Analysis-main

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1. What is the reason for log transforming protein levels in `biomarker-raw.csv`?

```
set.seed(1234)
library(tidyverse)
library(here)
rawdata <- read.csv(here("data", "biomarker-raw.csv"))

# random sample of 4 proteins to look at distributions of their levels
rand_indices <- sample(3:ncol(rawdata), 4)

prot1d <- as.numeric(rawdata[2:nrow(rawdata), rand_indices[1]])
prot1 <- prot1d[!is.na(prot1d)]

prot2d <- as.numeric(rawdata[2:nrow(rawdata), rand_indices[2]])
prot2 <- prot2d[!is.na(prot2d)]

prot3d <- as.numeric(rawdata[2:nrow(rawdata), rand_indices[3]])
prot3 <- prot3d[!is.na(prot3d)]

prot4d <- as.numeric(rawdata[2:nrow(rawdata), rand_indices[4]])
prot4 <- prot4d[!is.na(prot4d)]

summary(prot1)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##    2568    4649    5169    5243    5668    7435
```

```
summary(prot2)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##    3317    8790   12179   17164   18800  122168
```

```
summary(prot3)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   283.1   378.7   414.2   457.8   496.9  1894.7
```

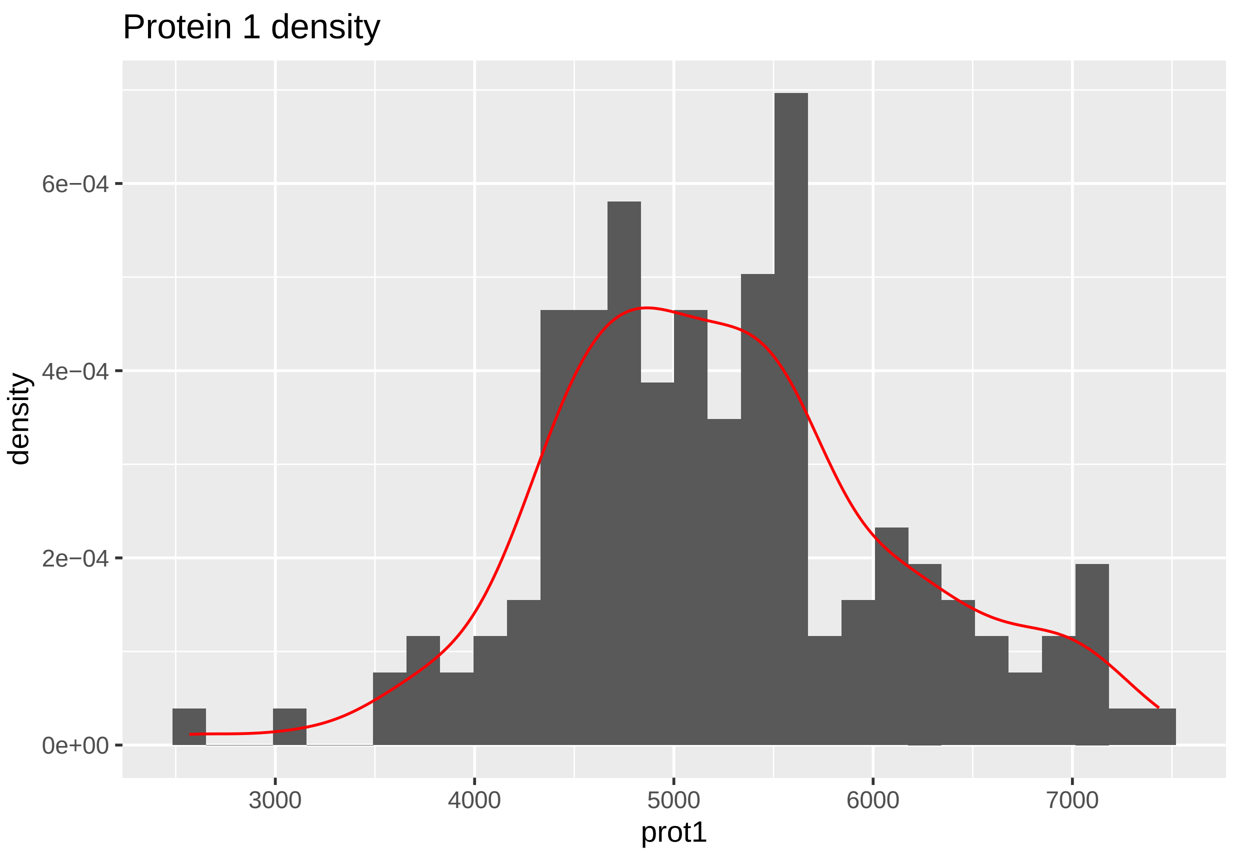
```r
summary(prot4)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   954.9  5434.7  6539.2  6551.7  7719.1 10917.8
```

