882         0.863340         2.961642         3.886277
      3            9.549683         0.096771         2.112367         2.341393
      4            2.541464         0.085581         1.142914         1.746493
      5            4.478528         0.350495         1.645951         2.773706
      ..                …                …                …                …
      185          7.831961        -0.678662        -2.749051        -3.486017
      186          7.730441        -2.342361        -5.149716         2.584994
      188          7.990447        -0.632825        -4.539715         1.241645
      190          4.256733        -2.002540        -1.517113         4.977028
      191          4.089949        -3.173712        -1.951319        -1.299243

      gene  ENSG00000104044  ENSG00000104435  ENSG00000112852  ENSG00000128313  \
      0            1.956820         1.440422         0.809067         0.265925
      1            2.372354         1.460581         0.863340         1.132527
      3            1.503946         1.354568         1.127797         0.617603
      4            0.490838         0.430717         0.161530         0.005411
```

```
5          1.414625         0.920810         0.435384         -0.445972
..              …                …                …                …
185       -1.901054        -3.486017        -2.263624        -2.263624
186       -2.827788        -1.979791        -1.062253        -5.149716
188       -4.539715        -2.217787        -2.954753        -0.839275
190       -2.002540        -2.739505        -1.517113        -0.624028
191       -4.758674        -0.851784        -1.588749         0.667591

gene  ENSG00000129824  ENSG00000131080  …  ENSG00000221890  ENSG00000223609  \
0           -3.278396         2.758693  …         1.706497         0.181036
1           -2.837100         2.890821  …         1.952977         0.787391
3           -4.426791         2.314676  …         1.913059        -0.726352
4           -5.123872         2.555608  …         0.233680        -0.265891
5           -4.693899         3.132649  …         1.681140        -0.606437
..              …                …   …            …                …
185         -5.070979        -5.070979  …        -0.316092        -5.070979
186         -3.564753        -0.901788  …         0.059737        -3.564753
188          2.201752         0.952138  …        -1.732360        -4.539715
190         -4.324468        -0.865036  …         0.067849        -2.002540
191         -3.173712         1.391073  …        -0.671211        -3.173712

gene  ENSG00000236882  ENSG00000237693  ENSG00000239839  ENSG00000250361  \
0            3.960009         1.245166         3.000828         1.019285
1            7.135880         2.020881        -0.334599         0.935490
3            5.746886         1.127797        -0.339329        -0.339329
4            5.103744         0.302393        -0.265891        -0.600310
5            6.590925        -0.050043        -2.371971         0.060988
..              …                …                …                …
185          4.631193        -5.070979        -0.427123        -2.749051
186          7.257286        -3.564753        -1.979791        -2.827788
188          6.416661        -0.632825         1.903228        -2.954753
190          6.016495        -4.324468         1.942319        -2.739505
191          6.967970        -4.758674        -0.235112        -4.758674

gene  ENSG00000251209  ENSG00000255798  ENSG00000256618  ENSG00000258484
0            0.751351         2.007006         6.124616        -0.219502
1            1.460581         1.600305         6.772079        -0.721623
3            0.431190        -0.967360         6.704422        -0.339329
4            0.657488        -0.875945         5.328369         0.430717
5            1.188744         2.486010         6.479153        -1.886544
..              …                …                …                …
185         -0.427123         2.148189         8.637566        -1.901054
186         -2.827788        -1.062253         9.595696        -1.979791
188          0.504679        -0.839275         9.543349        -0.839275
190         -0.865036        -0.417577         9.142755        -2.739505
191         -1.588749        -4.758674        10.316011        -1.058235
```

```
[154 rows x 44 columns]
```

It's time for regression. I have used three types of regressors in this part to ensure consistency. These regressors are well-established in machine learning and are well-suited for this task. The regressors used are: Linear Regression, Ridge Regression, and ElasticNet Regression.

[56]:
```
models = {
    "Linear Regression": LinearRegression(),
    "Ridge Regression": Ridge(alpha=1.0),
    "ElasticNet Regression": ElasticNet(alpha=1.0, l1_ratio=0.5),
}

results = {}

for name, model in models.items():
    model.fit(X_train_filtered[female_important_genes], y_train['Age'])
    y_pred = model.predict(X_test[female_important_genes])

    mse = mean_squared_error(y_test['Age'], y_pred)
    r2 = r2_score(y_test['Age'], y_pred)
    results[name] = {
        "MSE": mse,
        "R²": r2
    }

for model_name, metrics in results.items():
    print(f"{model_name}:\n\tMSE: {metrics['MSE']}\n\tR²: {metrics['R²']}\n")
```

