

qPCR Transporter Figures

Analysis of chronic morphine effect on P-gp and Bcrp

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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", "Fhi", "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", va

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

```

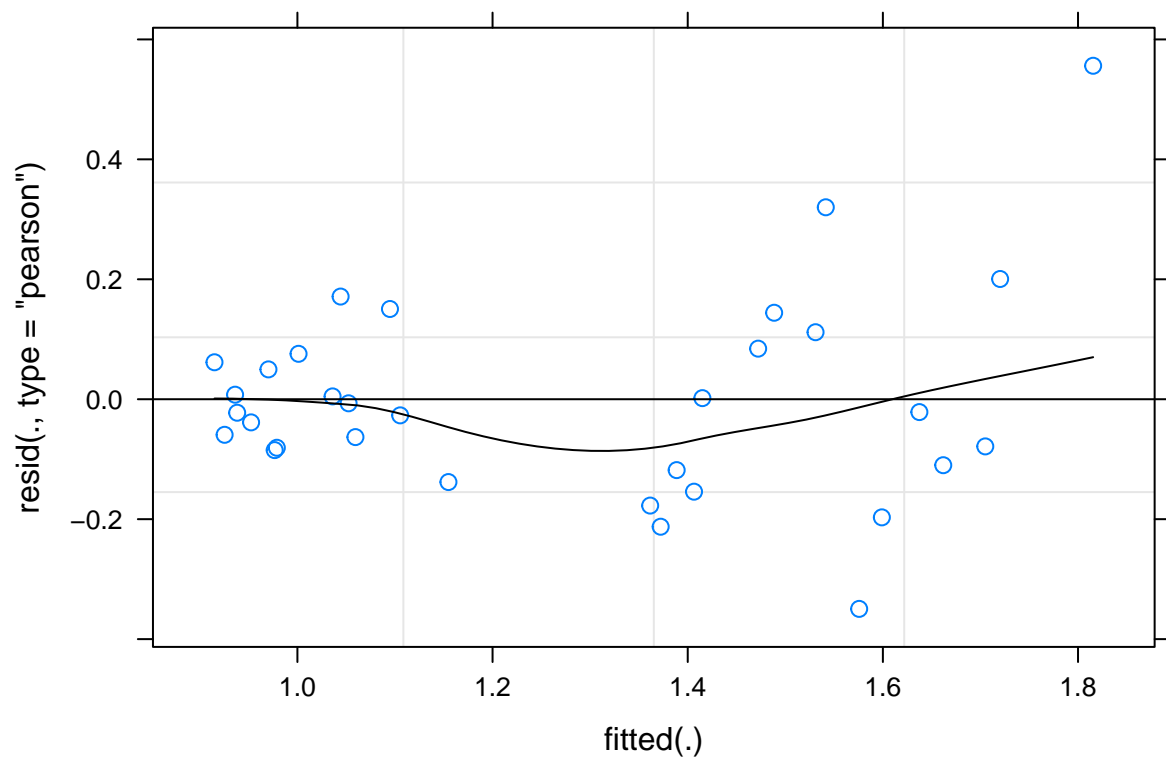
```

##   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

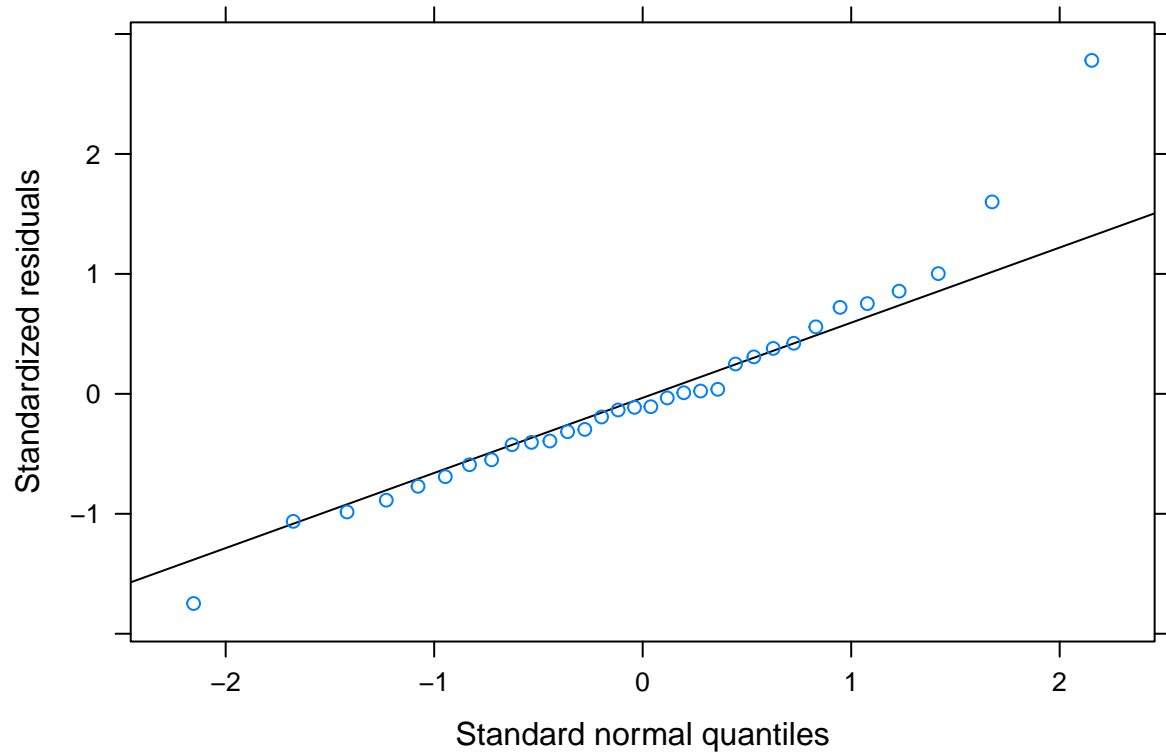
```

```
## 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
```

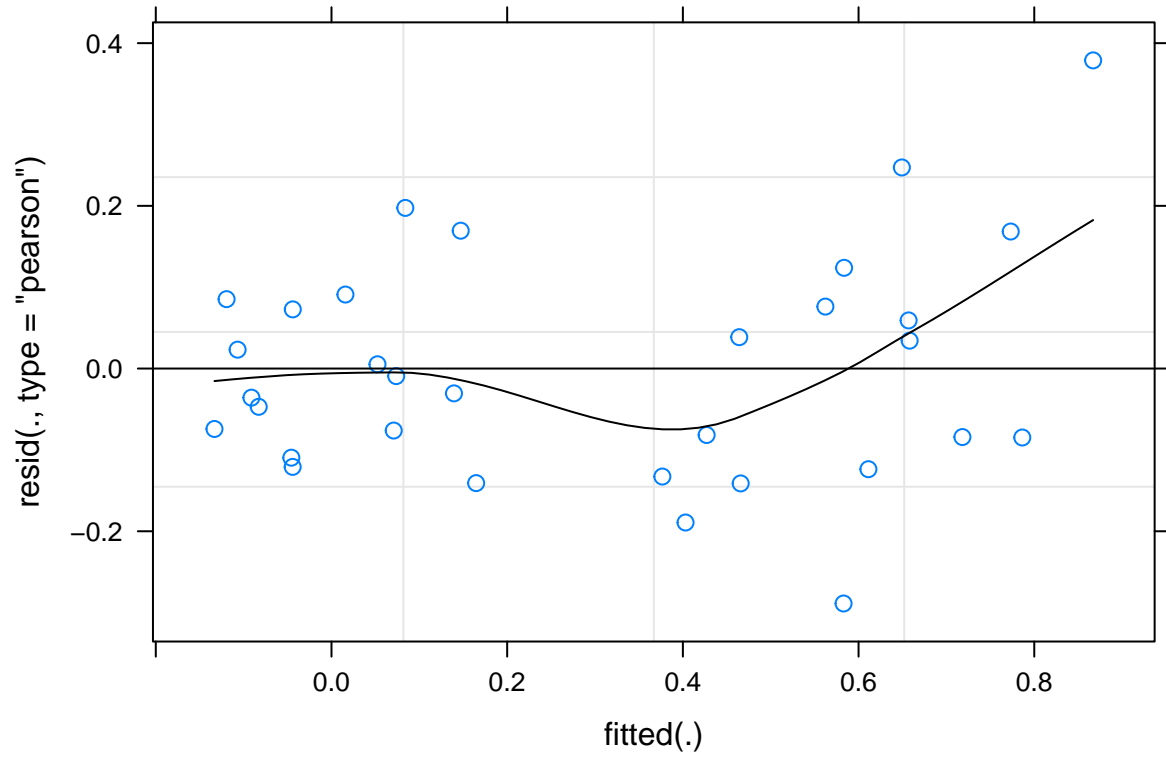
```
c_bcrp_lm <- lmer(Bcrp.RGE ~ Tissue * Treatment + (1|Sample), data=chronic)
plot(c_bcrp_lm, type=c("p","smooth"), col.line=1)
```



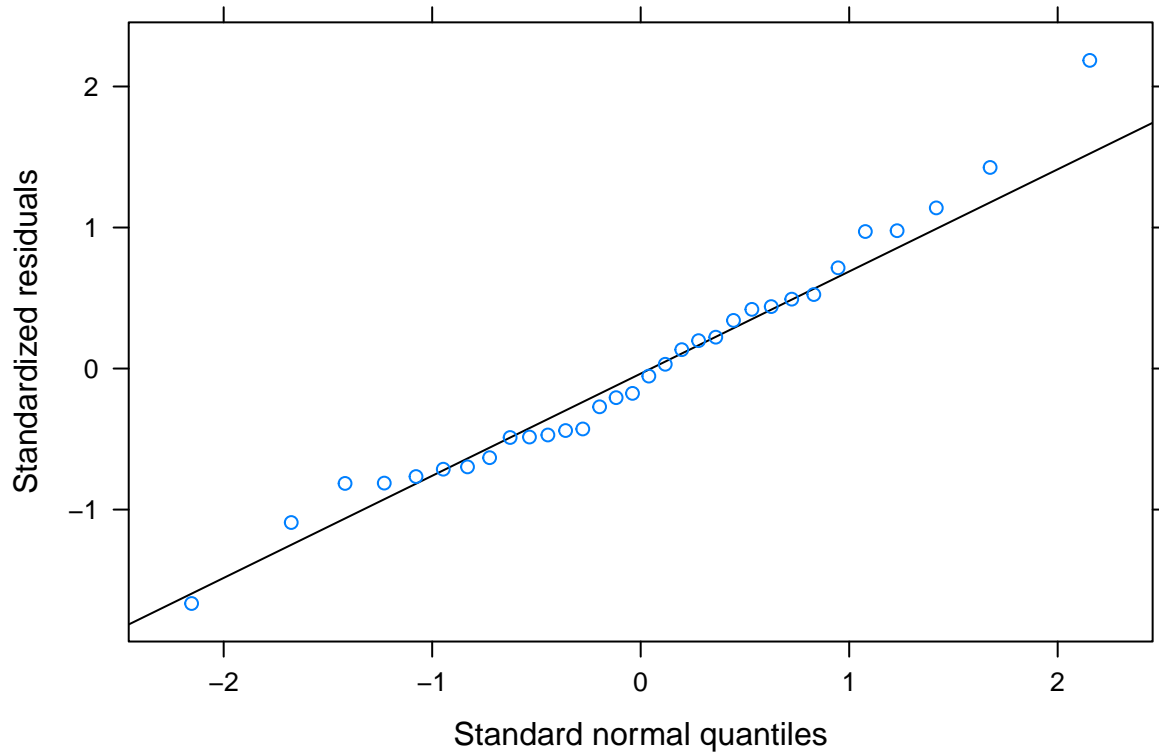
```
lattice::qqmath(c_bcrp_lm)
```



