

# 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)
```

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

### 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)
```

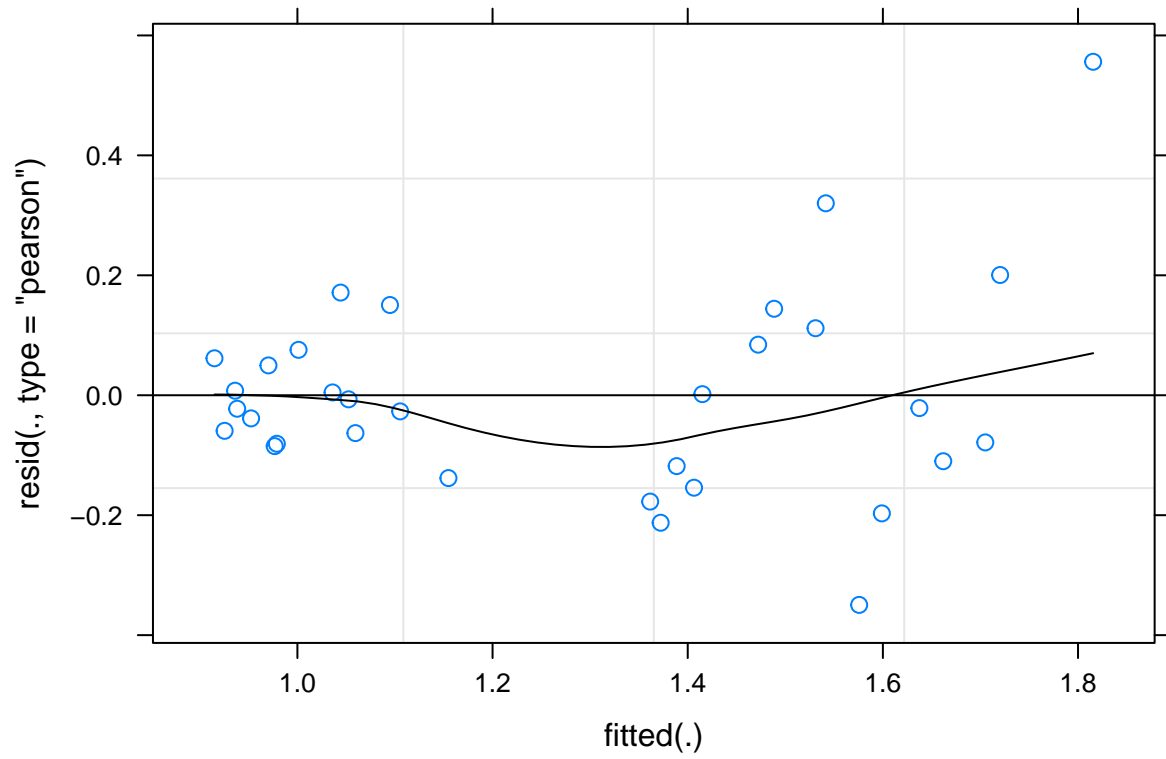
```
## # A tibble: 6 x 5
##   Sample Tissue Treatment gene    rge
##   <chr>   <fct>   <fct>   <fct> <dbl>
## 1 C1     hyp     C       Pgp   1.77
## 2 C1     hyp     C       Bcrp  1.62
## 3 C1     ret     C       Pgp   0.421
## 4 C1     ret     C       Bcrp  0.892
## 5 C2     hyp     C       Pgp   1.75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

### Stats on Bcrp only (chronic vs saline)

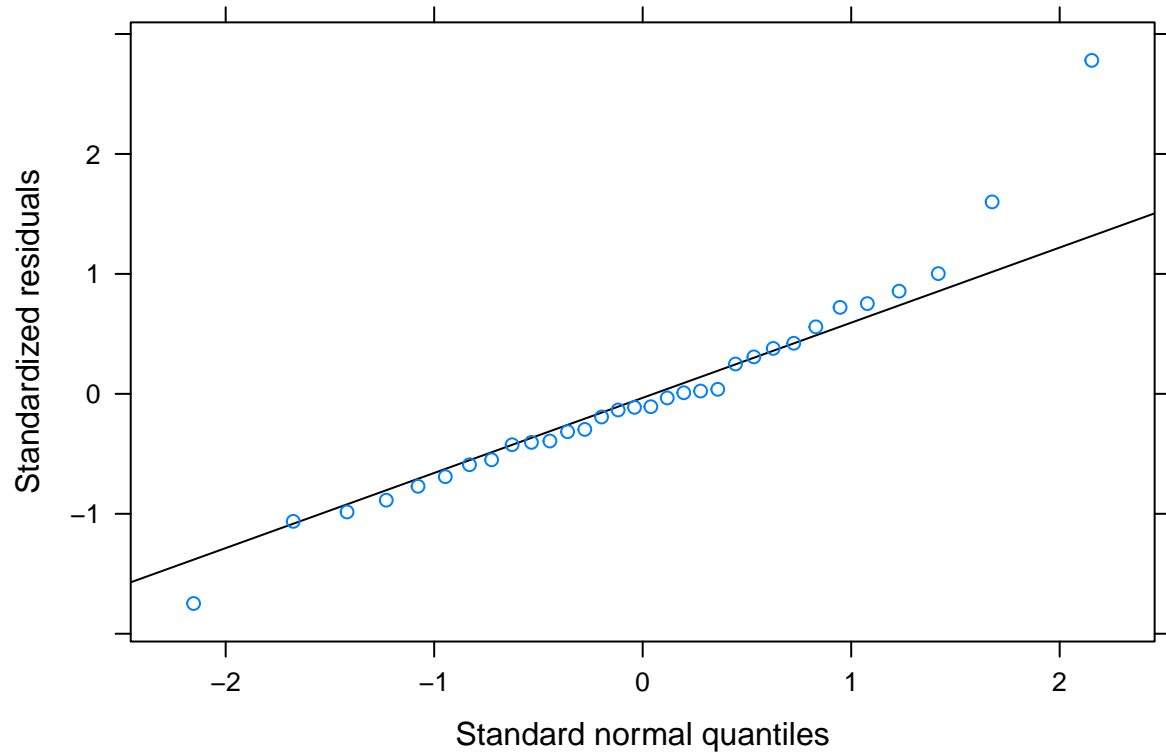
```
#bcrp only
chronic <- transporters %>% filter(Treatment %in% c("C", "S"))
chronic
```

```
##   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
```

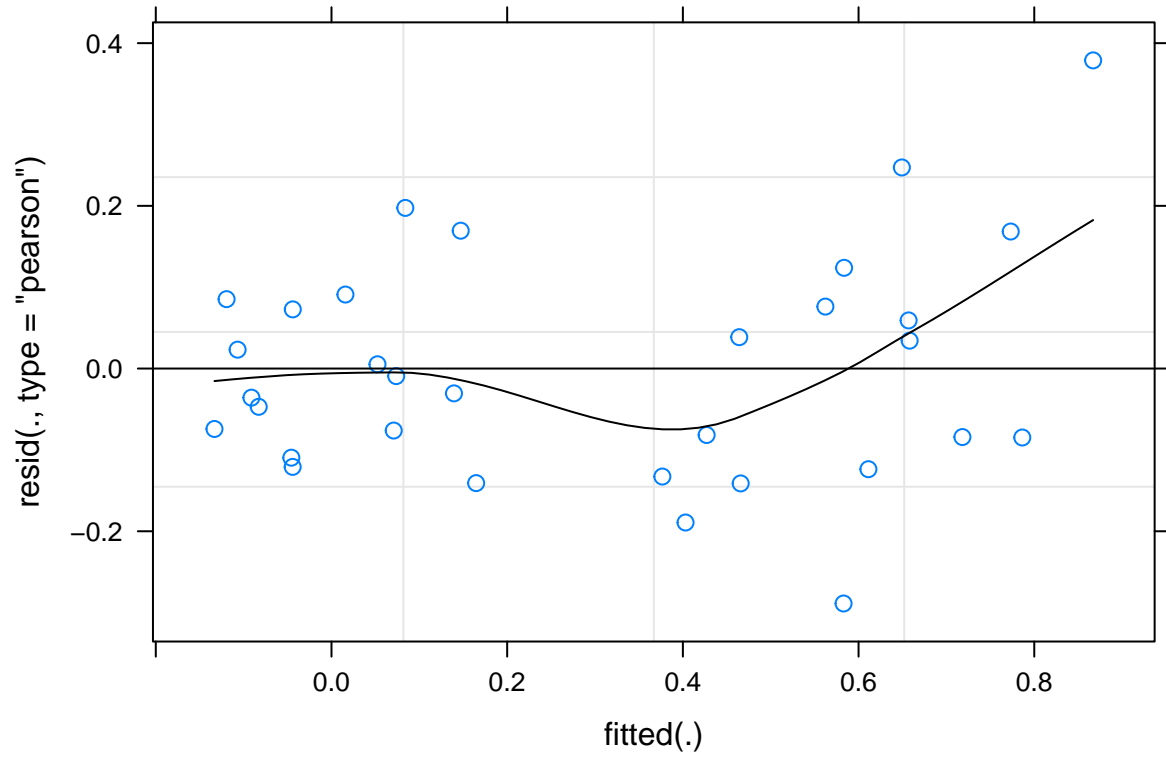
```
c_bcrp_lm <- lmer(Bcrp.RGE ~ Tissue * Treatment + (1|Sample), data=chronic)
plot(c_bcrp_lm, type=c("p","smooth"), col.line=1)
```



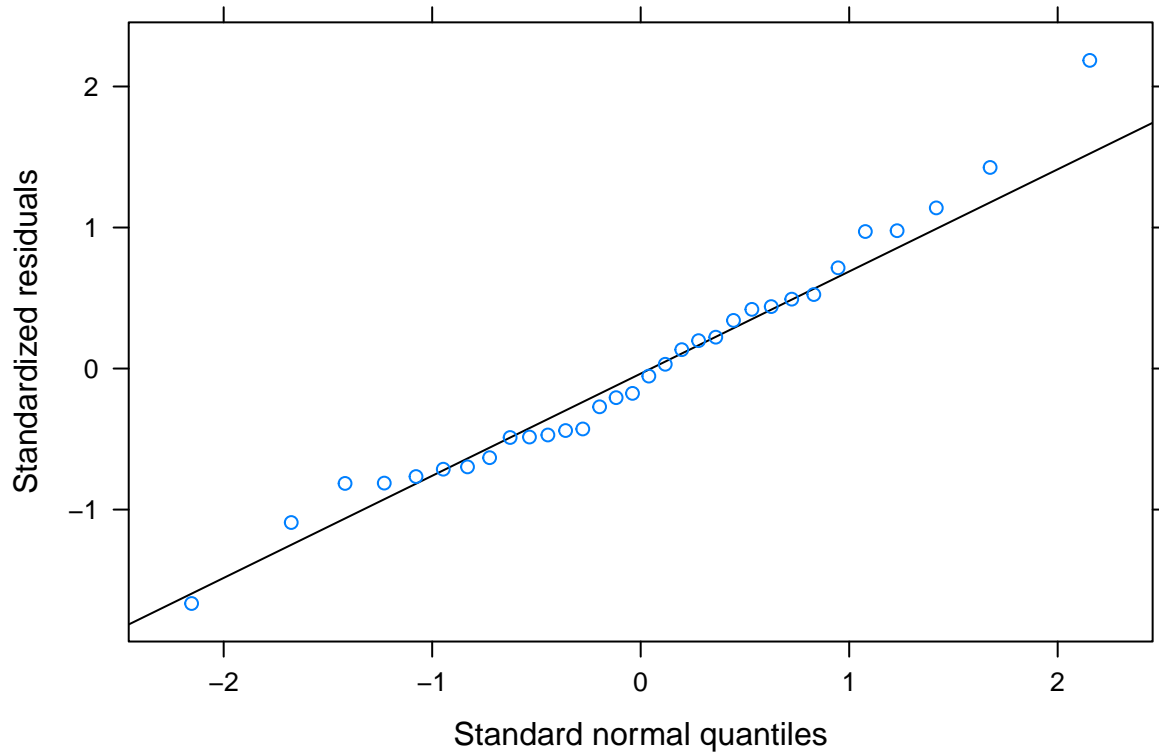
```
lattice::qqmath(c_bcrp_lm)
```



```
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
```

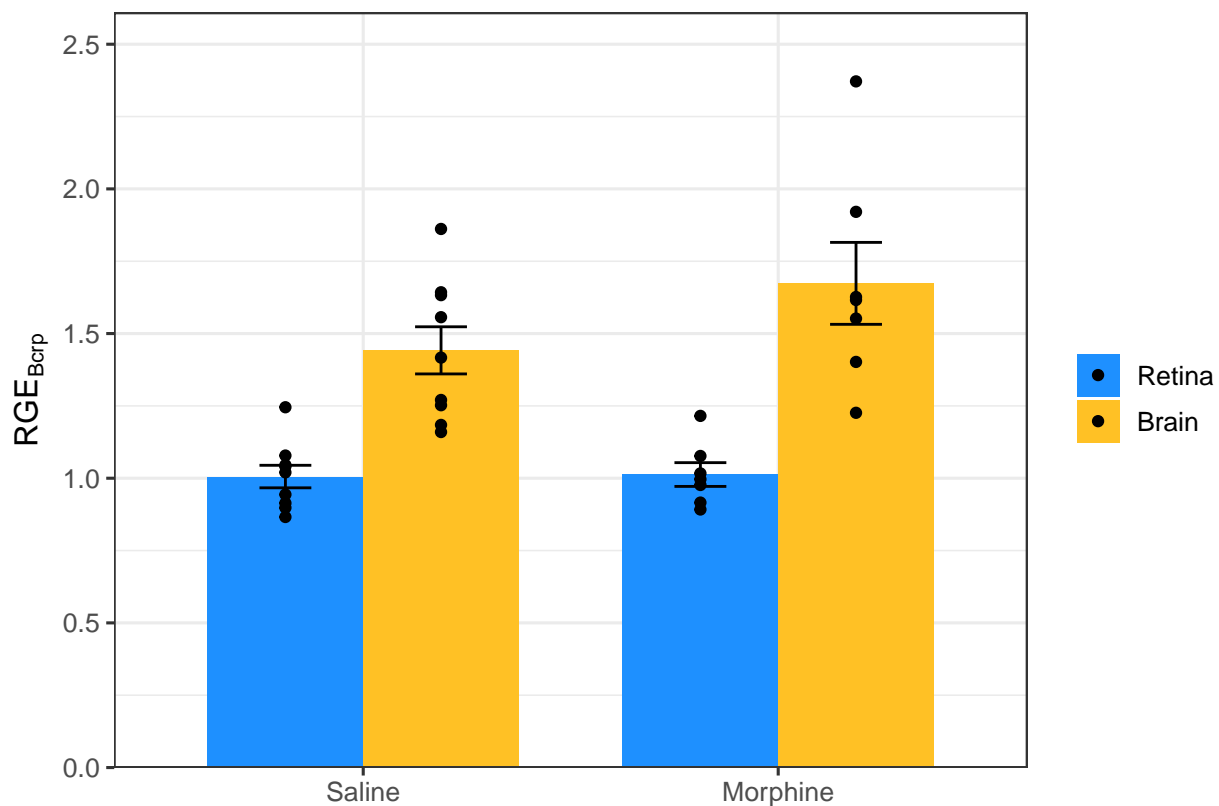
## Visualizing chronic vs saline Bcrp

