

qpCR Transporter Figures

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```
library(tidyverse)
library(ggplot2)
library(ggthemes)
library(forcats)
library(ggpubr)
#library(writexl)
library(emmeans)
library(lme4)
library(car)
library(lmerTest)
library(svglite)
```

Reading in & tidying the data

```
transporters <- read.csv("../data/qRT-PCR/07222022-transporter-gene-study.csv", fileEncoding = 'UTF-8')

transporters <- transporters %>% filter(Sample != "calib")

transporters <- transporters %>% mutate(Tissue = str_sub(Sample, start=-3L, end=-1L),
                                         Treatment = str_extract(Sample, "[:alpha:]+(?=[[:digit:]])"),
                                         Sample = str_extract(Sample, "[:alnum:]+(?=-)"))

transporters <- transporters %>% mutate(Treatment = factor(Treatment, c("S", "M", "Flo", "Phi", "C")),
                                         Tissue = factor(Tissue, c("ret", "hyp")))

head(transporters)

##   Sample  Pgp.RGE  Bcrp.RGE Tissue Treatment
## 1     C1  1.7701376  1.6160126   hyp         C
## 2     C1  0.4207643  0.8918919   ret         C
## 3     C2  1.7514899  1.6263272   hyp         C
## 4     C2  0.9387229  1.2154637   ret         C
## 5     C3  1.2344328  2.3715652   hyp         C
## 6     C3  0.7343906  1.0166798   ret         C

transporters_long <- transporters %>% pivot_longer(cols=c("Pgp.RGE", "Bcrp.RGE"), names_to = "gene", values_to = "RGE")

transporters_long <- transporters_long %>% mutate(gene = str_extract(gene, "[:alpha:]+(?=\\.)"))
transporters_long <- transporters_long %>% mutate(gene = factor(as.factor(gene), c("Pgp", "Bcrp")))

head(transporters_long)
```

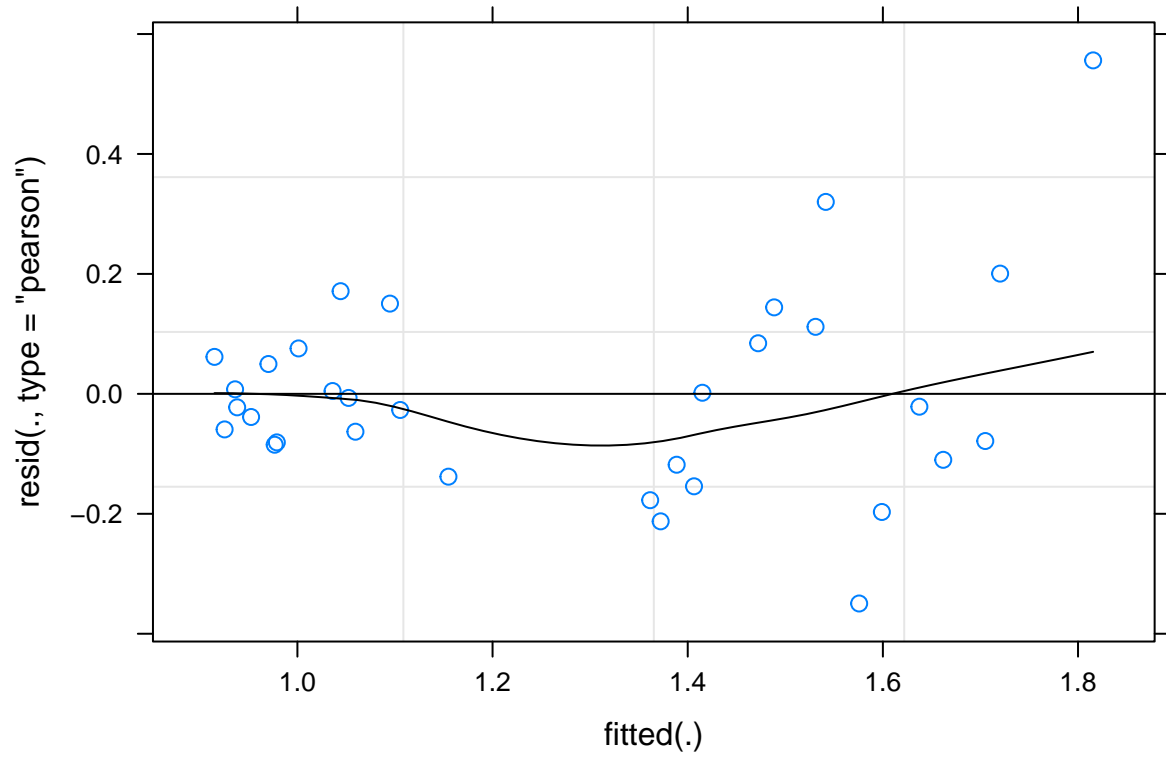
```
## # A tibble: 6 x 5
##   Sample Tissue Treatment gene    rge
##   <chr>   <fct>   <fct>   <fct> <dbl>
## 1 C1     hyp     C       Pgp    1.77
## 2 C1     hyp     C       Bcrp   1.62
## 3 C1     ret     C       Pgp    0.421
## 4 C1     ret     C       Bcrp   0.892
## 5 C2     hyp     C       Pgp    1.75
## 6 C2     hyp     C       Bcrp   1.63
```

Comparing chronic saline/morphine for Bcrp (rerun of samples for P-gp from Bergum 2022)

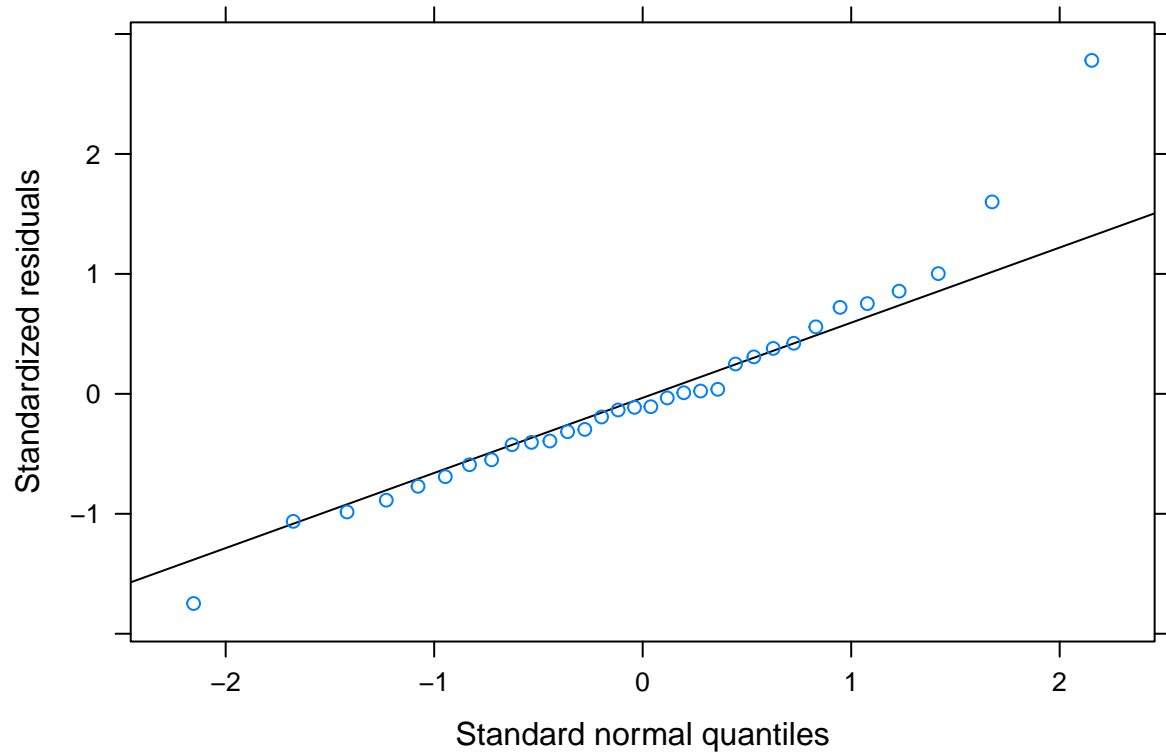
```
#bcrp only
chronic <- transporters %>% filter(Treatment %in% c("C", "S"))
chronic
```

```
##   Sample  Pgp.RGE  Bcrp.RGE Tissue Treatment
## 1      C1 1.7701376 1.6160126   hyp         C
## 2      C1 0.4207643 0.8918919   ret         C
## 3      C2 1.7514899 1.6263272   hyp         C
## 4      C2 0.9387229 1.2154637   ret         C
## 5      C3 1.2344328 2.3715652   hyp         C
## 6      C3 0.7343906 1.0166798   ret         C
## 7      C4 1.8650058 1.9206809   hyp         C
## 8      C4 0.6869529 0.9963086   ret         C
## 9      C5 1.3727992 1.2261280   hyp         C
## 10     C5 1.2258085 0.9766218   ret         C
## 11     C6 1.4958638 1.5518807   hyp         C
## 12     C6 0.7876697 1.0768726   ret         C
## 13     C7 2.0301205 1.4020048   hyp         C
## 14     C7 1.1768876 0.9157910   ret         C
## 15     S1 1.8016888 1.5565636   hyp         S
## 16     S1 1.0200728 1.0407493   ret         S
## 17     S2 1.3689686 1.8615469   hyp         S
## 18     S2 0.8222355 1.0784274   ret         S
## 19     S3 1.5582238 1.1842034   hyp         S
## 20     S3 1.0081232 0.8660578   ret         S
## 21     S4 1.0379047 1.2523247   hyp         S
## 22     S4 0.7776565 1.0200962   ret         S
## 23     S5 1.6163239 1.2704658   hyp         S
## 24     S5 0.8109336 0.9139269   ret         S
## 25     S6 1.7206882 1.1597168   hyp         S
## 26     S6 1.0835666 0.9436934   ret         S
## 27     S7 2.3260412 1.6427723   hyp         S
## 28     S7 1.4357874 1.2453351   ret         S
## 29     S8 2.0696005 1.6327948   hyp         S
## 30     S8 1.0679296 1.0456424   ret         S
## 31     S9 2.4339414 1.4168757   hyp         S
## 32     S9 1.1287493 0.8979728   ret         S
```

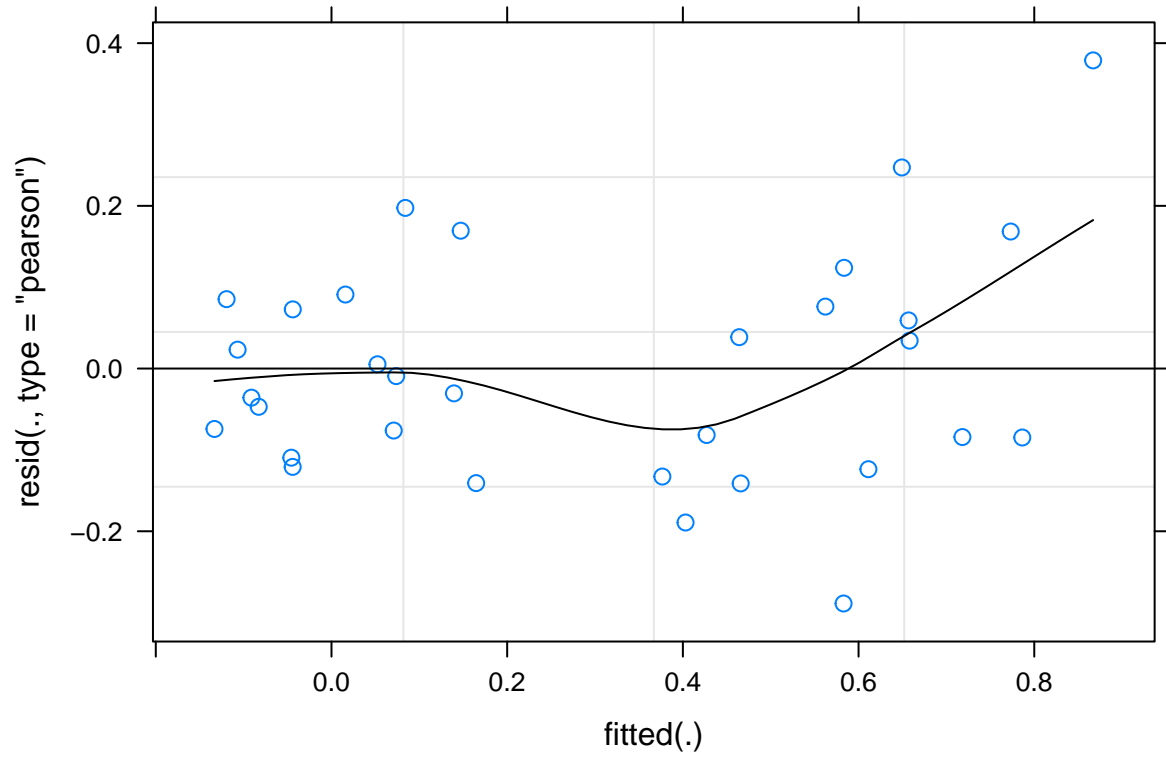
```
c_bcrp_lm <- lmer(Bcrp.RGE ~ Tissue * Treatment + (1|Sample), data=chronic)
plot(c_bcrp_lm, type=c("p", "smooth"), col.line=1)
```



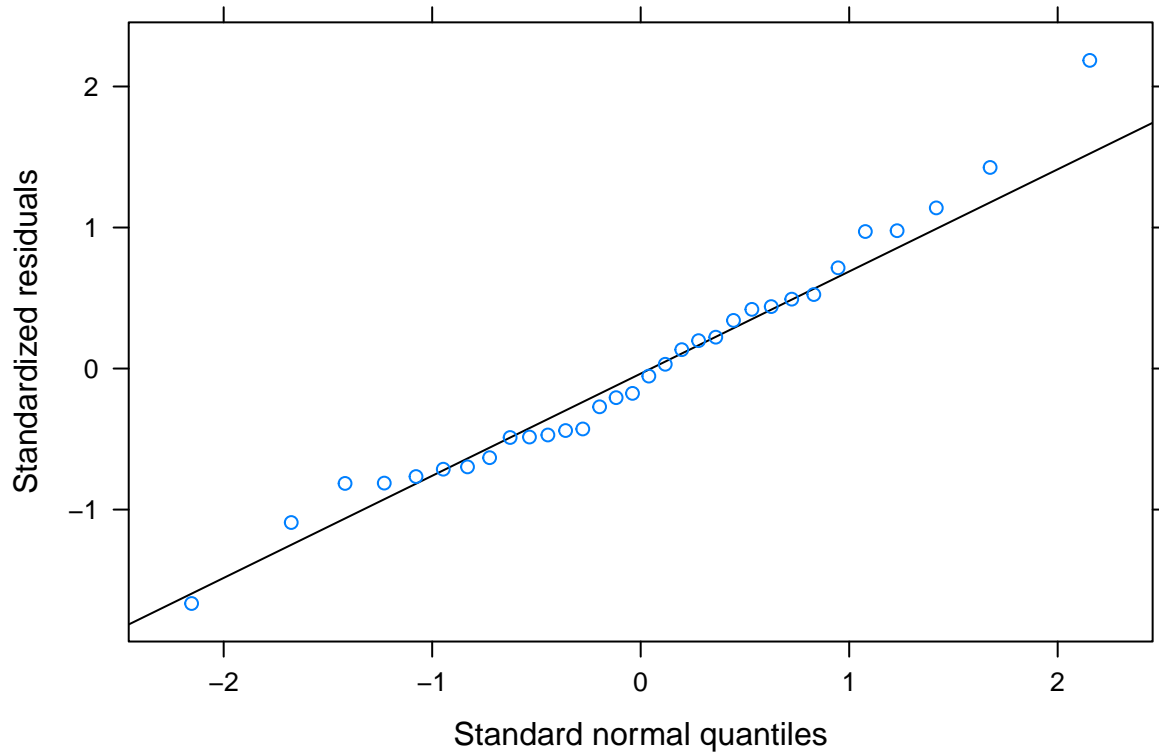
```
lattice::qqmath(c_bcrp_lm)
```