```
Linear Regression:
        MSE: 88.31876196989404
        R²: 0.3969937886987882

Ridge Regression:
        MSE: 88.56495689237829
        R²: 0.39531286536904964

ElasticNet Regression:
        MSE: 97.7445429895872
        R²: 0.3326382160609207
```

2.0.2 Male

[34]:
```
male_df_deg = df_deg[df_deg['SEX'] == 0]
```

[35]:
```
male_df_deg.shape
```

[35]:
```
(38, 17399)
```

```
[36]: male_meta_data = meta_data[meta_data['SEX'] == 'Male']
```

```
[37]: male_meta_data.shape
```

```
[37]: (38, 7)
```

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

```
[38]: X_train, X_test, y_train, y_test = train_test_split(male_df_deg,␣
       ↪male_meta_data, test_size=0.2, random_state = 10101)
```

First, we applied a variance threshold to filter out features that do not have sufficient variation.

```
[39]: threshold = 0.8
      selector = VarianceThreshold(threshold)
      filtered_data = selector.fit_transform(X_train)
      selected_features = X_train.columns[selector.get_support()]
      X_train_filtered = pd.DataFrame(filtered_data, columns=selected_features)
```

```
[40]: X_train_filtered.shape
```

```
[40]: (30, 5016)
```

Next, we performed LASSO to extract the most relevant features for this task.

```
[45]: lasso = LassoCV(cv=5).fit(X_train_filtered, y_train['Age'])
      # Extract the non-zero coefficients
      male_important_genes = X_train_filtered.columns[(lasso.coef_ != 0)]
```

```
[42]: male_important_genes
```

```
[42]: Index(['ENSG00000091138', 'ENSG00000095596', 'ENSG00000106236',
             'ENSG00000122121', 'ENSG00000131080', 'ENSG00000134184',
             'ENSG00000140379', 'ENSG00000141668', 'ENSG00000147257',
             'ENSG00000152726', 'ENSG00000168398', 'ENSG00000184674',
             'ENSG00000198074', 'ENSG00000215700', 'ENSG00000235098',
             'ENSG00000235961', 'ENSG00000244694'],
            dtype='object', name='gene')
```

```
[43]: len(male_important_genes)
```

```
[43]: 17
```

```
[44]: X_test[male_important_genes].shape
```

```
[44]: (8, 17)
```

It's time for regression. I have used three types of regressors in this part to ensure consistency. These regressors are well-established in machine learning and are well-suited for this task. The regressors used are: Linear Regression, Ridge Regression, and ElasticNet Regression.

[48]:
```python
models = {
    "Linear Regression": LinearRegression(),
    "Ridge Regression": Ridge(alpha=1.0),
    "ElasticNet Regression": ElasticNet(alpha=1.0, l1_ratio=0.5),
}

results = {}

for name, model in models.items():
    model.fit(X_train_filtered[male_important_genes], y_train['Age'])
    y_pred = model.predict(X_test[male_important_genes])

    mse = mean_squared_error(y_test['Age'], y_pred)
    r2 = r2_score(y_test['Age'], y_pred)
    results[name] = {
        "MSE": mse,
        "R²": r2
    }

for model_name, metrics in results.items():
    print(f"{model_name}:\n\tMSE: {metrics['MSE']}\n\tR²: {metrics['R²']}\n")
```

```
Linear Regression:
        MSE: 120.18807385165366
        R²: -0.6186945973286688

