

morphine_regression

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```
regression <- read.csv("../data/morphine__sex_diff_transporters.csv", fileEncoding = 'UTF-8-BOM')

regression$conc_wt <- (regression$raw_conc/regression$tissue_weight)

str(regression)
```

```
## 'data.frame': 48 obs. of 11 variables:
## $ animal : chr "1M" "2M" "4M" "5M" ...
## $ raw_conc : num 56.6 22.1 49.5 87.4 36.6 ...
## $ tissue_weight: num 20.1 10.3 15.1 31.4 14.7 14.3 20.8 15.7 43.7 14.6 ...
## $ body_wt : num 27.2 30.1 25.8 26.1 27.3 26.2 23.7 27.2 21.3 22.9 ...
## $ dose : num 20.6 18.6 21.7 20 19.1 19.9 22 19.2 20.4 19 ...
## $ pgp : num 2.43 2.7 2.92 2.77 2.46 ...
## $ bcrp : num 1.037 1.249 1.151 0.986 1.205 ...
## $ stage : chr "male" "male" "male" "male" ...
## $ group : chr "male" "male" "male" "male" ...
## $ tissue : chr "brain" "brain" "brain" "brain" ...
## $ conc_wt : num 2.82 2.14 3.28 2.78 2.49 ...
```

```
regression <- regression %>% mutate(stage = as.factor(stage),
                                     group = as.factor(group),
                                     tissue = factor(as.factor(tissue), c("retina", "brain")))
head(regression)
```

```
## animal raw_conc tissue_weight body_wt dose pgp bcrp stage group
## 1 1M 56.5895 20.1 27.2 20.6 2.429027 1.0366970 male male
## 2 2M 22.0798 10.3 30.1 18.6 2.699340 1.2487060 male male
## 3 4M 49.5114 15.1 25.8 21.7 2.923597 1.1505545 male male
## 4 5M 87.4161 31.4 26.1 20.0 2.770560 0.9860451 male male
## 5 6M 36.5960 14.7 27.3 19.1 2.464103 1.2046594 male male
## 6 7M 41.6730 14.3 26.2 19.9 2.381759 1.1407836 male male
## tissue conc_wt
## 1 brain 2.815398
## 2 brain 2.143670
## 3 brain 3.278901
## 4 brain 2.783952
## 5 brain 2.489524
## 6 brain 2.914196
```

```
pgp_plot <- ggplot(regression, aes(pgp, conc_wt, col=tissue)) +
  geom_point() +
  geom_smooth(method = "lm", se = T) +
  xlim(0,5) +
  theme_bw()

bcrp_plot <- ggplot(regression, aes(bcrp, conc_wt, col=tissue)) +
  geom_point() +
  geom_smooth(method = "lm", se = T) +
  xlim(0,5) +
  ylab("") +
  theme_bw() %+replace%
  theme(axis.text.y=element_blank())

combined_correlation_plots <- ggarrange(pgp_plot, bcrp_plot, ncol=2, common.legend = TRUE, legend="bottom")
```

```
## 'geom_smooth()' using formula 'y ~ x'
## 'geom_smooth()' using formula 'y ~ x'
## 'geom_smooth()' using formula 'y ~ x'
```

```
ggsave("../figures/combined_correlation_plots.png", plot=combined_correlation_plots, width=6, height =4)
```

```
regression_brain <- subset(regression, tissue == "brain")
head(regression_brain)
```