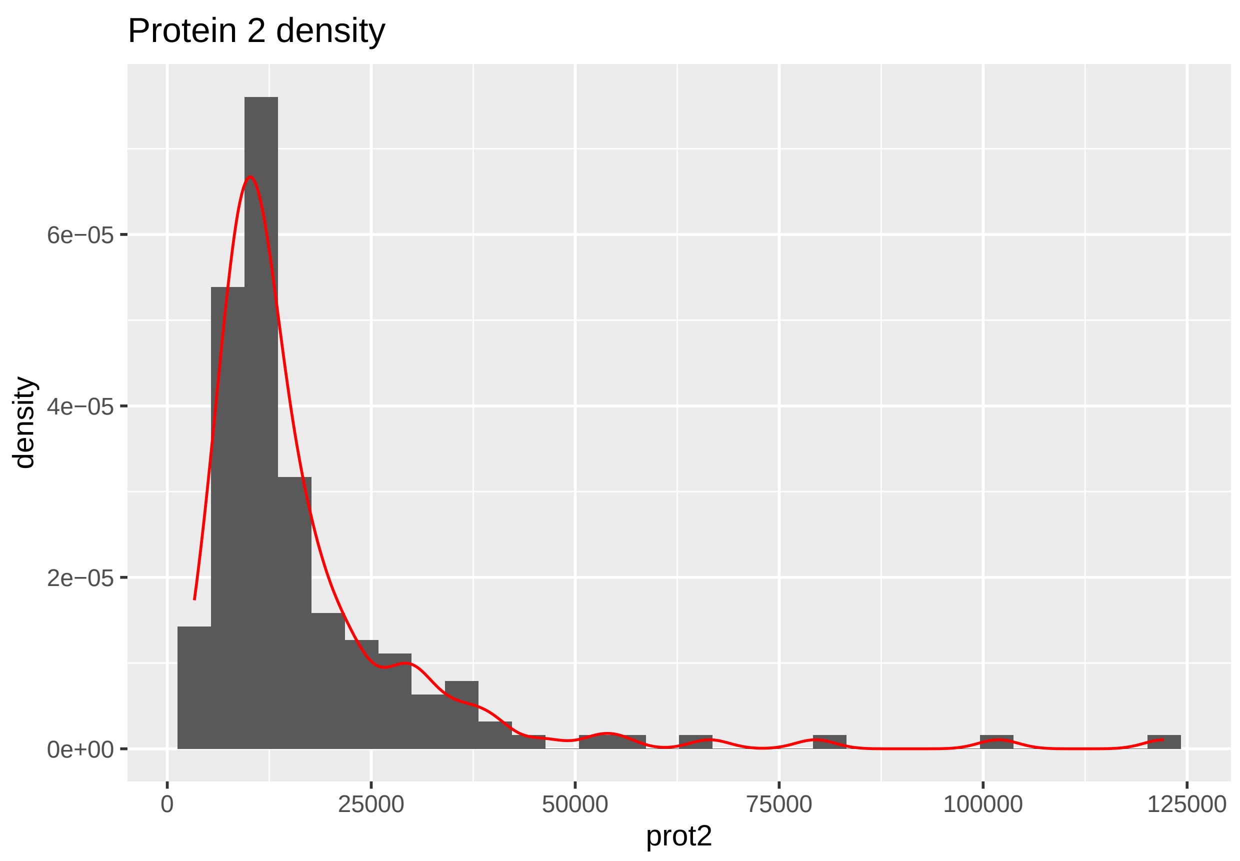
Looking at the summary statistics for 4 randomly selected proteins, the mean values differ significantly, with `prot1`'s mean being 17,164 and `prot3`'s mean being ≈ 458. `prot2`'s mean is roughly 5000 units greater than its median as well, indicating a right skew.

The log transformation of the protein levels helps compress the scale of protein levels since we have a wide range of positive values, where some are very large. Additionally, the logarithm helps with skewed data, and we have evidence that some of the data is skewed, since `prot2` has a mean much larger than its median.