Ridge Regression:
        MSE: 121.76068452045129
        R²: -0.639874538995977

ElasticNet Regression:
        MSE: 132.0734434710684
        R²: -0.7787669154352648
```

So far, we have obtained only a single result for each regressor, but these results are not statistically valid. To test for reproducibility, we have put the entire process into a loop to run it 10 times.

[61]:
```python
# 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, 2, 4]]
clinical_data.columns = ['BMI_surg', 'Sex', 'Diabet']
gene_names = normal_counts.iloc[:,0]
gene_names.at[17396] = 'BMI_surg'
gene_names.at[17397] = 'SEX'
gene_names.at[17398] = 'Diabet'

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

LR_R2s = []
LR_MSEs = []
f_LR_R2s = []
f_LR_MSEs = []
m_LR_R2s = []
m_LR_MSEs = []

RR_R2s = []
RR_MSEs = []
f_RR_R2s = []
f_RR_MSEs = []
m_RR_R2s = []
m_RR_MSEs = []

ENR_R2s = []
ENR_MSEs = []
f_ENR_R2s = []
f_ENR_MSEs = []
m_ENR_R2s = []
m_ENR_MSEs = []

for i in range(10):
    print('iteration',i)
    X_train, X_test, y_train, y_test = train_test_split(df_deg,␣
 ↪meta_data['Age'], test_size=0.2, random_state = i)
    threshold = 0.8
    selector = VarianceThreshold(threshold)
    filtered_data = selector.fit_transform(X_train)
    selected_features = X_train.columns[selector.get_support()]
    X_train_filtered = pd.DataFrame(filtered_data, columns=selected_features)
    lasso = LassoCV(cv=5).fit(X_train_filtered, y_train)
    # Extract the non-zero coefficients
    important_genes = X_train_filtered.columns[(lasso.coef_ != 0)]
```

```
# Linear Regression
LR_model = LinearRegression()
LR_model.fit(X_train_filtered[important_genes], y_train)
y_pred_LR = LR_model.predict(X_test[important_genes])
LR_MSE = mean_squared_error(y_test, y_pred_LR)
LR_R2 = r2_score(y_test, y_pred_LR)
LR_MSEs.append(LR_MSE)
LR_R2s.append(LR_R2)

# Ridge Regression
RR_model = Ridge(alpha=0.5)
RR_model.fit(X_train_filtered[important_genes], y_train)
y_pred_RR = RR_model.predict(X_test[important_genes])
RR_MSE = mean_squared_error(y_test, y_pred_RR)
RR_R2 = r2_score(y_test, y_pred_RR)
RR_MSEs.append(RR_MSE)
RR_R2s.append(RR_R2)

# ElasticNet Regression
ENR_model = ElasticNet(alpha=1.0, l1_ratio=0.5)
ENR_model.fit(X_train_filtered[important_genes], y_train)
y_pred_ENR = ENR_model.predict(X_test[important_genes])
ENR_MSE = mean_squared_error(y_test, y_pred_ENR)
ENR_R2 = r2_score(y_test, y_pred_ENR)
ENR_MSEs.append(ENR_MSE)
ENR_R2s.append(ENR_R2)


female_df_deg = df_deg[df_deg['SEX'] == 1]
female_meta_data = meta_data[meta_data['SEX'] == 'Female']
X_train, X_test, y_train, y_test = train_test_split(female_df_deg,␣
↪female_meta_data, test_size=0.2, random_state = i)
threshold = 0.8
selector = VarianceThreshold(threshold)
filtered_data = selector.fit_transform(X_train)
selected_features = X_train.columns[selector.get_support()]
X_train_filtered = pd.DataFrame(filtered_data, columns=selected_features)
lasso = LassoCV(cv=5).fit(X_train_filtered, y_train['Age'])
# Extract the non-zero coefficients
female_important_genes = X_train_filtered.columns[(lasso.coef_ != 0)]

# Linear Regression
LR_model = LinearRegression()
LR_model.fit(X_train_filtered[female_important_genes], y_train['Age'])
y_pred_LR = LR_model.predict(X_test[female_important_genes])
LR_MSE = mean_squared_error(y_test['Age'], y_pred_LR)
```

```
    LR_R2 = r2_score(y_test['Age'], y_pred_LR)
    f_LR_MSEs.append(LR_MSE)
    f_LR_R2s.append(LR_R2)