```
##   animal raw_conc tissue_weight body_wt dose      pgp      bcrp stage group
## 1      1M  56.5895         20.1    27.2 20.6 2.429027 1.0366970  male  male
## 2      2M  22.0798         10.3    30.1 18.6 2.699340 1.2487060  male  male
## 3      4M  49.5114         15.1    25.8 21.7 2.923597 1.1505545  male  male
## 4      5M  87.4161         31.4    26.1 20.0 2.770560 0.9860451  male  male
## 5      6M  36.5960         14.7    27.3 19.1 2.464103 1.2046594  male  male
## 6      7M  41.6730         14.3    26.2 19.9 2.381759 1.1407836  male  male
##   tissue conc_wt
## 1  brain 2.815398
## 2  brain 2.143670
## 3  brain 3.278901
## 4  brain 2.783952
## 5  brain 2.489524
## 6  brain 2.914196
```

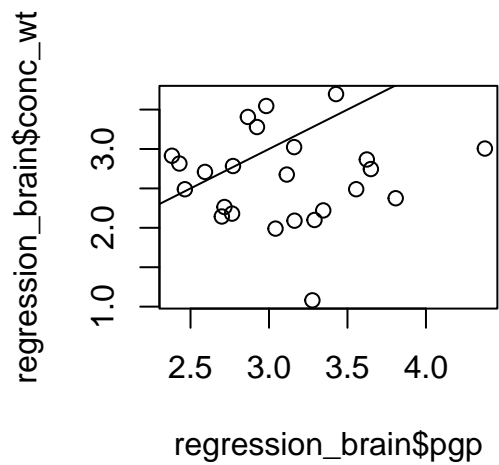
```
shapiro.test(regression_brain$conc_wt)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  regression_brain$conc_wt
## W = 0.9661, p-value = 0.5723
```

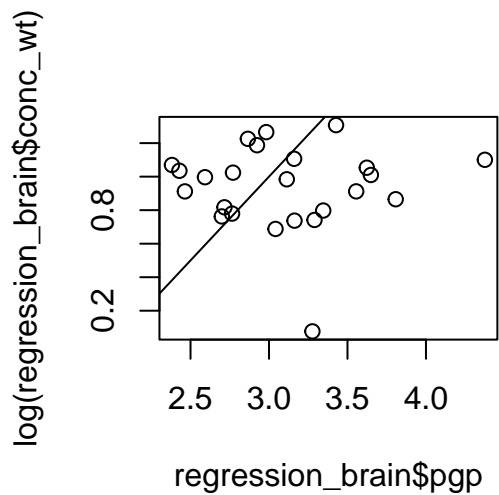
```
shapiro.test(regression_brain$pgp)
```

```
##
## Shapiro-Wilk normality test
##
## data: regression_brain$pgp
## W = 0.96357, p-value = 0.5143
```

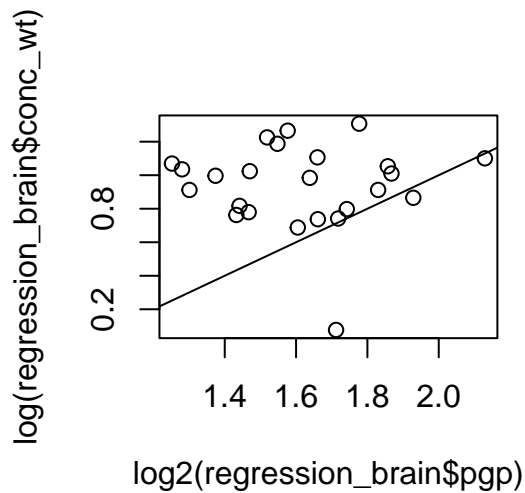
```
plot(regression_brain$pgp, regression_brain$conc_wt)
abline(0,1)
```



```
plot(regression_brain$pgp, log(regression_brain$conc_wt))
abline(-2,1)
```



```
plot(log2(regression_brain$pgp), log(regression_brain$conc_wt))
abline(-1,1)
```



```
cor.test(regression_brain$pgp, regression_brain$conc_wt, method = "pearson")
```