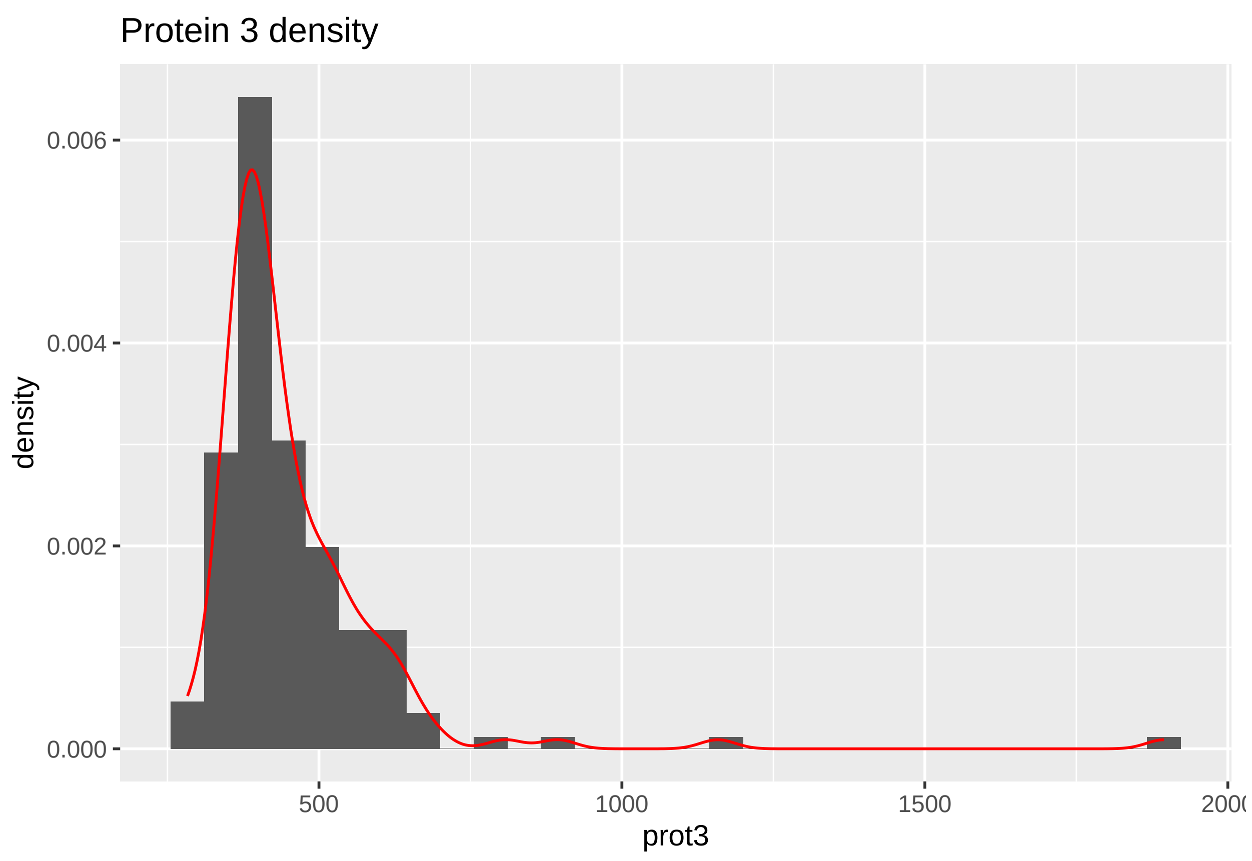
```r
library(ggplot2)
ggplot(as.data.frame(prot1), aes(x = prot1)) +
  geom_histogram(aes(y=after_stat(density)), bins = 30) +
  geom_density(color="red") +
  ggtitle('Protein 1 density')
```



