

# 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", 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

```

## Stats on Bcrp only (chronic vs saline)

```

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

```

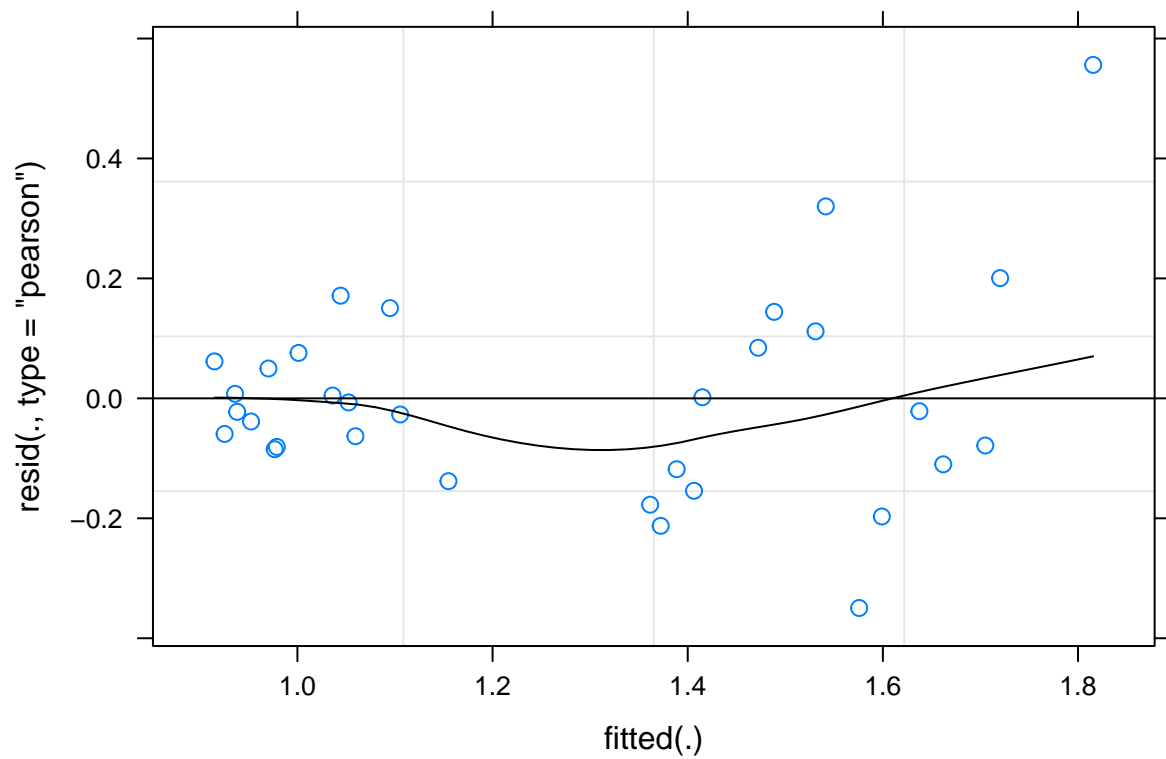
```