    # Ridge Regression
    RR_model = Ridge(alpha=0.5)
    RR_model.fit(X_train_filtered[female_important_genes], y_train['Age'])
    y_pred_RR = RR_model.predict(X_test[female_important_genes])
    RR_MSE = mean_squared_error(y_test['Age'], y_pred_RR)
    RR_R2 = r2_score(y_test['Age'], y_pred_RR)
    f_RR_MSEs.append(RR_MSE)
    f_RR_R2s.append(RR_R2)

    # ElasticNet Regression
    ENR_model = ElasticNet(alpha=1.0, l1_ratio=0.5)
    ENR_model.fit(X_train_filtered[female_important_genes], y_train['Age'])
    y_pred_ENR = ENR_model.predict(X_test[female_important_genes])
    ENR_MSE = mean_squared_error(y_test['Age'], y_pred_ENR)
    ENR_R2 = r2_score(y_test['Age'], y_pred_ENR)
    f_ENR_MSEs.append(ENR_MSE)
    f_ENR_R2s.append(ENR_R2)


    male_df_deg = df_deg[df_deg['SEX'] == 0]
    male_meta_data = meta_data[meta_data['SEX'] == 'Male']
    X_train, X_test, y_train, y_test = train_test_split(male_df_deg,␣
↪male_meta_data, test_size=0.2, random_state = i)
    threshold = 0.8
    selector = VarianceThreshold(threshold)
    filtered_data = selector.fit_transform(X_train)
    selected_features = X_train.columns[selector.get_support()]
    X_train_filtered = pd.DataFrame(filtered_data, columns=selected_features)
    lasso = LassoCV(cv=5).fit(X_train_filtered, y_train['Age'])
    # Extract the non-zero coefficients
    male_important_genes = X_train_filtered.columns[(lasso.coef_ != 0)]

    # Linear Regression
    LR_model = LinearRegression()
    LR_model.fit(X_train_filtered[male_important_genes], y_train['Age'])
    y_pred_LR = LR_model.predict(X_test[male_important_genes])
    LR_MSE = mean_squared_error(y_test['Age'], y_pred_LR)
    LR_R2 = r2_score(y_test['Age'], y_pred_LR)
    m_LR_MSEs.append(LR_MSE)
    m_LR_R2s.append(LR_R2)

    # Ridge Regression
    RR_model = Ridge(alpha=0.5)
    RR_model.fit(X_train_filtered[male_important_genes], y_train['Age'])
    y_pred_RR = RR_model.predict(X_test[male_important_genes])
    RR_MSE = mean_squared_error(y_test['Age'], y_pred_RR)
    RR_R2 = r2_score(y_test['Age'], y_pred_RR)
    m_RR_MSEs.append(RR_MSE)
    m_RR_R2s.append(RR_R2)

    # ElasticNet Regression
    ENR_model = ElasticNet(alpha=1.0, l1_ratio=0.5)
    ENR_model.fit(X_train_filtered[male_important_genes], y_train['Age'])
    y_pred_ENR = ENR_model.predict(X_test[male_important_genes])
    ENR_MSE = mean_squared_error(y_test['Age'], y_pred_ENR)
    ENR_R2 = r2_score(y_test['Age'], y_pred_ENR)
    m_ENR_MSEs.append(ENR_MSE)
    m_ENR_R2s.append(ENR_R2)
```

```
iteration 0
iteration 1
iteration 2
iteration 3
iteration 4
iteration 5
iteration 6
iteration 7
iteration 8
iteration 9
```

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 splitting the data based on sex will affect the performance or not

[70]:
```
def evaluation(LR_MSEs,LR_R2s,RR_MSEs,RR_R2s,ENR_MSEs,ENR_R2s):

  LR_mean_MSE = np.mean(LR_MSEs)
  LR_mean_R2 = np.mean(LR_R2s)
  LR_MSE_conf_interval = np.percentile(LR_MSEs, [2.5, 97.5])
  LR_R2_conf_interval = np.percentile(LR_R2s, [2.5, 97.5])
  print(f'Mean MSE for Linear Regression: {LR_mean_MSE}, 95% CI:␣
 ↪{LR_MSE_conf_interval}')
  print(f'Mean R2 for Linear Regression: {LR_mean_R2}, 95% CI:␣
 ↪{LR_R2_conf_interval}')
 ␣
 ↪print("------------------------------------------------------------------------------------------