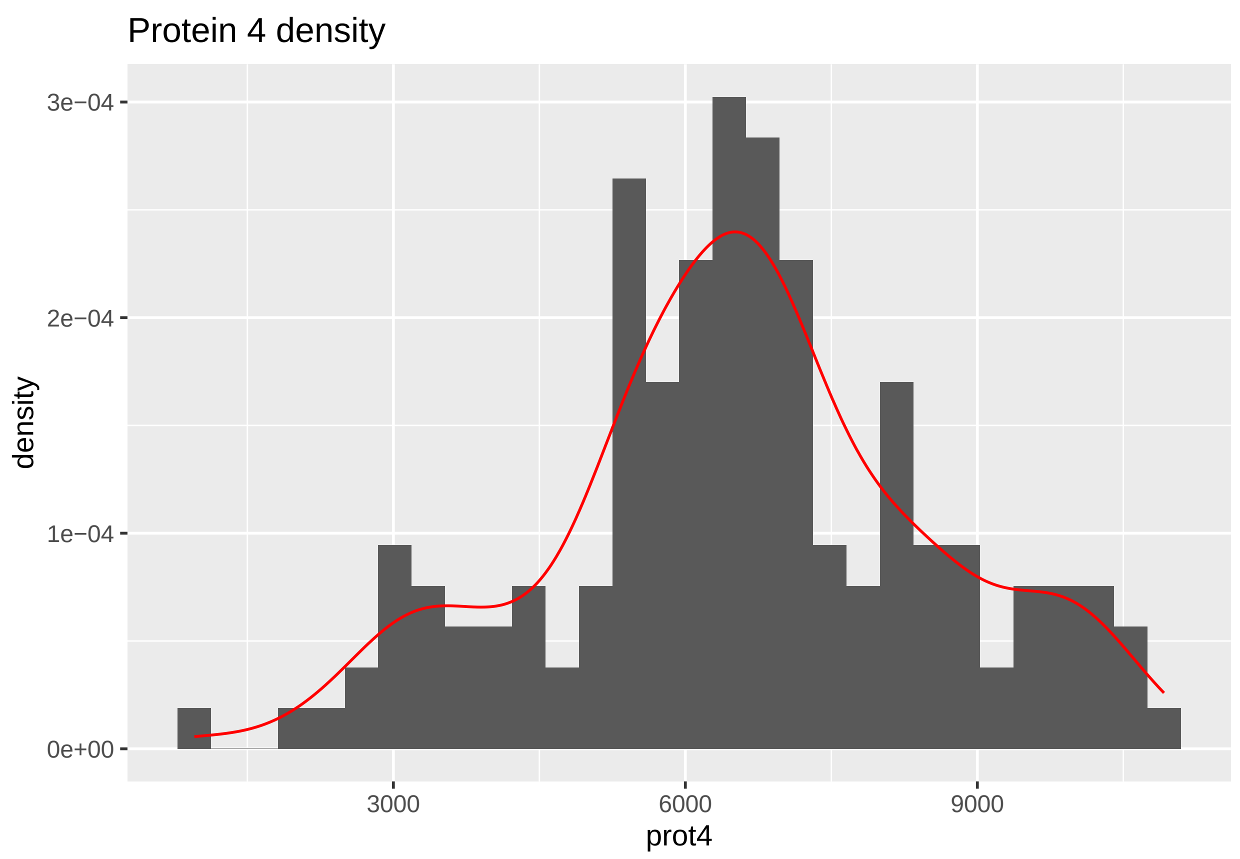
```r
ggplot(as.data.frame(prot2), aes(x = prot2)) +
  geom_histogram(aes(y=after_stat(density)), bins = 30) +
  geom_density(color="red") +
  ggtitle('Protein 2 density')
```



```r
ggplot(as.data.frame(prot3), aes(x = prot3)) +
  geom_histogram(aes(y=after_stat(density)), bins = 30) +
  geom_density(color="red") +
  ggtitle('Protein 3 density')
```