##   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

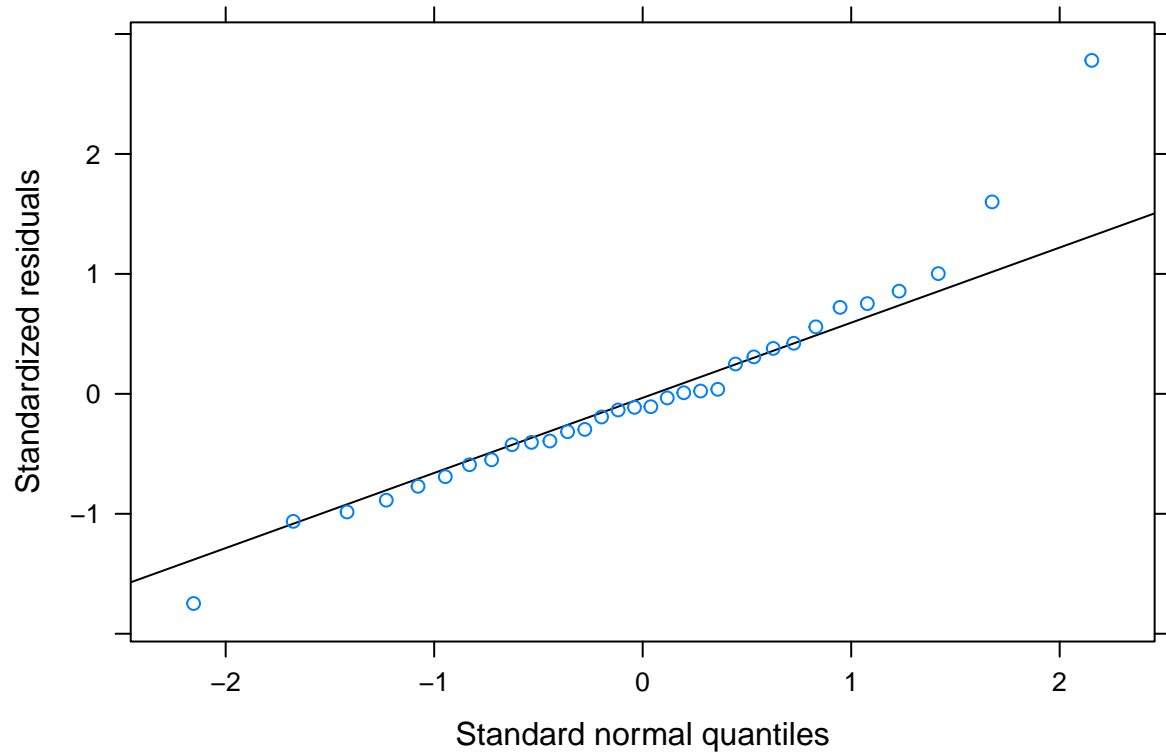
```

```
## 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
```

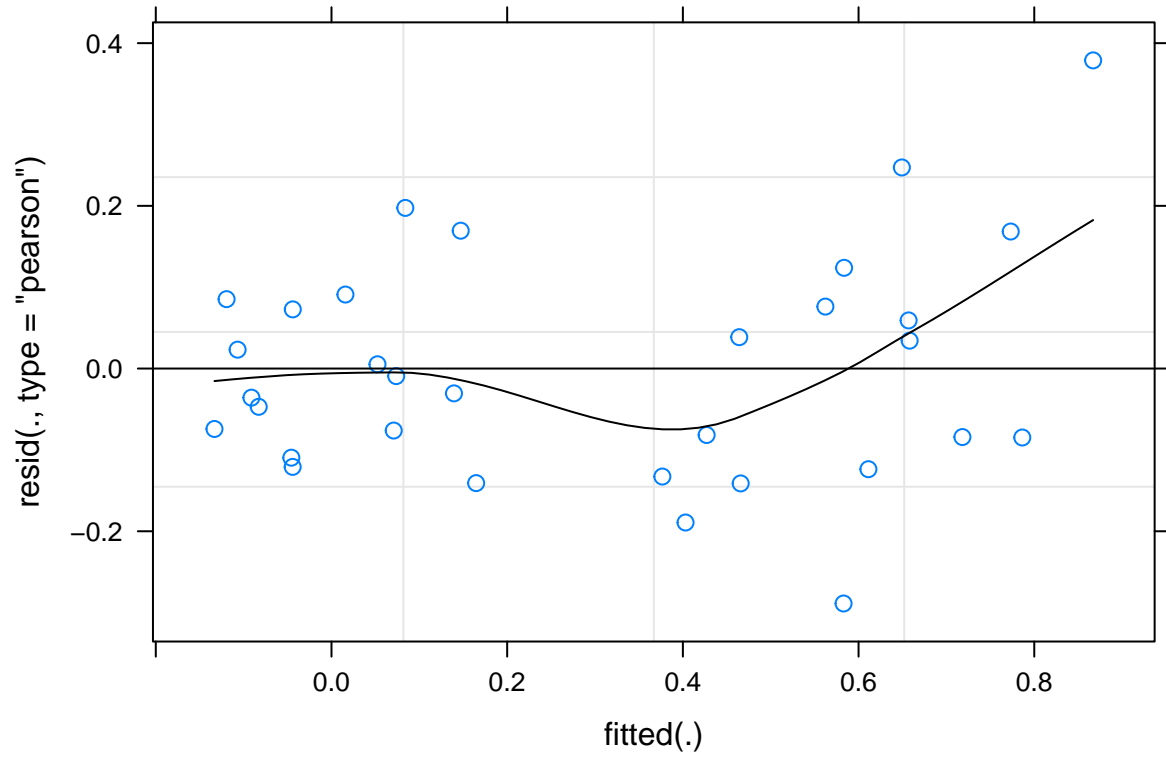
```
c_bcrp_lm <- lmer(Bcrp.RGE ~ Tissue * Treatment + (1|Sample), data=chronic)
plot(c_bcrp_lm, type=c("p","smooth"), col.line=1)
```



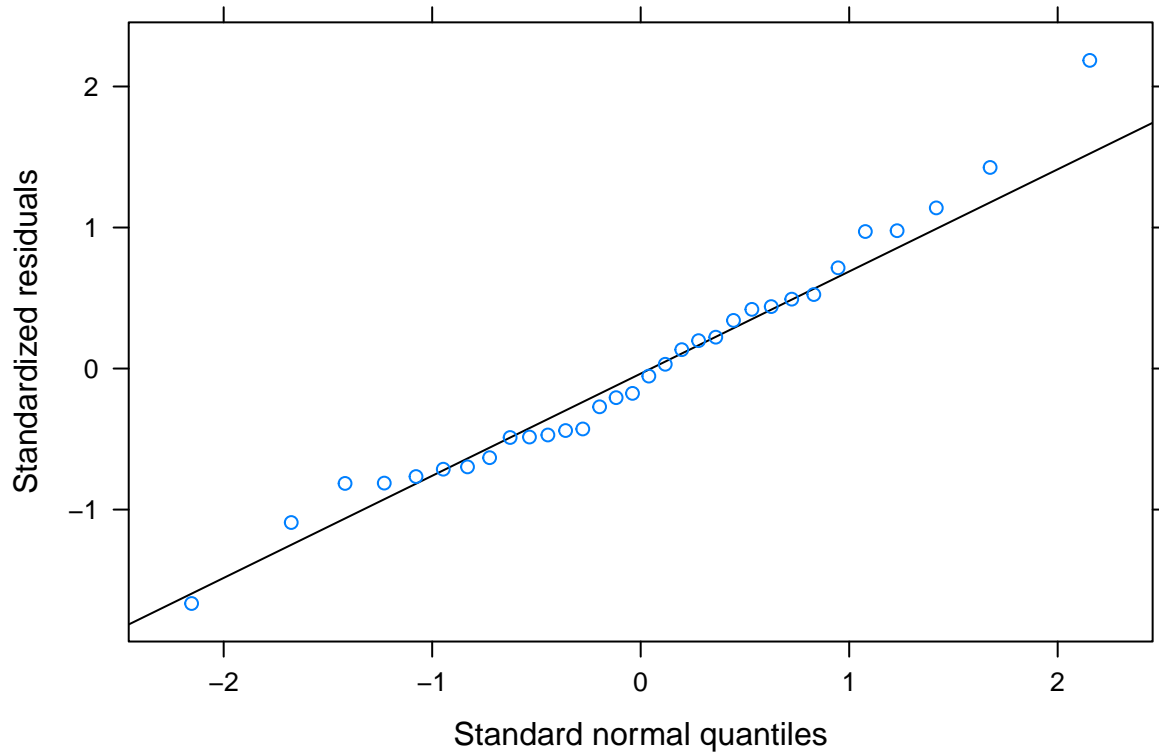
```
lattice::qqmath(c_bcrp_lm)
```