```
c_bcrp_log2_lm <- lmer(log2(Bcrp.RGE) ~ Tissue * Treatment + (1|Sample), data=chronic)
plot(c_bcrp_log2_lm, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(c_bcrp_log2_lm)
```



```
shapiro.test(chronic$Bcrp.RGE)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  chronic$Bcrp.RGE
## W = 0.89092, p-value = 0.003667
```

```
shapiro.test(log2(chronic$Bcrp.RGE))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(chronic$Bcrp.RGE)
## W = 0.94026, p-value = 0.07615
```

```
anova(c_bcrp_log2_lm)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Tissue        2.89290  2.89290     1    14  96.2633 1.182e-07 ***
## Treatment      0.04000  0.04000     1    14   1.3311  0.2679
## Tissue:Treatment 0.07294  0.07294     1    14   2.4271  0.1416
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(c_bcrp_log2_lm, pairwise ~ Treatment | Tissue)$contrasts
```

```
## Tissue = ret:
```

```
## contrast estimate SE df t.ratio p.value
## S - C -0.0115 0.112 24.3 -0.103 0.9188
##
## Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## S - C -0.2040 0.112 24.3 -1.822 0.0808
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
emmeans::emmeans(c_bcrp_log2_lm, pairwise ~ Tissue | Treatment)$contrasts

## Treatment = S:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.510 0.0817 14 -6.239 <.0001
##
## Treatment = C:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.702 0.0927 14 -7.580 <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
c_bcrp_sumstats <- chronic %>% group_by(Treatment, Tissue) %>% summarise(
  n = n(),
  mean = mean(Bcrp.RGE),
  sd = sd(Bcrp.RGE),
  se = sd/sqrt(n),
  log2_mean = mean(log2(Bcrp.RGE)),
  log2_sd = sd(log2(Bcrp.RGE)),
  log2_se = log2_sd/sqrt(n)
)

## `summarise()` has grouped output by 'Treatment'. You can override using the
## `.groups` argument.
c_bcrp_sumstats

## # A tibble: 4 x 9
## # Groups: Treatment [2]
## Treatment Tissue n mean sd se log2_mean log2_sd log2_se
## <fct> <fct> <int> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
## 1 S ret 9 1.01 0.117 0.0389 -4.39e-11 0.163 0.0542
## 2 S hyp 9 1.44 0.244 0.0815 5.10e- 1 0.242 0.0806
## 3 C ret 7 1.01 0.109 0.0411 1.15e- 2 0.150 0.0567
## 4 C hyp 7 1.67 0.375 0.142 7.14e- 1 0.308 0.116

#visualizing tissue effect per treatment
c_bcrp_plot <- ggplot() +
  stat_summary(data=chronic, aes(x=Treatment, y=Bcrp.RGE, fill=Tissue), fun='mean',
    geom="bar", width=0.75, position=position_dodge(width=0.75)) +
  geom_errorbar(data=c_bcrp_sumstats, aes(x=Treatment, ymin=mean-se,
    ymax=mean+se, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=chronic, aes(x=Treatment, y=Bcrp.RGE, fill=Tissue),
    position=position_dodge(width=0.75)) +
  #facet_grid(~Tissue, scales="free_y") +
```

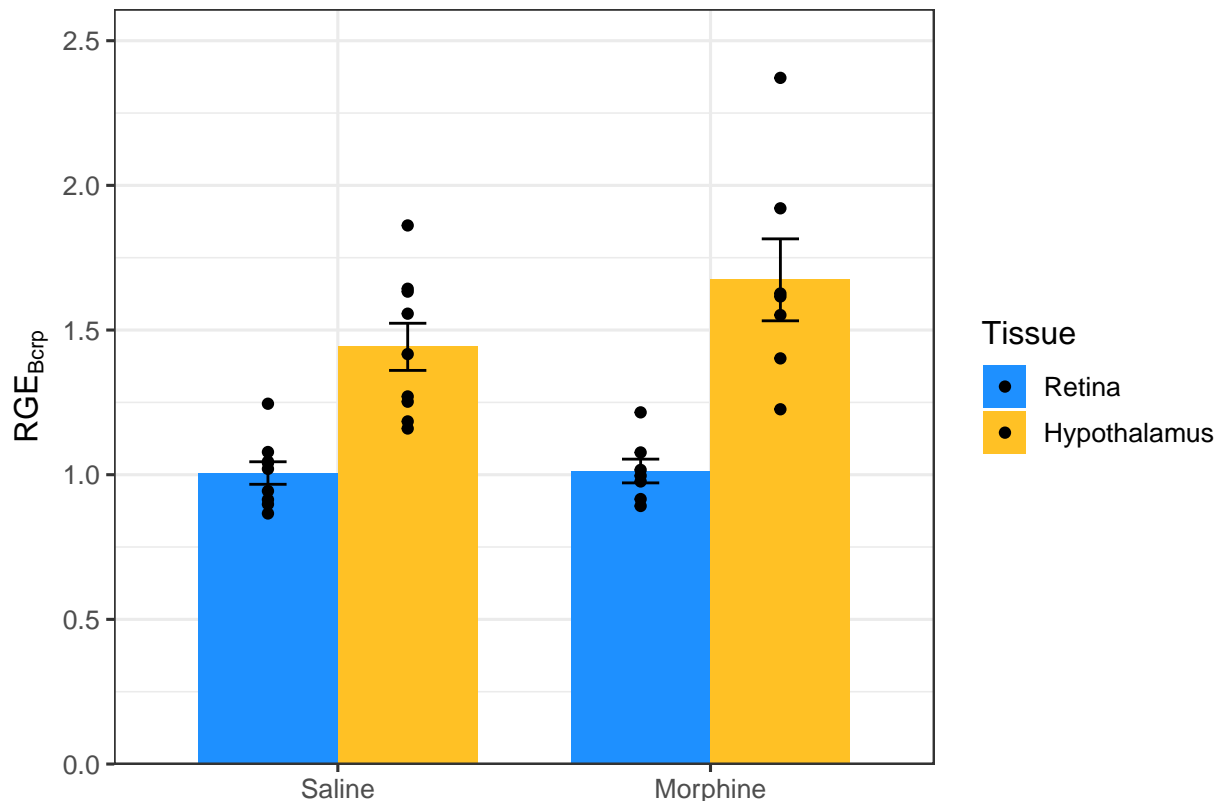
```

scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
theme_bw(base_size = 12) +
scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
                  labels=c("Retina", "Hypothalamus")) +
scale_x_discrete(labels=c("S" = "Saline", "C" = "Morphine")) +
xlab("") +
ylab(expression(RGEBcrp))

```

Warning: Ignoring unknown aesthetics: fill

c_bcrp_plot



#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_effect_plot.svg", plot=c_bcrp_plot, height=4, width=6)

```

c_bcrp_log_plot <- ggplot() +
  stat_summary(data=chronic, aes(x=Treatment, y=log2(Bcrp.RGE), fill=Tissue),
              fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=c_bcrp_sumstats, aes(x=Treatment, ymin=log2_mean-log2_se,
                                          ymax=log2_mean+log2_se, fill=Tissue),
               width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=chronic, aes(x=Treatment, y=log2(Bcrp.RGE), fill=Tissue),
             stat="identity", position=position_dodge(width=0.75)) +
  #facet_grid(~Tissue, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.2))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw(base_size = 12) +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),

```



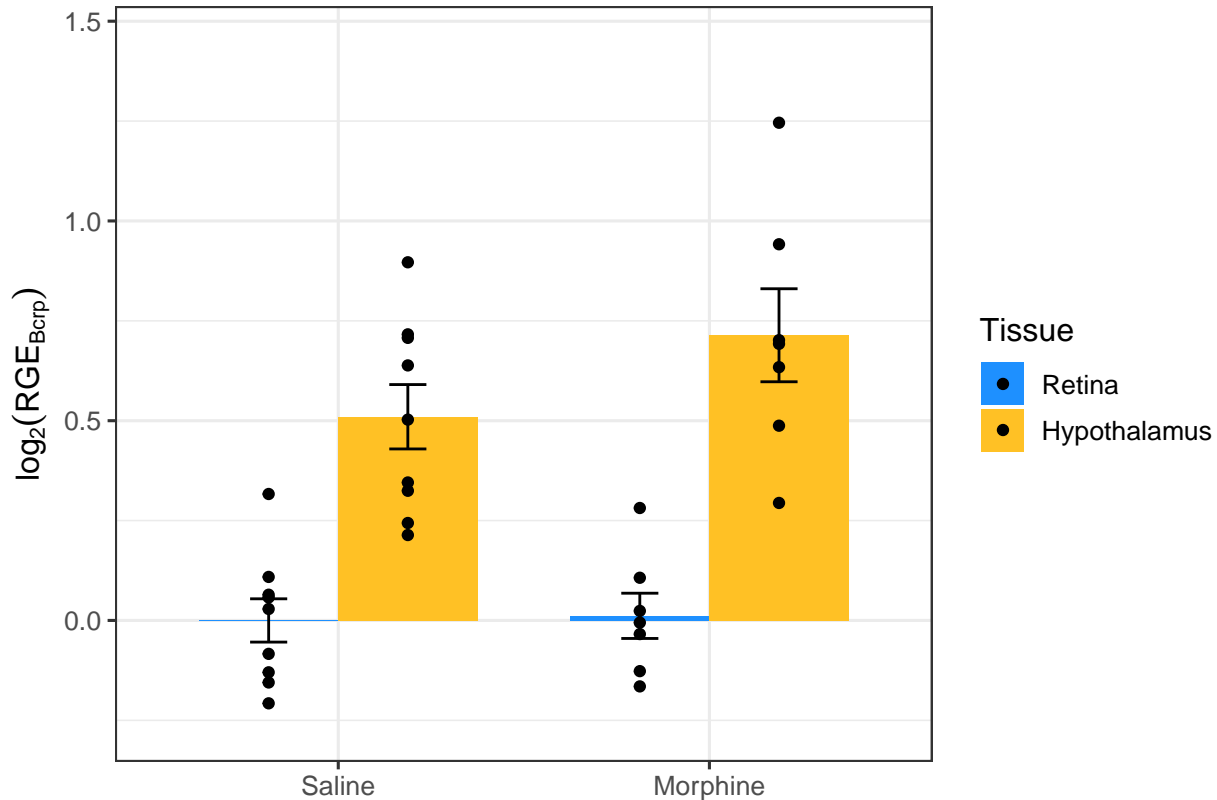
```