```
##
## Pearson's product-moment correlation
##
## data: regression_brain$pgp and regression_brain$conc_wt
## t = 0.05577, df = 22, p-value = 0.956
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## -0.3933942 0.4133038
## sample estimates:
## cor
## 0.01188931
```

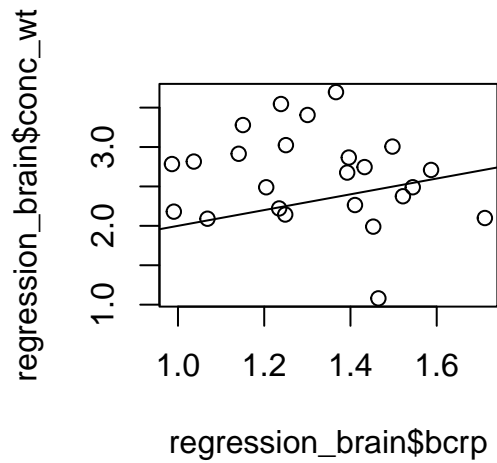
```
cor.test(regression_brain$pgp, regression_brain$conc_wt, method = "spearman")
```

```
##
## Spearman's rank correlation rho
##
## data: regression_brain$pgp and regression_brain$conc_wt
## S = 2306, p-value = 0.9919
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
## rho
## -0.002608696
```

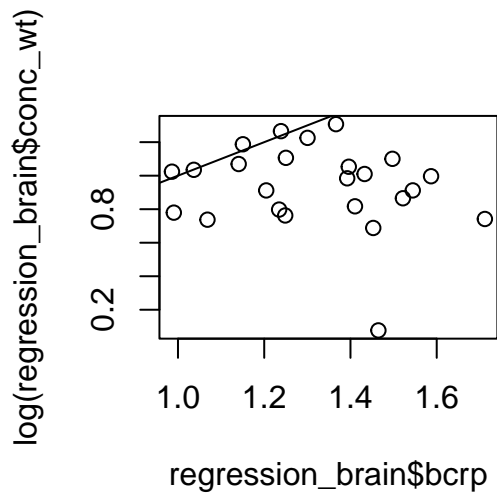
```
shapiro.test(regression_brain$bcrp)
```

```
##
## Shapiro-Wilk normality test
##
## data: regression_brain$bcrp
## W = 0.9744, p-value = 0.7749
```

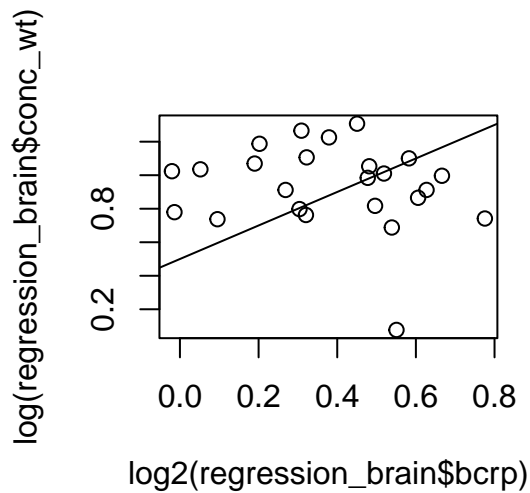
```
plot(regression_brain$bcrp, regression_brain$conc_wt)
abline(1,1)
```



```
plot(regression_brain$bcrp, log(regression_brain$conc_wt))
abline(0,1)
```



```
plot(log2(regression_brain$bcrp), log(regression_brain$conc_wt))
abline(0.5,1)
```



```
cor.test(regression_brain$bcrp, regression_brain$conc_wt, method = "pearson")
```

```
##
## Pearson's product-moment correlation
##
## data: regression_brain$bcrp and regression_brain$conc_wt
## t = -0.86512, df = 22, p-value = 0.3963
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## -0.5449099 0.2395396
## sample estimates:
## cor
## -0.1813841
```

```
cor.test(regression_brain$bcrp, regression_brain$conc_wt, method = "spearman")
```

```
##
## Spearman's rank correlation rho
##
## data: regression_brain$bcrp and regression_brain$conc_wt
## S = 2736, p-value = 0.3733
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
## rho
## -0.1895652
```