```
#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(RGEBcrp))
```

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

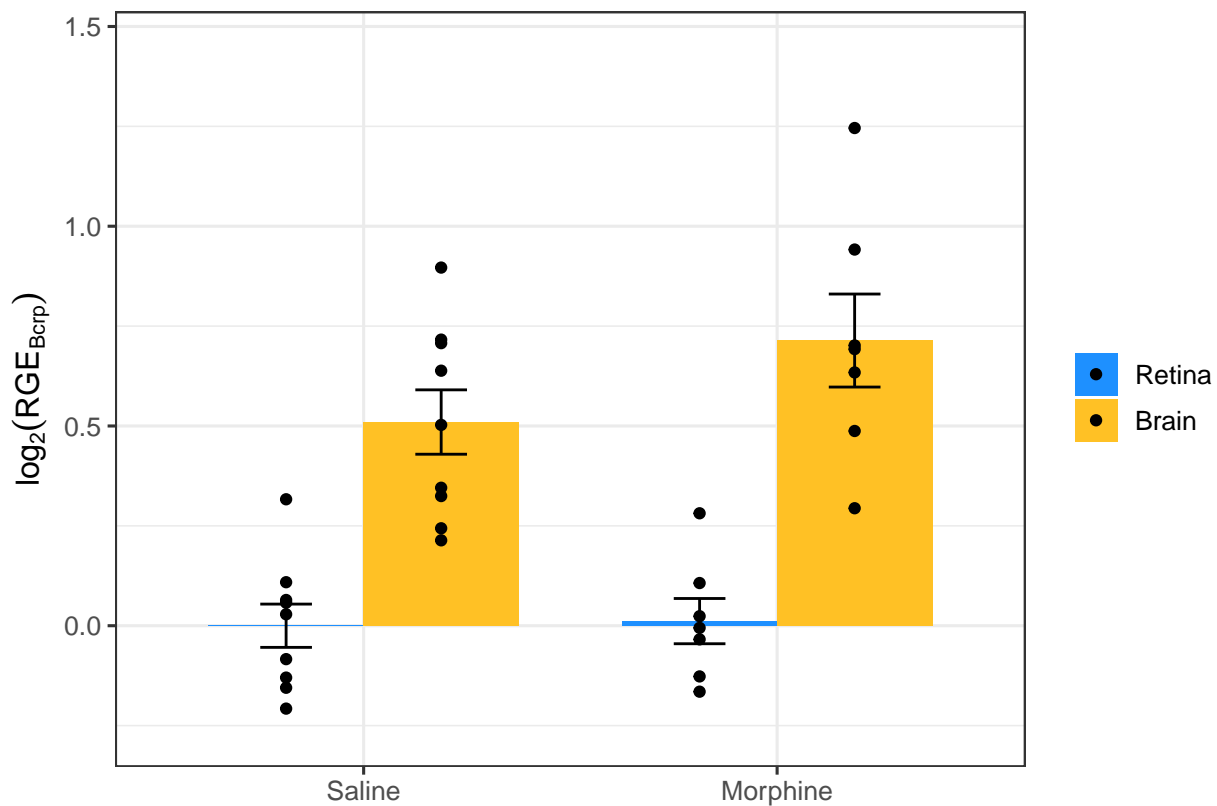
```
c_bcrp_log_plot <- ggplot() +
  stat_summary(data=chronic, aes(x=Treatment, y=log2(Bcrp.RGE), fill=Tissue),
              fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=c_bcrp_sumstats, aes(x=Treatment, ymin=log2_mean-log2_se,
                                          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),
```

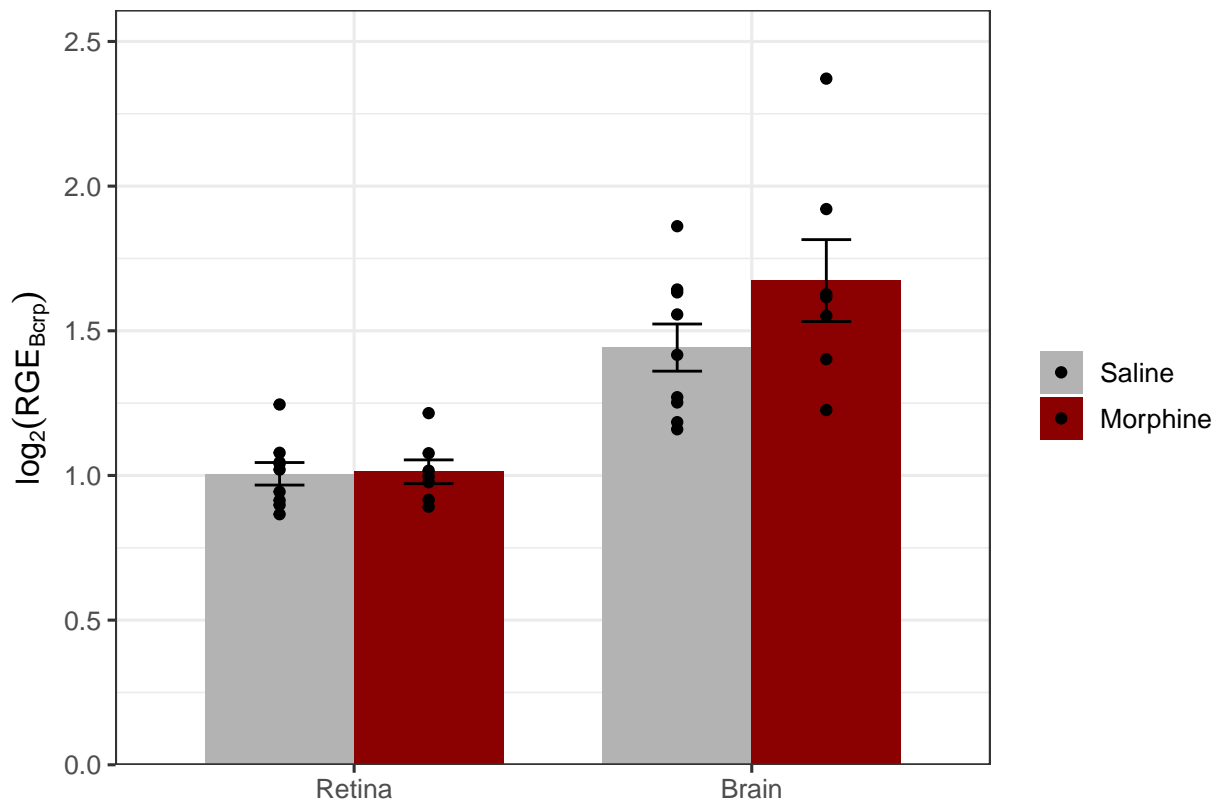
```

    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(RGEBcrp)))

```

## 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%

```

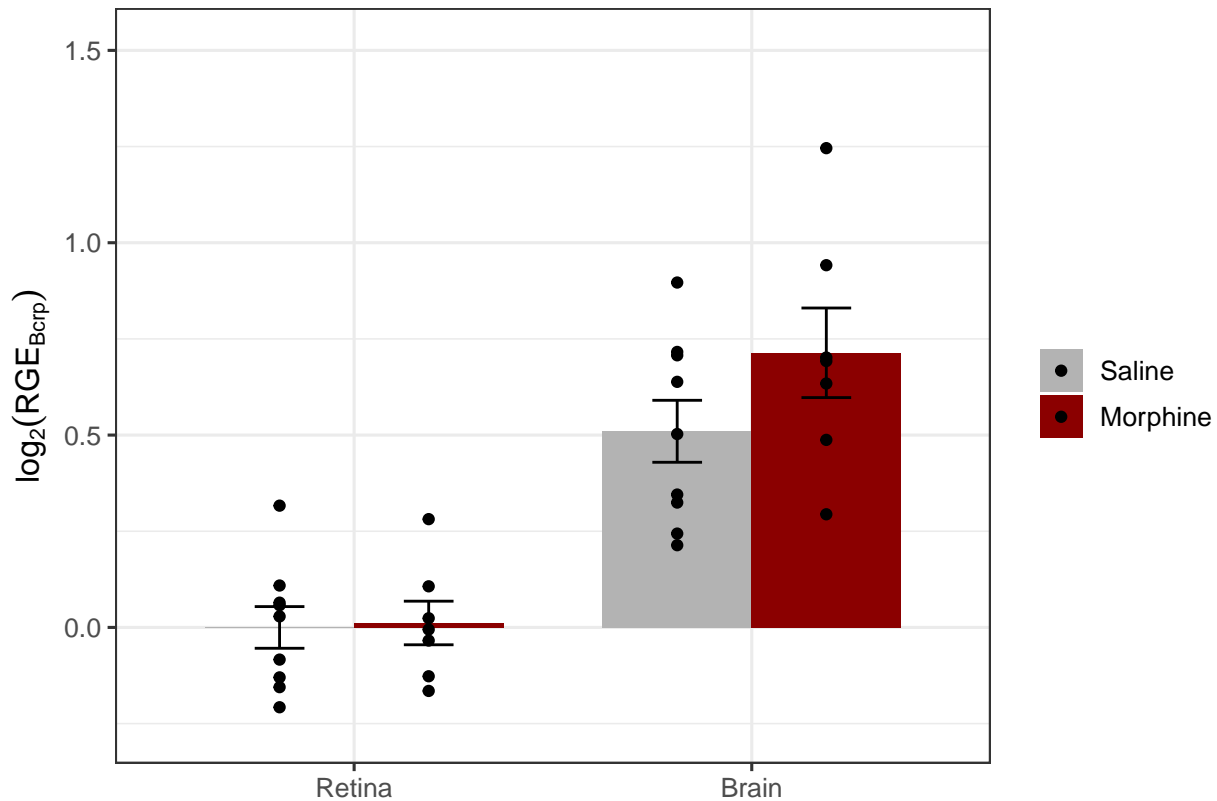
```

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

```

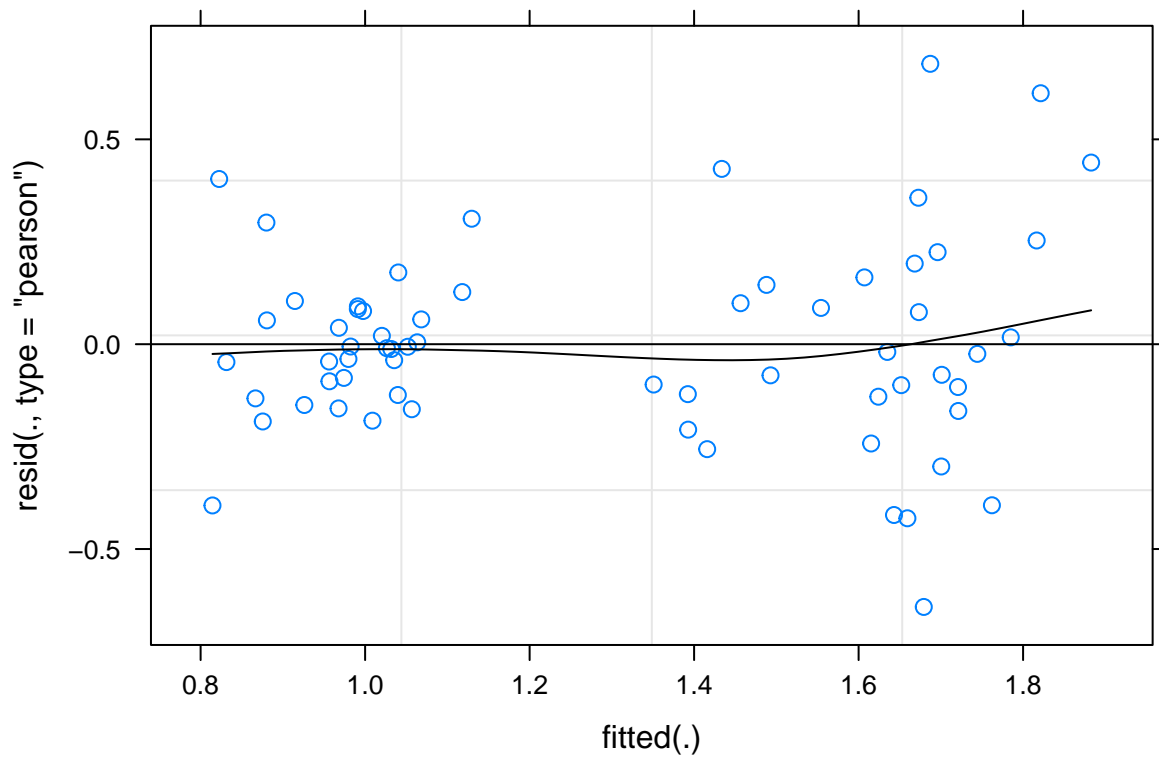
```

## # 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

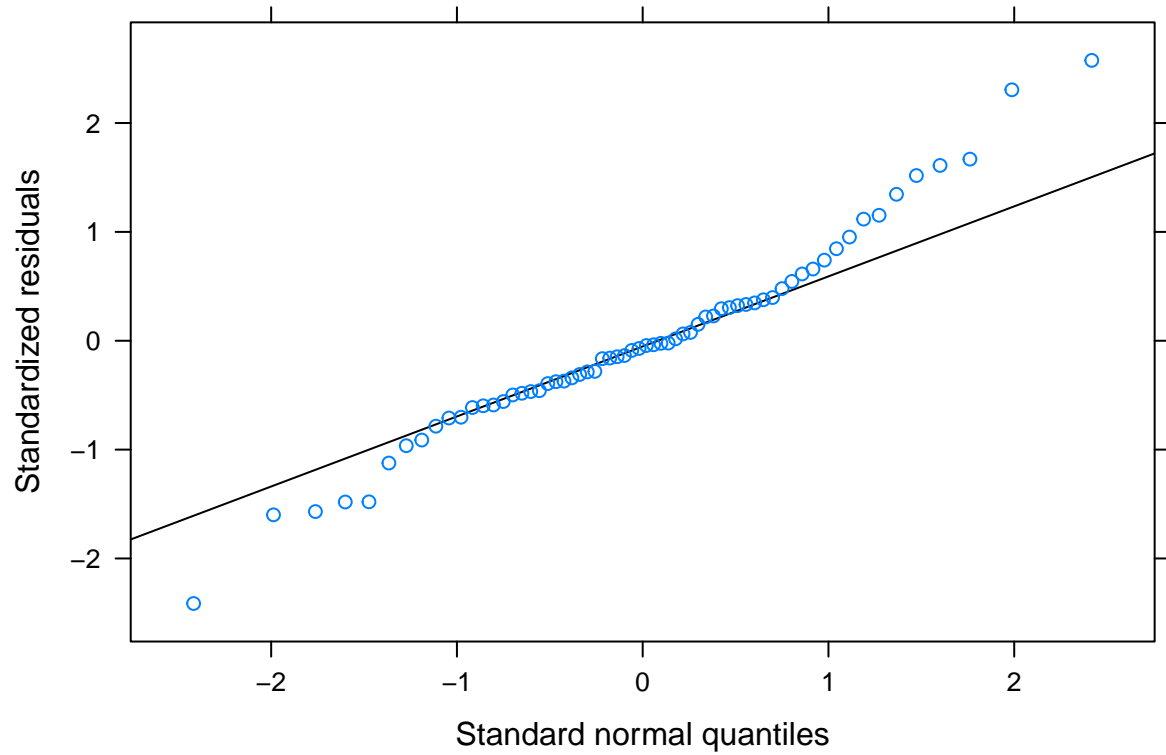
```

```
## 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
## # i Use `print(n = ...)` to see more rows
```