      labels=c("Retina", "Hypothalamus")) +
scale_x_discrete(labels=c("S" = "Saline", "C" = "Morphine")) +
xlab("") +
ylab(expression(log2(RGE[Bcrp])))

```

Warning: Ignoring unknown aesthetics: fill

c_bcrp_log_plot



#NEED TO SAVE THIS!!!!

#ggsave(filename="../../figures/qRT-PCR/c_bcrp_log_plot.g", plot=c_bcrp_log_plot, height=4, width=6, bg

#edit starting here

#tissue plots

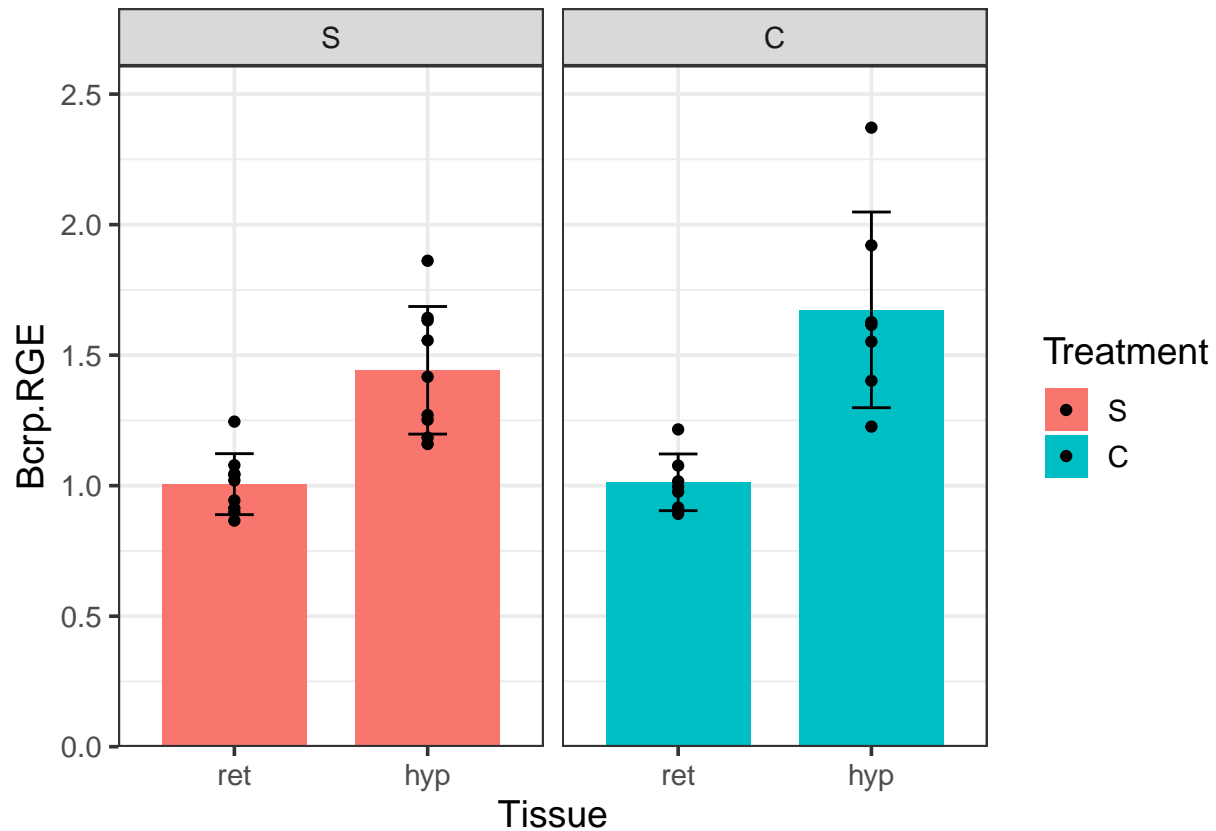
```

c_bcrp_tiss_plot <- ggplot() +
  stat_summary(data=chronic, aes(x=Tissue, y=Bcrp.RGE, fill=Treatment),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=c_bcrp_sumstats, aes(x=Tissue, ymin=mean-sd,
    ymax=mean+sd, fill=Treatment),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=chronic, aes(x=Tissue, y=Bcrp.RGE, fill=Treatment),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(~Treatment, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw(base_size = 14)

```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
c_bcrp_tiss_plot
```

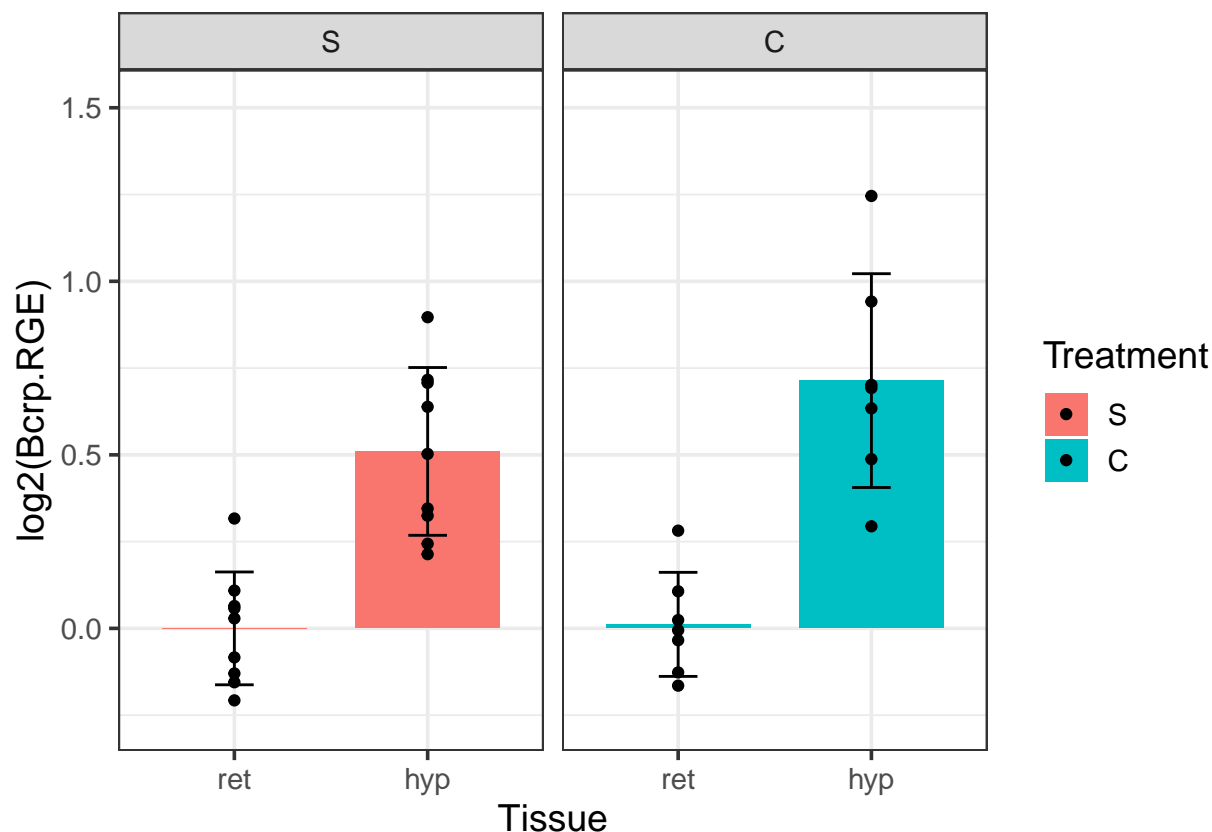


```
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_plot.svg", plot=c_bcrp_tiss_plot, height=4, width=6)
```

```
c_bcrp_tiss_log_plot <- ggplot() +
  stat_summary(data=chronic, aes(x=Tissue, y=log2(Bcrp.RGE), fill=Treatment),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=c_bcrp_sumstats, aes(x=Tissue, ymin=log2_mean-log2_sd,
    ymax=log2_mean+log2_sd, fill=Treatment),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=chronic, aes(x=Tissue, y=log2(Bcrp.RGE), fill=Treatment),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(~Treatment, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw(base_size = 14)
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
c_bcrp_tiss_log_plot
```



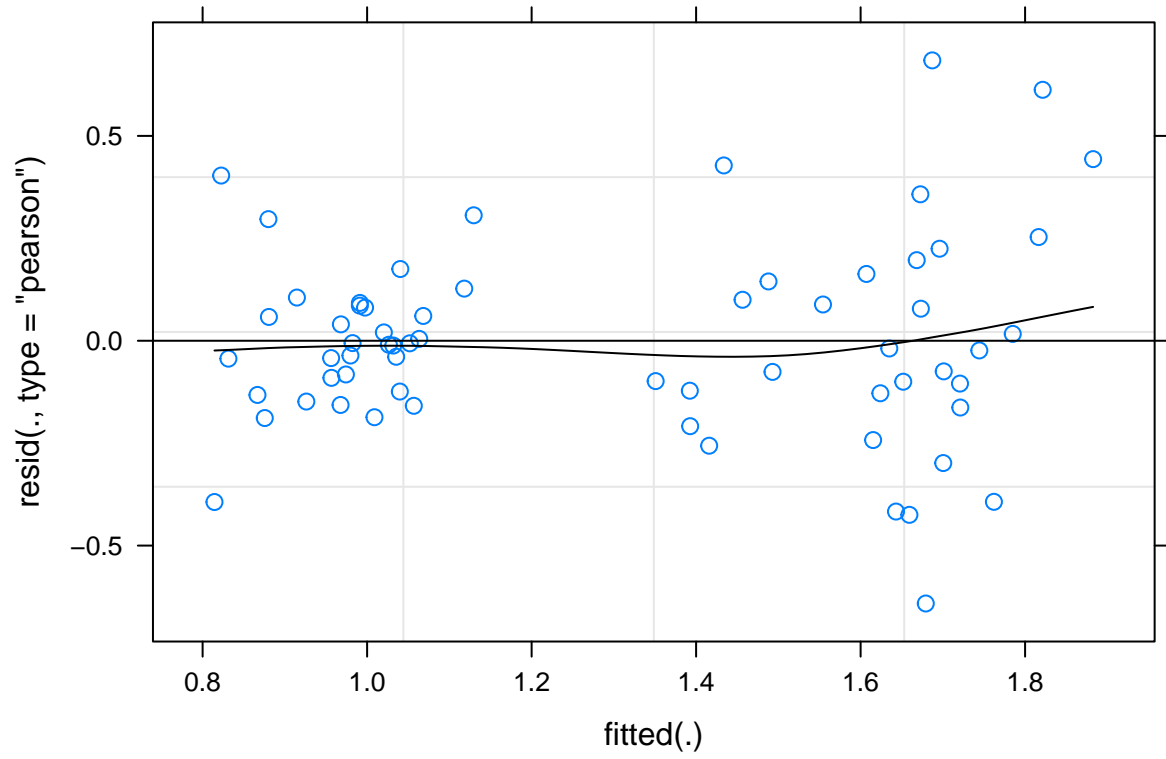
```
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_log_plot.svg", plot=c_bcrp_tiss_log_plot, height=4,
```

```
#bcrp & pgp together
```