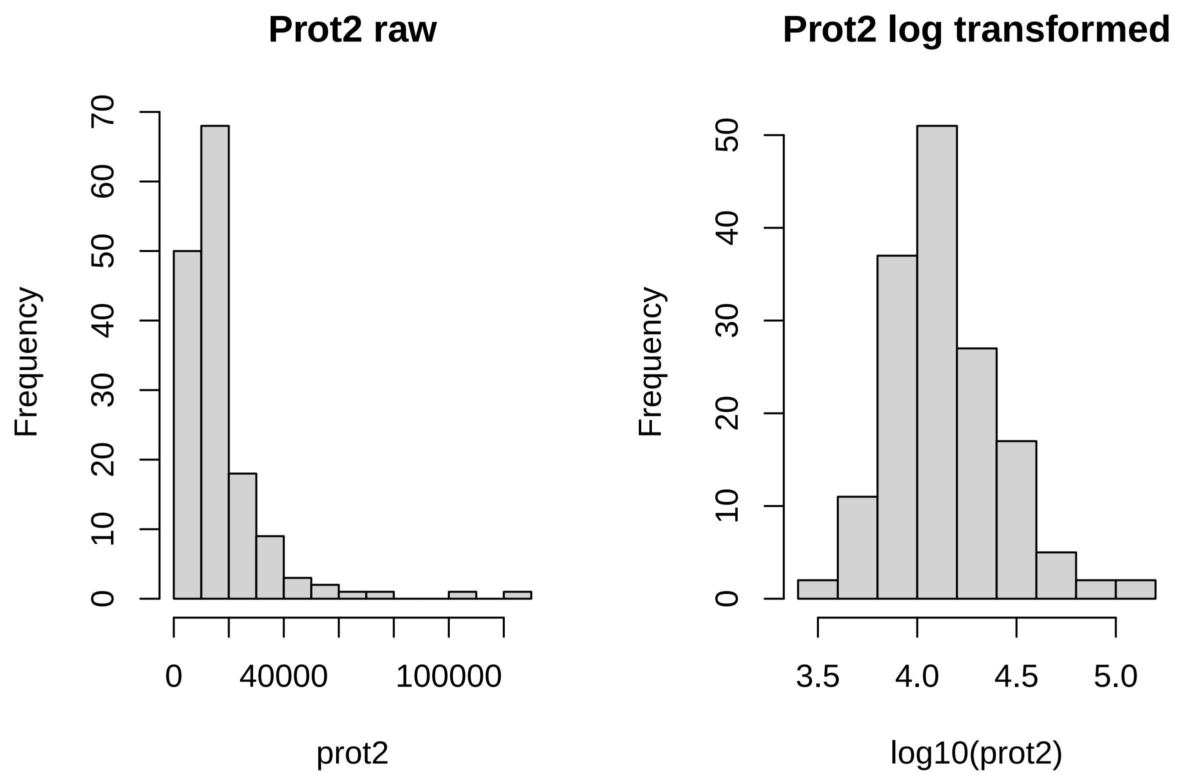
```r
ggplot(as.data.frame(prot4), aes(x = prot4)) +
  geom_histogram(aes(y=after_stat(density)), bins = 30) +
  geom_density(color="red") +
  ggtitle('Protein 4 density')
```



From the density plots, `prot2` looks the most skewed (strongly right-skewed) `prot3` looks slightly right-skewed as well. Both proteins contain large outliers, however the scale of `prot2`'s protein level is much higher, so the same follows for its outliers.

Comparison of `prot2` histogram to its log transformed counterpart:

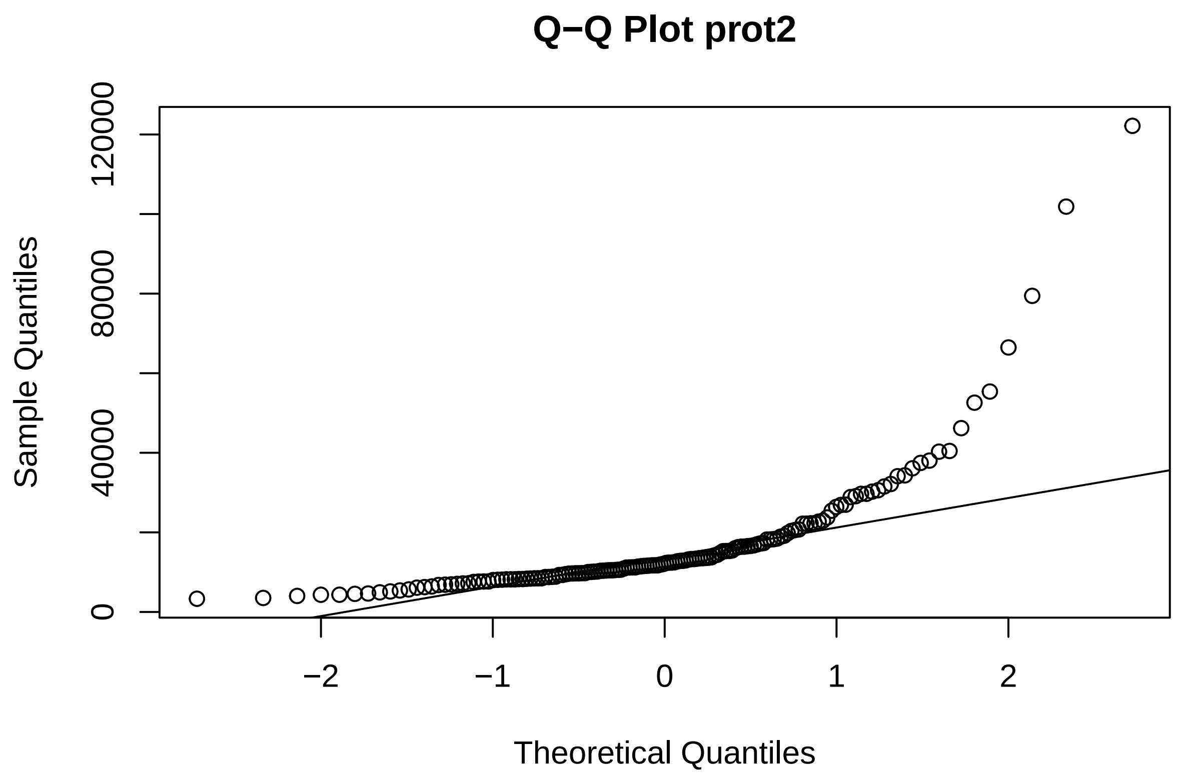
```
par(mfrow=c(1,2))
hist(prot2, main="Prot2 raw")
hist(log10(prot2), main="Prot2 log transformed")
```