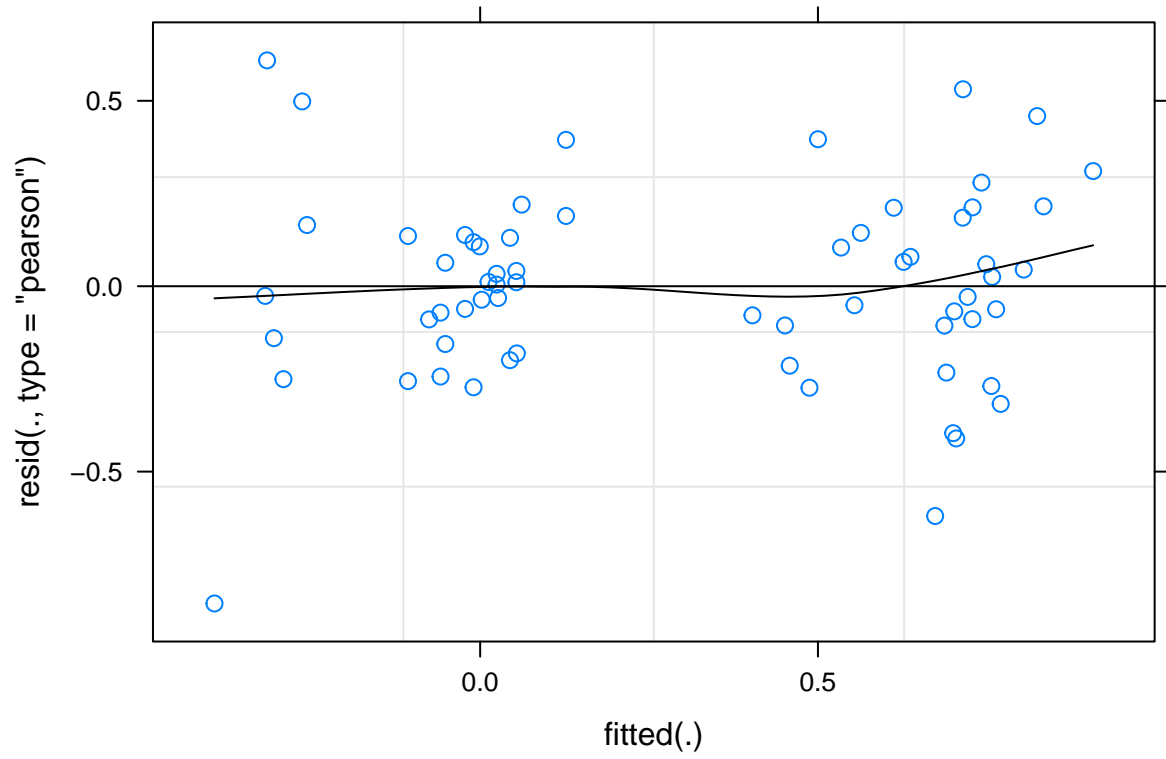
```
chronic_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=chronic_long)
plot(chronic_lm, type=c("p", "smooth"), col.line=1)
```



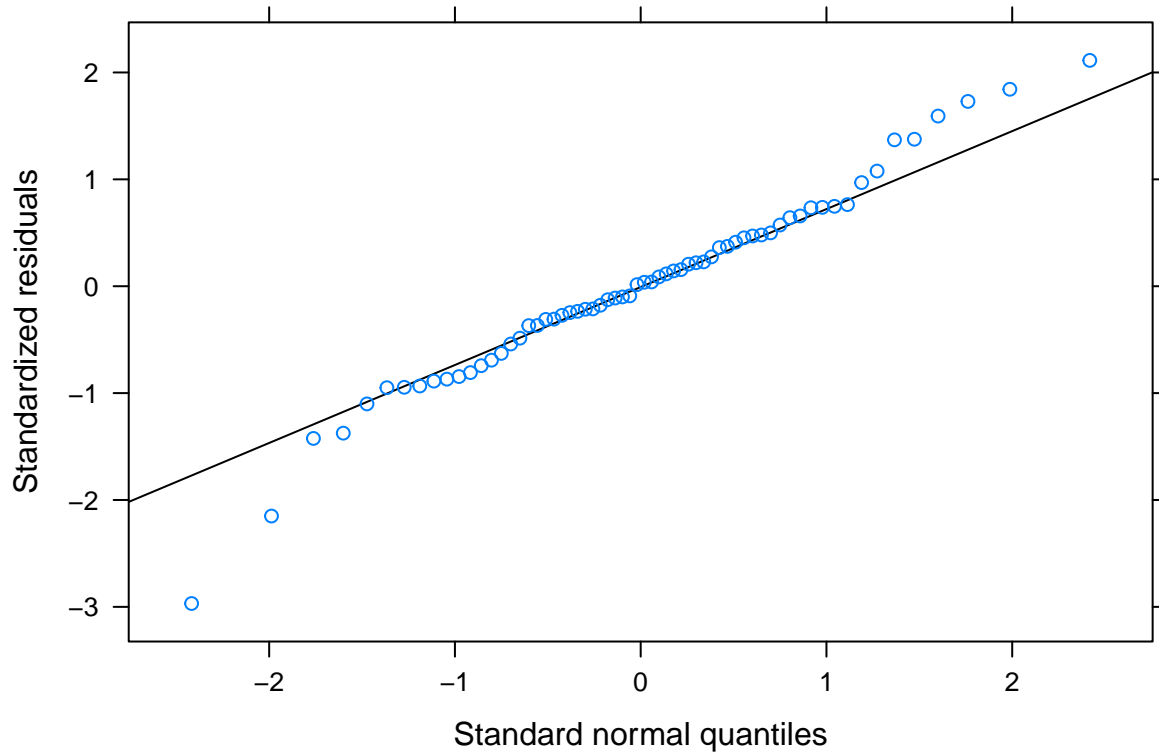
```
lattice::qqmath(chronic_lm)
```



```
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),
  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.
```

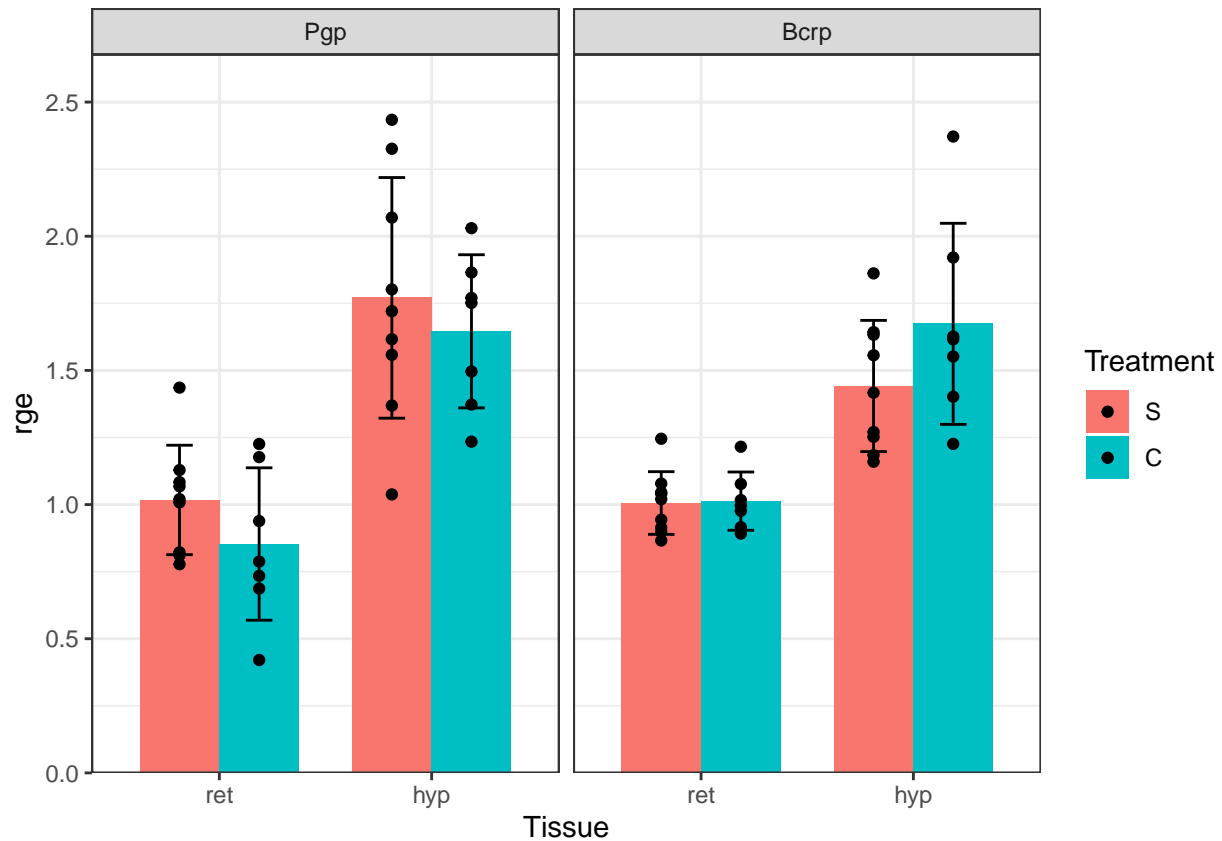
```
chronic_sumstats
```

```
## # 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
```

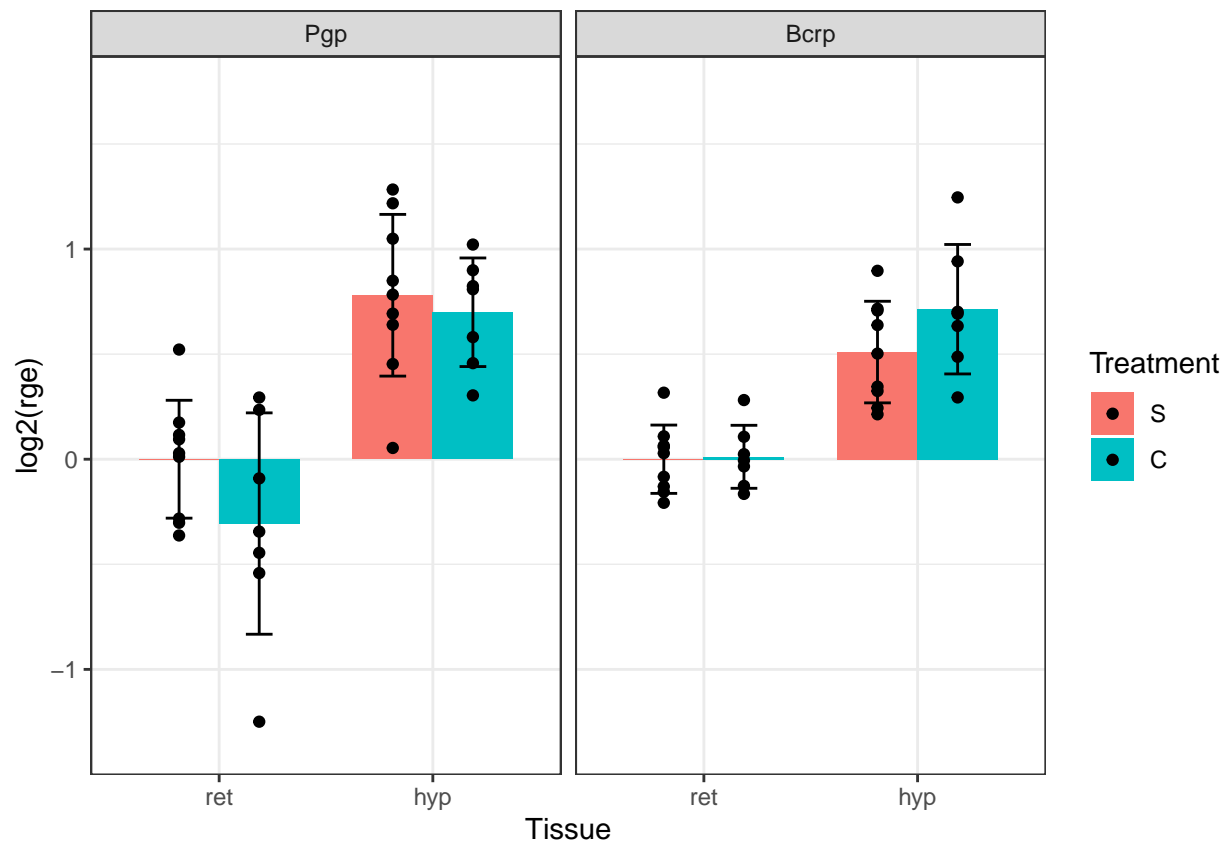


```
#ggsave(filename="../../figures/qRT-PCR/chronic_trt_plot.svg", plot=chronic_plot, height=4, width=6)
```

```
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)
```

Looking at only the 1 injection samples (males, low e/p females, high e/p females)

Reading in the new dataset

```
transporters <- read.csv("../data/qRT-PCR/07222022-transporter-gene-study-1inj-only.csv", fileEncoding="UTF-8")
#transporters <- transporters %>% filter(Sample != "calib")

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

transporters <- transporters %>% mutate(Group = factor(Group, c("M", "Flo", "Phi")),
                                       Tissue = factor(Tissue, c("ret", "hyp")),
                                       Sample = as.factor(Sample))

transporters_long <- transporters %>% pivot_longer(cols=c("Pgp.RGE", "Bcrp.RGE"), names_to = "gene", values_to = "log2(rge)")

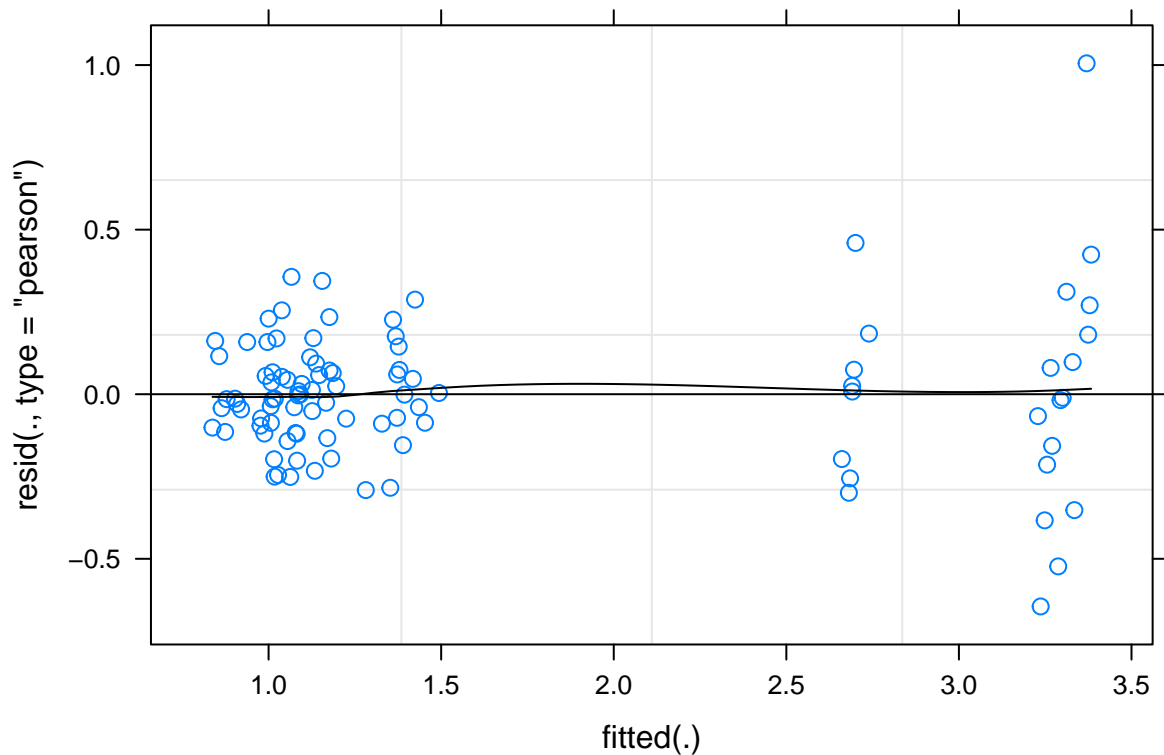
transporters_long <- transporters_long %>% mutate(gene = str_extract(gene, "[:alpha:]+(?:[:digit:])"))
transporters_long <- transporters_long %>% mutate(gene = factor(as.factor(gene), c("Pgp", "Bcrp")))

transporters_long
```