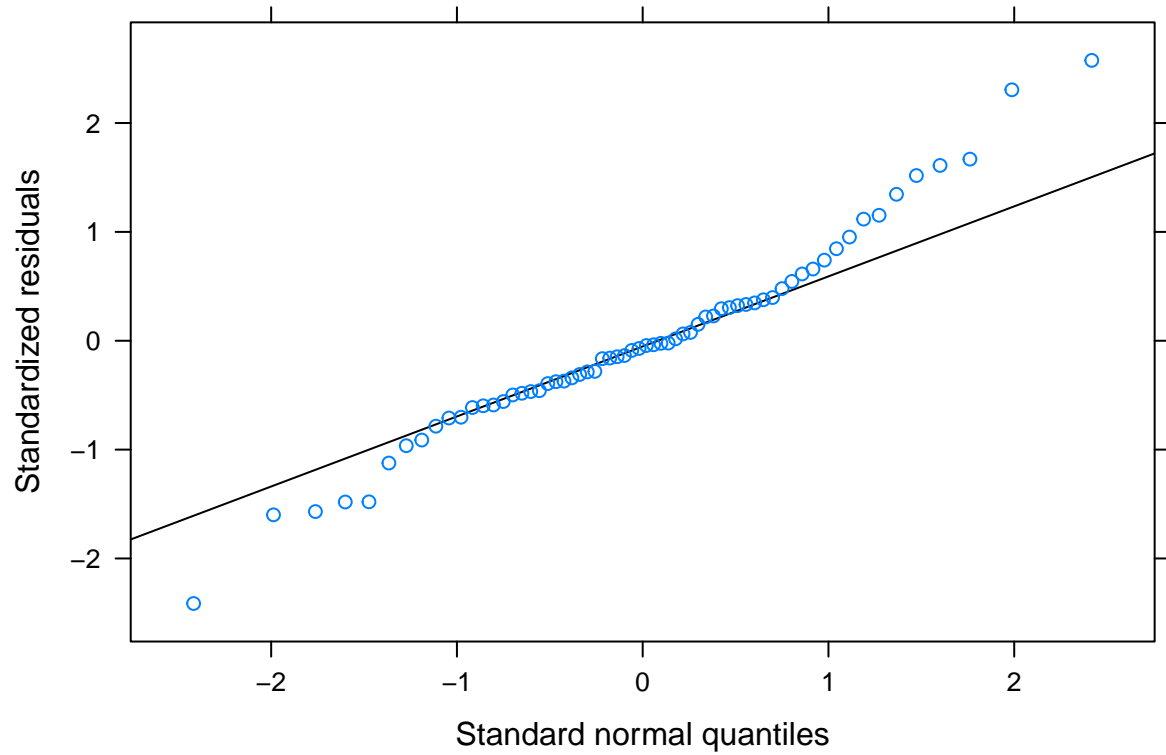
```
chronic_long <- transporters_long %>% filter(Treatment %in% c("C", "S"))
chronic_long
```

```
## # A tibble: 64 x 5
##   Sample Tissue Treatment gene    rge
##   <chr>   <fct>   <fct>   <fct> <dbl>
## 1 C1     hyp      C       Pgp    1.77
## 2 C1     hyp      C       Bcrp   1.62
## 3 C1     ret      C       Pgp    0.421
## 4 C1     ret      C       Bcrp   0.892
## 5 C2     hyp      C       Pgp    1.75
## 6 C2     hyp      C       Bcrp   1.63
## 7 C2     ret      C       Pgp    0.939
## 8 C2     ret      C       Bcrp   1.22
## 9 C3     hyp      C       Pgp    1.23
## 10 C3    hyp      C       Bcrp   2.37
## # ... with 54 more rows
## # i Use `print(n = ...)` to see more rows
```

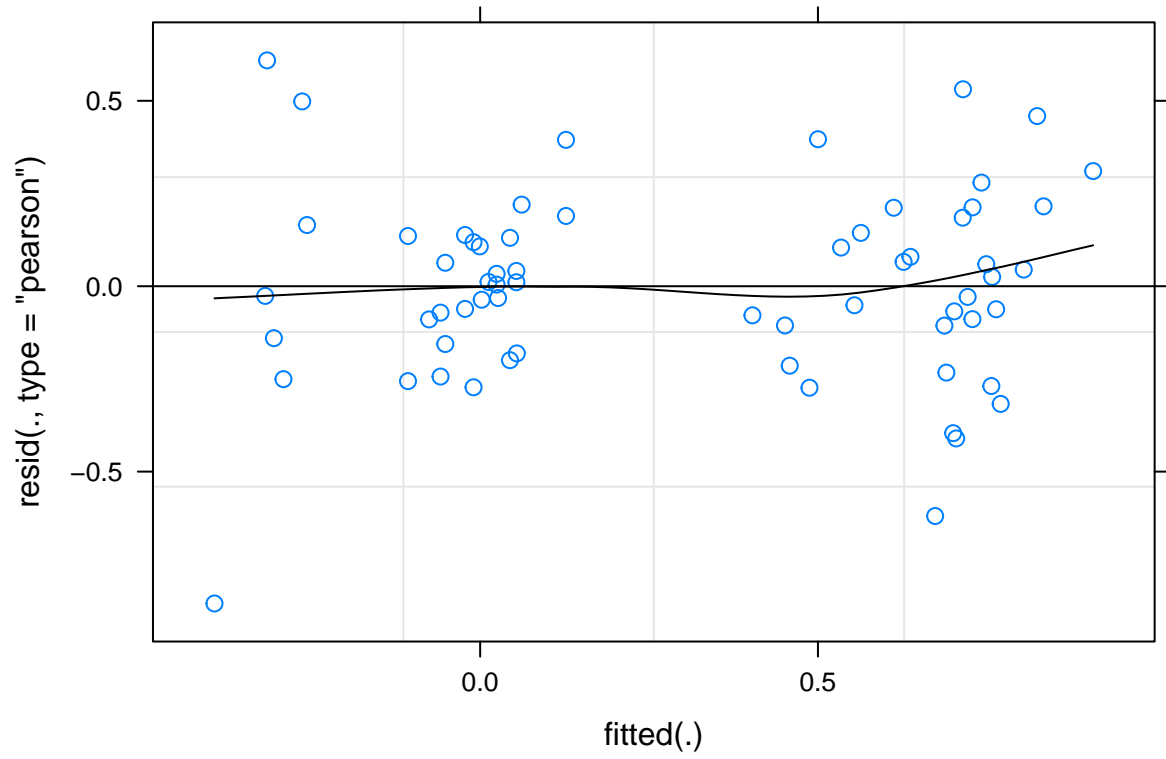
```
chronic_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=chronic_long)
plot(chronic_lm, type=c("p", "smooth"), col.line=1)
```



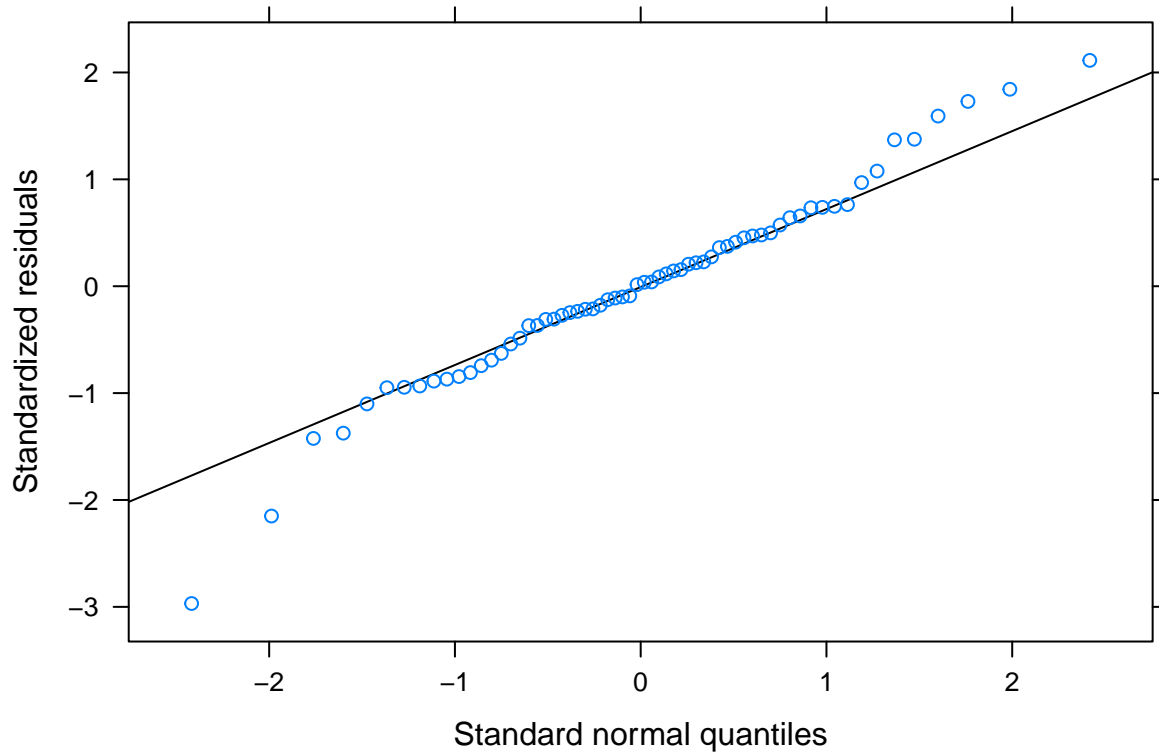
```
lattice::qqmath(chronic_lm)
```



```
chronic_log2_lm <- lmer(log2(rge) ~ gene * Tissue * Treatment + (1|Sample), data=chronic_long)
plot(chronic_log2_lm, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(chronic_log2_lm)
```



```
shapiro.test(chronic_long$rge)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  chronic_long$rge
## W = 0.95417, p-value = 0.01844
```

```
shapiro.test(log2(chronic_long$rge))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(chronic_long$rge)
## W = 0.98001, p-value = 0.3853
```

```
anova(chronic_log2_lm)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##
```

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
gene	0.0038	0.0038	1	42	0.0453	0.83247
Tissue	8.8468	8.8468	1	42	106.4695	4.365e-13 ***
Treatment	0.0190	0.0190	1	14	0.2290	0.63967
gene:Tissue	0.3237	0.3237	1	42	3.8962	0.05500 .
gene:Treatment	0.3575	0.3575	1	42	4.3023	0.04423 *
Tissue:Treatment	0.1717	0.1717	1	42	2.0665	0.15797
gene:Tissue:Treatment	0.0011	0.0011	1	42	0.0127	0.91092

```
## ---
```

```

## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

emmeans::emmeans(chronic_log2_lm, pairwise ~ Treatment | Tissue, by="gene")$contrasts

## Tissue = ret, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## S - C          0.3061 0.154 53.9   1.982  0.0526
##
## Tissue = hyp, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## S - C          0.0809 0.154 53.9   0.524  0.6024
##
## Tissue = ret, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## S - C         -0.0115 0.154 53.9  -0.075  0.9407
##
## Tissue = hyp, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## S - C         -0.2040 0.154 53.9  -1.321  0.1921
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.

emmeans::emmeans(chronic_log2_lm, pairwise ~ Tissue | Treatment, by="gene")$contrasts

## Treatment = S, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -0.780 0.136 42   -5.742  <.0001
##
## Treatment = C, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.005 0.154 42   -6.525  <.0001
##
## Treatment = S, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -0.510 0.136 42   -3.752  0.0005
##
## Treatment = C, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -0.702 0.154 42   -4.558  <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.

emmeans::emmeans(chronic_log2_lm, pairwise ~ gene | Treatment, by="Tissue")$contrasts

## Treatment = S, Tissue = ret:
## contrast estimate      SE df t.ratio p.value
## Pgp - Bcrp    0.0000 0.136 42    0.000  1.0000
##
## Treatment = C, Tissue = ret:
## contrast estimate      SE df t.ratio p.value
## Pgp - Bcrp   -0.3177 0.154 42   -2.062  0.0455
##
## Treatment = S, Tissue = hyp:
## contrast estimate      SE df t.ratio p.value

```



```
## Pgp - Bcrp    0.2704 0.136 42    1.990  0.0531
##
## Treatment = C, Tissue = hyp:
## contrast      estimate      SE df t.ratio p.value
## Pgp - Bcrp   -0.0146 0.154 42   -0.095  0.9251
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
chronic_sumstats <- chronic_long %>% group_by(Treatment, Tissue, gene) %>% summarise(
  n = n(),
  mean = mean(rge),
  sd = sd(rge),
  log2_mean = mean(log2(rge)),
  log2_sd = sd(log2(rge))
)
```

```
## `summarise()` has grouped output by 'Treatment', 'Tissue'. You can override
## using the `.groups` argument.
```