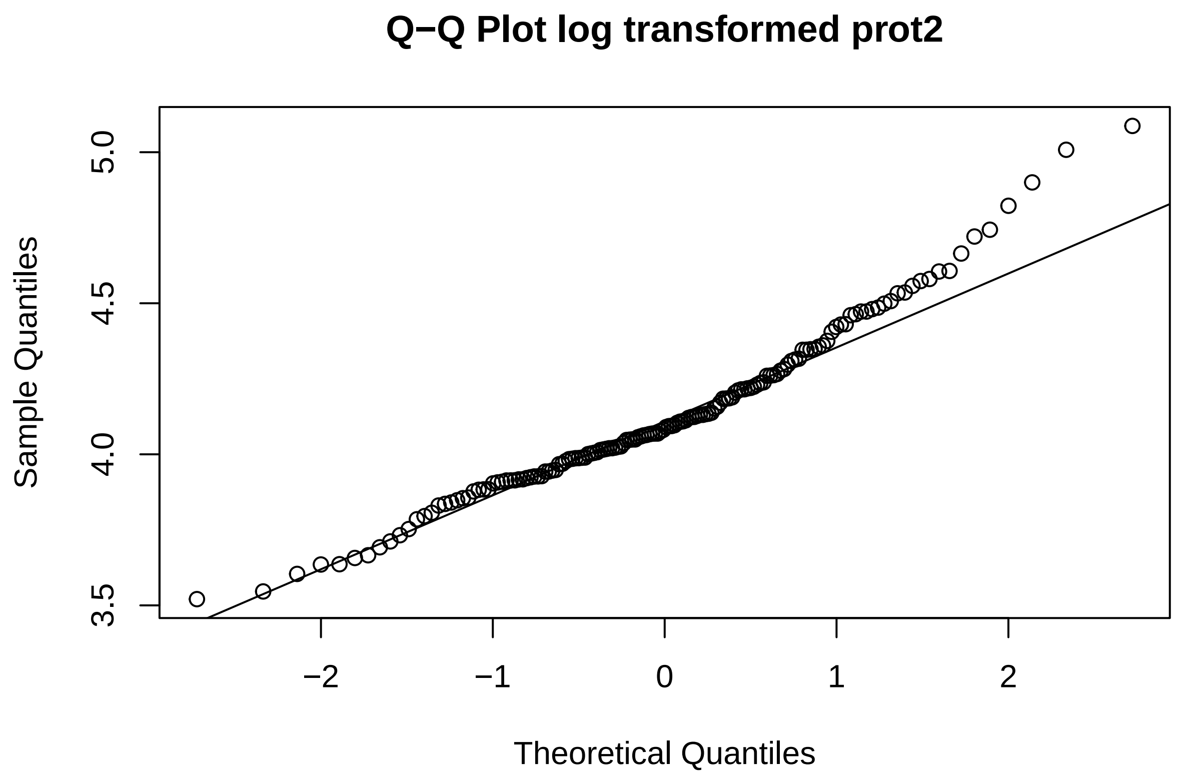
As we can see, after log transforming, the right-skewed `prot2` now appears more symmetric and of a much smaller and more readable scale.

We can check more rigorously to see how normality differs between the raw and log transformed `prot2` with a QQ-Plot.

```r
# normality check for raw data
qqnorm(prot2, main = 'Q-Q Plot prot2')
qqline(prot2)
```



```r
# normality check for log transformed data
qqnorm(log10(prot2), main = 'Q-Q Plot log transformed prot2')
qqline(log10(prot2))
```



After transforming `prot2`, the protein values appear slightly more normal, reducing the influential outliers. Overall, log transforming the protein levels acted as a way to reduce the large scale of the values, and make the data more symmetric.