```
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
```

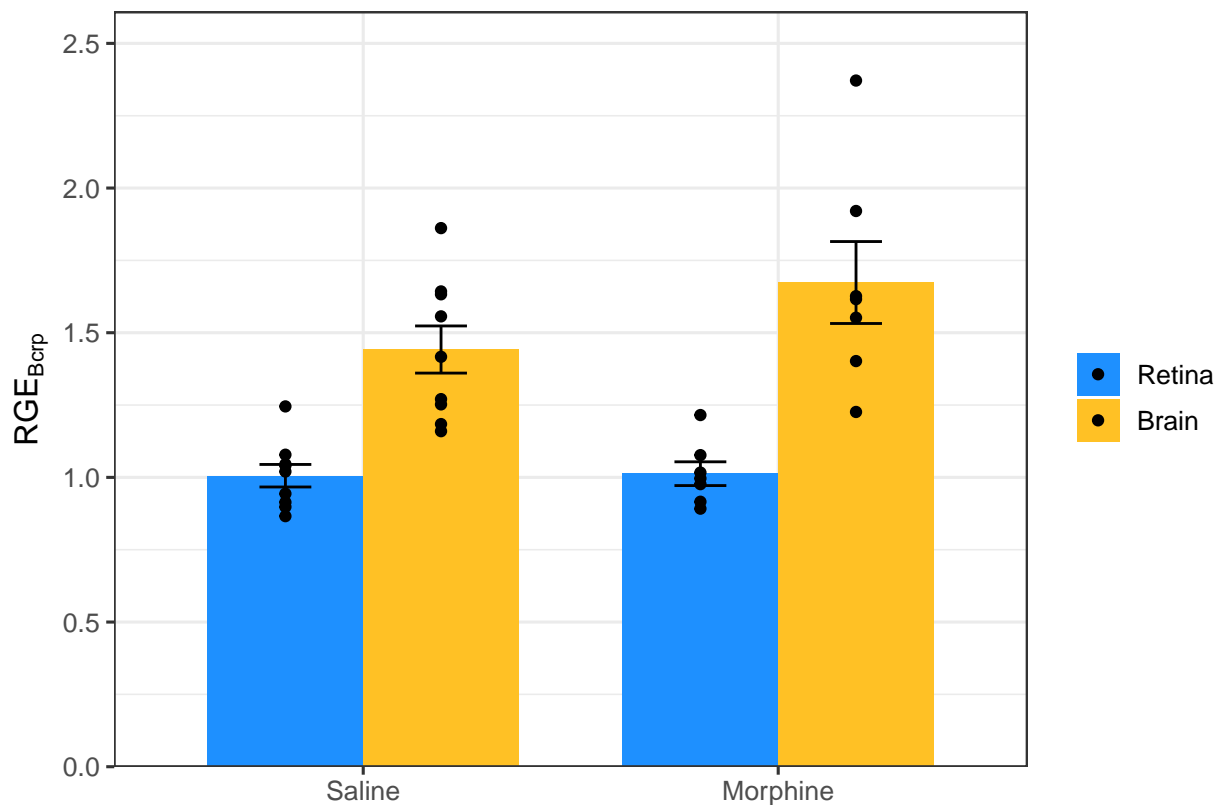
```
## # A tibble: 4 x 9
## # Groups: Treatment [2]
## Treatment Tissue n mean sd se log2_mean log2_sd log2_se
## <fct> <fct> <int> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
## 1 S ret 9 1.01 0.117 0.0389 -4.39e-11 0.163 0.0542
## 2 S hyp 9 1.44 0.244 0.0815 5.10e- 1 0.242 0.0806
## 3 C ret 7 1.01 0.109 0.0411 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_tiss_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\_tiss\_plot