  RR_mean_MSE = np.mean(RR_MSEs)
  RR_mean_R2 = np.mean(RR_R2s)
  RR_MSE_conf_interval = np.percentile(RR_MSEs, [2.5, 97.5])
```

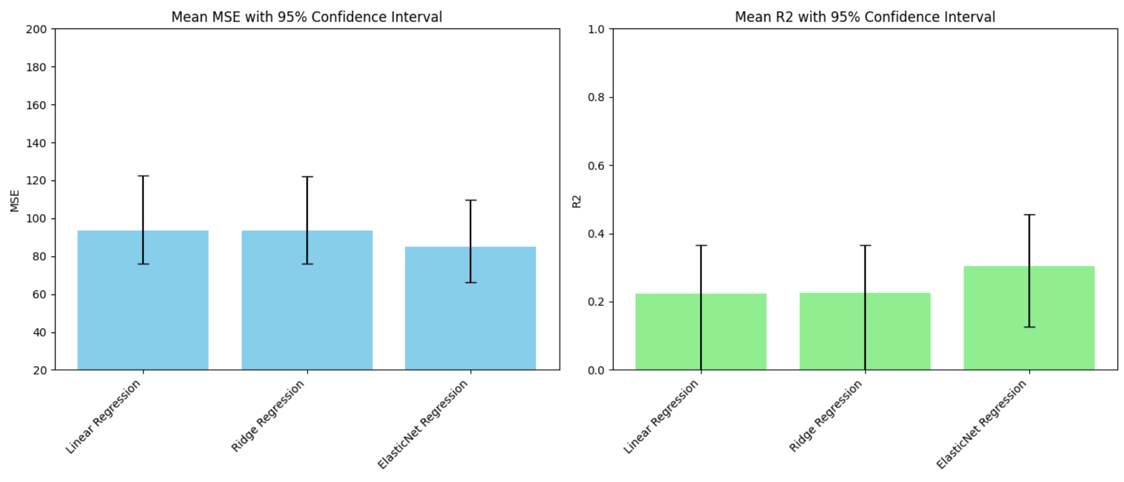
```
RR_R2_conf_interval = np.percentile(RR_R2s, [2.5, 97.5])
print(f'Mean MSE for Ridge Regression: {RR_mean_MSE}, 95% CI:␣
↪{RR_MSE_conf_interval}')
print(f'Mean R2 for Ridge Regression: {RR_mean_R2}, 95% CI:␣
↪{RR_R2_conf_interval}')
␣
↪print("-----------------------------------------------------------------------------------

ENR_mean_MSE = np.mean(ENR_MSEs)
ENR_mean_R2 = np.mean(ENR_R2s)
ENR_MSE_conf_interval = np.percentile(ENR_MSEs, [2.5, 97.5])
ENR_R2_conf_interval = np.percentile(ENR_R2s, [2.5, 97.5])
print(f'Mean MSE for ElasticNet Regression: {ENR_mean_MSE}, 95% CI:␣
↪{ENR_MSE_conf_interval}')
print(f'Mean R2 for ElasticNet Regression: {ENR_mean_R2}, 95% CI:␣
↪{ENR_R2_conf_interval}')

model_results = {
    'Linear Regression': {
        'mean_MSE': LR_mean_MSE,
        'MSE_ci': LR_MSE_conf_interval,
        'mean_R2': LR_mean_R2,
        'R2_ci': LR_R2_conf_interval
    },
    'Ridge Regression': {
        'mean_MSE': RR_mean_MSE,
        'MSE_ci': RR_MSE_conf_interval,
        'mean_R2': RR_mean_R2,
        'R2_ci': RR_R2_conf_interval
    },
    'ElasticNet Regression': {
        'mean_MSE': ENR_mean_MSE,
        'MSE_ci': ENR_MSE_conf_interval,
        'mean_R2': ENR_mean_R2,
        'R2_ci': ENR_R2_conf_interval
    }
}

models = list(model_results.keys())
mean_MSEs = [model_results[model]['mean_MSE'] for model in models]
MSE_cis = [model_results[model]['MSE_ci'] for model in models]
mean_R2s = [model_results[model]['mean_R2'] for model in models]
R2_cis = [model_results[model]['R2_ci'] for model in models]
MSE_errors = np.array([[mean - ci[0], ci[1] - mean] for mean, ci in␣
↪zip(mean_MSEs, MSE_cis)]).T
```