2. Temporarily remove the outlier trimming from preprocessing and do some exploratory analysis of the outlying values. Are there specific subjects (not values) that seem to be outliers? If so, are outliers more frequent in one group or the other?

——————————-ANNA ADD SECTION——————————-

3. Repeat the analysis but carry out the entire selection procedure on a training partition – in other words, set aside some testing data at the very beginning and don't use it until you are evaluating accuracy at the very end

Choose a larger number (more than ten) of top predictive proteins using each selection method

Use a fuzzy intersection instead of a hard intersection to combine the sets of top predictive proteins across selection methods

——————————-Nathan/Minu SECTION——————————-

4. Find an alternative panel that achieves improved classification accuracy

Benchmark your results against the in-class analysis.

Goal: Explore alternative feature selection

```r
library(tidyverse)
library(infer)
library(randomForest)
library(tidymodels)
library(modelr)
library(yardstick)
library(glmnet)
library(here)

load(here("data", "biomarker-clean.RData")) # loads data as biomarker_clean
```

```r
## MULTIPLE TESTING
####################
# function to compute tests
test_fn <- function(.df){
  t_test(.df,
         formula = level ~ group,
         order = c('ASD', 'TD'),
         alternative = 'two-sided',
         var.equal = F)
}

ttests_out <- biomarker_clean %>%
  # drop ADOS score
  select(-ados) %>%
  # arrange in long format
  pivot_longer(-group,
               names_to = 'protein',
               values_to = 'level') %>%
  # nest by protein
  nest(data = c(level, group)) %>%
  # compute t tests
  mutate(ttest = map(data, test_fn)) %>%
  unnest(ttest) %>%
  # sort by p-value
  arrange(p_value) %>%
  # multiple testing correction
  mutate(m = n(),
         hm = log(m) + 1/(2*m) - digamma(1),
         rank = row_number(),
         p.adj = m*hm*p_value/rank)

# select significant proteins
proteins_s1 <- ttests_out %>%
  slice_min(p.adj, n = 10) %>%
  pull(protein)

## RANDOM FOREST
```

```r
##################
# store predictors and response separately
predictors <- biomarker_clean %>%
  select(-c(group, ados))

response <- biomarker_clean %>% pull(group) %>% factor()

# fit RF
set.seed(101422)
rf_out <- randomForest(x = predictors,
                       y = response,
                       ntree = 1000,
                       importance = T)

# compute importance scores
proteins_s2 <- rf_out$importance %>%
  as_tibble() %>%
  mutate(protein = rownames(rf_out$importance)) %>%
  slice_max(MeanDecreaseGini, n = 10) %>%
  pull(protein)

## LASSO with guided penalty
###########################################

# Get the intersection of important proteins
proteins_sstar <- intersect(proteins_s1, proteins_s2)
cat("Selected proteins from intersection:", proteins_sstar, "\n")
```

```
## Selected proteins from intersection: DERM RELT MRC2 IgD Cadherin-5
```

```r
cat("Number of selected proteins:", length(proteins_sstar), "\n")
```

```
## Number of selected proteins: 5
```

```r
# LASSO with all proteins, choosing different penalties for sstar vs x_train

# full dataset (all proteins except ADOS)
biomarker_full <- biomarker_clean %>%
  select(-ados) %>%
  mutate(class = (group == 'ASD')) %>%
  select(-group)

# training and test set
set.seed(101422)
biomarker_split <- biomarker_full %>%
  initial_split(prop = 0.8)

# matrices for glmnet
x_train <- training(biomarker_split) %>%
  select(-class) %>%
  as.matrix()
```

```r
y_train <- training(biomarker_split) %>%
  pull(class) %>%
  as.numeric()

x_test <- testing(biomarker_split) %>%
  select(-class) %>%
  as.matrix()

y_test <- testing(biomarker_split) %>%
  pull(class) %>%
  as.numeric()

# penalty factors: lower penalty for pre-selected proteins
penalty_factors <- rep(1, ncol(x_train))  # Start with penalty = 1 for all
names(penalty_factors) <- colnames(x_train)

# no penalty for sstar proteins (ones selected by t-test and RF)
penalty_factors[proteins_sstar] <- 0

cat("\nPenalty factors:\n")
```

```
##
## Penalty factors:
```

```r
print(table(penalty_factors))
```

```
## penalty_factors
##    0    1
##    5 1312
```

```r