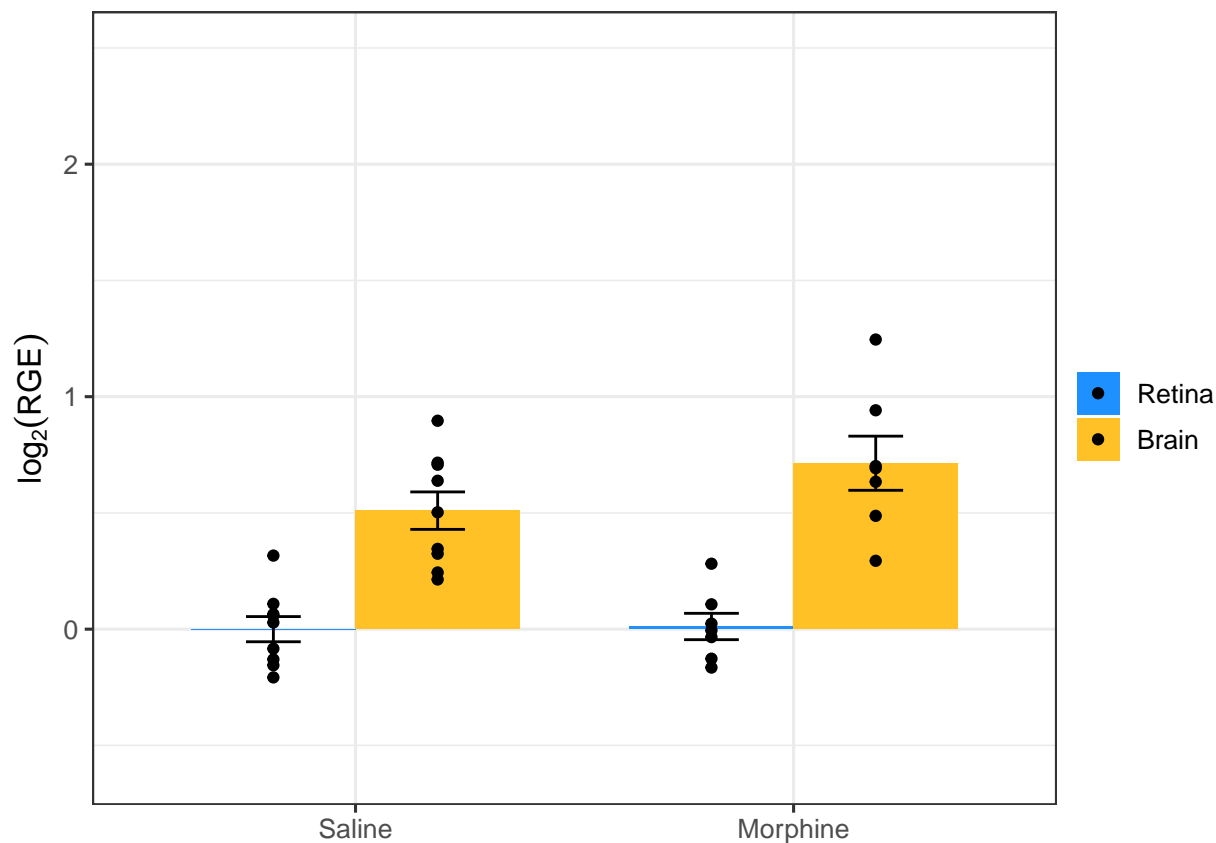


```
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_plot.png", plot=c_bcrp_tiss_plot, height=4, width=4)
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_plot.svg", plot=c_bcrp_tiss_plot, height=4, width=4)
```

```
c_bcrp_tiss_log_plot <- ggplot() +
  stat_summary(data=chronic, aes(x=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))) +
  ylim(-0.6, 2.5) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw(base_size = 12) %+replace%
  theme(legend.title = element_blank(),
    legend.box.spacing = unit(1, "pt"),
    axis.title.x=element_blank(),
    plot.title = element_text(hjust = 0.5, vjust=1.5)
  ) +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
    labels=c("Retina", "Brain")) +
  scale_x_discrete(labels=c("S" = "Saline", "C" = "Morphine")) +
  xlab("") +
  ylab(expression(log2(RGE)))
```

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

```
c_bcrp_tiss_log_plot
```



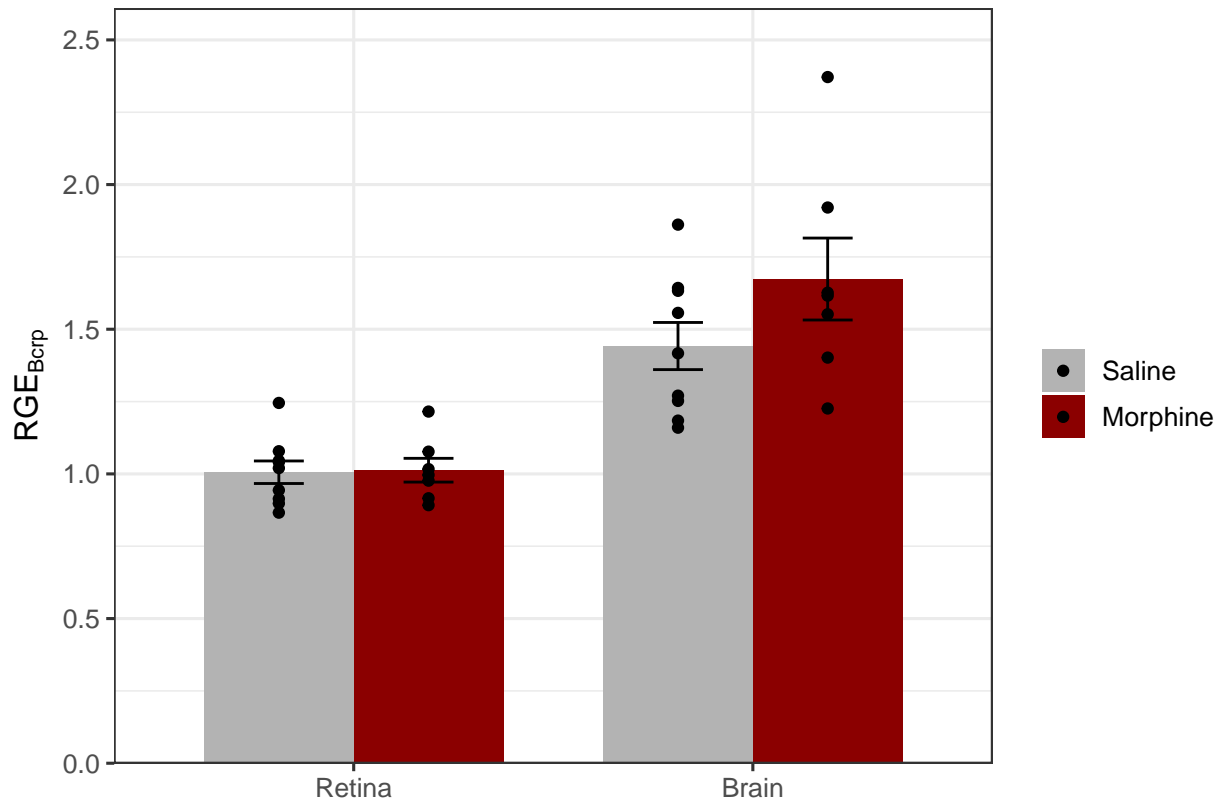
```
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_log_plot.png", plot=c_bcrp_tiss_log_plot, height=4,
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_tiss_log_plot.svg", plot=c_bcrp_tiss_log_plot, height=4,
```

```
#visualizing treatment effect per tissue
```

```
c_bcrp_trt_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(RGE[Bcrp]))
```