```
regression_retina <- subset(regression, tissue == "retina")
head(regression_retina)
```

```
## animal raw_conc tissue_weight body_wt dose pgp bcrp stage group
## 25 1M 104.7333 3.8 27.2 20.55 1.0782765 1.2298197 male male
## 26 2M 86.2347 5.0 30.1 18.57 1.0045893 0.9196512 male male
## 27 4M 88.3202 4.0 25.8 21.67 1.4228127 1.0984593 male male
```

```
## 28      5M 99.2028          4.3    26.1 20.00 1.1925447 0.9961628 male male
## 29      6M 97.7141          7.0    27.3 19.12 0.8683951 0.8810145 male male
## 30      7M 99.8877          5.2    26.2 19.92 1.0440075 1.1553400 male male
##      tissue conc_wt
## 25 retina 27.56139
## 26 retina 17.24694
## 27 retina 22.08005
## 28 retina 23.07042
## 29 retina 13.95916
## 30 retina 19.20917
```

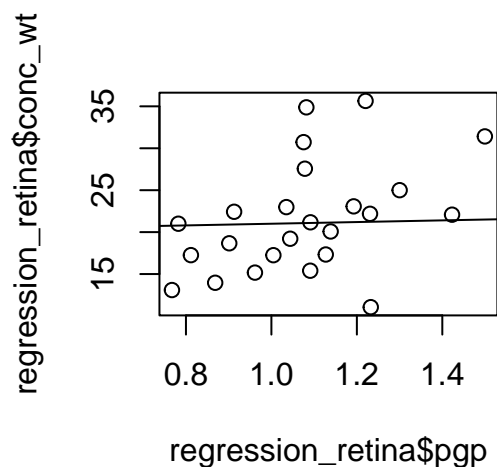
```
shapiro.test(regression_retina$conc_wt)
```

```
##
## Shapiro-Wilk normality test
##
## data:  regression_retina$conc_wt
## W = 0.94676, p-value = 0.2303
```

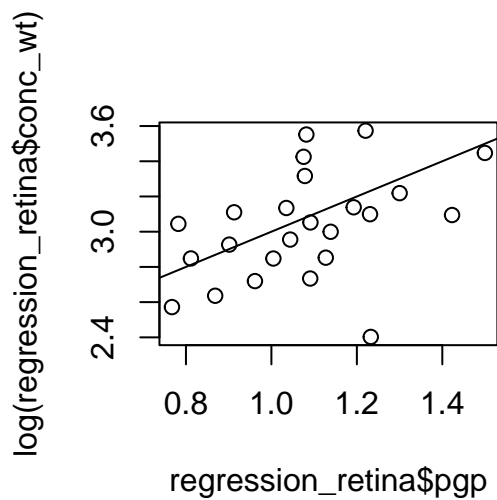
```
shapiro.test(regression_retina$pgp)
```

```
##
## Shapiro-Wilk normality test
##
## data:  regression_retina$pgp
## W = 0.973, p-value = 0.7409
```

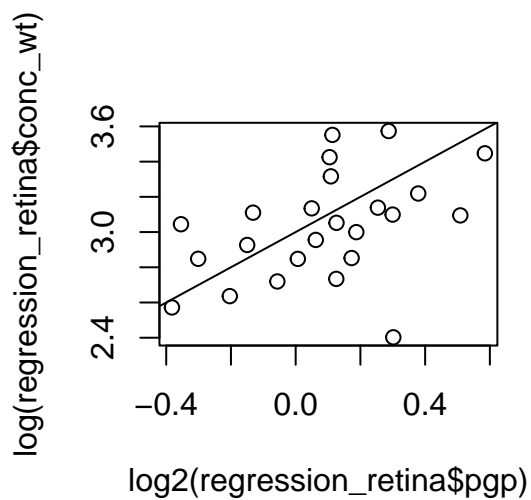
```
plot(regression_retina$pgp, regression_retina$conc_wt)
abline(20,1)
```