```
    R2_errors = np.array([[mean - ci[0], ci[1] - mean] for mean, ci in␣
    ↪zip(mean_R2s, R2_cis)]).T


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

    # Plot MSE
    ax[0].bar(models, mean_MSEs, yerr=MSE_errors, capsize=5, color='skyblue')
    ax[0].set_title('Mean MSE with 95% Confidence Interval')
    ax[0].set_ylabel('MSE')
    ax[0].set_ylim([20, 200])
    ax[0].set_xticklabels(models, rotation=45, ha="right")

    # Plot R2
    ax[1].bar(models, mean_R2s, yerr=R2_errors, capsize=5, color='lightgreen')
    ax[1].set_title('Mean R2 with 95% Confidence Interval')
    ax[1].set_ylabel('R2')
    ax[1].set_ylim([0, 1])
    ax[1].set_xticklabels(models, rotation=45, ha="right")

    plt.tight_layout()
    plt.show()
```

1- Men and women

```
[71]: evaluation(LR_MSEs,LR_R2s,RR_MSEs,RR_R2s,ENR_MSEs,ENR_R2s)
```

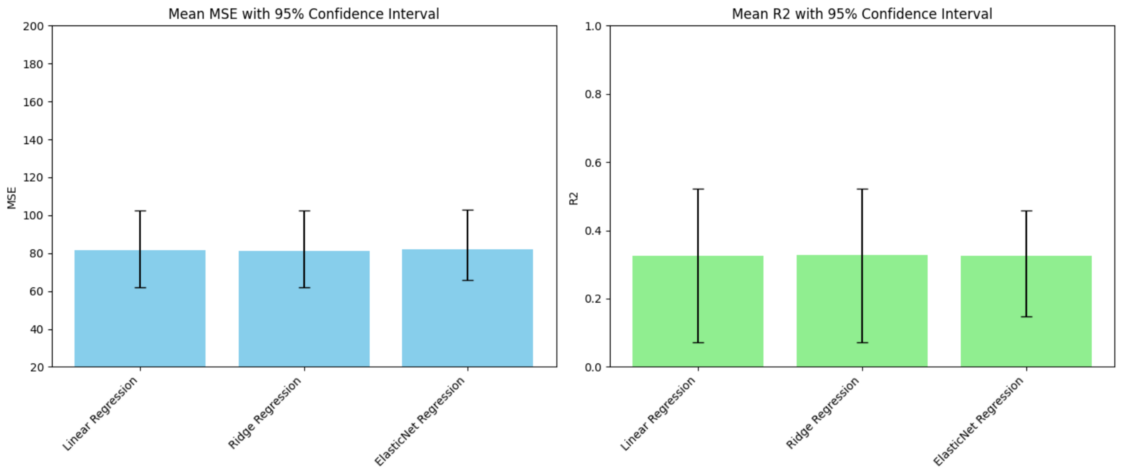
```
Mean MSE for Linear Regression: 93.72387171724446, 95% CI: [ 76.13892279
122.3389011 ]
Mean R2 for Linear Regression: 0.22398519282954946, 95% CI: [-0.15693711
0.3652658 ]
--------------------------------------------------------------------------------
-----------------
Mean MSE for Ridge Regression: 93.58325500454752, 95% CI: [ 75.99978915
122.15512382]
Mean R2 for Ridge Regression: 0.2252074305987804, 95% CI: [-0.15420639
0.36603938]
--------------------------------------------------------------------------------
-----------------
Mean MSE for ElasticNet Regression: 85.03072068605735, 95% CI: [ 66.24003299
109.73508623]
Mean R2 for ElasticNet Regression: 0.30500370984307895, 95% CI: [0.12720519
0.4558891 ]
```



2- Women only

[72]:
```
evaluation(f_LR_MSEs,f_LR_R2s,f_RR_MSEs,f_RR_R2s,f_ENR_MSEs,f_ENR_R2s)
```

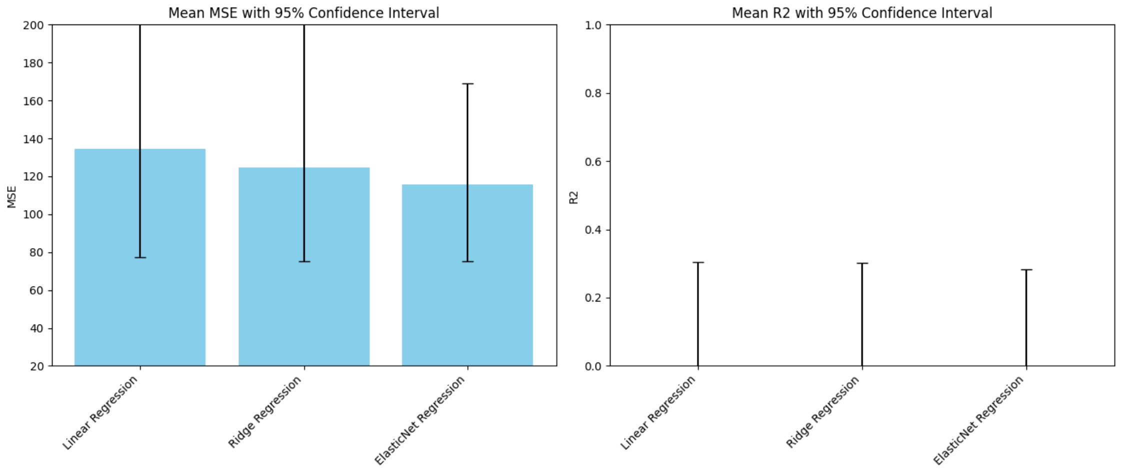
```
Mean MSE for Linear Regression: 81.4039767743786, 95% CI: [ 61.99474362
102.30060272]
Mean R2 for Linear Regression: 0.32655859857434866, 95% CI: [0.07109799
0.52107335]
--------------------------------------------------------------------------------