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

c\_bcrp\_trt\_plot

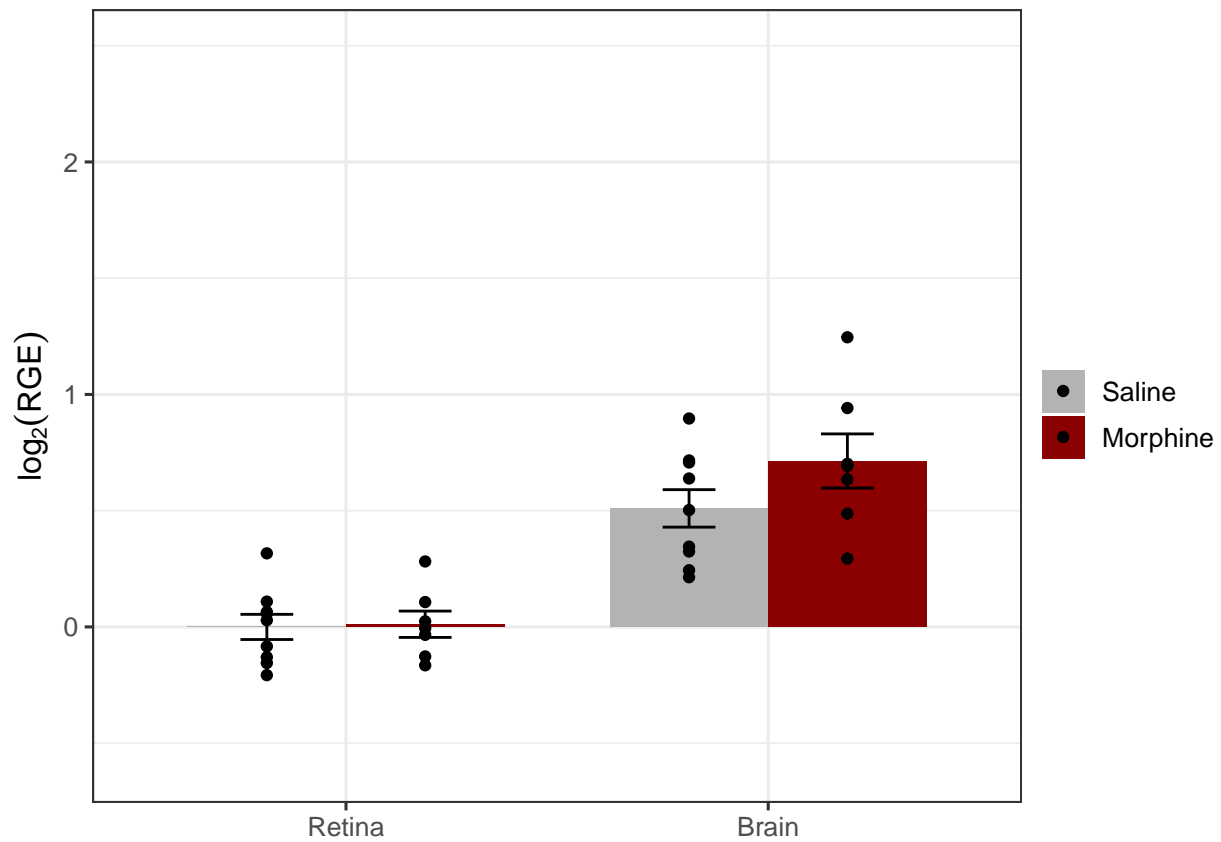


```
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_trt_plot.png", plot=c_bcrp_trt_plot, height=4, width=4)
#ggsave(filename="../../figures/qRT-PCR/c_bcrp_trt_plot.svg", plot=c_bcrp_trt_plot, height=4, width=4)

c_bcrp_trt_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))) +
  ylim(-0.6, 2.5) +
  theme_bw(base_size = 12) %+replace%
  theme(legend.title = element_blank(),
    legend.box.spacing = unit(1, "pt"),
    axis.title.x=element_blank(),
    plot.title = element_text(hjust = 0.5, vjust=1.5)) +
  scale_fill_manual(values=c("gray70", "red4"),
    labels=c("Saline", "Morphine")) +
  scale_x_discrete(labels=c("ret" = "Retina", "hyp" = "Brain")) +
  xlab("") +
  ylab(expression(log[2](RGE)))
```