```
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
```

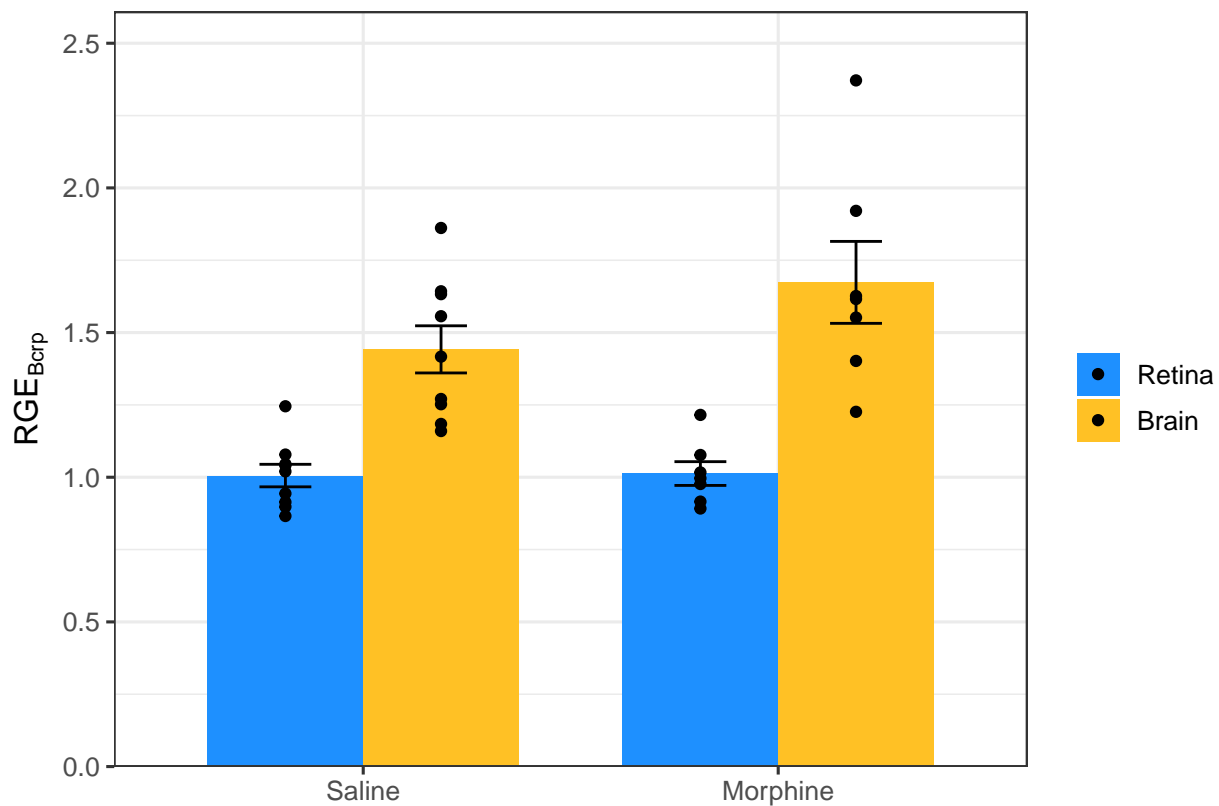
```

#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=0.25, 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) %+replace%
  theme(legend.title = element_blank()) +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
    labels=c("Retina", "Brain")) +
  scale_x_discrete(labels=c("S" = "Saline", "C" = "Morphine")) +
  xlab("") +
  ylab(expression(RGE[Bcrp]))

```

Warning: Ignoring unknown aesthetics: fill

c_bcrp_plot

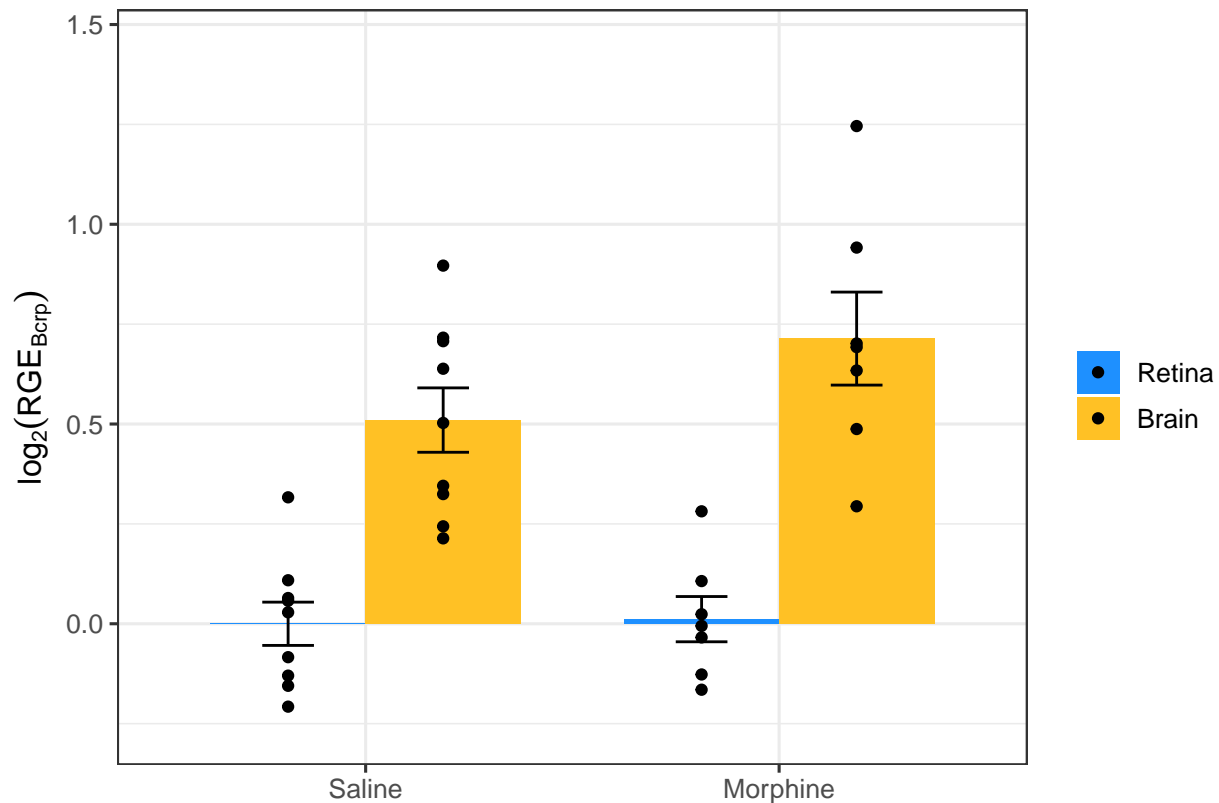



```
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_effect_plot.png", plot=c_bcrp_plot, height=4, width=10)
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_effect_plot.svg", plot=c_bcrp_plot, height=4, width=10)
```

```
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=0.25, 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) %+replace%
  theme(legend.title = element_blank()) +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
    labels=c("Retina", "Brain")) +
  scale_x_discrete(labels=c("S" = "Saline", "C" = "Morphine")) +
  xlab("") +
  ylab(expression(log2[RGEBcrp]))
```

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