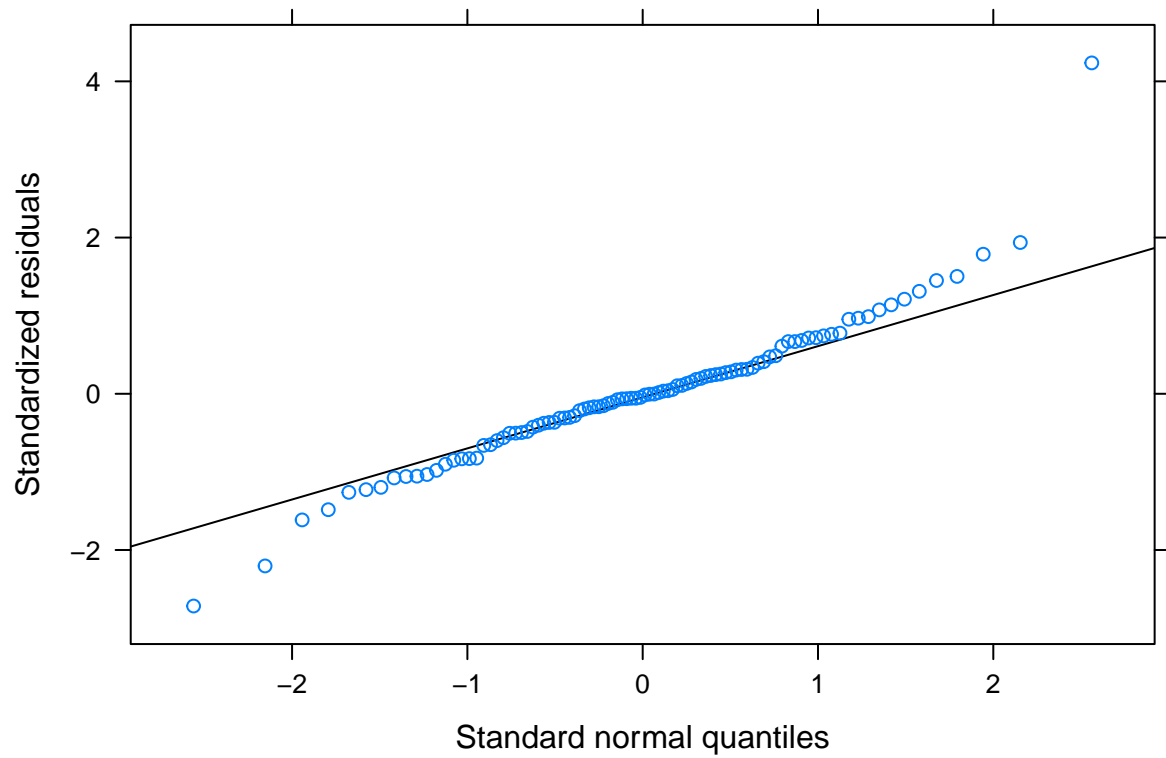
```
## # A tibble: 96 x 5
##   Sample Tissue Group gene    rge
##   <fct>  <fct>  <fct> <fct> <dbl>
## 1 Fhi10  hyp    Fhi    Pgp    4.38
## 2 Fhi10  hyp    Fhi    Bcrp    1.50
## 3 Fhi10  ret    Fhi    Pgp    1.22
## 4 Fhi10  ret    Fhi    Bcrp    0.905
## 5 Fhi11  hyp    Fhi    Pgp    3.29
## 6 Fhi11  hyp    Fhi    Bcrp    1.71
## 7 Fhi11  ret    Fhi    Pgp    1.08
## 8 Fhi11  ret    Fhi    Bcrp    0.880
## 9 Fhi1   hyp    Fhi    Pgp    3.11
## 10 Fhi1  hyp    Fhi    Bcrp    1.39
## # ... with 86 more rows
## # i Use `print(n = ...)` to see more rows
```

## Stats

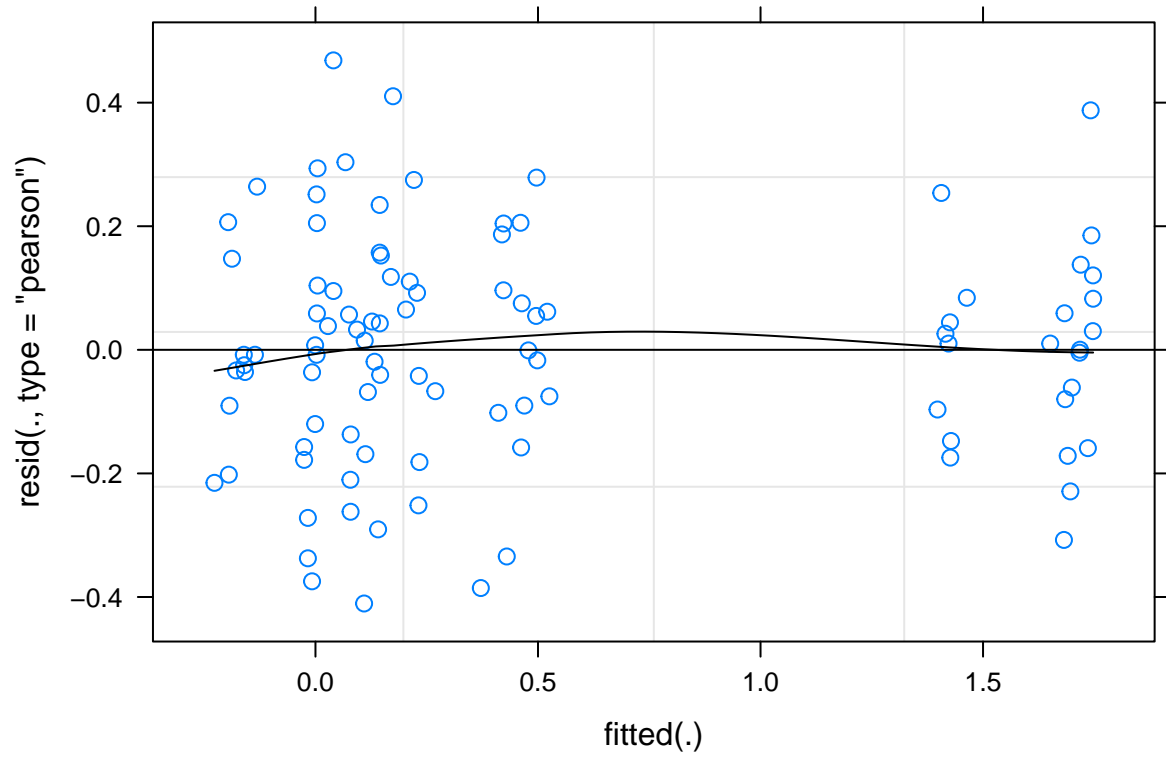
```
transporter_lm <- lmer(rge ~ gene * Tissue * Group + (1|Sample), data=transporters_long)
plot(transporter_lm, type=c("p","smooth"), col.line=1)
```



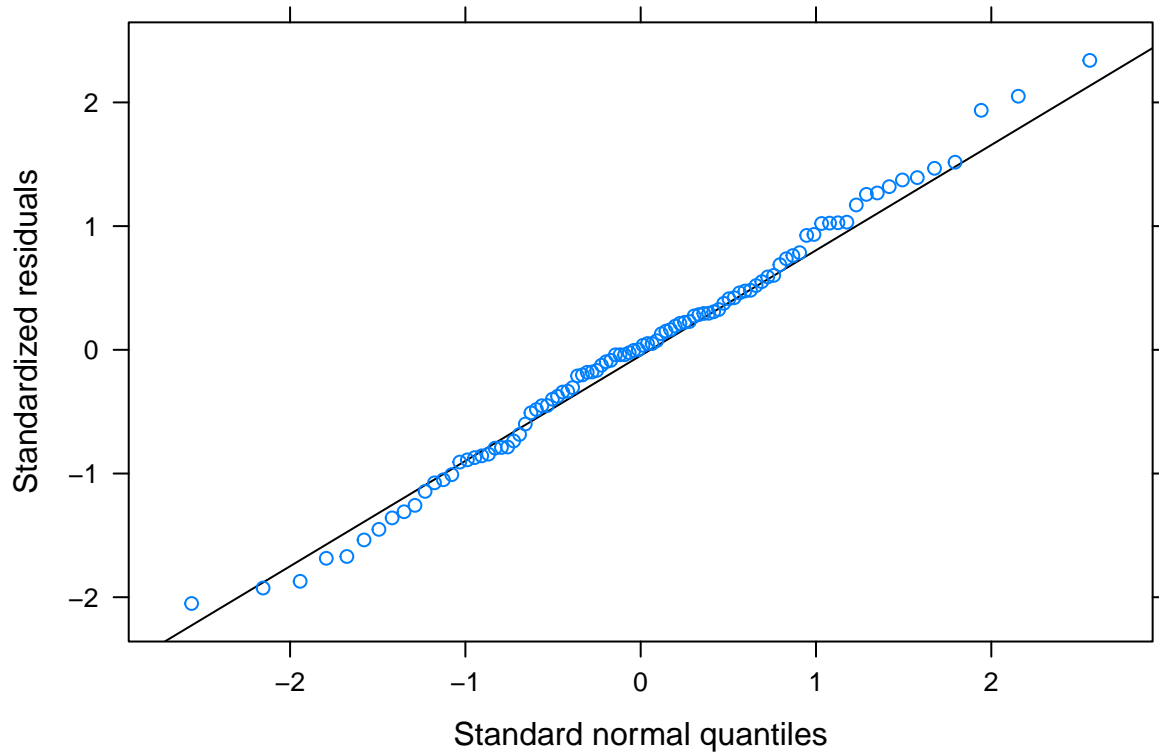
```
lattice::qqmath(transporter_lm)
```



```
transporter_log2_lm <- lmer(log2(rge) ~ gene * Tissue * Group + (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 = 0.77877, p-value = 1.044e-10
```

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

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(transporters_long$rge)
## W = 0.87148, p-value = 1.285e-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	10.1573	10.1573	1	63	253.4878	< 2e-16 ***
Tissue	20.4143	20.4143	1	63	509.4664	< 2e-16 ***
Group	0.3434	0.1717	2	21	4.2854	0.02749 *
gene:Tissue	6.8233	6.8233	1	63	170.2831	< 2e-16 ***
gene:Group	0.1278	0.0639	2	63	1.5945	0.21109
Tissue:Group	0.3872	0.1936	2	63	4.8315	0.01117 *
gene:Tissue:Group	0.1405	0.0703	2	63	1.7536	0.18149

```
## ---
```

```

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

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

## Tissue = ret, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.1312 0.1180 83  -1.111  0.5098
## M - Fhi      -0.1306 0.0962 83  -1.357  0.3680
## Flo - Fhi      0.0006 0.1117 83   0.005  1.0000
##
## Tissue = hyp, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.3108 0.1180 83  -2.633  0.0270
## M - Fhi      -0.2798 0.0962 83  -2.909  0.0128
## Flo - Fhi      0.0310 0.1117 83   0.277  0.9585
##
## Tissue = ret, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.0658 0.1180 83  -0.557  0.8430
## M - Fhi       0.1748 0.0962 83   1.818  0.1702
## Flo - Fhi      0.2406 0.1117 83   2.155  0.0852
##
## Tissue = hyp, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.1804 0.1180 83  -1.528  0.2830
## M - Fhi      -0.2530 0.0962 83  -2.630  0.0272
## Flo - Fhi     -0.0726 0.1117 83  -0.651  0.7926
##
## 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 3 estimates

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

## Group = M, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.423 0.1001 63 -14.219  <.0001
##
## Group = Flo, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.603 0.1266 63 -12.660  <.0001
##
## Group = Fhi, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.572 0.0854 63 -18.422  <.0001
##
## Group = M, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -0.229 0.1001 63  -2.287  0.0255
##
## Group = Flo, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -0.344 0.1266 63  -2.714  0.0086
##
## Group = Fhi, gene = Bcrp:

```



```
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.657 0.0854 63 -7.695 <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

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

```
## Group = M, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.0000 0.1001 63 0.000 1.0000
##
## Group = Flo, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.0654 0.1266 63 0.516 0.6073
##
## Group = Fhi, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.3054 0.0854 63 3.578 0.0007
##
## Group = M, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.1942 0.1001 63 11.932 <.0001
##
## Group = Flo, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.3247 0.1266 63 10.463 <.0001
##
## Group = Fhi, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.2211 0.0854 63 14.306 <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

```
trans_sumstats <- transporters_long %>% group_by(Group, 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 'Group', 'Tissue'. You can override using
## the `.groups` argument.
```

```
trans_sumstats
```

```
## # A tibble: 12 x 10
## # Groups:   Group, Tissue [6]
##   Group Tissue gene      n mean    sd    se log2_mean log2_sd log2_se
##   <fct> <fct> <fct> <int> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
## 1 M     ret    Pgp      8 1.02  0.220 0.0779 2.02e-10 0.305 0.108
## 2 M     ret    Bcrp      8 1.01  0.142 0.0501 6.58e-11 0.202 0.0713
```

```
## 3 M    hyp    Pgp      8 2.69 0.266 0.0942 1.42e+ 0 0.141 0.0498
## 4 M    hyp    Bcrp     8 1.18 0.133 0.0470 2.29e- 1 0.163 0.0576
## 5 Flo  ret    Pgp      5 1.10 0.143 0.0638 1.31e- 1 0.190 0.0848
## 6 Flo  ret    Bcrp     5 1.06 0.156 0.0699 6.58e- 2 0.209 0.0935
## 7 Flo  hyp    Pgp      5 3.35 0.452 0.202 1.73e+ 0 0.201 0.0897
## 8 Flo  hyp    Bcrp     5 1.35 0.232 0.104 4.09e- 1 0.268 0.120
## 9 Fhi  ret    Pgp     11 1.11 0.187 0.0565 1.31e- 1 0.242 0.0731
## 10 Fhi ret    Bcrp     11 0.891 0.105 0.0316 -1.75e- 1 0.168 0.0507
## 11 Fhi hyp    Pgp     11 3.28 0.457 0.138 1.70e+ 0 0.193 0.0580
## 12 Fhi hyp    Bcrp     11 1.41 0.172 0.0519 4.82e- 1 0.182 0.0548
```