# fit LASSO with guided penalties
set.seed(101422)
lasso_guided <- cv.glmnet(
  x_train, y_train,
  alpha = 1,
  family = "binomial",
  penalty.factor = penalty_factors,
  type.measure = "auc",
  nfolds = 10
)

# get predictions
pred_prob_guided <- predict(lasso_guided, newx = x_test,
                           s = "lambda.min", type = "response")[,1]

# metrics
test_results_guided <- data.frame(
  pred = pred_prob_guided,
  pred_class = factor(pred_prob_guided > 0.5,
                      levels = c(FALSE, TRUE),
                      labels = c("TD", "ASD")),
  class_factor = factor(y_test,
                       levels = c(0, 1),
                       labels = c("TD", "ASD"))
)

# class-based metrics
class_metrics <- metric_set(sensitivity, specificity, accuracy)
class_output_guided <- test_results_guided %>%
  class_metrics(estimate = pred_class,
                truth = class_factor,
                event_level = 'second')

# AUC
prob_output_guided <- test_results_guided %>%
  roc_auc(truth = class_factor,
          pred,
          event_level = 'second')

# Combine all metrics
all_metrics_guided <- bind_rows(class_output_guided, prob_output_guided)
print("\nGuided LASSO Results:")
```

```
## [1] "\nGuided LASSO Results:"
```

```r
print(all_metrics_guided)
```

```
## # A tibble: 4 x 3
##   .metric     .estimator .estimate
##   <chr>       <chr>          <dbl>
## 1 sensitivity binary         0.75
## 2 specificity binary         0.933
## 3 accuracy    binary         0.839
## 4 roc_auc     binary         0.858
```

```r
# which coefficients are non-zero
coef_guided <- coef(lasso_guided, s = "lambda.min")
selected_features <- rownames(coef_guided)[which(coef_guided != 0)]
selected_features <- selected_features[selected_features != "(Intercept)"]

cat("\nSelected features by guided LASSO:\n")
```

```
##
## Selected features by guided LASSO:
```

```r
print(selected_features)
```

```
##  [1] "CD59"                     "4-1BB"
##  [3] "Dtk"                      "Cadherin-5"
##  [5] "HAI-1"                    "Kallikrein 11"
##  [7] "PAI-1"                    "Growth hormone receptor"
##  [9] "IGFBP-4"                  "MRC2"
## [11] "CRDL1"                    "IL-17 RD"
## [13] "TPSG1"                          "MP2K2"
## [15] "ENPP7"                          "MFGM"
## [17] "PCSK7"                          "ITI heavy chain H4"
## [19] "IgD"                            "DBNL"
## [21] "DERM"                           "Elafin"
## [23] "RELT"                           "PPID"
## [25] "Semaphorin 3E"                  "CD27"
## [27] "CNDP1"                          "IL-17 RC"
## [29] "SRCN1"                          "Epo"
## [31] "GDNF"                           "14-3-3 protein zeta/delta"
## [33] "a-Synuclein"                    "CSRP3"
## [35] "MIG"
```

```
cat("\nFeatures from proteins_sstar that were kept:\n")
```

```
##
## Features from proteins_sstar that were kept:
```

```
print(intersect(selected_features, proteins_sstar))
```

```
## [1] "Cadherin-5" "MRC2"       "IgD"         "DERM"        "RELT"
```

We chose to find an alternative panel that achieves improved classification accuracy by utilizing the pure LASSO (Least Absolute Shrinkage and Selection Operator) penalized regression model.

The original analysis used the intersection of three methods (multiple t-test, Random Forest, and LASSO) followed by an unregularized Logistic Regression. For this alternative approach, we used the LASSO method alone to perform both feature selection and classification. The LASSO model was trained on the 80% training partition, and the optimal penalty (λ_{min}) automatically selected the feature panel.

Results:

1. Alternative Panel: The LASSO model selected a panel of 44 proteins listed above under the Alternate Panel.

2. Test Set Performance: The LASSO classifier achieved a Test Set AUC ROC (Area Under the Receiver Operating Characteristic curve) of 0.858, accuracy of 0.767, sensitivity of 0.800, and specificity of 0.733.

Comparison to the benchmark:

$$\text{roc auc} = 0.883 \text{ ; accuracy} = 0.774 \text{ ; sensitivity} = 0.812 \text{ ; specificity} = 0.733$$

Metrics are similar to the baseline. Panel is more compelex though (compared to panel of 5 proteins in the baseline).