```
c_bcrp_log_plot
```

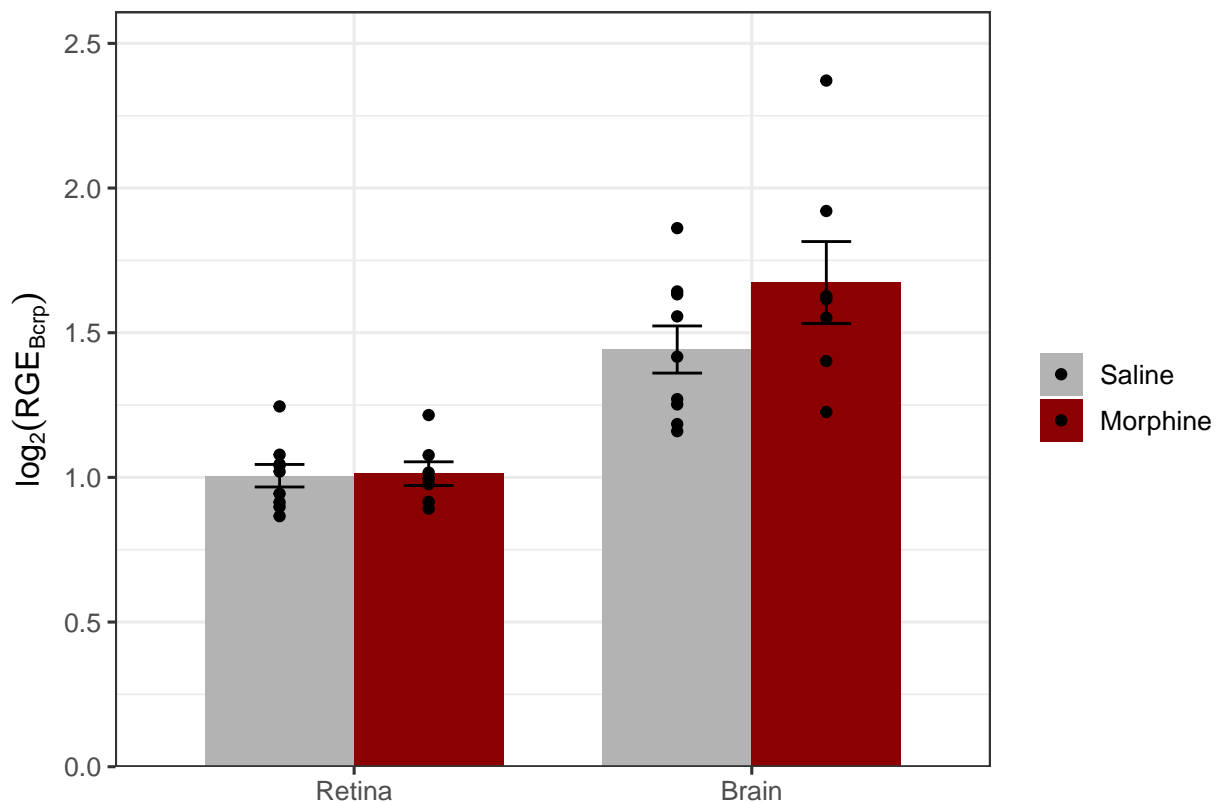


```
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_log_plot.png", plot=c_bcrp_log_plot, height=4, width=4)
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_log_plot.svg", plot=c_bcrp_log_plot, height=4, width=4)
```

```
#visualizing treatment effect per tissue
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-se,
    ymax=mean+se, fill=Treatment),
    width=0.25, 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)) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  theme_bw(base_size = 12) %+replace%
  theme(legend.title = element_blank()) +
  scale_fill_manual(values=c("gray70", "red4"),
    labels=c("Saline", "Morphine")) +
  scale_x_discrete(labels=c("ret" = "Retina", "hyp" = "Brain")) +
  xlab("") +
  ylab(expression(log2(RGE[Bcrp])))
```

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

```
c_bcrp_tiss_plot
```

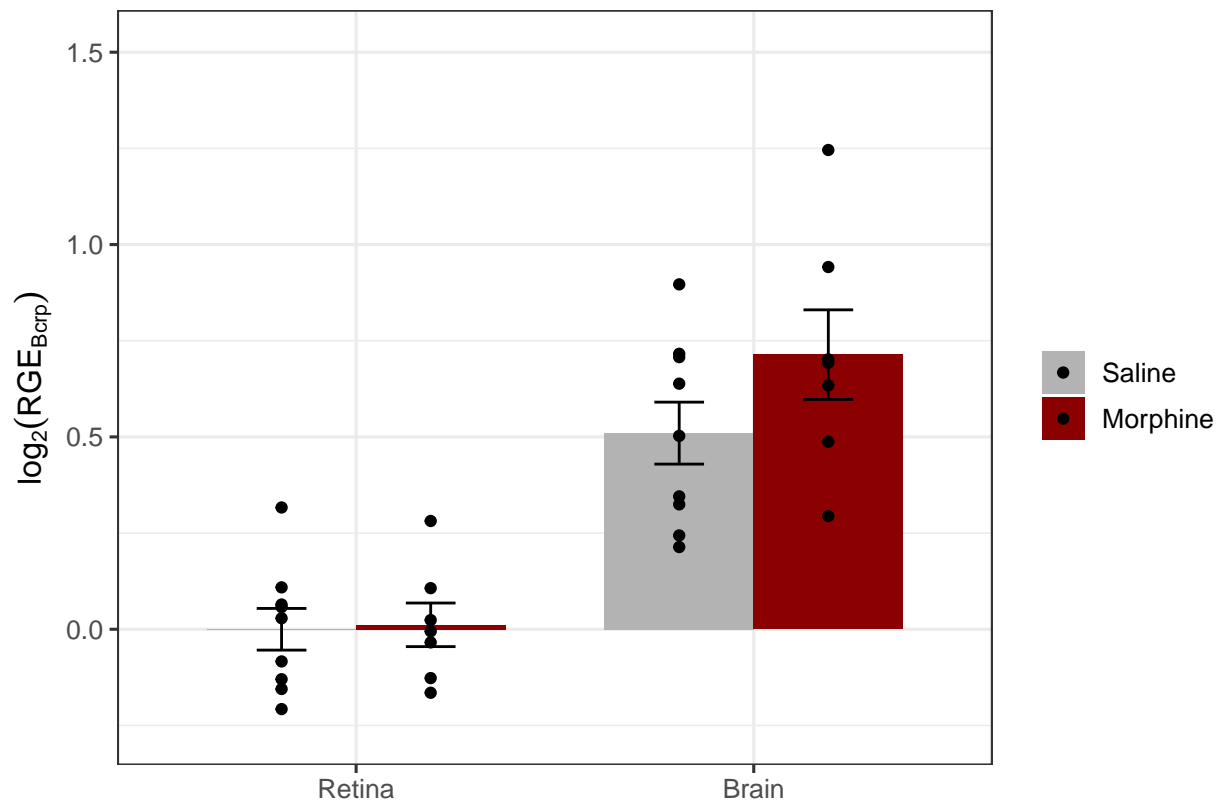


```
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_plot.png", plot=c_bcrp_tiss_plot, height=4, width=4)
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_plot.svg", plot=c_bcrp_tiss_plot, height=4, width=4)
```

```
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_se,
    ymax=log2_mean+log2_se, fill=Treatment),
    width=0.25, 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)) +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  theme_bw(base_size = 12) %+replace%
  theme(legend.title = element_blank()) +
  scale_fill_manual(values=c("gray70", "red4"),
    labels=c("Saline", "Morphine")) +
  scale_x_discrete(labels=c("ret" = "Retina", "hyp" = "Brain")) +
  xlab("") +
  ylab(expression(log2[RGEBcrp]))
```

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

```
c_bcrp_tiss_log_plot
```



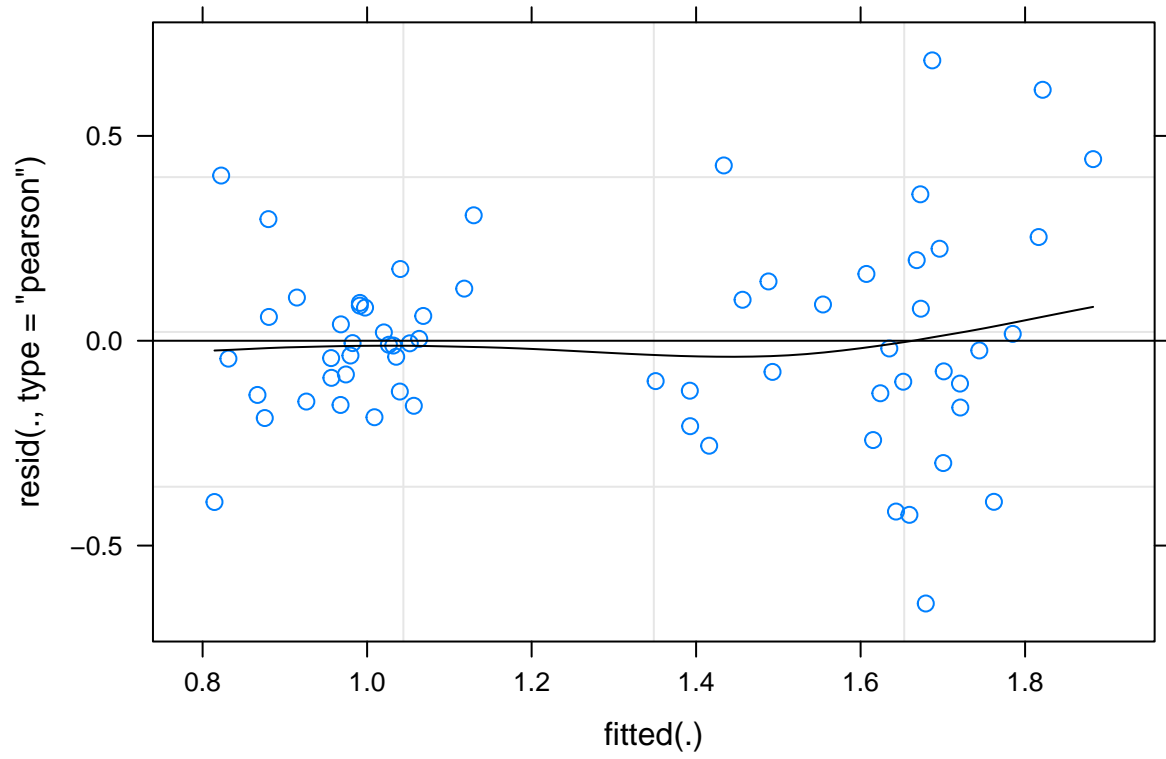
```
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_log_plot.png", plot=c_bcrp_tiss_log_plot, height=4,
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_log_plot.svg", plot=c_bcrp_tiss_log_plot, height=4,
```

Chronic morphine Bcrp test WITH P-gp in stats

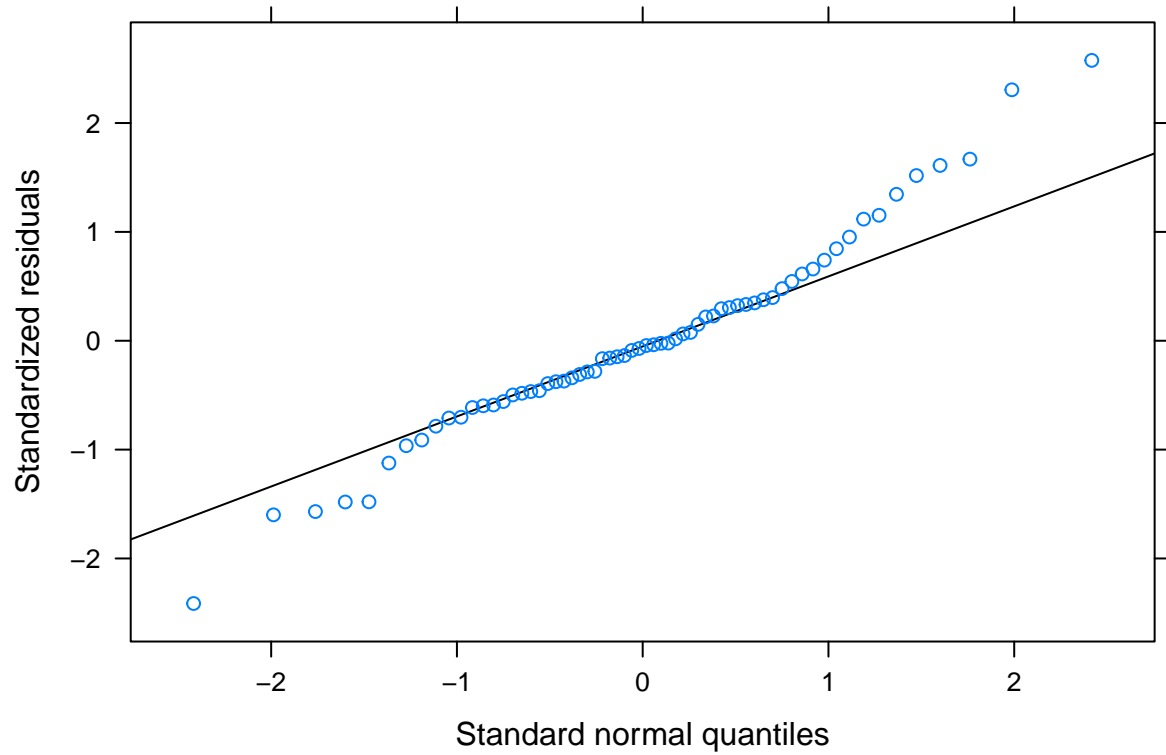
```
#bcrp & pgp together
chronic_long <- transporters_long %>% filter(Treatment %in% c("C", "S"))
chronic_long
```