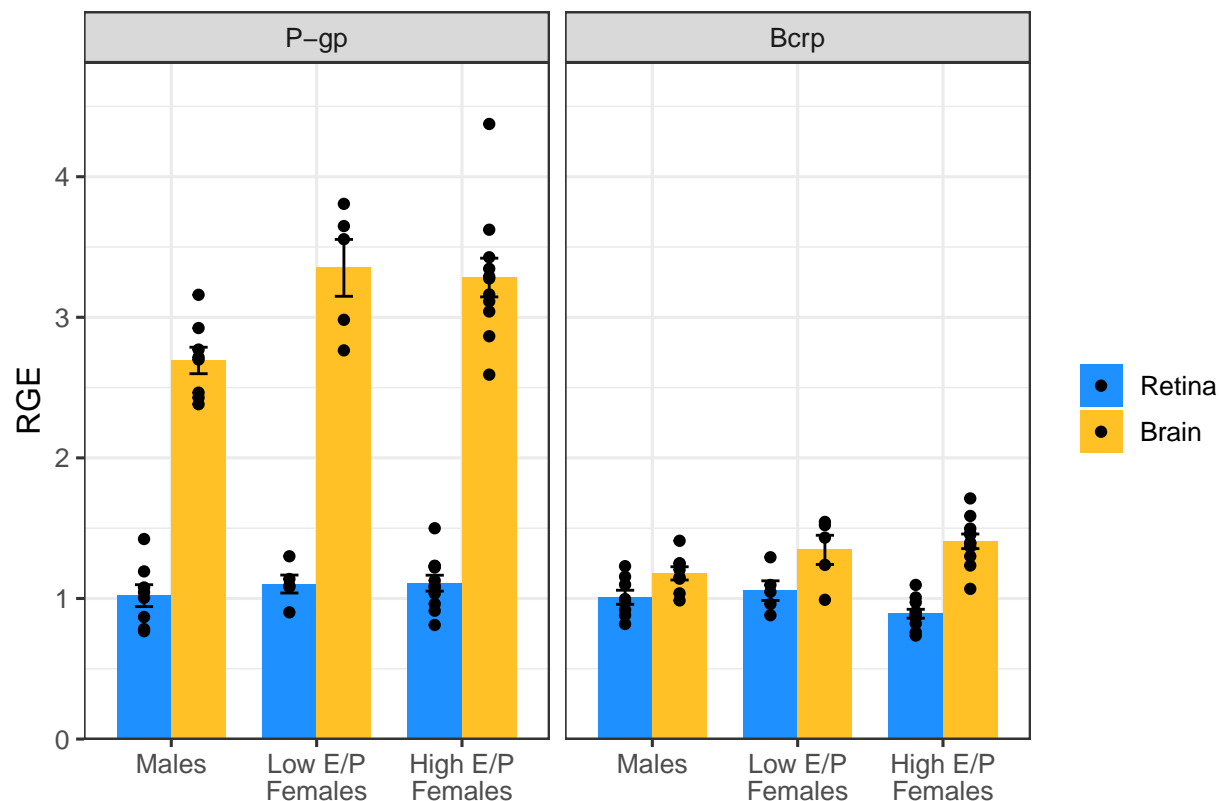
## Visualizing hyp vs ret within each treatment/gene

```
gene.labs <- c("P-gp", "Bcrp")
names(gene.labs) <- c("Pgp", "Bcrp")

trans_hyp_ret_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Group, y=rge, fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Group, ymin=mean-se, ymax=mean+se, fill=Tissue),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Group, y=rge, fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, labeller = labeller(gene = gene.labs), ncol=2) +
  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("M" = "Males", "Flo" = "Low E/P\nFemales",
    "Fhi" = "High E/P\nFemales")) +
  xlab("") +
  ylab("RGE")
```

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

```
trans_hyp_ret_plot
```

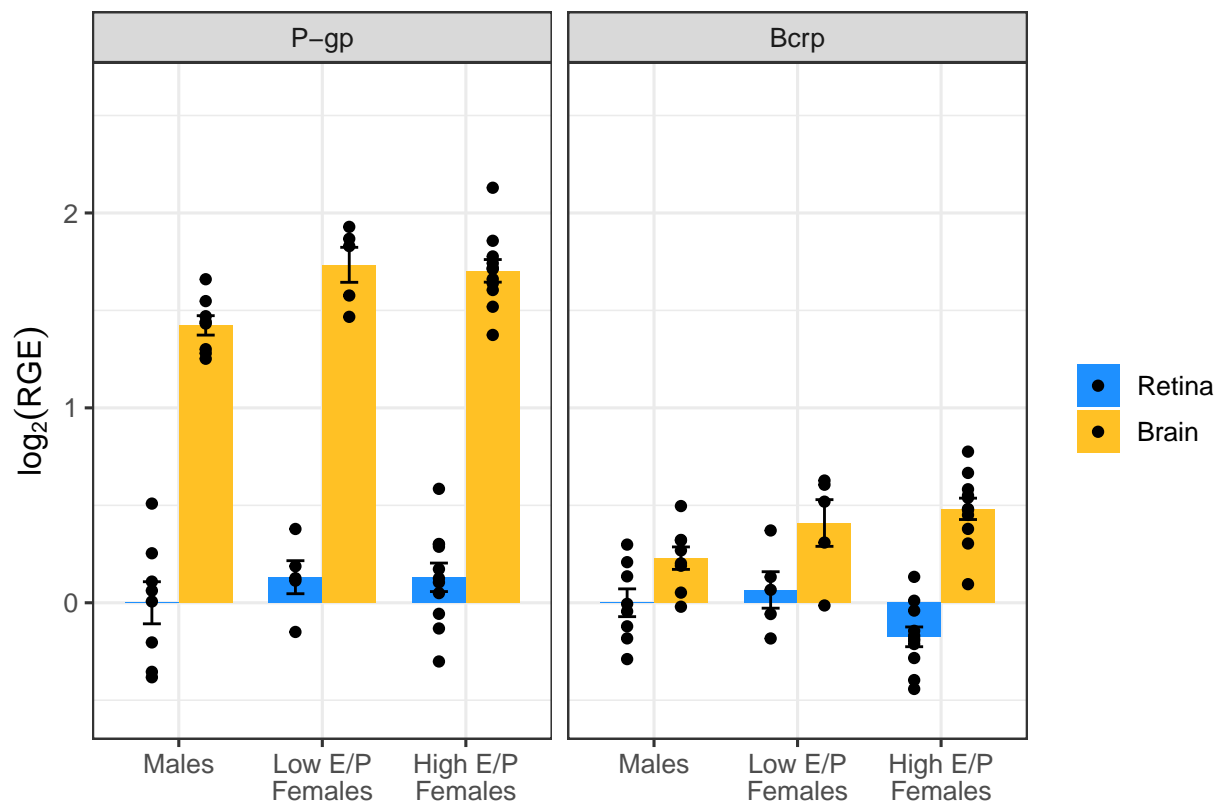


```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_1inj.png", plot=trans_hyp_ret_plot, height=4, width=10)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_1inj.svg", plot=trans_hyp_ret_plot, height=4, width=10)
```

```
trans_hyp_ret_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Group, y=log2(rge), fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Group, ymin=log2_mean-log2_se,
    ymax=log2_mean+log2_se, fill=Tissue),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Group, y=log2(rge), fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, labeller = labeller(gene = gene.labs), ncol=2) +
  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("dodgerblue", "goldenrod1"),
    labels=c("Retina", "Brain")) +
  scale_x_discrete(labels=c("M" = "Males", "Flo" = "Low E/P\nFemales",
    "Phi" = "High E/P\nFemales")) +
  xlab("") +
  ylab(expression(log[2](RGE)))
```

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

```
trans_hyp_ret_log_plot
```



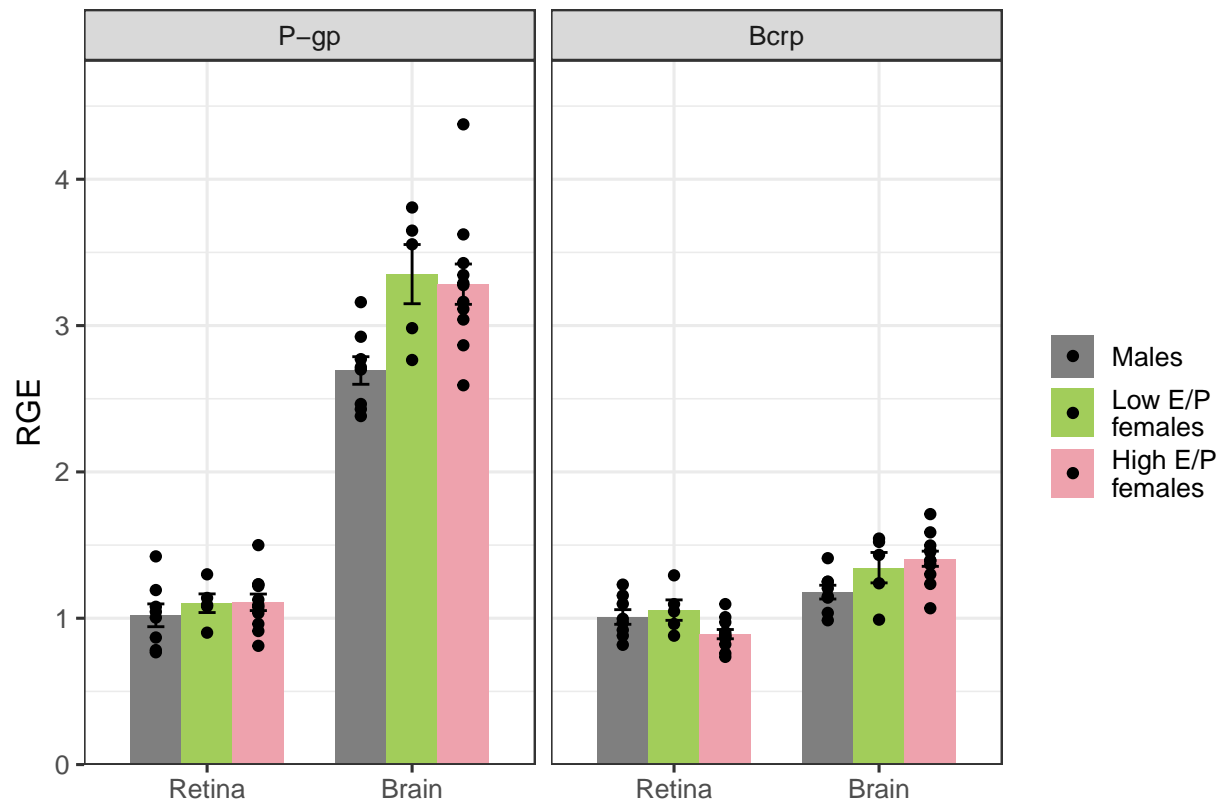
```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log_1inj.png", plot=trans_hyp_ret_log_plot, height=10)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log_1inj.svg", plot=trans_hyp_ret_log_plot, height=10)
```

## Visualizing sex diffs within each tissue/gene

```
trans_trt_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Tissue, y=rge, fill=Group),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Tissue, ymin=mean-se, ymax=mean+se, fill=Group),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Tissue, y=rge, fill=Group),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, labeller = labeller(gene = gene.labs), ncol=2) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  theme_bw(base_size = 12) %+replace%
  theme(legend.title = element_blank(),
    legend.spacing.y = unit(0.1, 'cm')) +
  ## next line needed to make previous line work
  guides(fill = guide_legend(byrow = TRUE)) +
  scale_fill_manual(values=c("gray50", "darkolivegreen3", "lightpink2"),
    labels=c("M" = "Males", "Flo" = "Low E/P\nfemales",
      "Phi" = "High E/P\nfemales")) +
  scale_x_discrete(labels=c("ret" = "Retina", "hyp" = "Brain")) +
  xlab("") +
  ylab("RGE")
```

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

```
trans_trt_plot
```



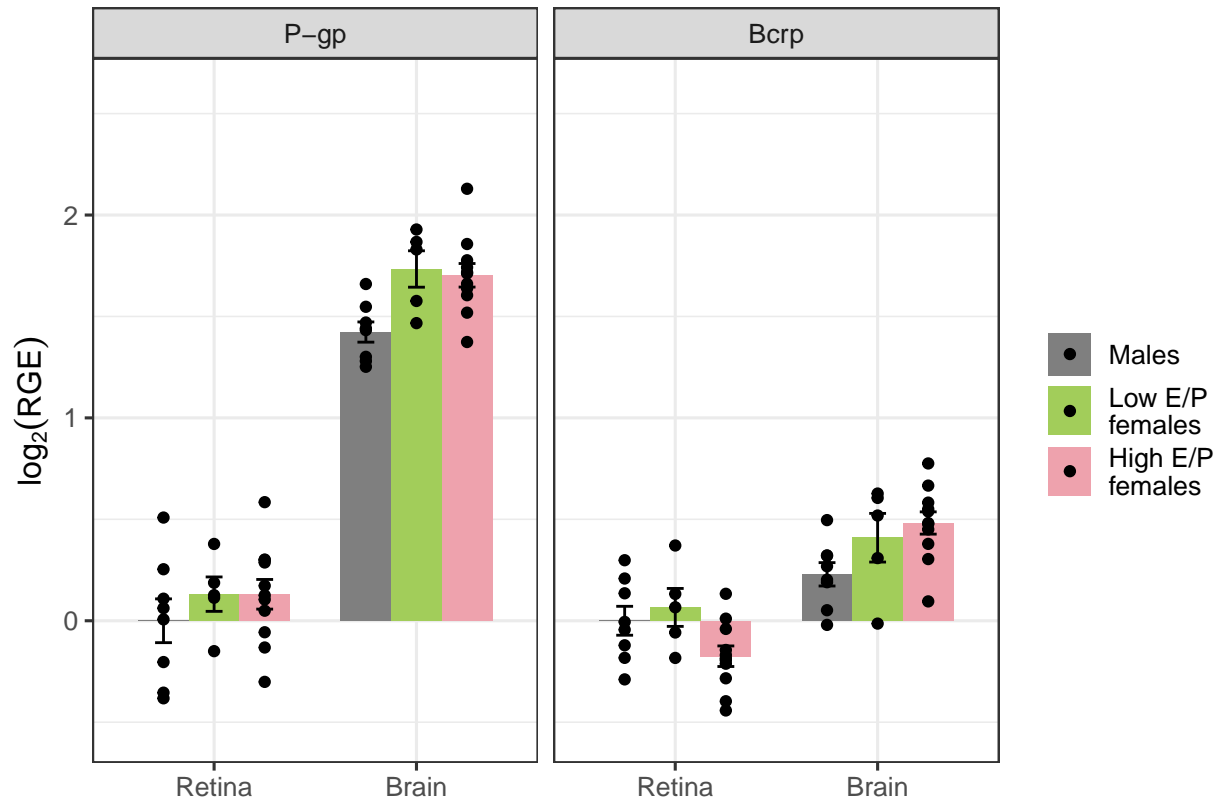
```
#ggsave(filename="../../figures/qRT-PCR/trans_trt_1inj.png", plot=trans_trt_plot, height=4, width=7)
#ggsave(filename="../../figures/qRT-PCR/trans_trt_1inj.svg", plot=trans_trt_plot, height=4, width=7)
```

```
trans_trt_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Tissue, y=log2(rge), fill=Group),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Tissue, ymin=log2_mean-log2_se,
    ymax=log2_mean+log2_se, fill=Group),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Tissue, y=log2(rge), fill=Group),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, labeller = labeller(gene = gene.labs), ncol=2) +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  theme_bw(base_size = 12) %+replace%
  theme(legend.title = element_blank(),
    legend.spacing.y = unit(0.1, 'cm')) +
  ## next line needed to make previous line work
  guides(fill = guide_legend(byrow = TRUE)) +
  scale_fill_manual(values=c("gray50", "darkolivegreen3", "lightpink2"),
    labels=c("M" = "Males", "Flo" = "Low E/P\nfemales",
      "Phi" = "High E/P\nfemales")) +
  scale_x_discrete(labels=c("ret" = "Retina", "hyp" = "Brain")) +
```

```
xlab("") +
ylab(expression(log[2](RGE)))
```