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

```
c_bcrp_trt_log_plot
```



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

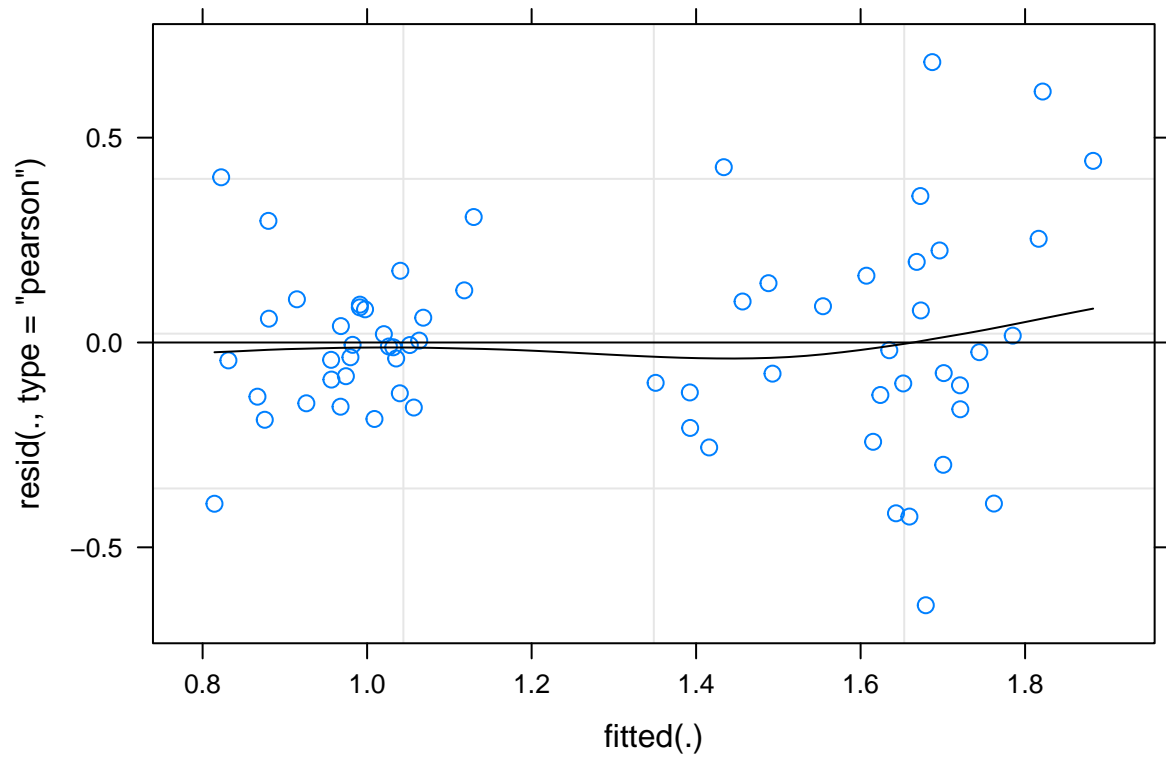
### Chronic morphine Bcrp test WITH P-gp in stats

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

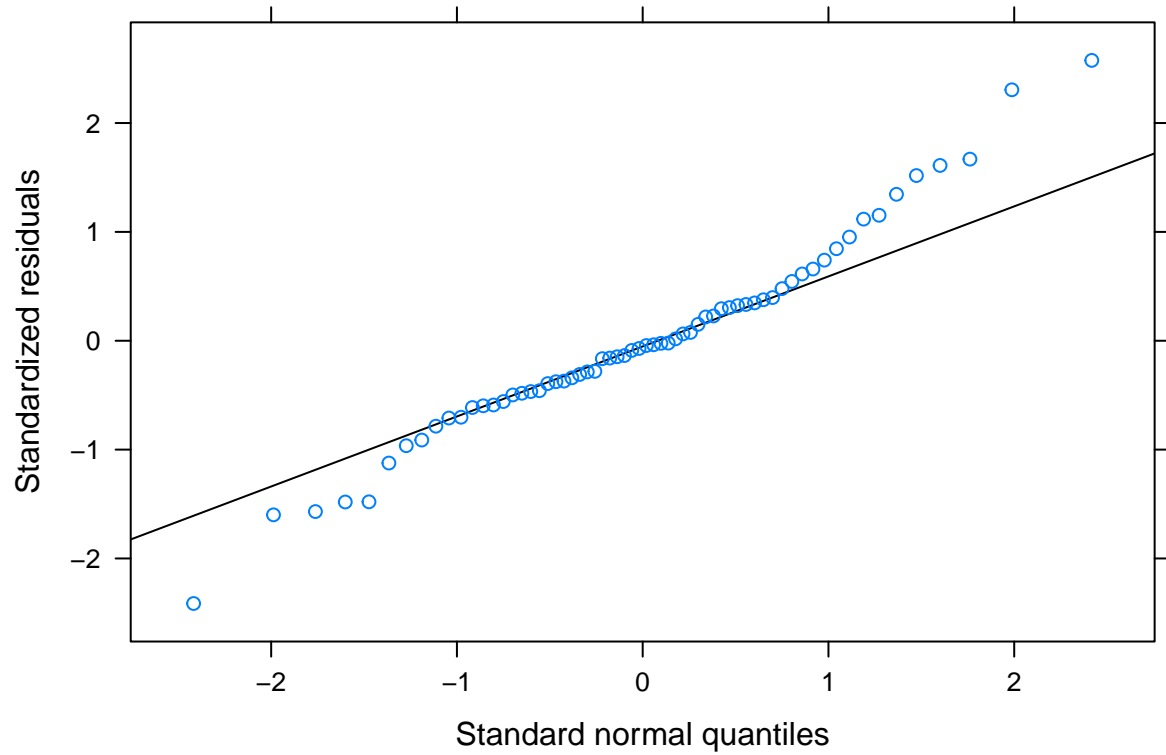
```
## # A tibble: 64 x 5
##   Sample Tissue Treatment gene    rge
##   <chr>   <fct>   <fct>   <fct> <dbl>
## 1 C1     hyp      C        Pgp   1.77
## 2 C1     hyp      C        Bcrp  1.62
## 3 C1     ret      C        Pgp   0.421
## 4 C1     ret      C        Bcrp  0.892
## 5 C2     hyp      C        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
```