```
## # 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
```

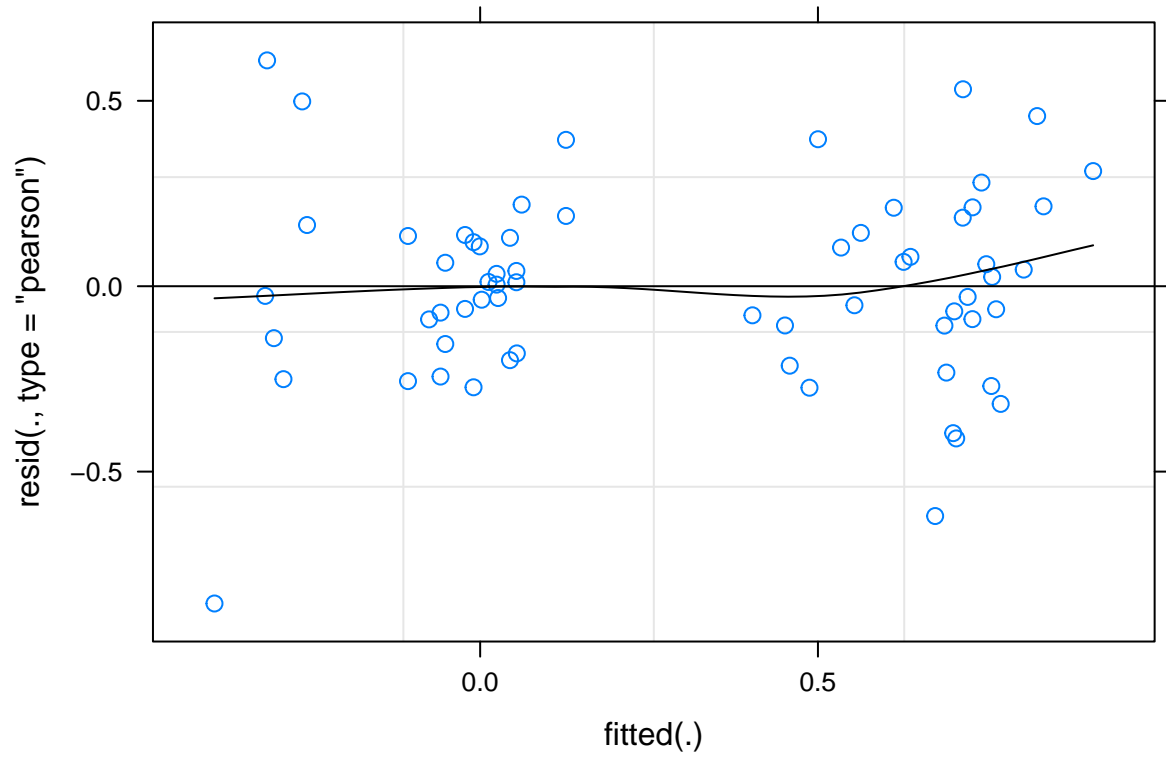
```
chronic_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=chronic_long)
plot(chronic_lm, type=c("p", "smooth"), col.line=1)
```



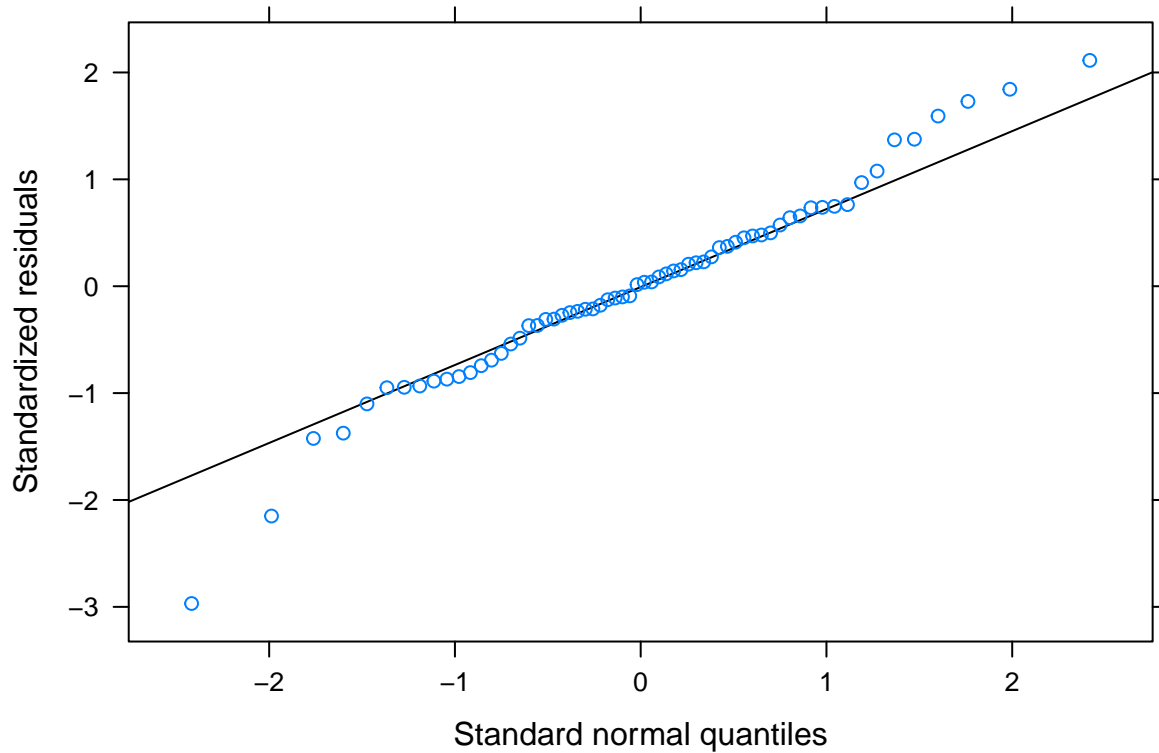
```
lattice::qqmath(chronic_lm)
```



```
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),
  se = sd/sqrt(n),
  log2_mean = mean(log2(rge)),
  log2_sd = sd(log2(rge)),
  log2_se = log2_sd/sqrt(n)
)
```

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

```
chronic_sumstats
```

```
## # A tibble: 8 x 10
## # Groups:   Treatment, Tissue [4]
##   Treatment Tissue gene      n mean      sd      se log2_mean log2_sd log2_se
##   <fct>      <fct> <fct> <int> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
## 1 S        ret    Pgp     9 1.02  0.204 0.0680 -1.11e-11  0.280  0.0935
## 2 S        ret    Bcrp     9 1.01  0.117 0.0389 -4.39e-11  0.163  0.0542
## 3 S        hyp    Pgp     9 1.77  0.448 0.149  7.80e- 1  0.385  0.128
## 4 S        hyp    Bcrp     9 1.44  0.244 0.0815  5.10e- 1  0.242  0.0806
## 5 C        ret    Pgp     7 0.853 0.284 0.107 -3.06e- 1  0.527  0.199
## 6 C        ret    Bcrp     7 1.01  0.109 0.0411  1.15e- 2  0.150  0.0567
## 7 C        hyp    Pgp     7 1.65  0.285 0.108  6.99e- 1  0.258  0.0976
## 8 C        hyp    Bcrp     7 1.67  0.375 0.142  7.14e- 1  0.308  0.116
```