```
plot(regression_retina$pgp, log(regression_retina$conc_wt))
abline(2,1)
```



```
plot(log2(regression_retina$pgp), log(regression_retina$conc_wt))
abline(3,1)
```



```
cor.test(regression_retina$pgp, regression_retina$conc_wt, method = "pearson")
```

```
##
## Pearson's product-moment correlation
##
## data: regression_retina$pgp and regression_retina$conc_wt
## t = 2.2598, df = 22, p-value = 0.03408
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.03714565 0.71265643
## sample estimates:
## cor
```



```
## 0.4340385
```

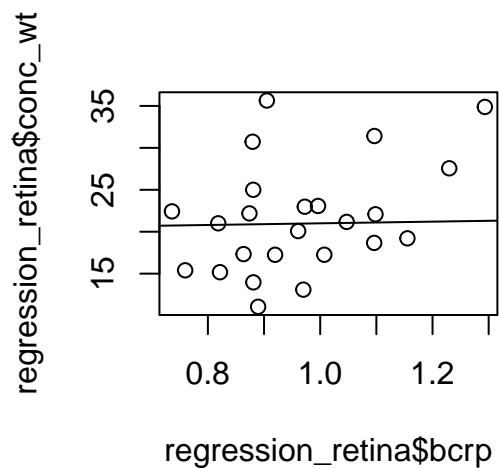
```
cor.test(regression_retina$pgp, regression_retina$conc_wt, method = "spearman")
```

```
##  
## Spearman's rank correlation rho  
##  
## data: regression_retina$pgp and regression_retina$conc_wt  
## S = 1304, p-value = 0.03568  
## alternative hypothesis: true rho is not equal to 0  
## sample estimates:  
##      rho  
## 0.4330435
```

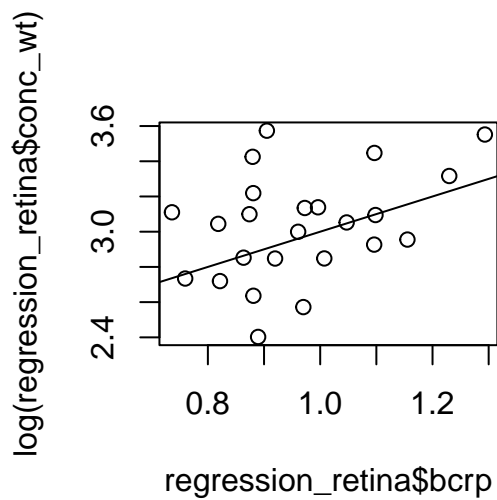
```
shapiro.test(regression_retina$bcrp)
```

```
##  
## Shapiro-Wilk normality test  
##  
## data: regression_retina$bcrp  
## W = 0.9579, p-value = 0.3978
```

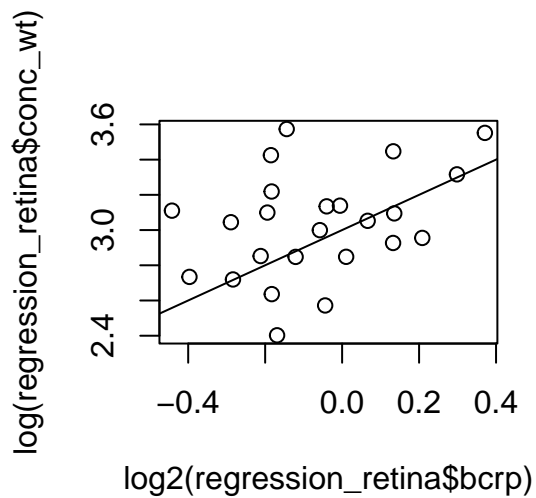
```
plot(regression_retina$bcrp, regression_retina$conc_wt)  
abline(20,1)
```