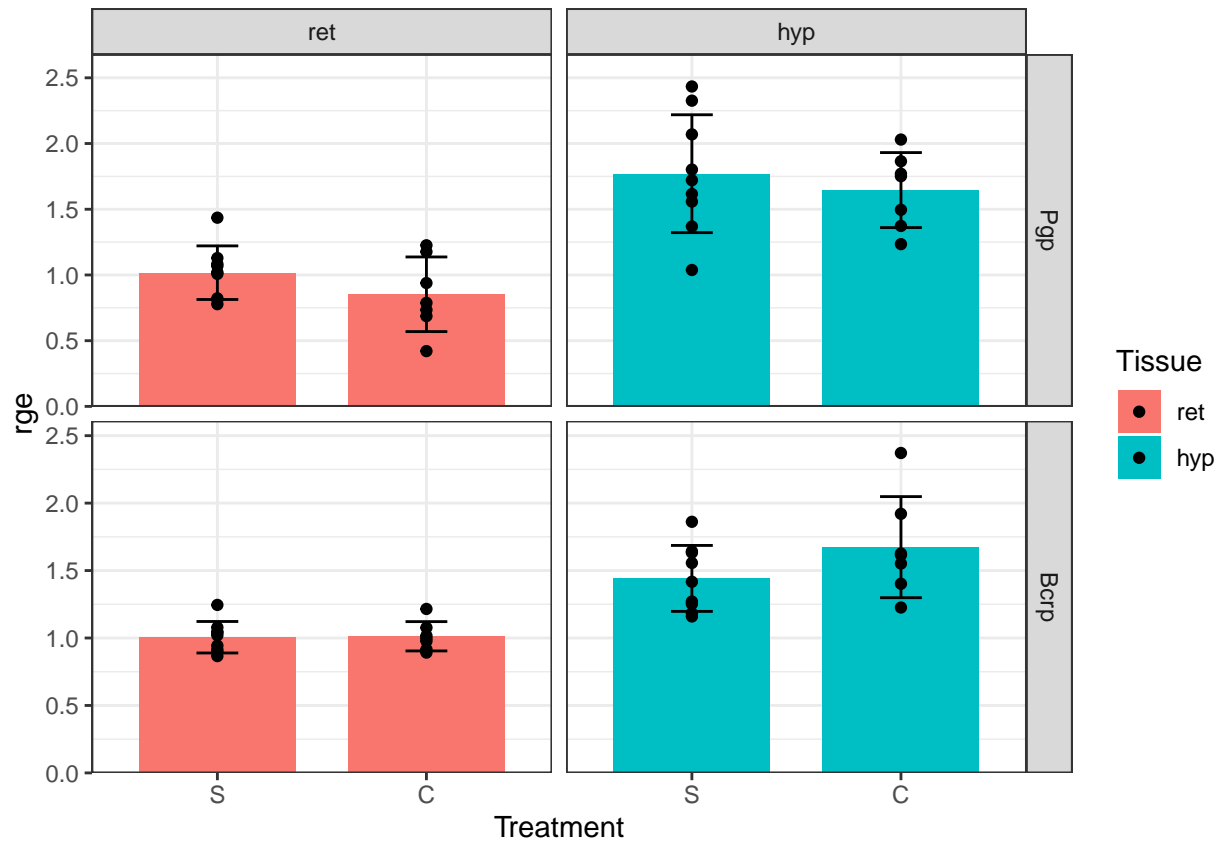
```
chronic_sumstats
```

```
## # A tibble: 8 x 8
## # Groups:   Treatment, Tissue [4]
##   Treatment Tissue gene      n mean    sd log2_mean log2_sd
##   <fct>      <fct> <fct> <int> <dbl> <dbl>      <dbl>      <dbl>
## 1 S        ret    Pgp     9 1.02  0.204 -1.11e-11  0.280
## 2 S        ret    Bcrp     9 1.01  0.117 -4.39e-11  0.163
## 3 S        hyp    Pgp     9 1.77  0.448  7.80e- 1  0.385
## 4 S        hyp    Bcrp     9 1.44  0.244  5.10e- 1  0.242
## 5 C        ret    Pgp     7 0.853 0.284 -3.06e- 1  0.527
## 6 C        ret    Bcrp     7 1.01  0.109  1.15e- 2  0.150
## 7 C        hyp    Pgp     7 1.65  0.285  6.99e- 1  0.258
## 8 C        hyp    Bcrp     7 1.67  0.375  7.14e- 1  0.308
```

```
chronic_plot <- ggplot() +
  stat_summary(data=chronic_long, aes(x=Treatment, y=rge, fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=chronic_sumstats, aes(x=Treatment, ymin=mean-sd,
    ymax=mean+sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=chronic_long, aes(x=Treatment, y=rge, fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(gene~Tissue, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
chronic_plot
```

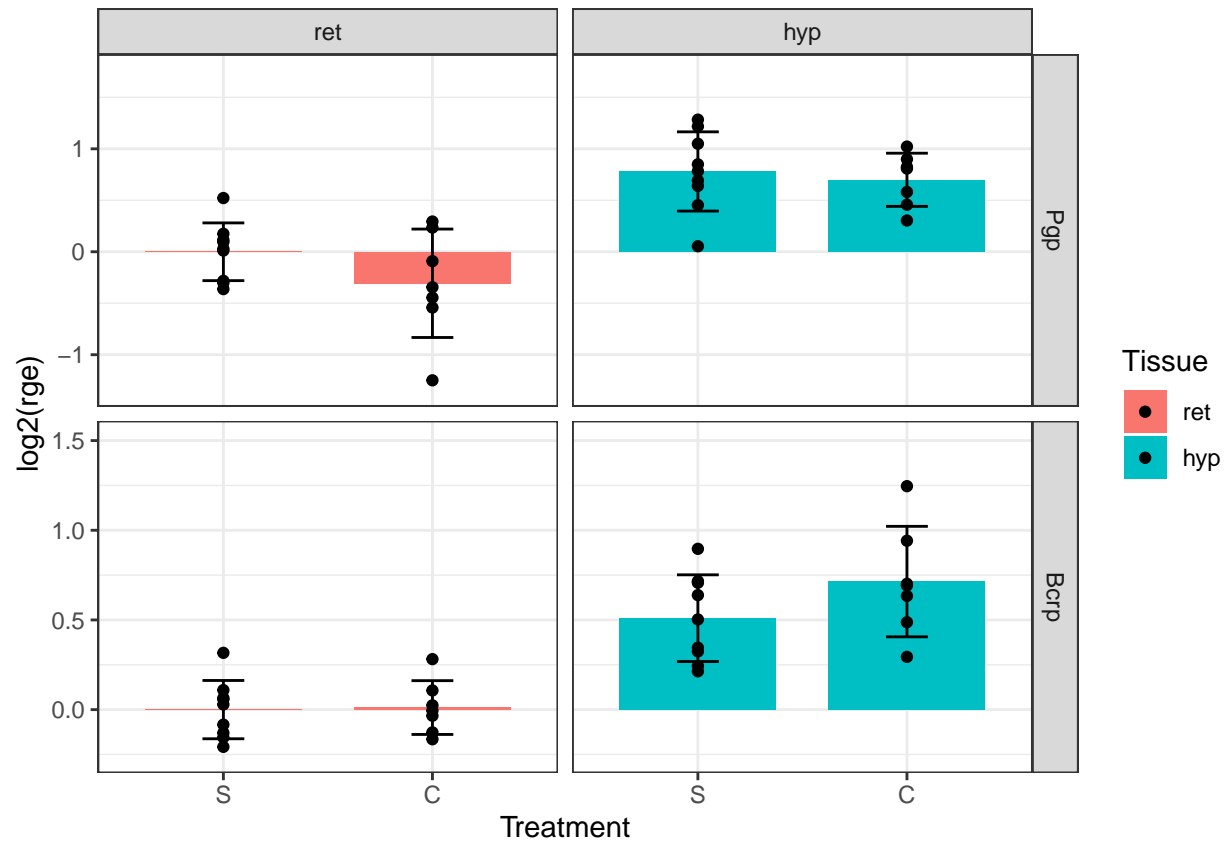


```
#ggsave(filename="../../figures/qRT-PCR/chronic_trt_plot.svg", plot=chronic_plot, height=4, width=6)
```

```
chronic_log_plot <- ggplot() +
  stat_summary(data=chronic_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=chronic_sumstats, aes(x=Treatment, ymin=log2_mean-log2_sd,
    ymax=log2_mean+log2_sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=chronic_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(gene~Tissue, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

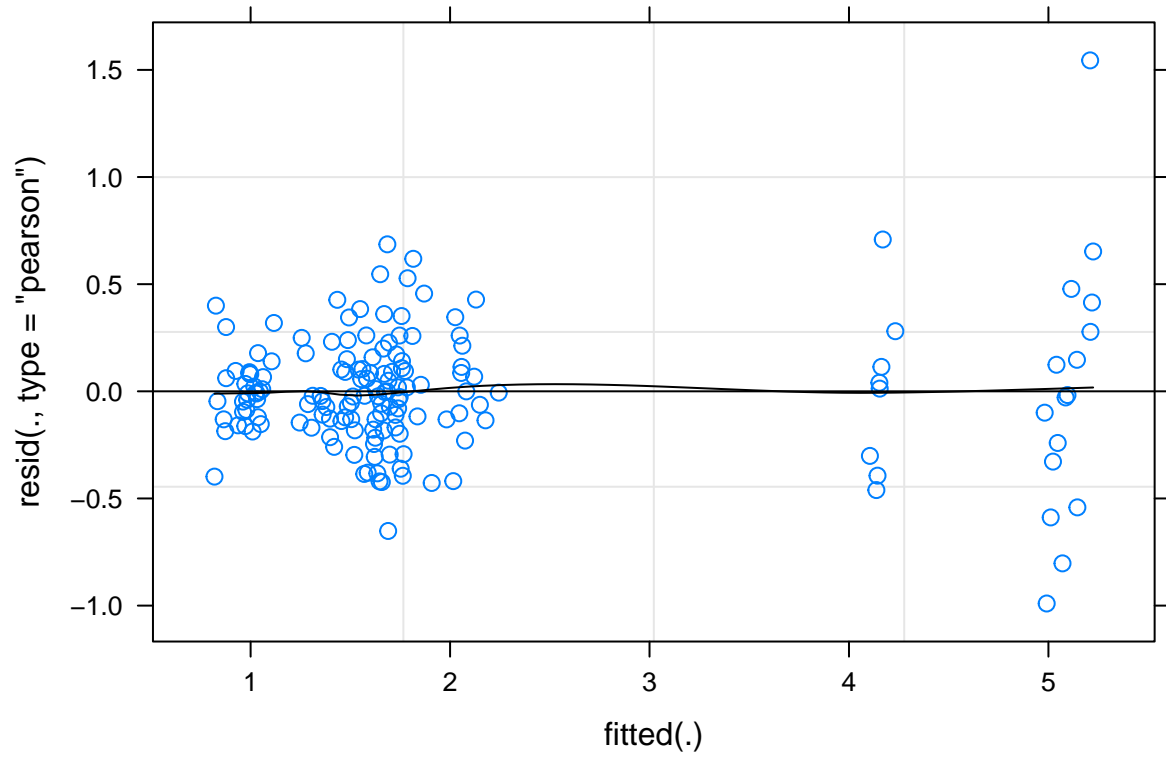
```
chronic_log_plot
```



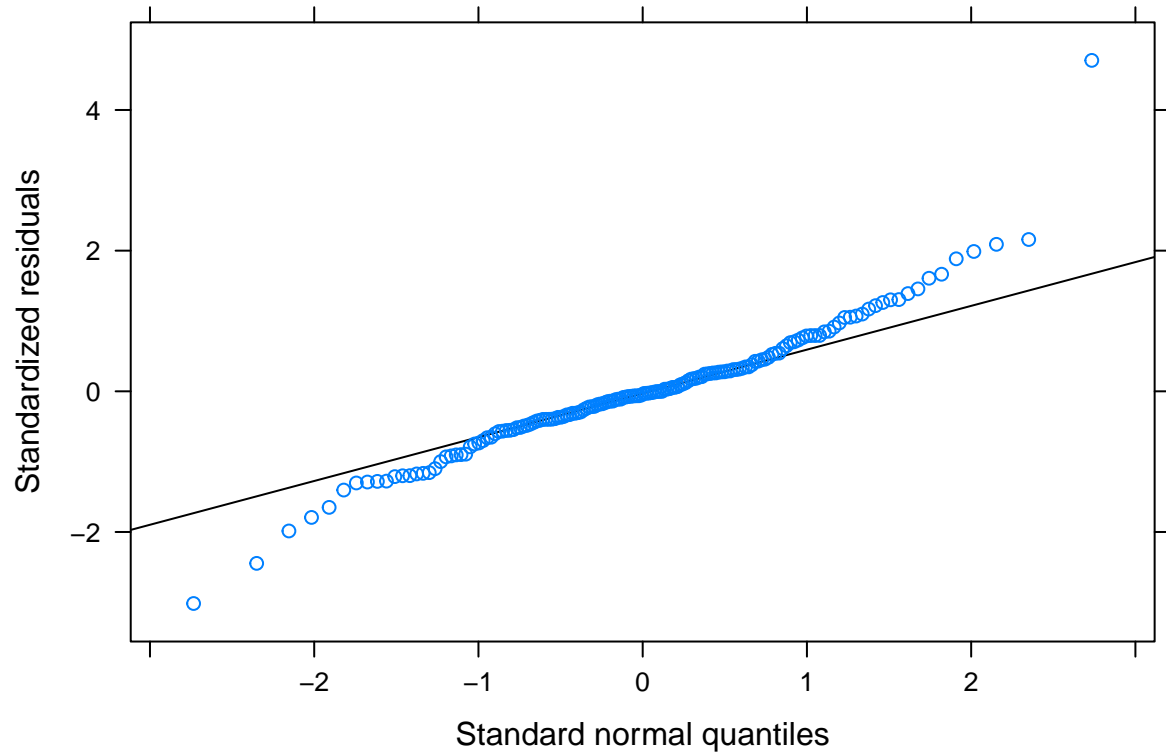
```
#ggsave(filename="../../figures/qRT-PCR/chronic_trt_log.svg", plot=chronic_log_plot, height=4, width=6)
```

Full analysis (all 5 groups) - not used

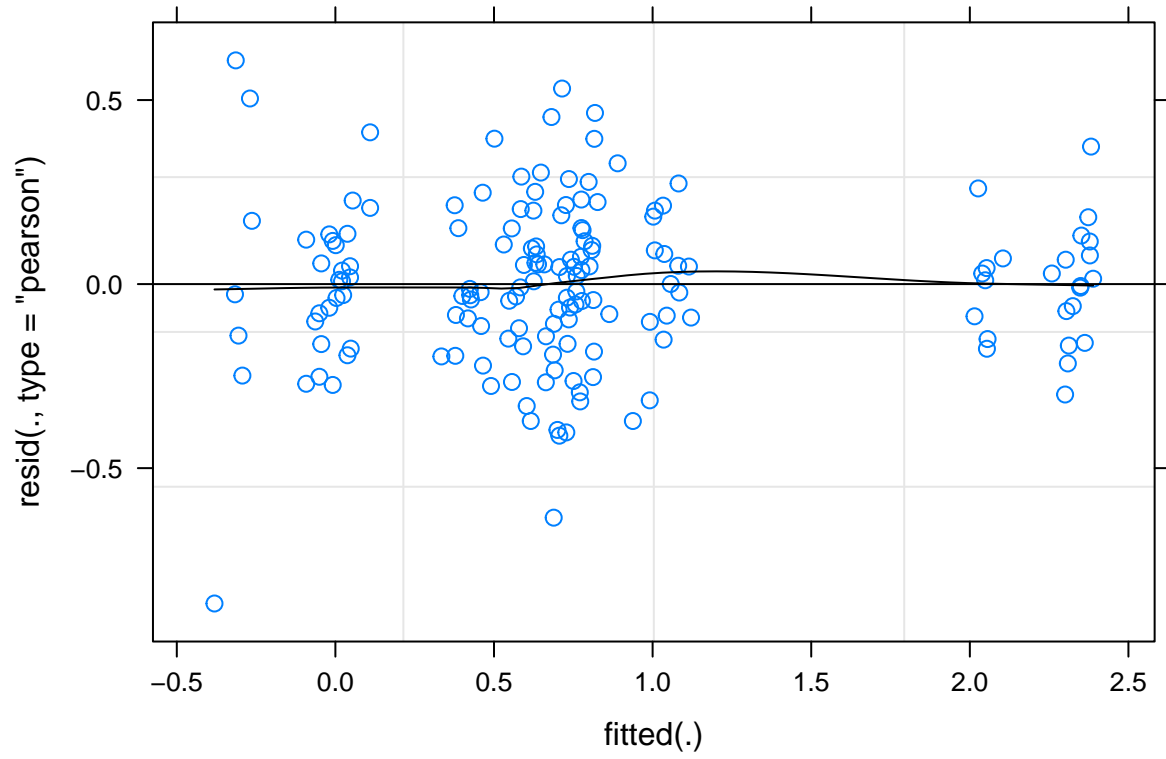
```
transporter_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=transporters_long)
plot(transporter_lm, type=c("p","smooth"), col.line=1)
```



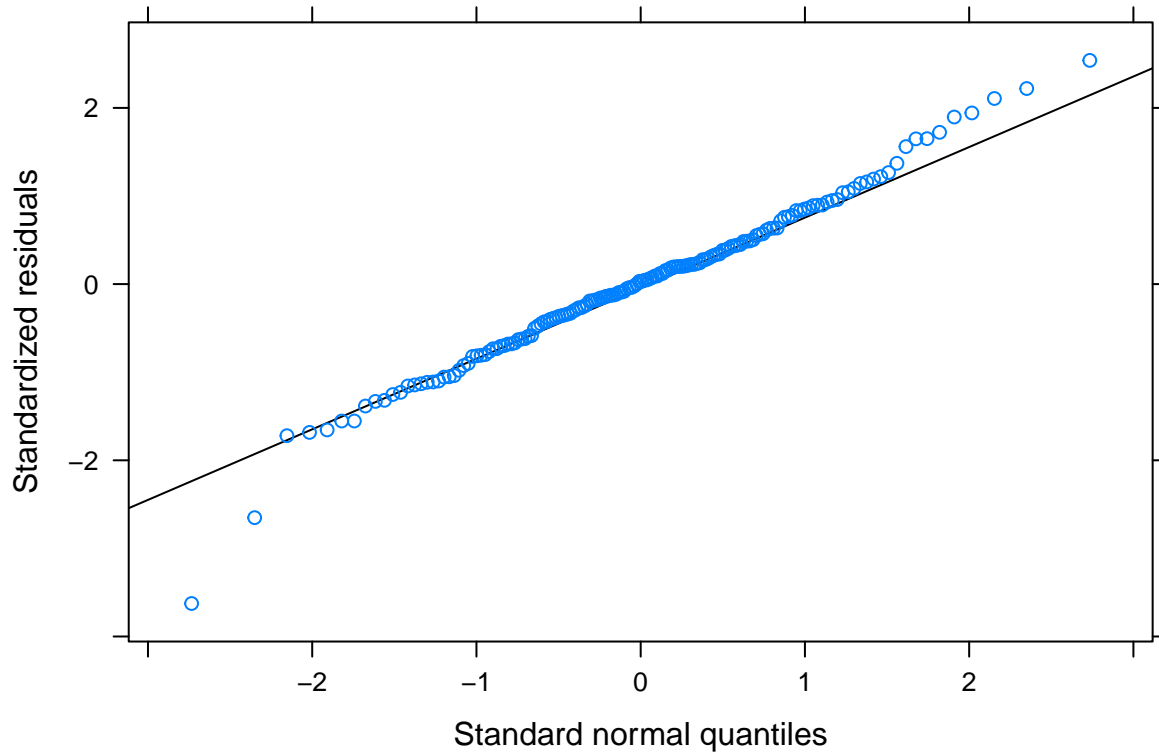
```
lattice::qqmath(transporter_lm)
```