```
chronic_plot <- ggplot() +
  stat_summary(data=chronic_long, aes(x=Tissue, y=rge, fill=Treatment),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=chronic_sumstats, aes(x=Tissue, ymin=mean-sd,
    ymax=mean+sd, fill=Treatment),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=chronic_long, aes(x=Tissue, y=rge, fill=Treatment),
```

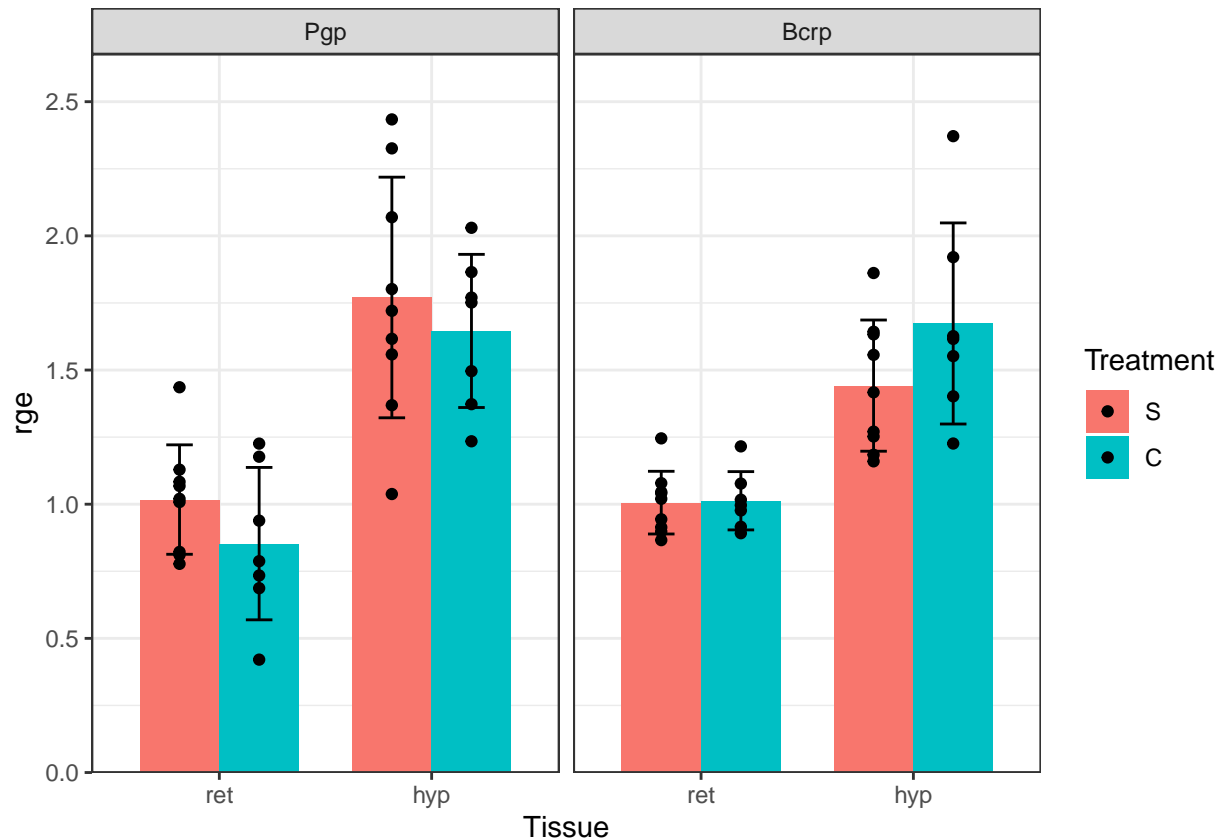
```

    stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(~gene, 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)

```

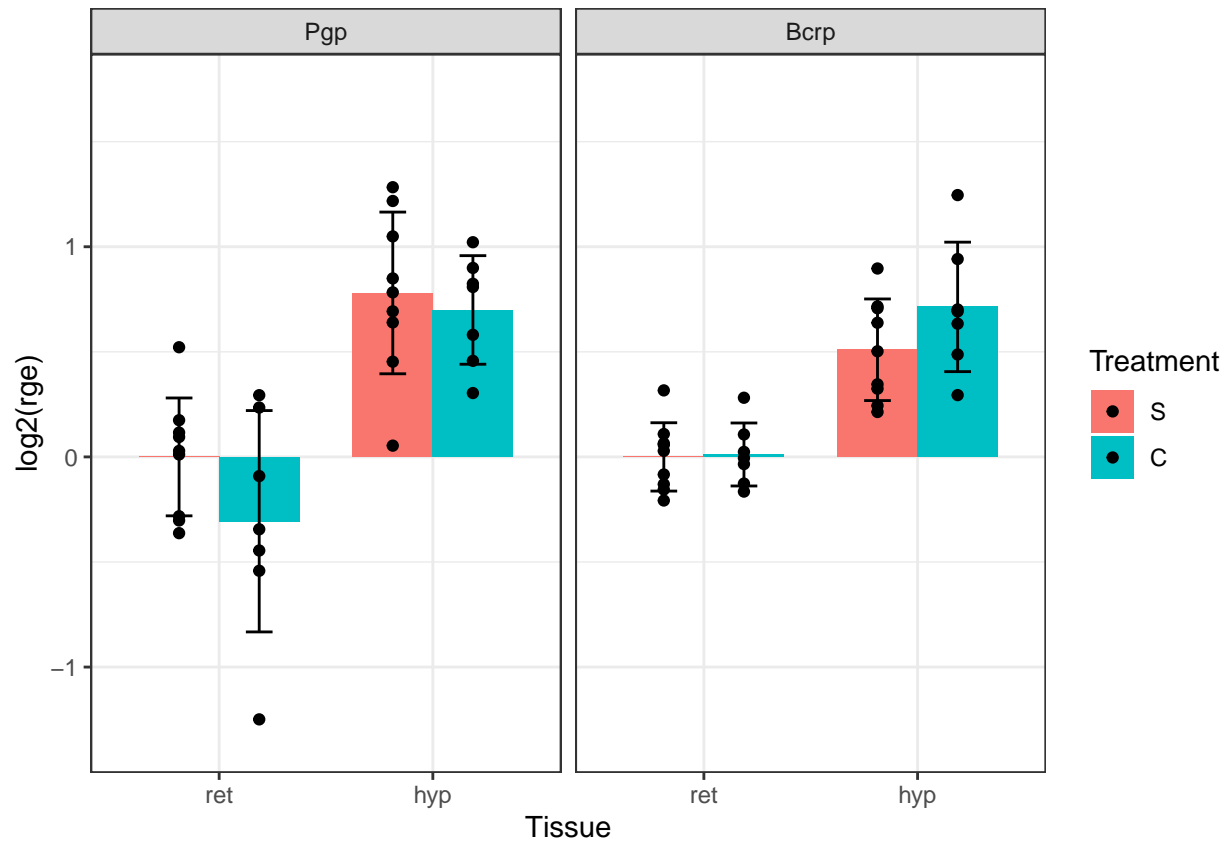
```