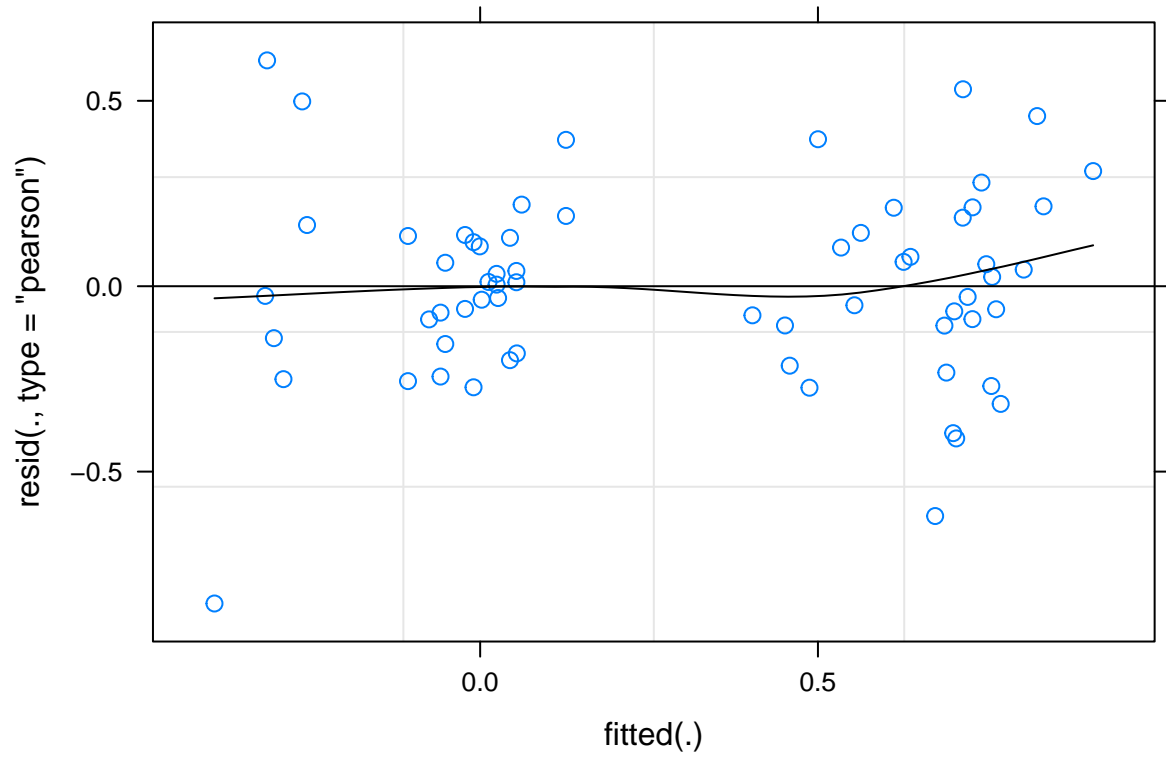
```
chronic_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=chronic_long)
plot(chronic_lm, type=c("p", "smooth"), col.line=1)
```