```
transporter_log2_lm <- lmer(log2(rge) ~ gene * Tissue * Treatment + (1|Sample), data=transporters_long)
plot(transporter_log2_lm, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(transporter_log2_lm)
```



```
shapiro.test(transporters_long$rge)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  transporters_long$rge
## W = 0.75086, p-value = 3.492e-15
```

```
shapiro.test(log2(transporters_long$rge))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(transporters_long$rge)
## W = 0.92972, p-value = 4.647e-07
```

```
anova(transporter_log2_lm)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##
```

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
gene	6.9904	6.9904	1	105	122.0416	< 2.2e-16 ***
Tissue	29.0690	29.0690	1	105	507.4983	< 2.2e-16 ***
Treatment	17.8233	4.4558	4	35	77.7915	< 2.2e-16 ***
gene:Tissue	5.8013	5.8013	1	105	101.2816	< 2.2e-16 ***
gene:Treatment	5.6966	1.4241	4	105	24.8634	1.699e-14 ***
Tissue:Treatment	1.1712	0.2928	4	105	5.1118	0.0008386 ***
gene:Tissue:Treatment	1.6600	0.4150	4	105	7.2453	3.424e-05 ***

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(transporter_log2_lm, pairwise ~ Treatment | Tissue, by="gene")$contrasts
```

```
## Tissue = ret, gene = Pgp:
```

contrast	estimate	SE	df	t.ratio	p.value
S - M	-0.6263	0.122	136	-5.124	<.0001
S - Flo	-0.7575	0.140	136	-5.398	<.0001
S - Fhi	-0.7569	0.113	136	-6.694	<.0001
S - C	0.3061	0.127	136	2.415	0.1176
M - Flo	-0.1312	0.143	136	-0.915	0.8909
M - Fhi	-0.1306	0.117	136	-1.117	0.7974
M - C	0.9324	0.130	136	7.162	<.0001
Flo - Fhi	0.0006	0.136	136	0.004	1.0000
Flo - C	1.0636	0.147	136	7.221	<.0001
Fhi - C	1.0630	0.122	136	8.740	<.0001

```
## Tissue = hyp, gene = Pgp:
```

contrast	estimate	SE	df	t.ratio	p.value
S - M	-1.2693	0.122	136	-10.383	<.0001
S - Flo	-1.5800	0.140	136	-11.260	<.0001
S - Fhi	-1.5491	0.113	136	-13.700	<.0001
S - C	0.0809	0.127	136	0.638	0.9685
M - Flo	-0.3108	0.143	136	-2.167	0.1986
M - Fhi	-0.2798	0.117	136	-2.394	0.1232
M - C	1.3502	0.130	136	10.370	<.0001
Flo - Fhi	0.0310	0.136	136	0.228	0.9994
Flo - C	1.6610	0.147	136	11.276	<.0001
Fhi - C	1.6300	0.122	136	13.401	<.0001

```
## Tissue = ret, gene = Bcrp:
```

contrast	estimate	SE	df	t.ratio	p.value
S - M	-0.5798	0.122	136	-4.743	0.0001
S - Flo	-0.6456	0.140	136	-4.601	0.0001
S - Fhi	-0.4050	0.113	136	-3.582	0.0043
S - C	-0.0115	0.127	136	-0.091	1.0000
M - Flo	-0.0658	0.143	136	-0.459	0.9908
M - Fhi	0.1748	0.117	136	1.496	0.5671
M - C	0.5683	0.130	136	4.365	0.0002
Flo - Fhi	0.2406	0.136	136	1.773	0.3934
Flo - C	0.6340	0.147	136	4.304	0.0003
Fhi - C	0.3934	0.122	136	3.235	0.0131

```
## Tissue = hyp, gene = Bcrp:
```

contrast	estimate	SE	df	t.ratio	p.value
S - M	-0.2989	0.122	136	-2.445	0.1097
S - Flo	-0.4793	0.140	136	-3.416	0.0074
S - Fhi	-0.5519	0.113	136	-4.881	<.0001
S - C	-0.2040	0.127	136	-1.609	0.4941
M - Flo	-0.1804	0.143	136	-1.258	0.7174
M - Fhi	-0.2530	0.117	136	-2.164	0.1996
M - C	0.0949	0.130	136	0.729	0.9495
Flo - Fhi	-0.0726	0.136	136	-0.535	0.9835
Flo - C	0.2752	0.147	136	1.869	0.3393
Fhi - C	0.3479	0.122	136	2.860	0.0387


```
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
## P value adjustment: tukey method for comparing a family of 5 estimates
emmeans::emmeans(transporter_log2_lm, pairwise ~ Tissue | Treatment, by="gene")$contrasts

## Treatment = S, gene = Pgp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.780 0.113 105 -6.916 <.0001
##
## Treatment = M, gene = Pgp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -1.423 0.120 105 -11.893 <.0001
##
## Treatment = Flo, gene = Pgp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -1.603 0.151 105 -10.589 <.0001
##
## Treatment = Fhi, gene = Pgp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -1.572 0.102 105 -15.408 <.0001
##
## Treatment = C, gene = Pgp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -1.005 0.128 105 -7.859 <.0001
##
## Treatment = S, gene = Bcrp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.510 0.113 105 -4.519 <.0001
##
## Treatment = M, gene = Bcrp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.229 0.120 105 -1.913 0.0584
##
## Treatment = Flo, gene = Bcrp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.344 0.151 105 -2.270 0.0253
##
## Treatment = Fhi, gene = Bcrp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.657 0.102 105 -6.436 <.0001
##
## Treatment = C, gene = Bcrp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.702 0.128 105 -5.490 <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
emmeans::emmeans(transporter_log2_lm, pairwise ~ gene | Treatment, by="Tissue")$contrasts

## Treatment = S, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.0000 0.113 105 0.000 1.0000
```

```
##
## Treatment = M, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.0465 0.120 105 0.389 0.6983
##
## Treatment = Flo, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.1119 0.151 105 0.739 0.4614
##
## Treatment = Fhi, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.3519 0.102 105 3.448 0.0008
##
## Treatment = C, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp -0.3177 0.128 105 -2.483 0.0146
##
## Treatment = S, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.2704 0.113 105 2.397 0.0183
##
## Treatment = M, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.2408 0.120 105 10.369 <.0001
##
## Treatment = Flo, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.3712 0.151 105 9.059 <.0001
##
## Treatment = Fhi, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.2676 0.102 105 12.421 <.0001
##
## Treatment = C, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp -0.0146 0.128 105 -0.114 0.9095
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.

trans_sumstats <- transporters_long %>% group_by(Treatment, Tissue, gene) %>% summarise(
  n = n(),
  mean = mean(rge),
  sd = sd(rge),
  log2_mean = mean(log2(rge)),
  log2_sd = sd(log2(rge))
)

## `summarise()` has grouped output by 'Treatment', 'Tissue'. You can override
## using the `.groups` argument.

trans_sumstats

## # A tibble: 20 x 8
## # Groups: Treatment, Tissue [10]
## Treatment Tissue gene n mean sd log2_mean log2_sd
```