chronic_log_plot <- ggplot() +
  stat_summary(data=chronic_long, aes(x=Tissue, y=log2(rge), fill=Treatment),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=chronic_sumstats, aes(x=Tissue, ymin=log2_mean-log2_sd,
    ymax=log2_mean+log2_sd, fill=Treatment),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=chronic_long, aes(x=Tissue, y=log2(rge), fill=Treatment),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(~gene, 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
```

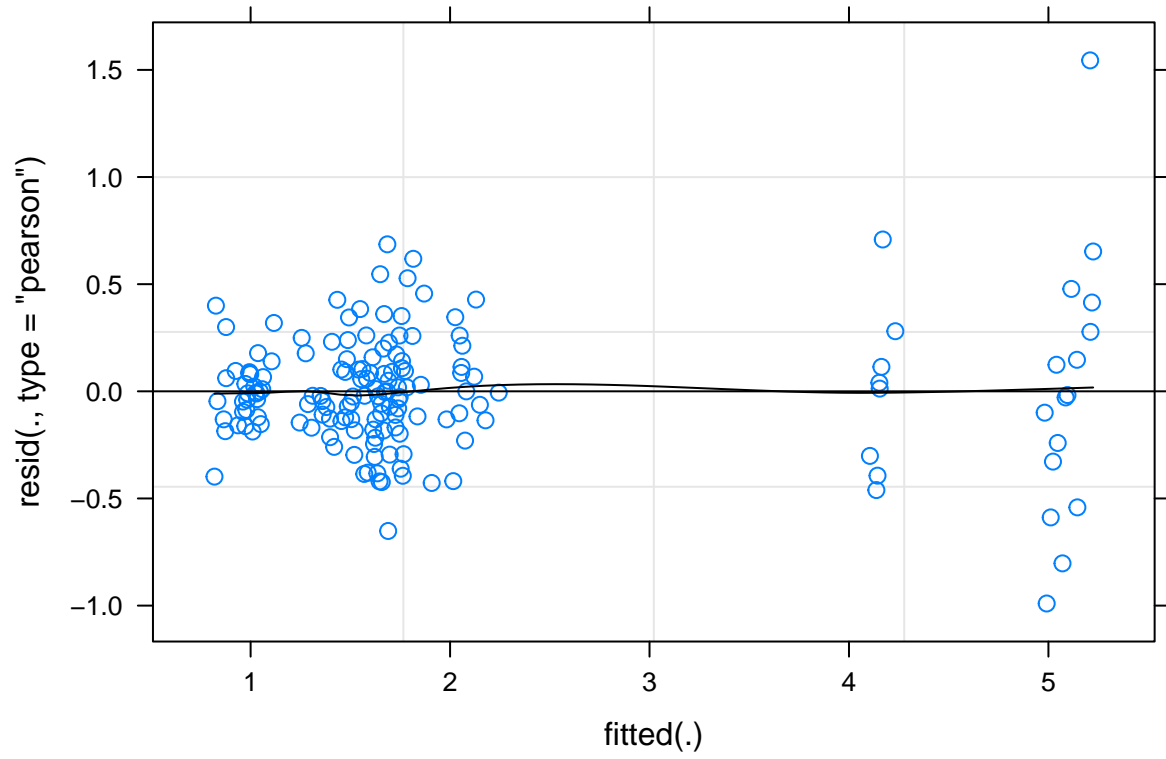
```
chronic_log_plot
```



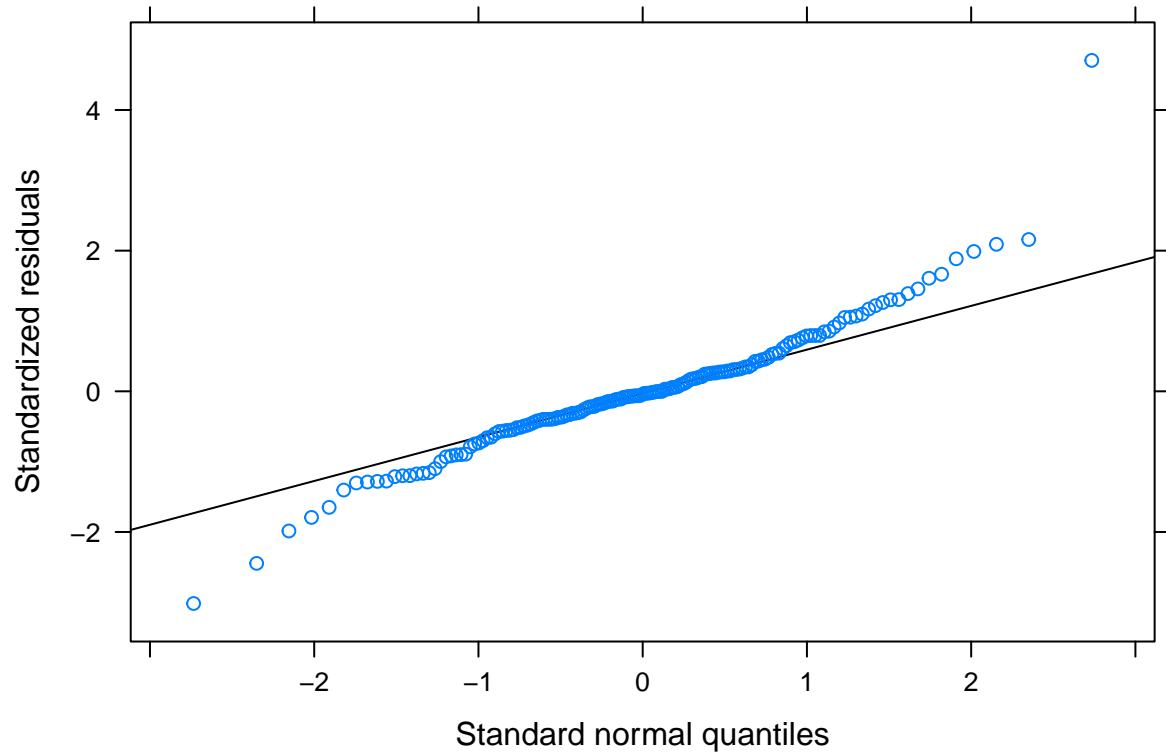
```
#ggsave(filename="../../figures/qRT-PCR/chronic_trt_log.svg", plot=chronic_log_plot, height=4, width=6)
```

To bottom: Full analysis (all 5 groups) - not used

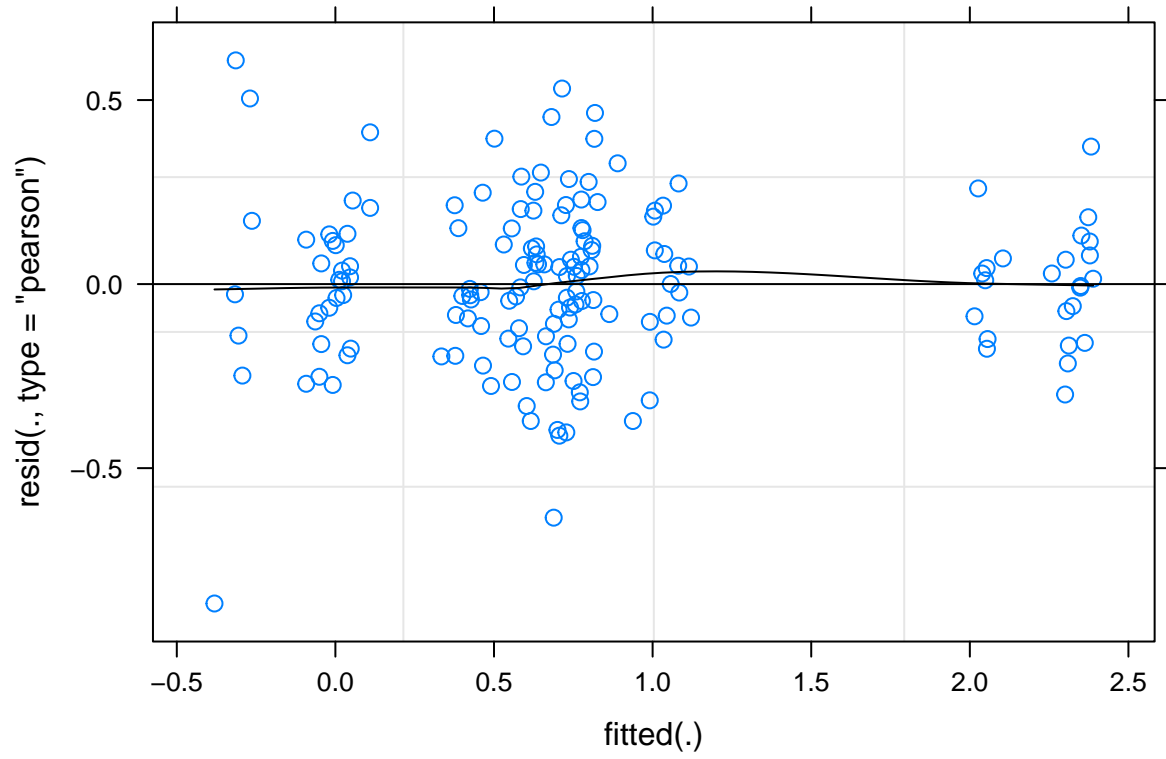
```
transporter_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=transporters_long)  
plot(transporter_lm, type=c("p","smooth"), col.line=1)
```



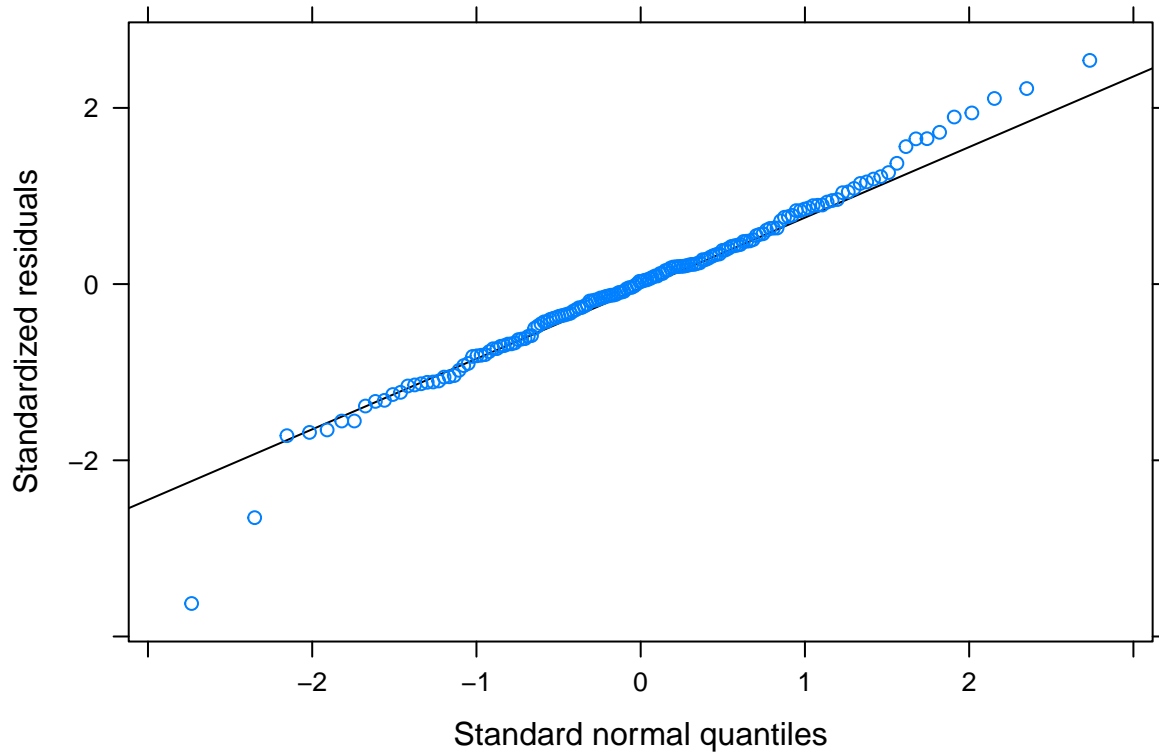
```
lattice::qqmath(transporter_lm)
```