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

```
trans_trt_log_plot
```



```
#ggsave(filename="../../figures/qRT-PCR/trans_trt_log_1inj.png", plot=trans_trt_log_plot, height=4, width=10)
#ggsave(filename="../../figures/qRT-PCR/trans_trt_log_1inj.svg", plot=trans_trt_log_plot, height=4, width=10)
```

## Visualizing P-gp vs Bcrp differences

```
group.labs <- c("Males", "Low E/P Females", "High E/P Females")
names(group.labs) <- c("M", "Flo", "Phi")

trans_g2g_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Tissue, y=rge, fill=gene),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Tissue, ymin=mean-se,
    ymax=mean+se, fill=gene),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Tissue, y=rge, fill=gene),
    position=position_dodge(width=0.75)) +
  facet_grid(~Group, labeller = labeller(Group = group.labs)) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  scale_fill_manual(values=c("darkorchid1", "orange1"),
```

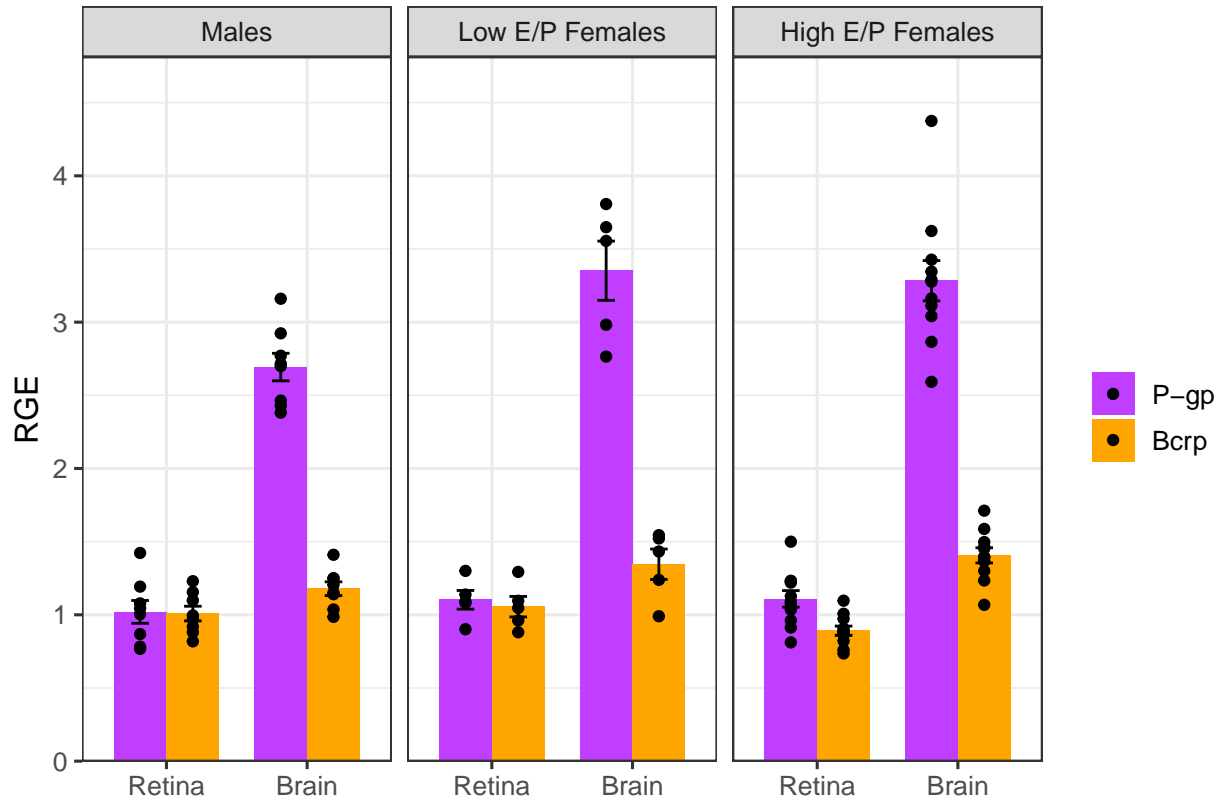
```

      labels = c("P-gp", "Bcrp")) +
theme_bw(base_size = 12) %+replace%
theme(legend.title = element_blank()) +
xlab("") +
ylab("RGE") +
scale_x_discrete(labels=c("ret" = "Retina", "hyp" = "Brain"))

```

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

```
trans_g2g_plot
```



```

#ggsave(filename="../../figures/qRT-PCR/trans_g2g_1inj.png", plot=trans_g2g_plot, height=4, width=7)
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_1inj.svg", plot=trans_g2g_plot, height=4, width=7)

```

```

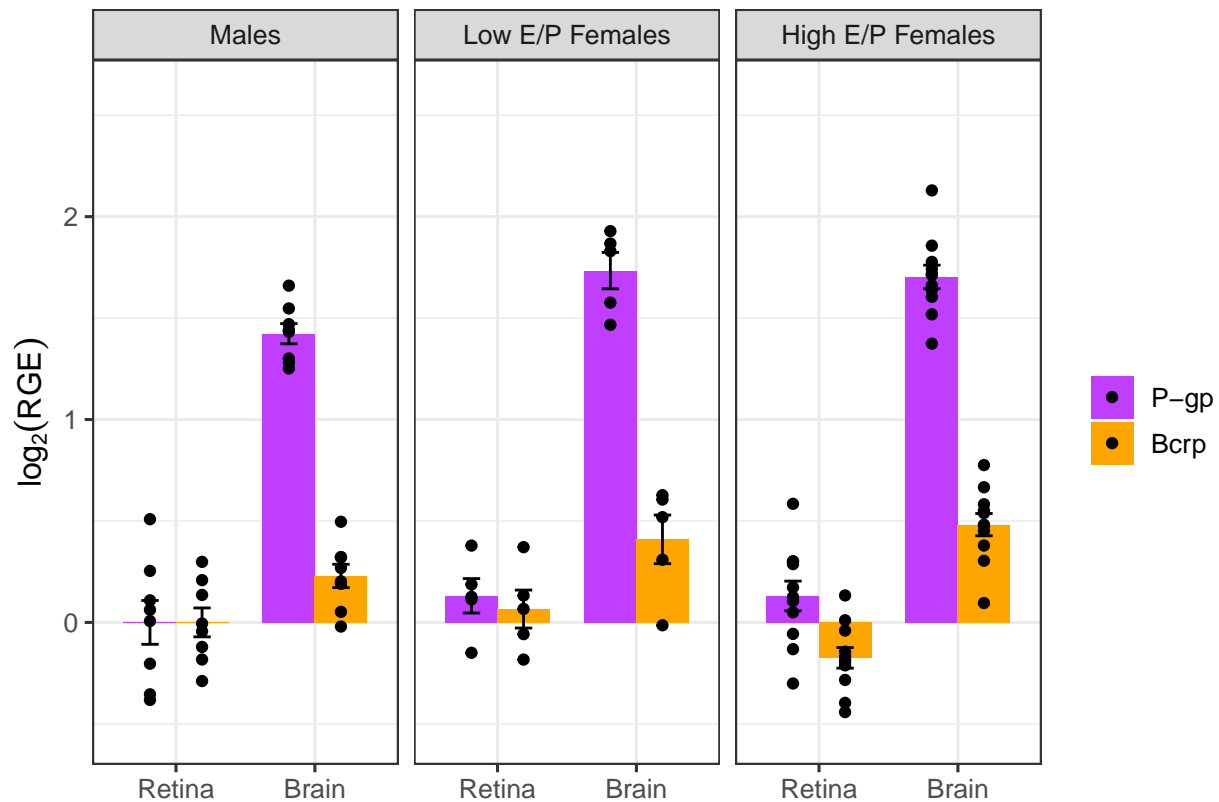
trans_g2g_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Tissue, y=log2(rge), fill=gene),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Tissue, ymin=log2_mean-log2_se,
    ymax=log2_mean+log2_se, fill=gene),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Tissue, y=log2(rge), fill=gene),
    position=position_dodge(width=0.75)) +
  facet_wrap(~Group, labeller = labeller(Group = group.labs)) +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  scale_fill_manual(values=c("darkorchid1", "orange1"),
    labels = c("P-gp", "Bcrp")) +

```

```
theme_bw(base_size = 12) %+replace%
theme(legend.title = element_blank()) +
xlab("") +
ylab(expression(log2(RGE))) +
scale_x_discrete(labels=c("ret" = "Retina", "hyp" = "Brain"))
```

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

```
trans_g2g_log_plot
```

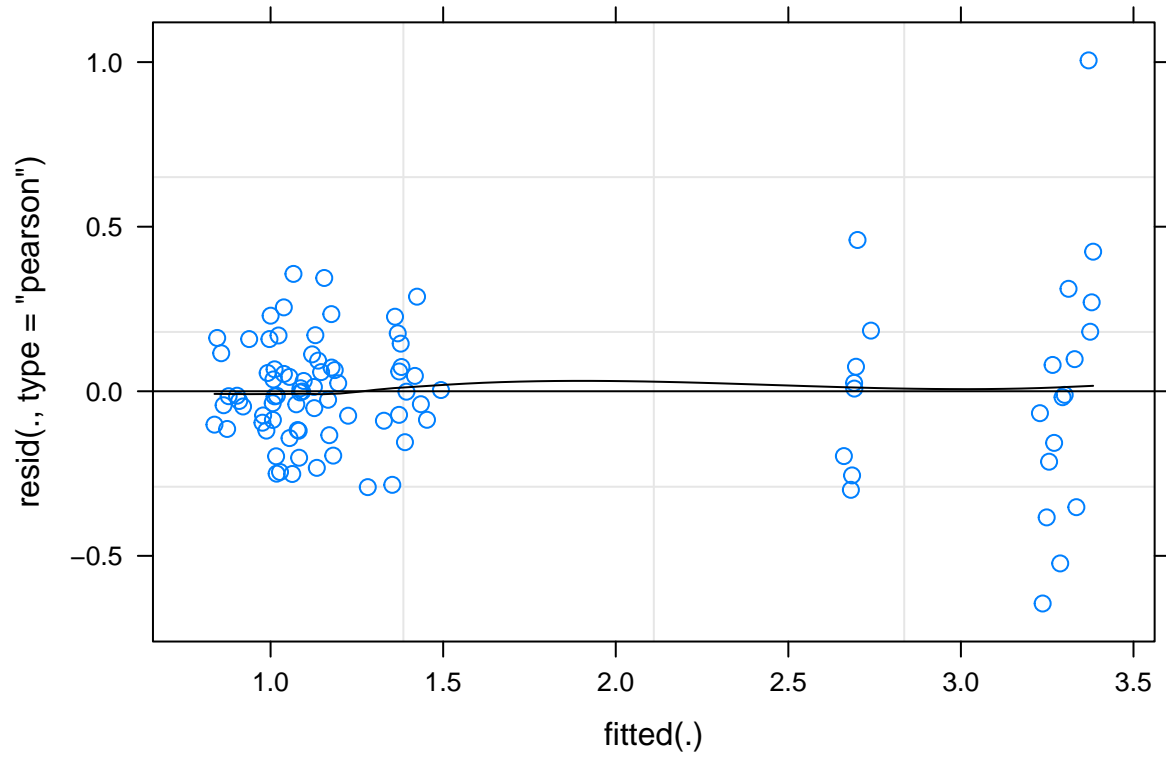


```
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_log_1inj.png", plot=trans_g2g_log_plot, height=4, width=10)
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_log_1inj.svg", plot=trans_g2g_log_plot, height=4, width=10)
```

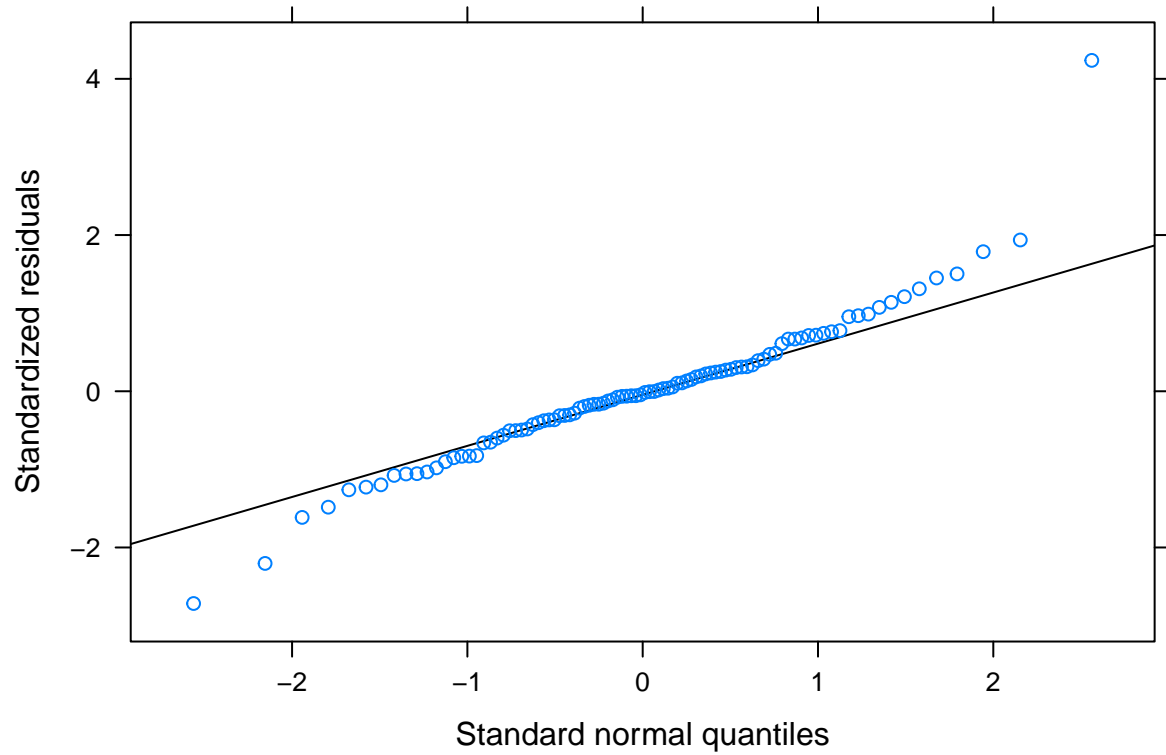
To bottom: Full analysis (all 5 groups) - not used