```
plot(regression_retina$bcrp, log(regression_retina$conc_wt))  
abline(2,1)
```



```
plot(log2(regression_retina$bcrp), log(regression_retina$conc_wt))
abline(3,1)
```



```
cor.test(regression_retina$bcrp, regression_retina$conc_wt, method = "pearson")
```

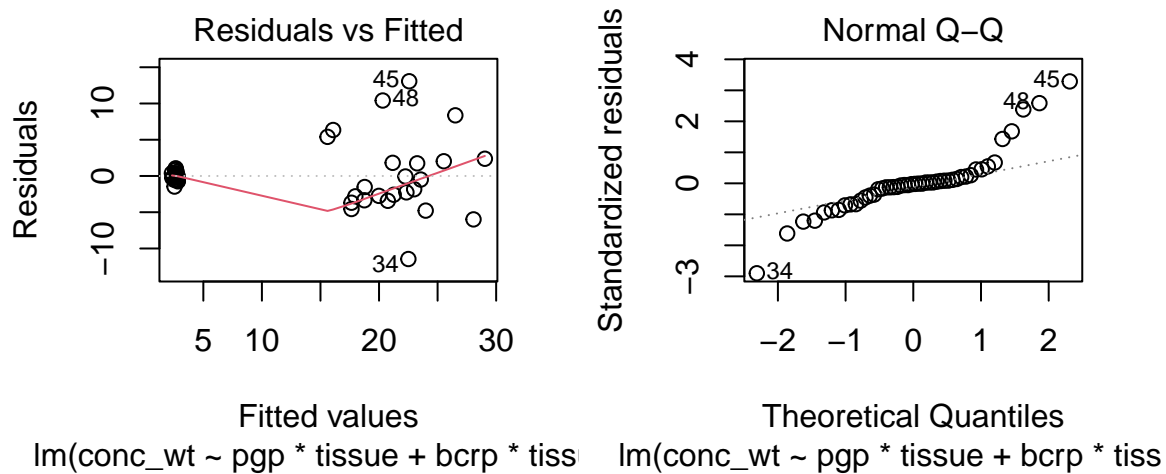
```
##
## Pearson's product-moment correlation
##
## data: regression_retina$bcrp and regression_retina$conc_wt
## t = 2.0013, df = 22, p-value = 0.05785
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## -0.01300906 0.68707216
## sample estimates:
## cor
```

```
## 0.3924471
```

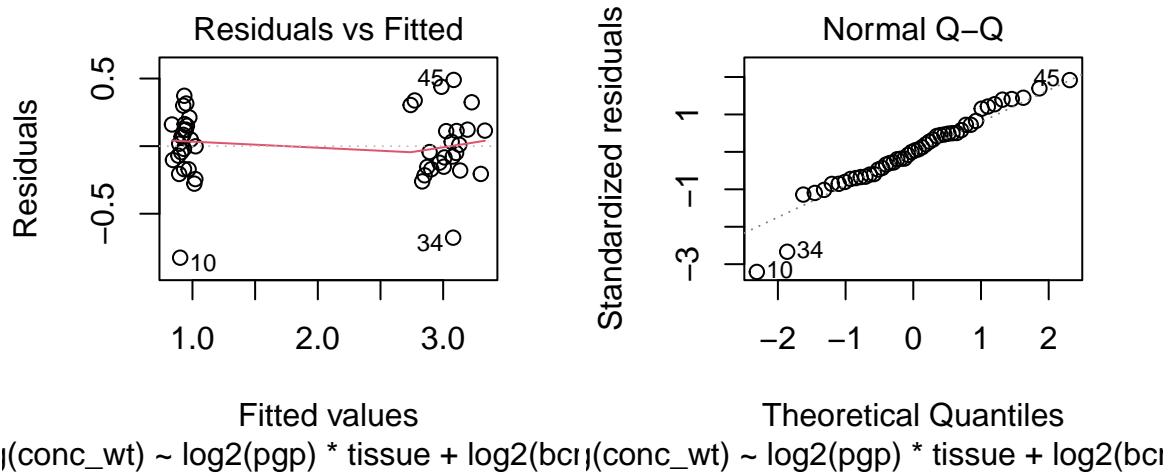
```
cor.test(regression_retina$bcrp, regression_retina$conc_wt, method = "spearman")
```