-----------------
Mean MSE for Ridge Regression: 81.26961196554569, 95% CI: [ 61.87980603
102.27423771]
Mean R2 for Ridge Regression: 0.3277495253969612, 95% CI: [0.07221842
0.52102313]
--------------------------------------------------------------------------------
-----------------
Mean MSE for ElasticNet Regression: 82.14879724899781, 95% CI: [ 65.95899767
102.71718425]
Mean R2 for ElasticNet Regression: 0.3258626266544995, 95% CI: [0.14689447
0.45795909]
```



3- Men only

[73]:
```
evaluation(m_LR_MSEs,m_LR_R2s,m_RR_MSEs,m_RR_R2s,m_ENR_MSEs,m_ENR_R2s)
```

```
Mean MSE for Linear Regression: 134.5769243894009, 95% CI: [ 77.24690153
277.44700226]
Mean R2 for Linear Regression: -0.4490993665624063, 95% CI: [-1.73943844
0.3035828 ]
--------------------------------------------------------------------------------
-----------------
Mean MSE for Ridge Regression: 124.60972408899434, 95% CI: [ 75.26276249
244.97374347]
Mean R2 for Ridge Regression: -0.33927324390218344, 95% CI: [-1.46493756
0.30066489]
--------------------------------------------------------------------------------
-----------------
Mean MSE for ElasticNet Regression: 115.57231466443727, 95% CI: [ 75.20562135
168.87937051]
Mean R2 for ElasticNet Regression: -0.25273830383631973, 95% CI: [-1.23332149
0.28383753]
```



As you can see, we were unable to achieve a satisfactory R^2 for any of the conditions. While it is biologically and computationally feasible to estimate human age based on gene expression data, our results suggest that the limited sample size was a significant factor in our lack of success. With fewer than 200 samples, there was insufficient data to effectively perform this regression task. Additionally, although we hypothesized that splitting the data based on sex might improve age prediction, this approach further reduced the number of samples (to fewer than 40 for men), making it impossible for us to accomplish our desired results.