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

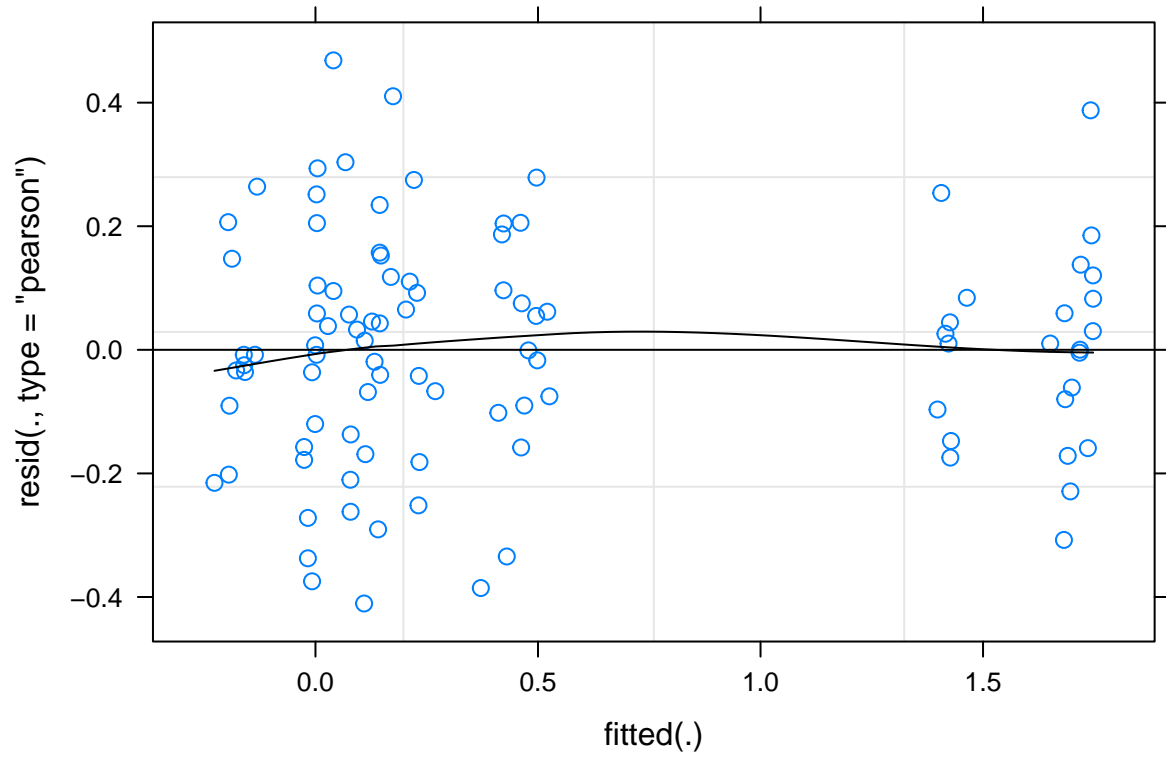




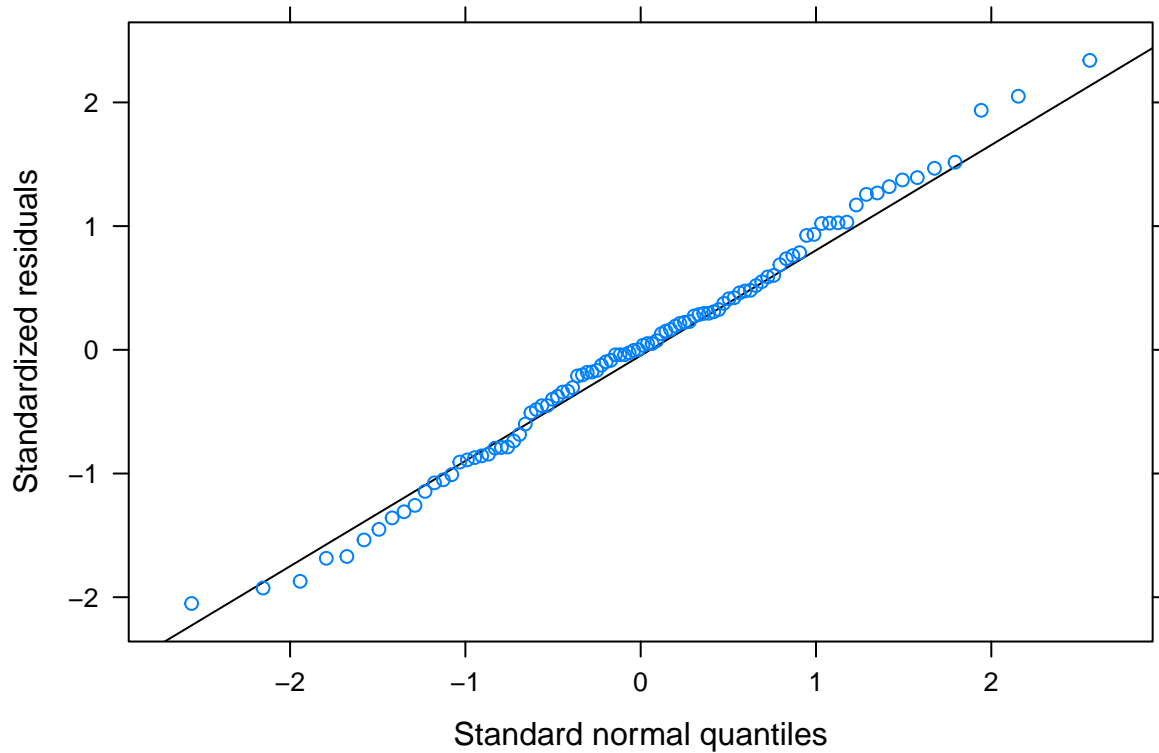
```
lattice::qqmath(transporter_lm)
```



```
transporter_log2_lm <- lmer(log2(rge) ~ gene * Tissue * Group + (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 = 0.77877, p-value = 1.044e-10
```

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

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(transporters_long$rge)
## W = 0.87148, p-value = 1.285e-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	10.1573	10.1573	1	63	253.4878	< 2e-16 ***
Tissue	20.4143	20.4143	1	63	509.4664	< 2e-16 ***
Group	0.3434	0.1717	2	21	4.2854	0.02749 *
gene:Tissue	6.8233	6.8233	1	63	170.2831	< 2e-16 ***
gene:Group	0.1278	0.0639	2	63	1.5945	0.21109
Tissue:Group	0.3872	0.1936	2	63	4.8315	0.01117 *
gene:Tissue:Group	0.1405	0.0703	2	63	1.7536	0.18149

```
## ---
```

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

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

## Tissue = ret, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.1312 0.1180 83  -1.111  0.5098
## M - Fhi      -0.1306 0.0962 83  -1.357  0.3680
## Flo - Fhi      0.0006 0.1117 83   0.005  1.0000
##
## Tissue = hyp, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.3108 0.1180 83  -2.633  0.0270
## M - Fhi      -0.2798 0.0962 83  -2.909  0.0128
## Flo - Fhi      0.0310 0.1117 83   0.277  0.9585
##
## Tissue = ret, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.0658 0.1180 83  -0.557  0.8430
## M - Fhi       0.1748 0.0962 83   1.818  0.1702
## Flo - Fhi      0.2406 0.1117 83   2.155  0.0852
##
## Tissue = hyp, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.1804 0.1180 83  -1.528  0.2830
## M - Fhi      -0.2530 0.0962 83  -2.630  0.0272
## Flo - Fhi     -0.0726 0.1117 83  -0.651  0.7926
##
## 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 3 estimates

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

## Group = M, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.423 0.1001 63 -14.219 <.0001
##
## Group = Flo, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.603 0.1266 63 -12.660 <.0001
##
## Group = Fhi, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.572 0.0854 63 -18.422 <.0001
##
## Group = M, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -0.229 0.1001 63  -2.287  0.0255
##
## Group = Flo, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -0.344 0.1266 63  -2.714  0.0086
##
## Group = Fhi, gene = Bcrp:
```

```
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.657 0.0854 63 -7.695 <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

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

```
## Group = M, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.0000 0.1001 63 0.000 1.0000
##
## Group = Flo, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.0654 0.1266 63 0.516 0.6073
##
## Group = Fhi, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.3054 0.0854 63 3.578 0.0007
##
## Group = M, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.1942 0.1001 63 11.932 <.0001
##
## Group = Flo, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.3247 0.1266 63 10.463 <.0001
##
## Group = Fhi, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.2211 0.0854 63 14.306 <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

```
trans_sumstats <- transporters_long %>% group_by(Group, 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 'Group', 'Tissue'. You can override using
## the `.groups` argument.
```

```
trans_sumstats
```

```
## # A tibble: 12 x 8
## # Groups:   Group, Tissue [6]
##   Group Tissue gene      n mean    sd log2_mean log2_sd
##   <fct> <fct> <fct> <int> <dbl> <dbl> <dbl> <dbl>
## 1 M     ret    Pgp      8 1.02 0.220 2.02e-10 0.305
## 2 M     ret    Bcrp      8 1.01 0.142 6.58e-11 0.202
## 3 M     hyp    Pgp      8 2.69 0.266 1.42e+ 0 0.141
## 4 M     hyp    Bcrp      8 1.18 0.133 2.29e- 1 0.163
```

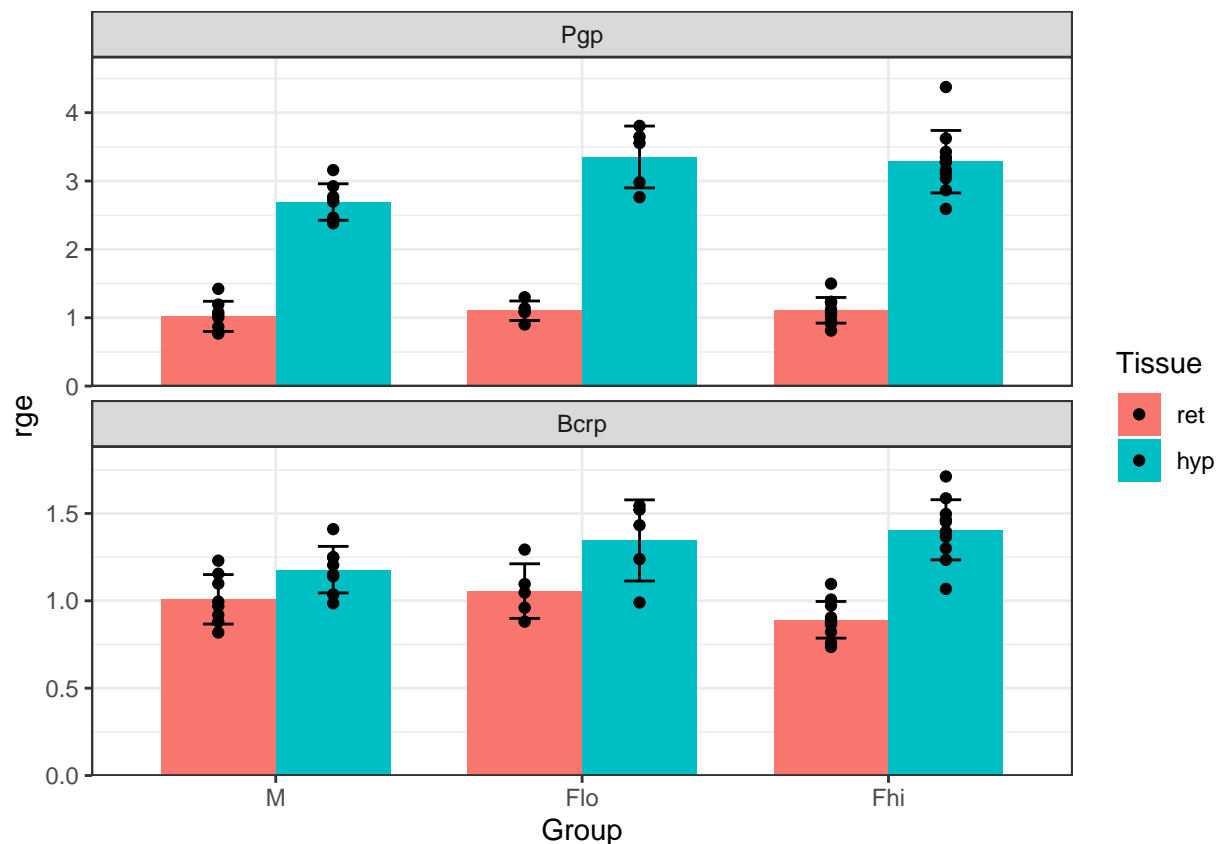
```
## 5 Flo ret Pgp 5 1.10 0.143 1.31e- 1 0.190
## 6 Flo ret Bcrp 5 1.06 0.156 6.58e- 2 0.209
## 7 Flo hyp Pgp 5 3.35 0.452 1.73e+ 0 0.201
## 8 Flo hyp Bcrp 5 1.35 0.232 4.09e- 1 0.268
## 9 Fhi ret Pgp 11 1.11 0.187 1.31e- 1 0.242
## 10 Fhi ret Bcrp 11 0.891 0.105 -1.75e- 1 0.168
## 11 Fhi hyp Pgp 11 3.28 0.457 1.70e+ 0 0.193
## 12 Fhi hyp Bcrp 11 1.41 0.172 4.82e- 1 0.182
```

Comparing hyp vs ret within each treatment/gene

```
trans_hyp_ret_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Group, y=rge, fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Group, ymin=mean-sd, ymax=mean+sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Group, 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
```

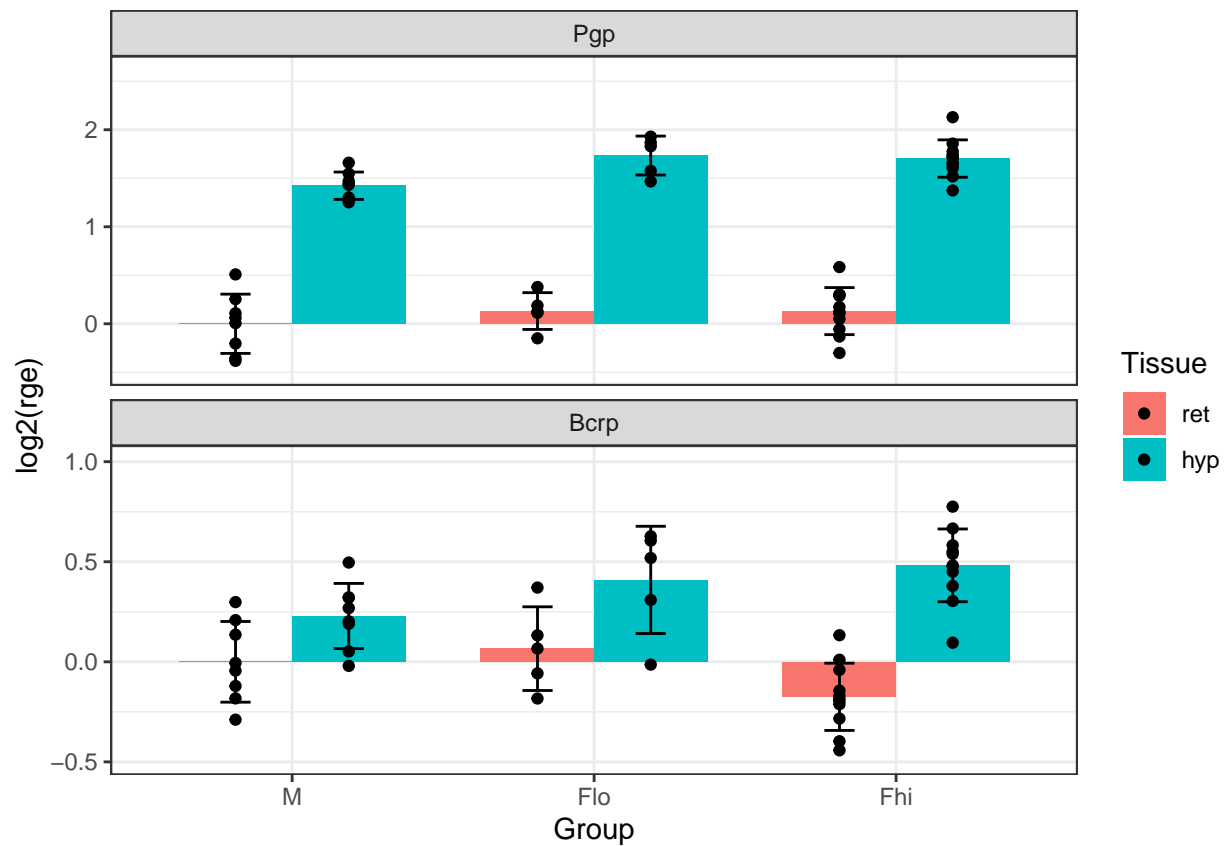