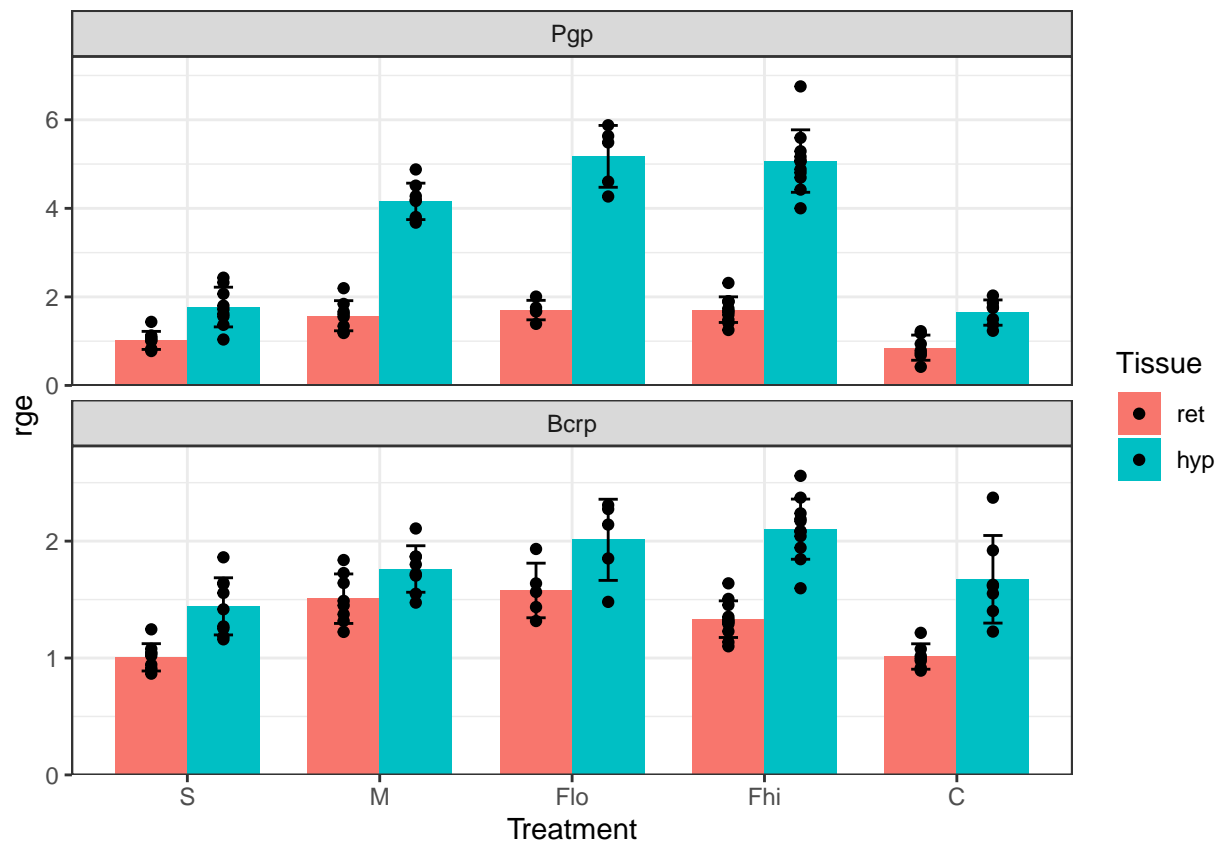
	<fct>	<fct>	<fct>	<int>	<dbl>	<dbl>	<dbl>	<dbl>	
##	1	S	ret	Pgp	9	1.02	0.204	-1.11e-11	0.280
##	2	S	ret	Bcrp	9	1.01	0.117	-4.39e-11	0.163
##	3	S	hyp	Pgp	9	1.77	0.448	7.80e- 1	0.385
##	4	S	hyp	Bcrp	9	1.44	0.244	5.10e- 1	0.242
##	5	M	ret	Pgp	8	1.57	0.340	6.26e- 1	0.305
##	6	M	ret	Bcrp	8	1.51	0.212	5.80e- 1	0.202
##	7	M	hyp	Pgp	8	4.16	0.411	2.05e+ 0	0.141
##	8	M	hyp	Bcrp	8	1.76	0.199	8.09e- 1	0.163
##	9	Flo	ret	Pgp	5	1.70	0.220	7.57e- 1	0.190
##	10	Flo	ret	Bcrp	5	1.58	0.234	6.46e- 1	0.209
##	11	Flo	hyp	Pgp	5	5.17	0.698	2.36e+ 0	0.201
##	12	Flo	hyp	Bcrp	5	2.01	0.347	9.89e- 1	0.268
##	13	Fhi	ret	Pgp	11	1.71	0.289	7.57e- 1	0.242
##	14	Fhi	ret	Bcrp	11	1.33	0.157	4.05e- 1	0.168
##	15	Fhi	hyp	Pgp	11	5.07	0.705	2.33e+ 0	0.193
##	16	Fhi	hyp	Bcrp	11	2.10	0.257	1.06e+ 0	0.182
##	17	C	ret	Pgp	7	0.853	0.284	-3.06e- 1	0.527
##	18	C	ret	Bcrp	7	1.01	0.109	1.15e- 2	0.150
##	19	C	hyp	Pgp	7	1.65	0.285	6.99e- 1	0.258
##	20	C	hyp	Bcrp	7	1.67	0.375	7.14e- 1	0.308

Comparing hyp vs ret within each treatment/gene

```
trans_hyp_ret_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=mean-sd, ymax=mean+sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, scales="free_y", ncol=1) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_hyp_ret_plot
```

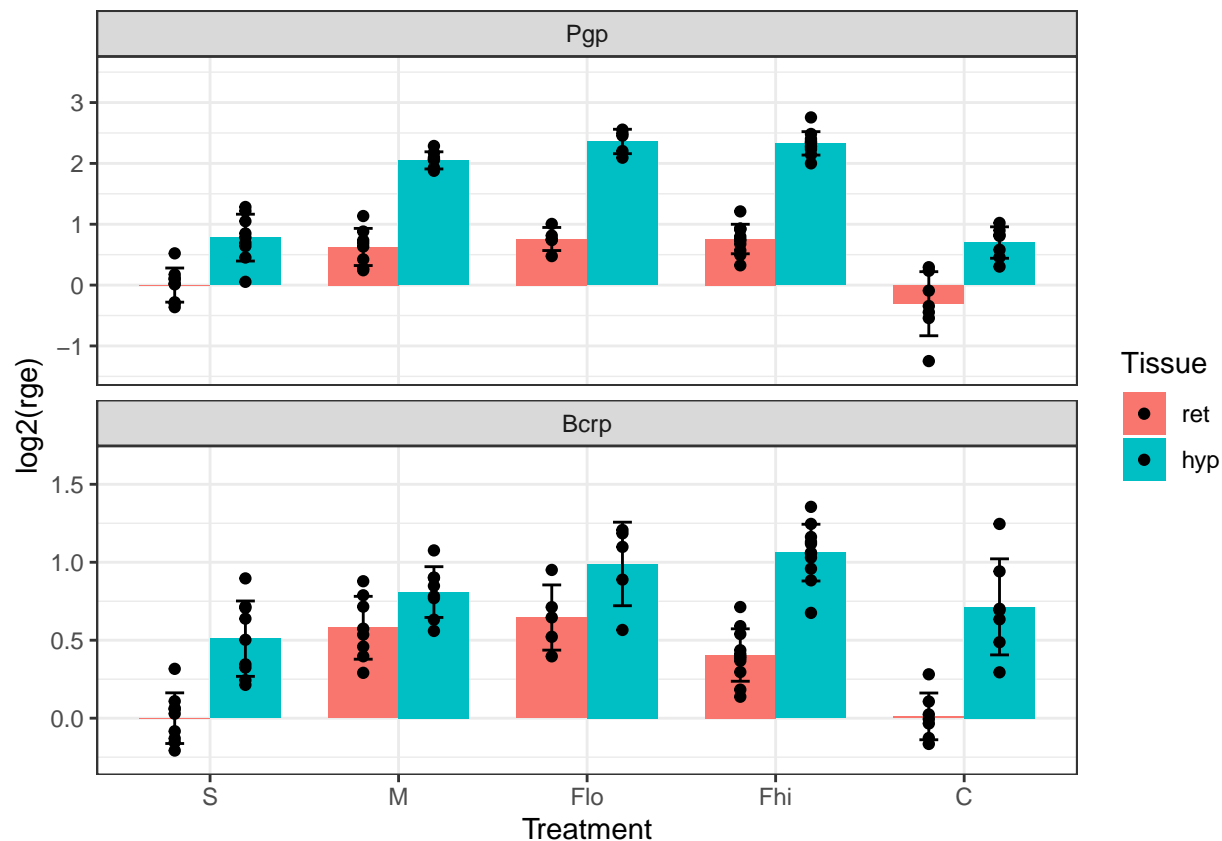


```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret.png", plot=trans_hyp_ret_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret.svg", plot=trans_hyp_ret_plot, height=4, width=6)
```

```
trans_hyp_ret_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=log2_mean-log2_sd,
    ymax=log2_mean+log2_sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, scales="free_y", ncol=1) +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_hyp_ret_log_plot
```



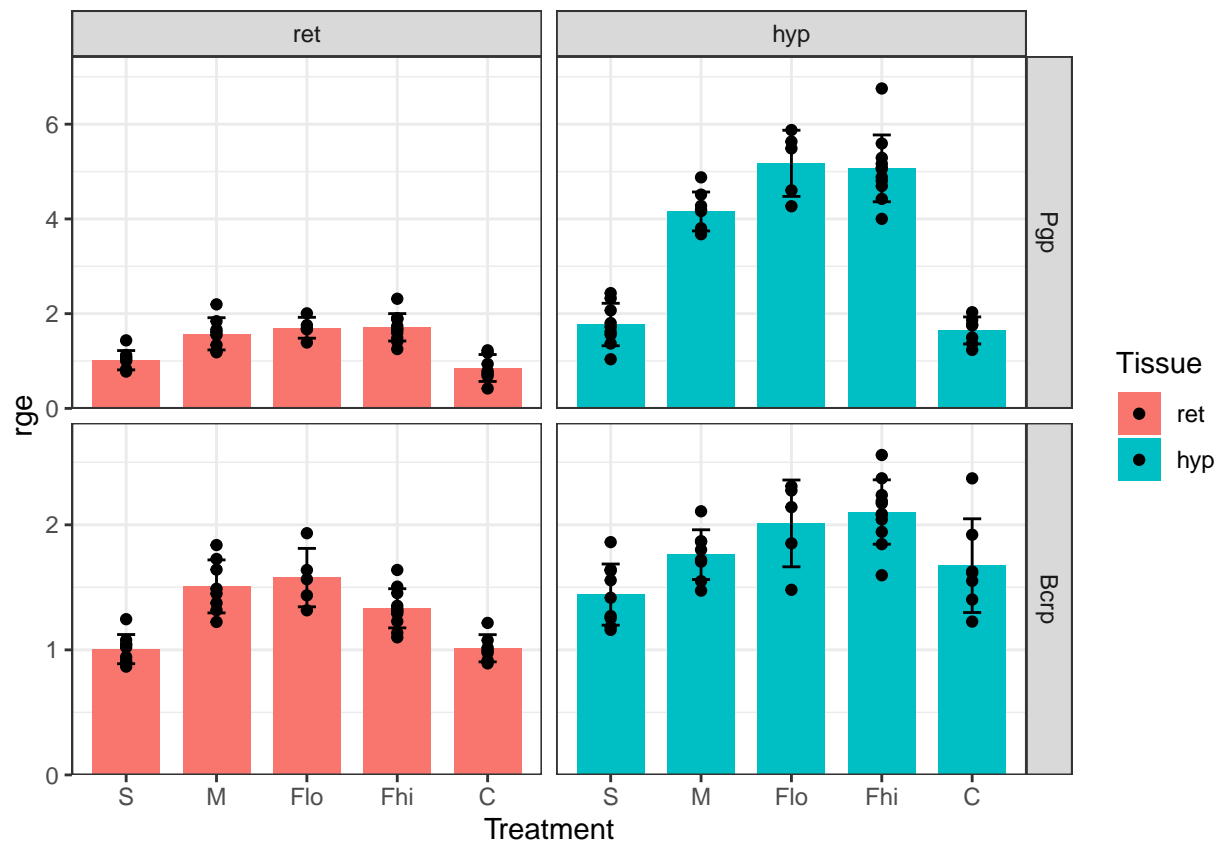
```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log.png", plot=trans_hyp_ret_log_plot, height=4, width=10)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log.svg", plot=trans_hyp_ret_log_plot, height=4, width=10)
```