```
##  
## Spearman's rank correlation rho  
##  
## data: regression_retina$bcrp and regression_retina$conc_wt  
## S = 1632, p-value = 0.1682  
## alternative hypothesis: true rho is not equal to 0  
## sample estimates:  
##      rho  
## 0.2904348
```

```
lm_ex1 <- lm(conc_wt ~ pgp*tissue + bcrp*tissue, data = regression)  
plot(lm_ex1, which=c(1,2))
```



```
lm_ex <- lm(log(conc_wt) ~ log2(pgp)*tissue + log2(bcrp)*tissue, data = regression)  
plot(lm_ex, which=c(1,2))
```



```
summary(lm_ex)$coefficient
```

```
##               Estimate Std. Error   t value    Pr(>|t|)
## (Intercept)    3.0193972 0.06176213 48.8875210 1.186804e-38
## log2(pgp)       0.4385311 0.22416220  1.9563116 5.709991e-02
## tissuebrain    -2.1538742 0.43938139 -4.9020608 1.462185e-05
## log2(bcrp)      0.4272982 0.27311844  1.5645161 1.252008e-01
## log2(pgp):tissuebrain -0.3335960 0.37326691 -0.8937197 3.765647e-01
## tissuebrain:log2(bcrp) -0.6872715 0.40615462 -1.6921424 9.802618e-02
```

```
pgp_subset <- regression %>% select(-bcrp)
bcrp_subset <- regression %>% select(-pgp)
```

```
#untransformed
```

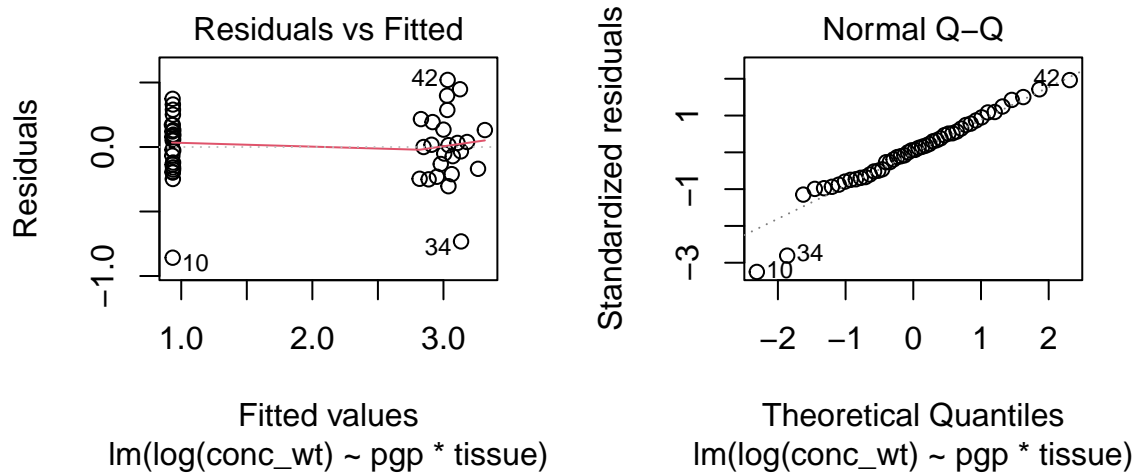
```
#lm_pgp <- lm(conc_wt ~ pgp * tissue, data=pgp_subset)
```