```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret.png", plot=trans_hyp_ret_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret.svg", plot=trans_hyp_ret_plot, height=4, width=6)
```

```
trans_hyp_ret_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Group, y=log2(rge), fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Group, 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=Group, 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=6)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log.svg", plot=trans_hyp_ret_log_plot, height=4, width=6)
```

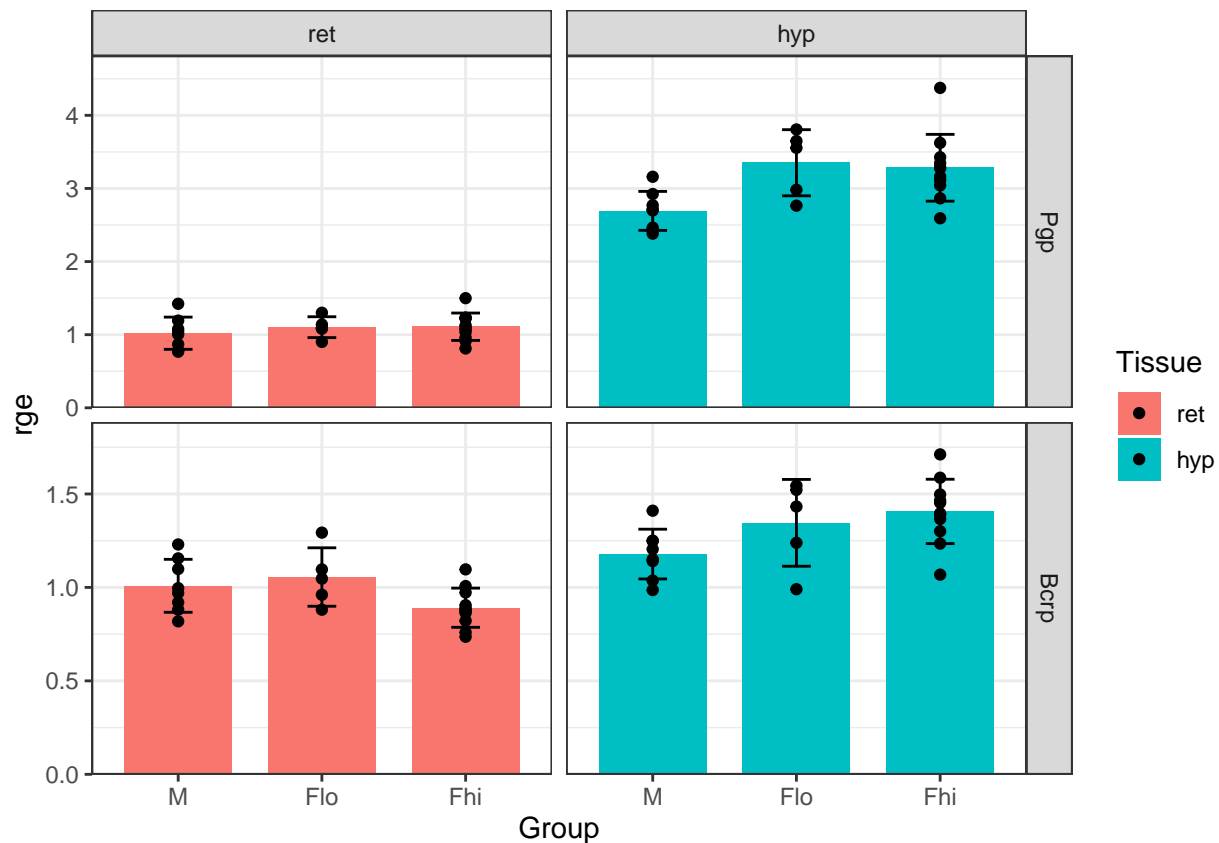


## Comparing treatments within each tissue/gene

```
trans_trt_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Group, y=rge, fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Group, ymin=mean-sd, ymax=mean+sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Group, 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=Group, y=log2(rge), fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Group, ymin=log2_mean-log2_sd,
    ymax=log2_mean+log2_sd, fill=Tissue),
```

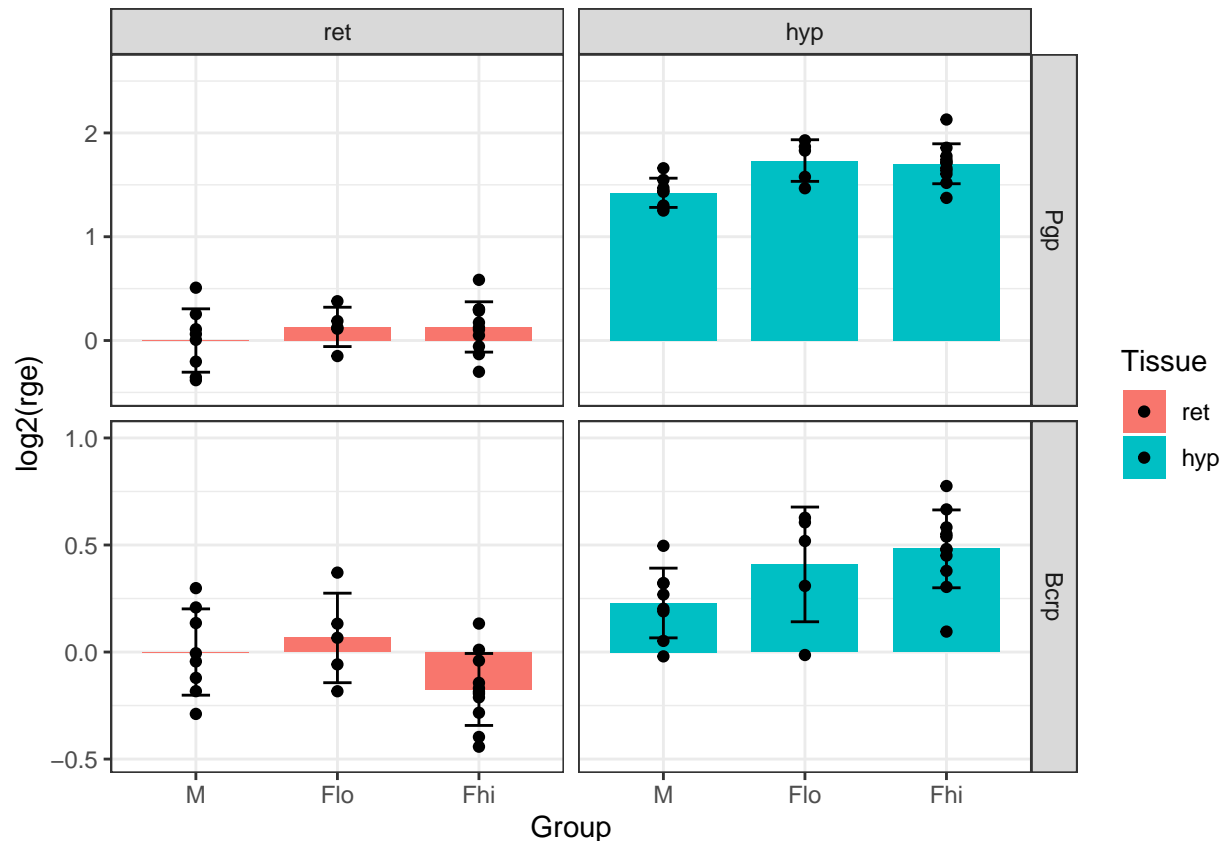
```

width=0.2, position=position_dodge(width=0.75)) +
geom_point(data=transporters_long, aes(x=Group, 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)

```

### Comparing P-gp to Bcrp

```

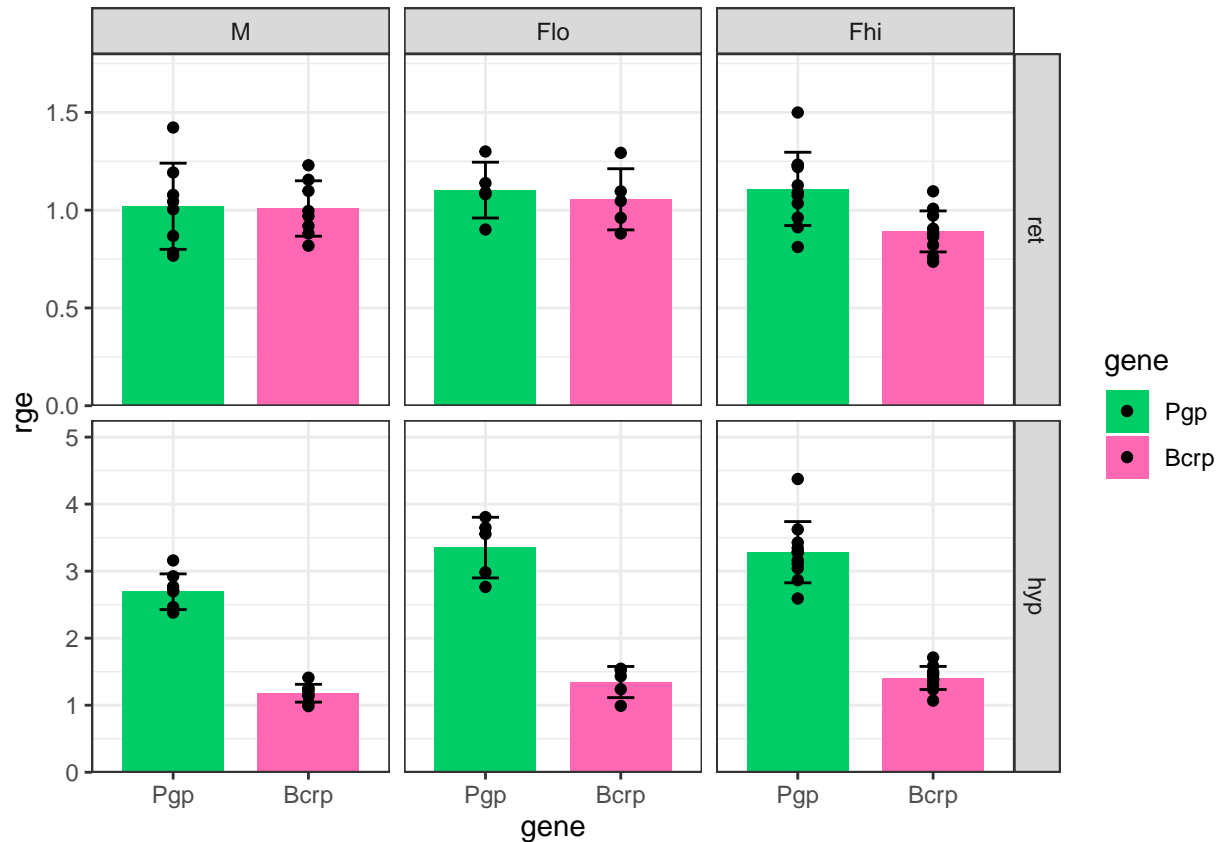
trans_g2g_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=gene, y=rge, fill=gene),
              fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=gene, ymin=mean-sd,
                                         ymax=mean+sd, fill=gene),
               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~Group, 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
```

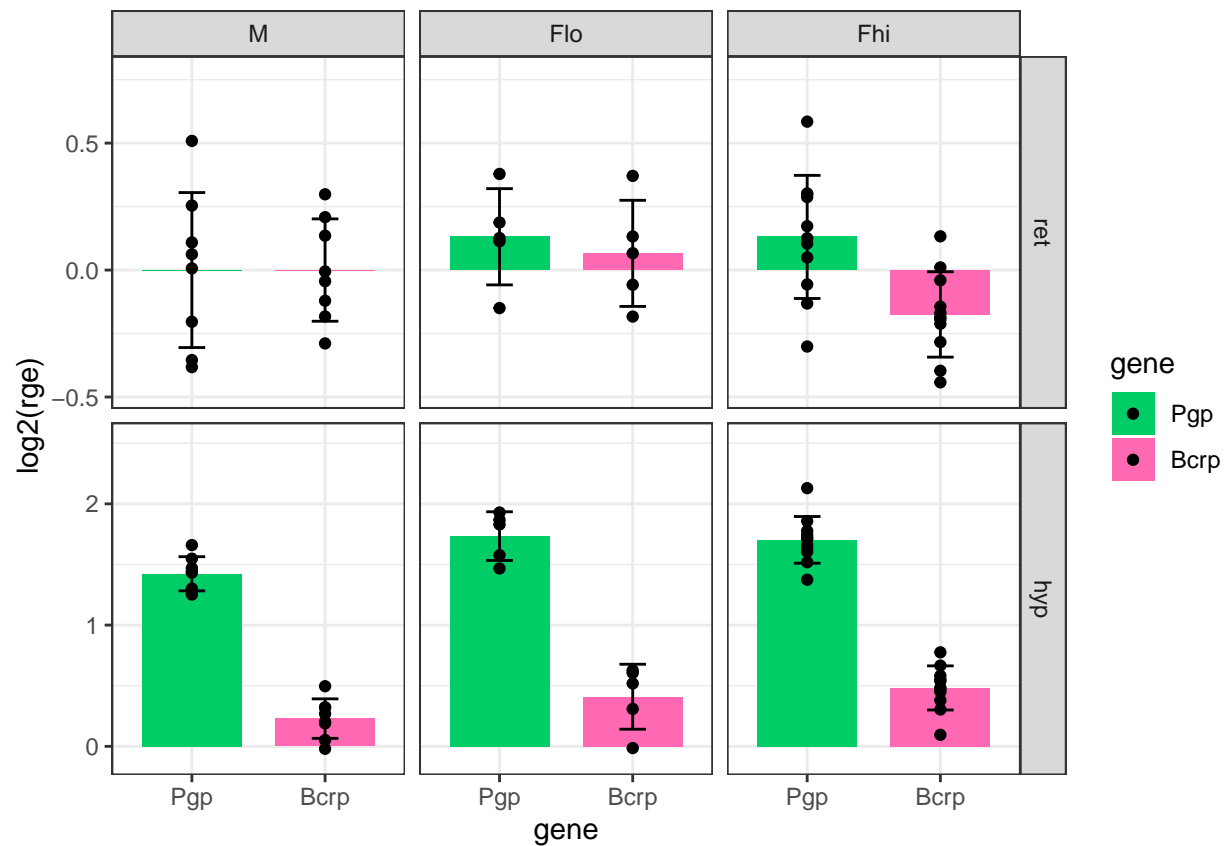


```
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene.png", plot=trans_g2g_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene.svg", plot=trans_g2g_plot, height=4, width=6)
```

```
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~Group, 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)
```