Comparing treatments within each tissue/gene

```
trans_trt_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=mean-sd, ymax=mean+sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(gene~Tissue, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_trt_plot
```

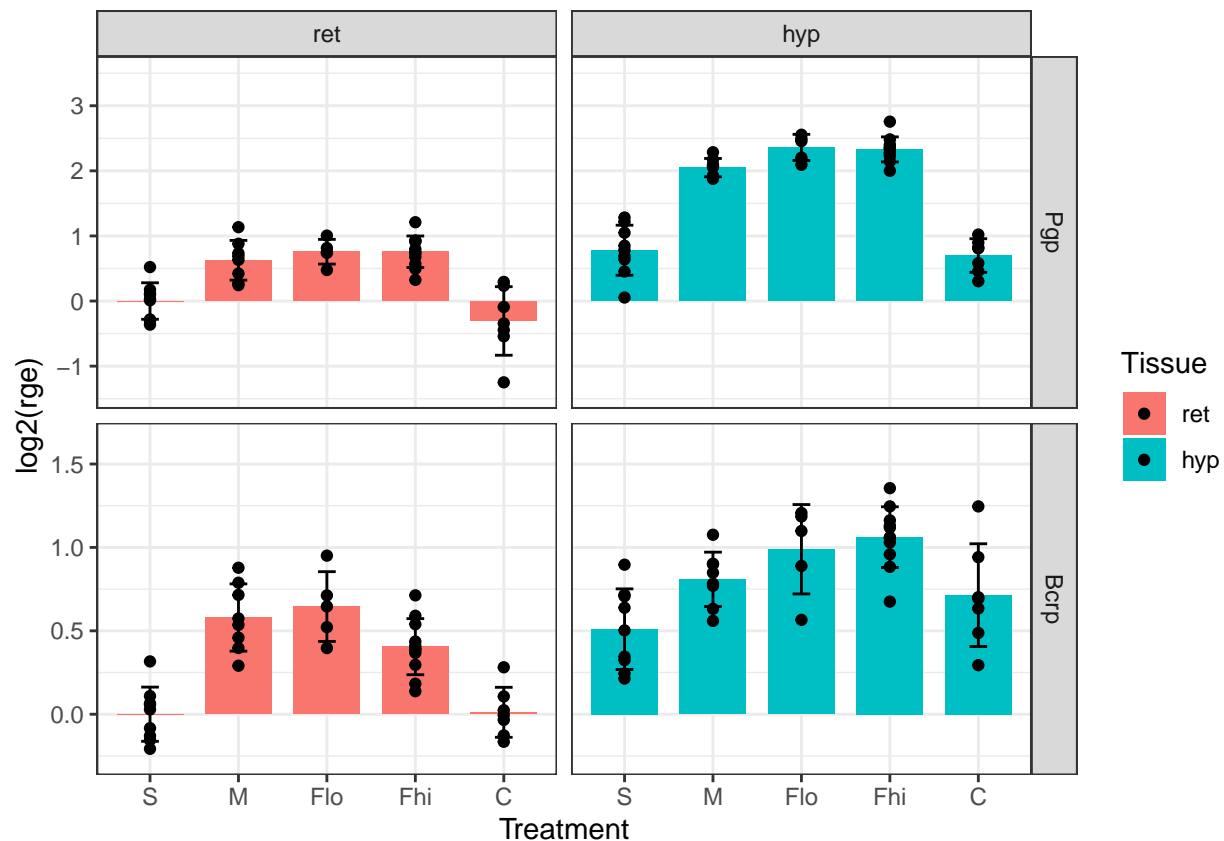


```
#ggsave(filename="../../figures/qRT-PCR/trans_trt.png", plot=trans_trt_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_trt.svg", plot=trans_trt_plot, height=4, width=6)
```

```
trans_trt_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=log2_mean-log2_sd,
    ymax=log2_mean+log2_sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(gene~Tissue, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_trt_log_plot
```

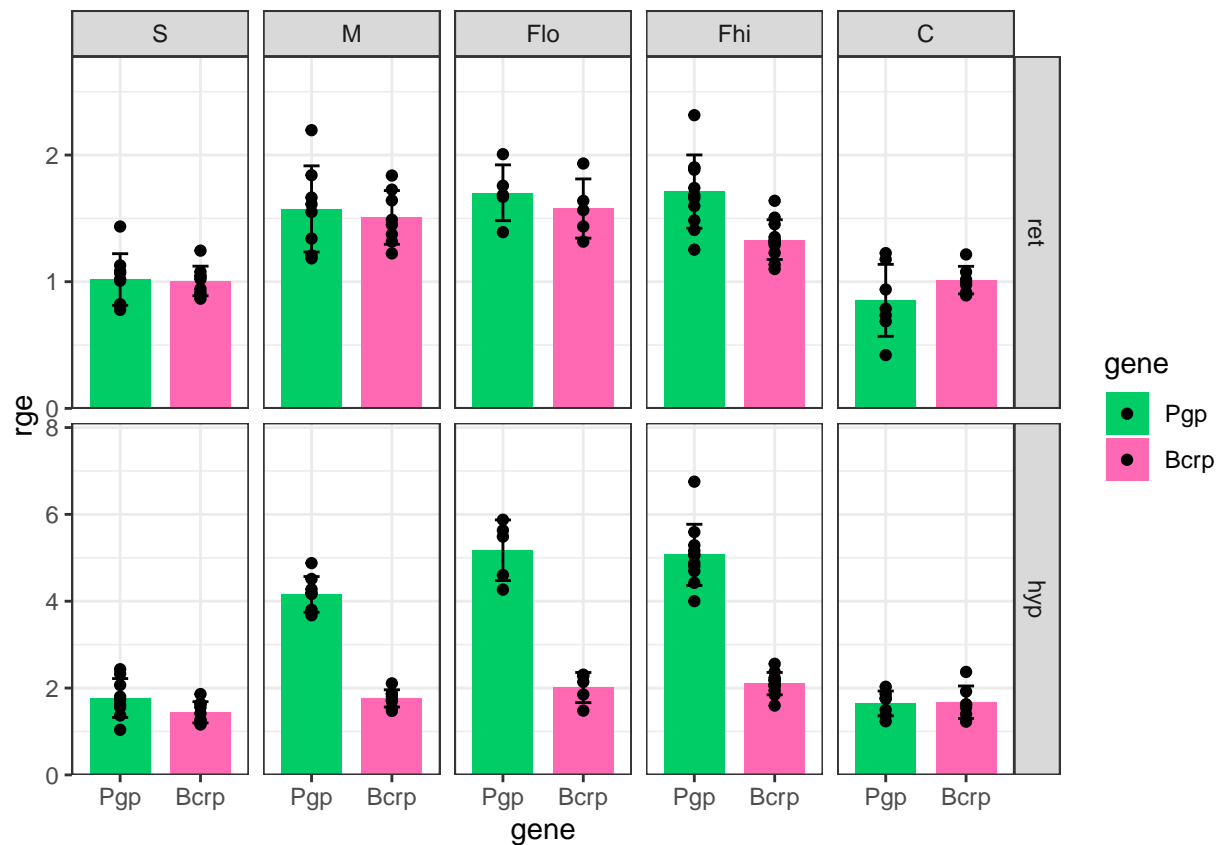


```
#ggsave(filename="../../figures/qRT-PCR/trans_trt_log.png", plot=trans_trt_log_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_trt_log.svg", plot=trans_trt_log_plot, height=4, width=6)
```

```
trans_g2g_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=gene, y=rge, fill=gene),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=gene, ymin=mean-sd,
    ymax=mean+sd, fill=gene),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=gene, y=rge, fill=gene),
    position=position_dodge(width=0.75)) +
  facet_grid(Tissue~Treatment, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.2))) +
  scale_fill_manual(values=c("springgreen3", "hotpink")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_g2g_plot
```

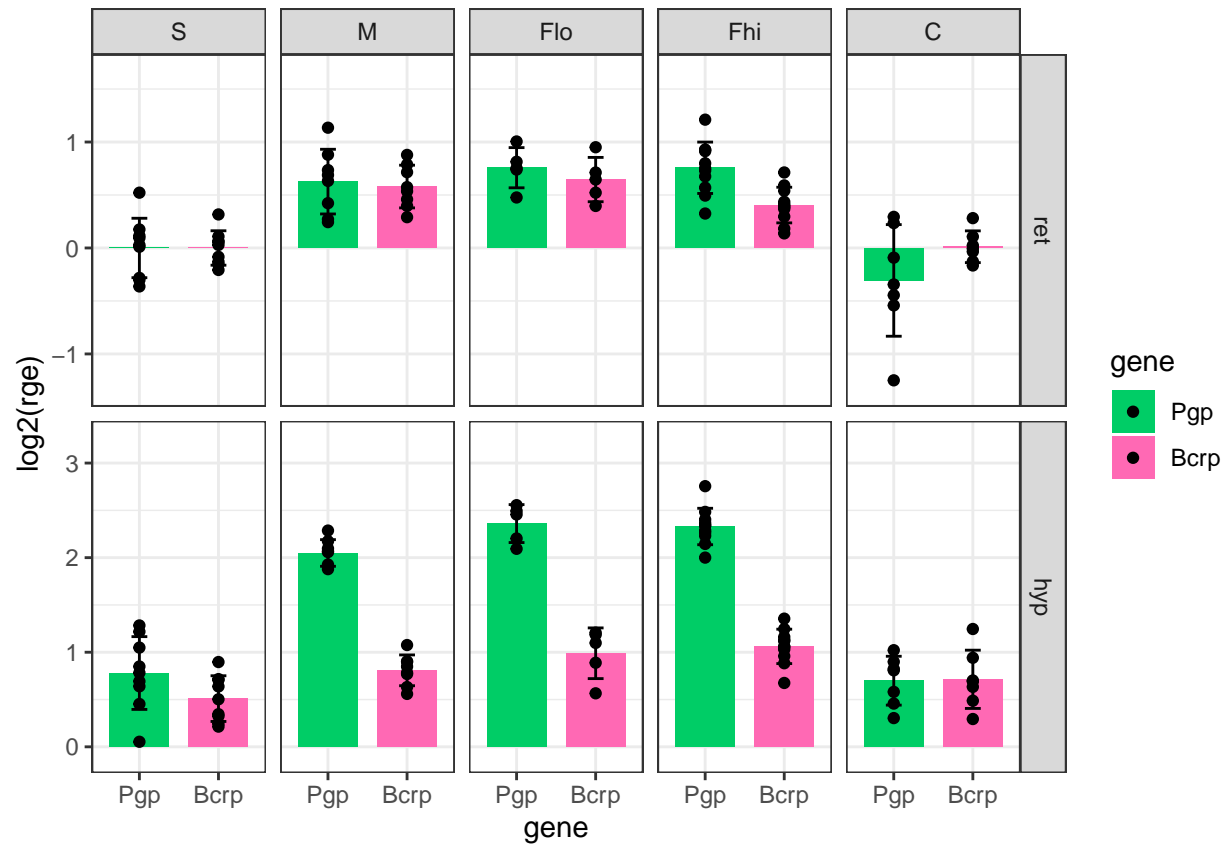


```
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene.png", plot=trans_g2g_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene.svg", plot=trans_g2g_plot, height=4, width=6)
```

```
trans_g2g_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=gene, y=log2(rge), fill=gene),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=gene, ymin=log2_mean-log2_sd,
    ymax=log2_mean+log2_sd, fill=gene),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=gene, y=log2(rge), fill=gene),
    position=position_dodge(width=0.75)) +
  facet_grid(Tissue~Treatment, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  scale_fill_manual(values=c("springgreen3", "hotpink")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_g2g_log_plot
```

```
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_log.png", plot=trans_g2g_log_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_log.svg", plot=trans_g2g_log_plot, height=4, width=6)
```