```
#plot(lm_pgp, which=c(1,2))
```

```
#concentration log transformed
```

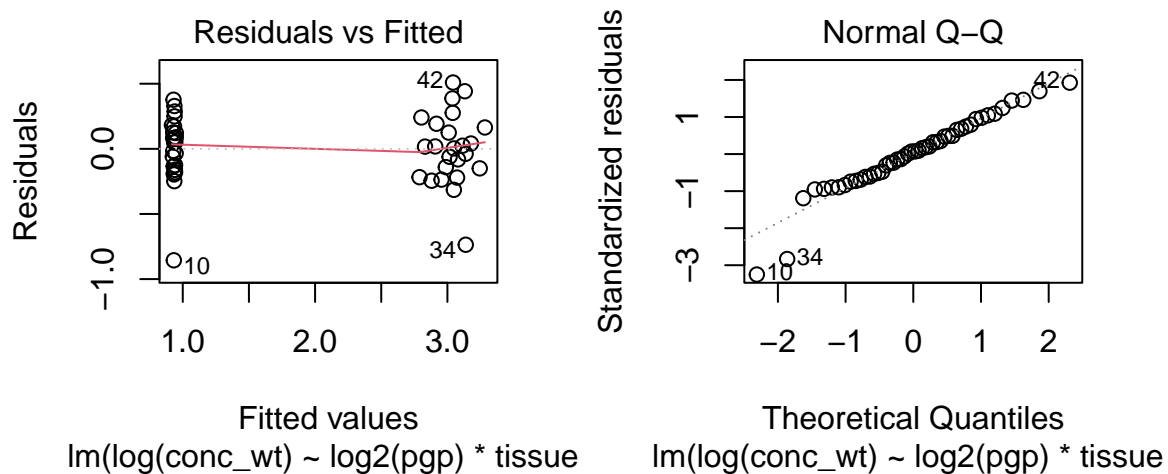
```
lm_pgp_log <- lm(log(conc_wt) ~ pgp * tissue, data=pgp_subset)
```

```
plot(lm_pgp_log, which=c(1,2))
```



```
#pgp log transformed
#lm_pgp_log1 <- lm(conc_wt ~ log2(pgp) * tissue, data=pgp_subset)
#plot(lm_pgp_loglog, which=c(1,2))

#both transformed
lm_pgp_loglog <- lm(log(conc_wt) ~ log2(pgp) * tissue, data=pgp_subset)
plot(lm_pgp_loglog, which=c(1,2))
```



```
summary(lm_pgp_log)
```

```
##
## Call:
## lm(formula = log(conc_wt) ~ pgp * tissue, data = pgp_subset)
##
## Residuals:
```

```
##      Min      1Q   Median      3Q      Max
## -0.85801 -0.16073  0.01583  0.14377  0.52006
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      2.2967     0.3281   7.000 1.14e-08 ***
## pgp              0.6796     0.3000   2.265  0.02850 *
## tissuebrain     -1.3453     0.4906  -2.742  0.00879 **
## pgp:tissuebrain  -0.6846     0.3218  -2.128  0.03901 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2705 on 44 degrees of freedom
## Multiple R-squared:  0.9427, Adjusted R-squared:  0.9388
## F-statistic: 241.4 on 3 and 44 DF,  p-value: < 2.2e-16
```

```
anova(lm_pgp_log)
```

```
## Analysis of Variance Table
##
## Response: log(conc_wt)
##      Df Sum Sq Mean Sq F value    Pr(>F)
## pgp      1 45.719  45.719 624.9245 < 2.2e-16 ***
## tissue    1  6.921   6.921  94.6050 1.56e-12 ***
## pgp:tissue 1  0.331   0.331   4.5267 0.03901 *
## Residuals 44  3.219   0.073
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(lm_pgp_log, pairwise ~ tissue)$contrasts
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
## contrast      estimate      SE df t.ratio p.value
## retina - brain      2.78 0.335 44   8.294 <.0001
##
## Results are given on the log (not the response) scale.
```