```
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
```

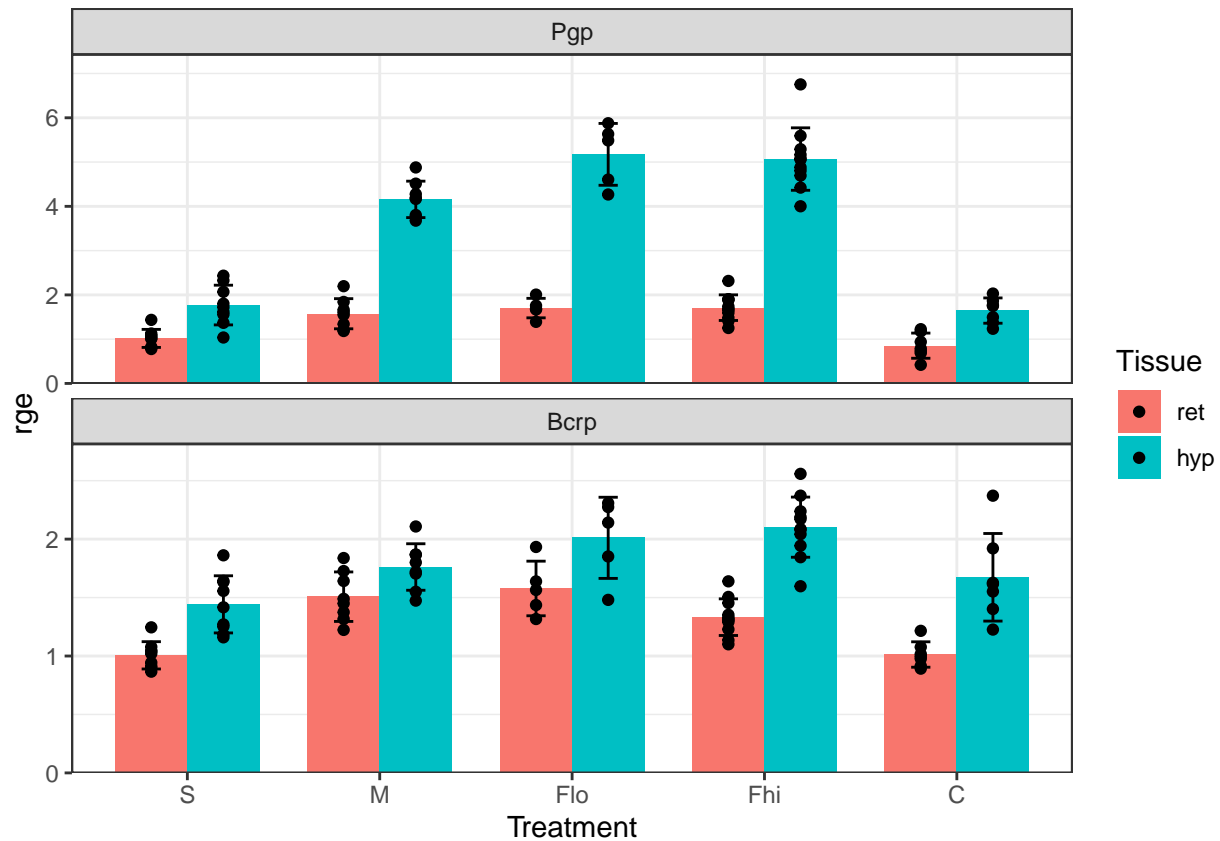
```
## # A tibble: 20 x 8
## # Groups:   Treatment, Tissue [10]
##   Treatment Tissue gene      n mean    sd log2_mean log2_sd
##   <fct>      <fct> <fct> <int> <dbl> <dbl>      <dbl>  <dbl>
## 1 S        ret    Pgp     9 1.02  0.204 -1.11e-11  0.280
## 2 S        ret    Bcrp     9 1.01  0.117 -4.39e-11  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
```

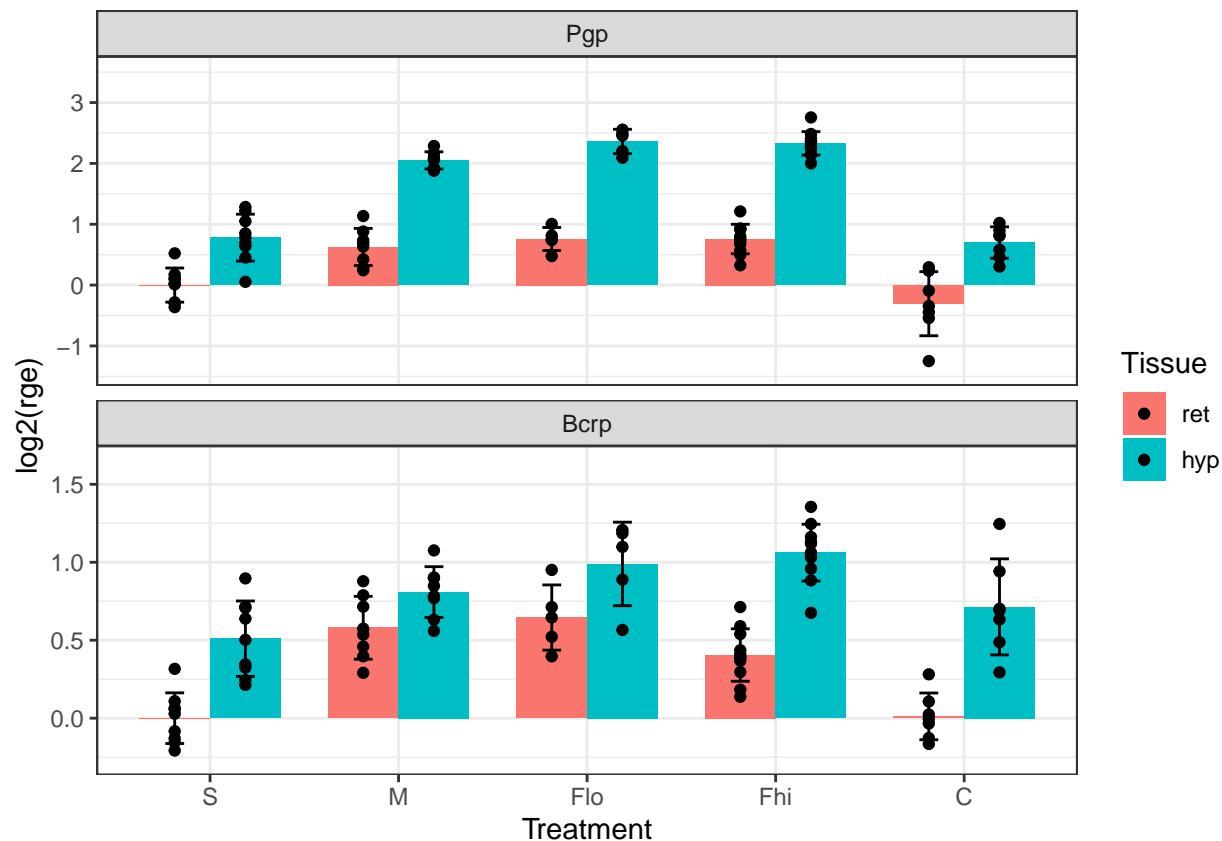


```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret.png", plot=trans_hyp_ret_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret.svg", plot=trans_hyp_ret_plot, height=4, width=6)
```

```
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
```



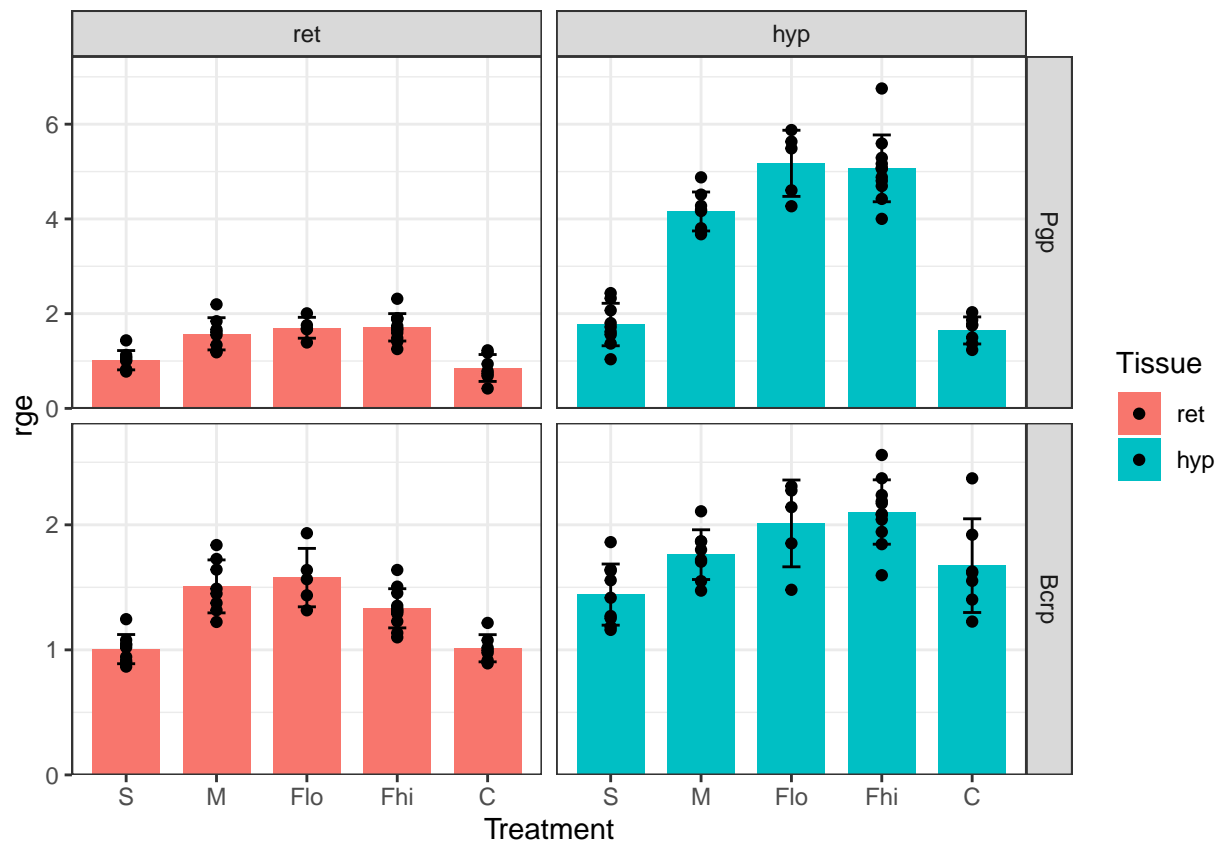
```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log.png", plot=trans_hyp_ret_log_plot, height=4, width=10)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log.svg", plot=trans_hyp_ret_log_plot, height=4, width=10)
```

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
```

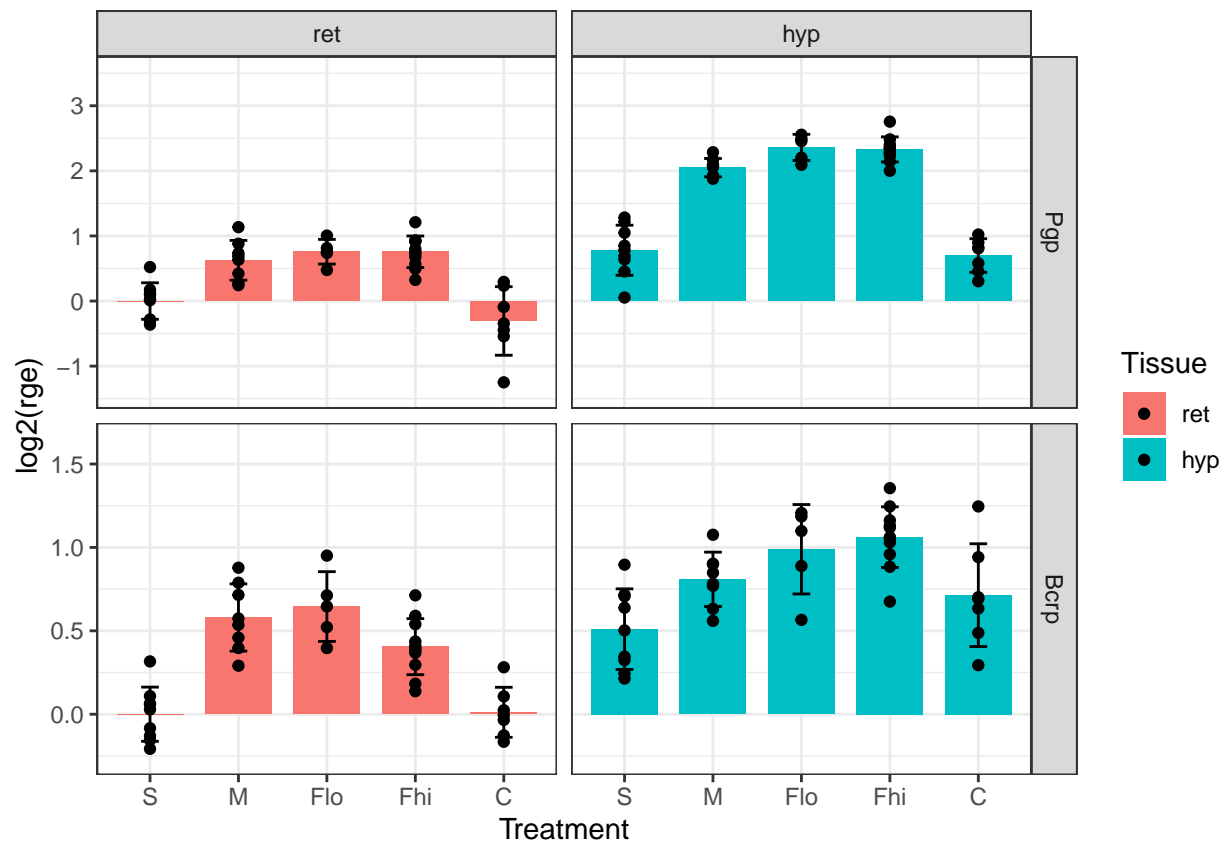


```
#ggsave(filename="../../figures/qRT-PCR/trans_trt.png", plot=trans_trt_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_trt.svg", plot=trans_trt_plot, height=4, width=6)
```

```
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
```

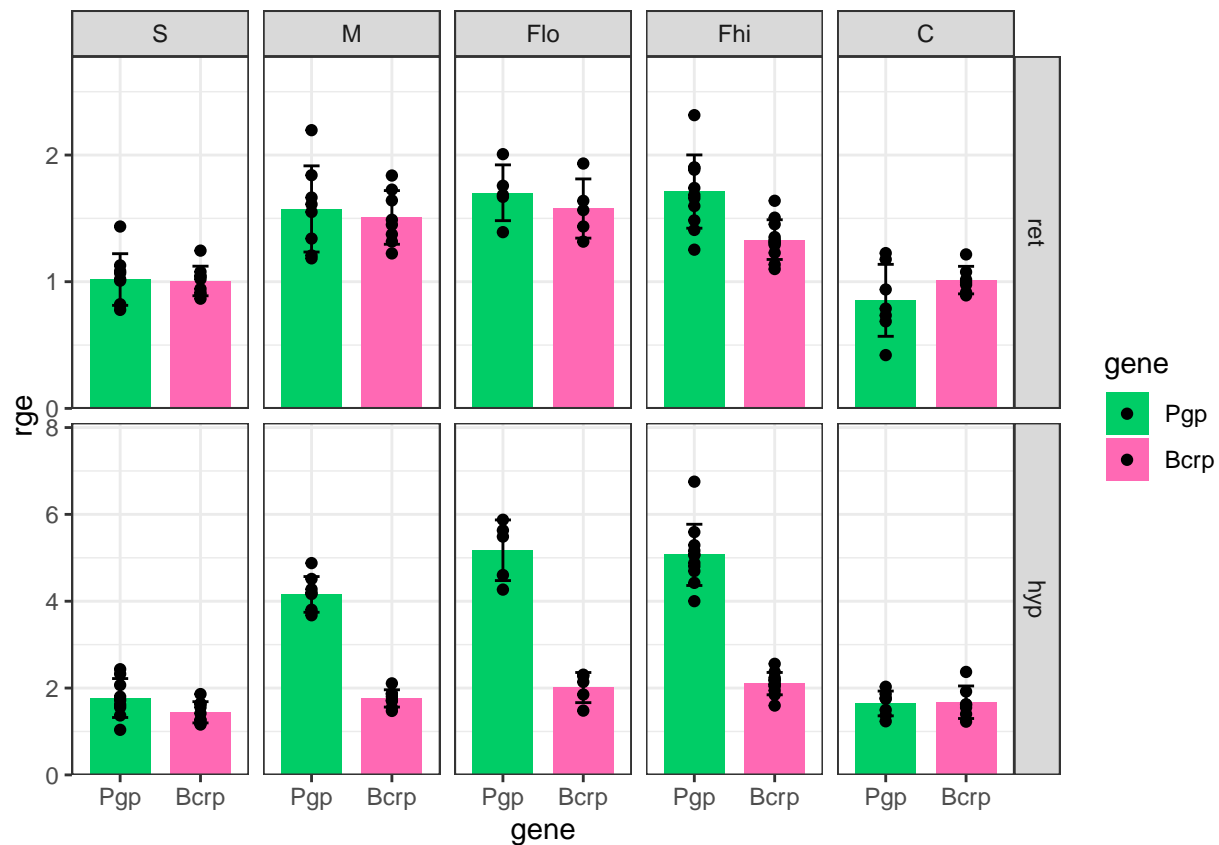


```
#ggsave(filename="../../figures/qRT-PCR/trans_trt_log.png", plot=trans_trt_log_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_trt_log.svg", plot=trans_trt_log_plot, height=4, width=6)
```

```
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
```

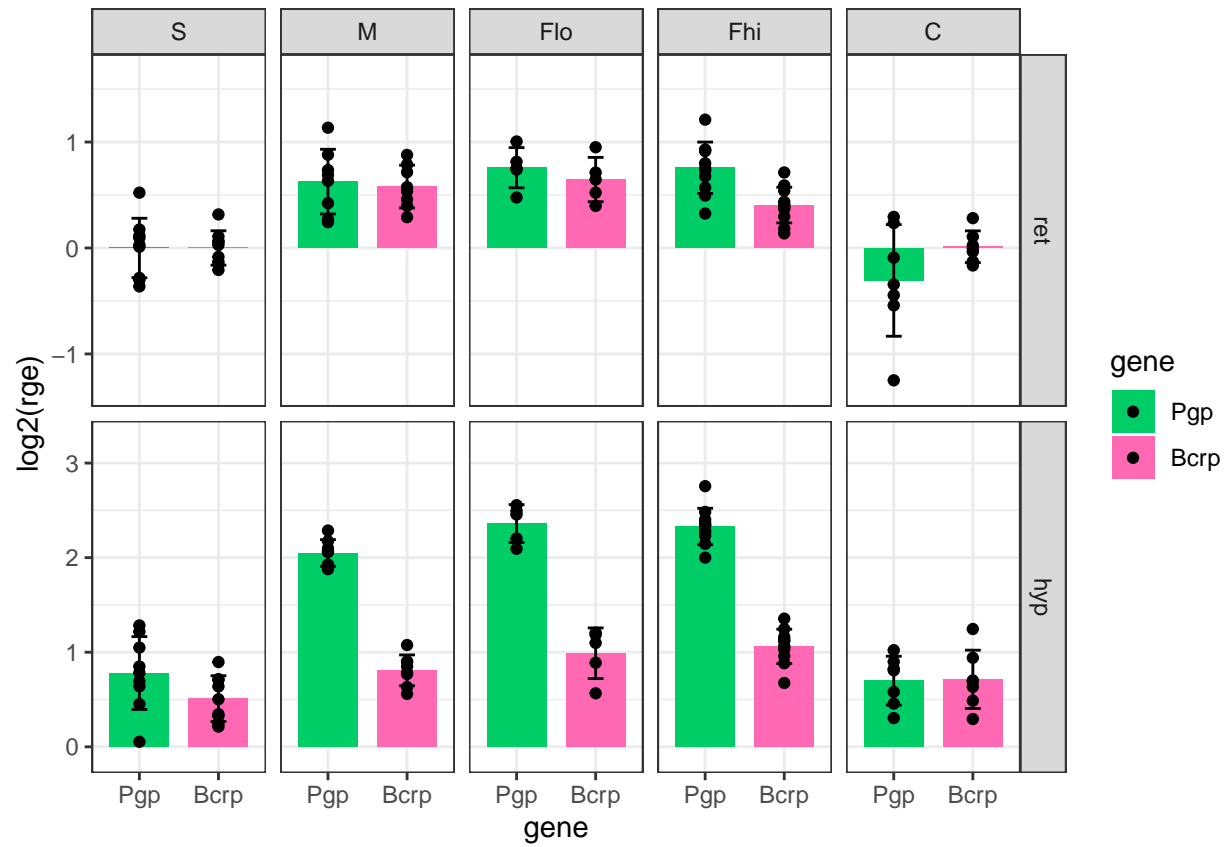



```
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene.png", plot=trans_g2g_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene.svg", plot=trans_g2g_plot, height=4, width=6)
```

```
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)
```