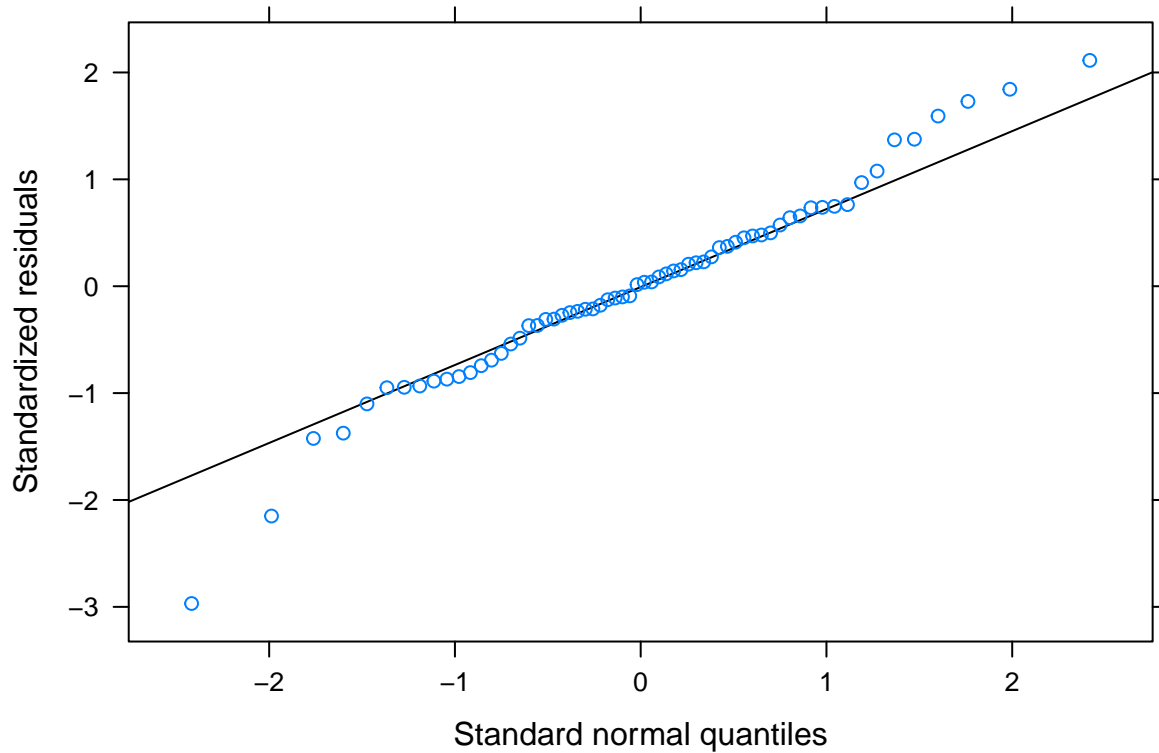
```
lattice::qqmath(chronic_lm)
```



```
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),
```

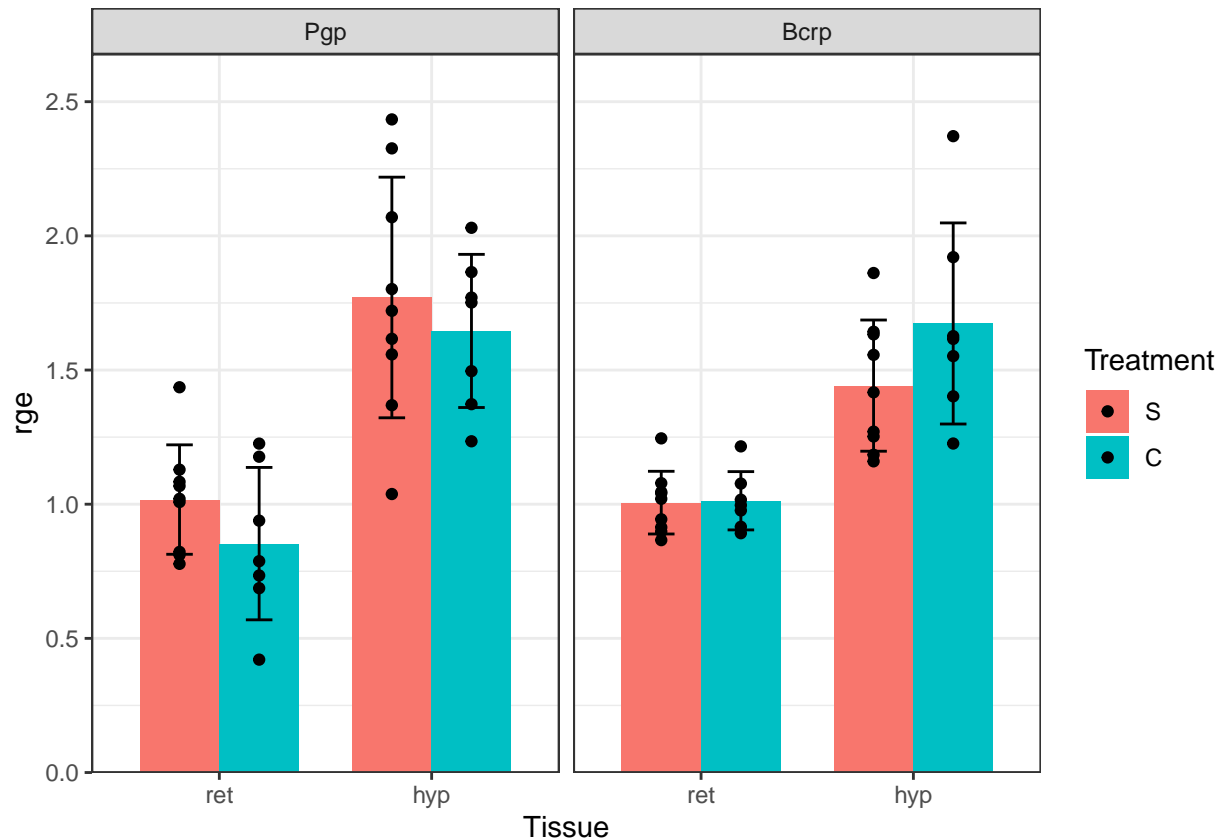
```

    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)

```

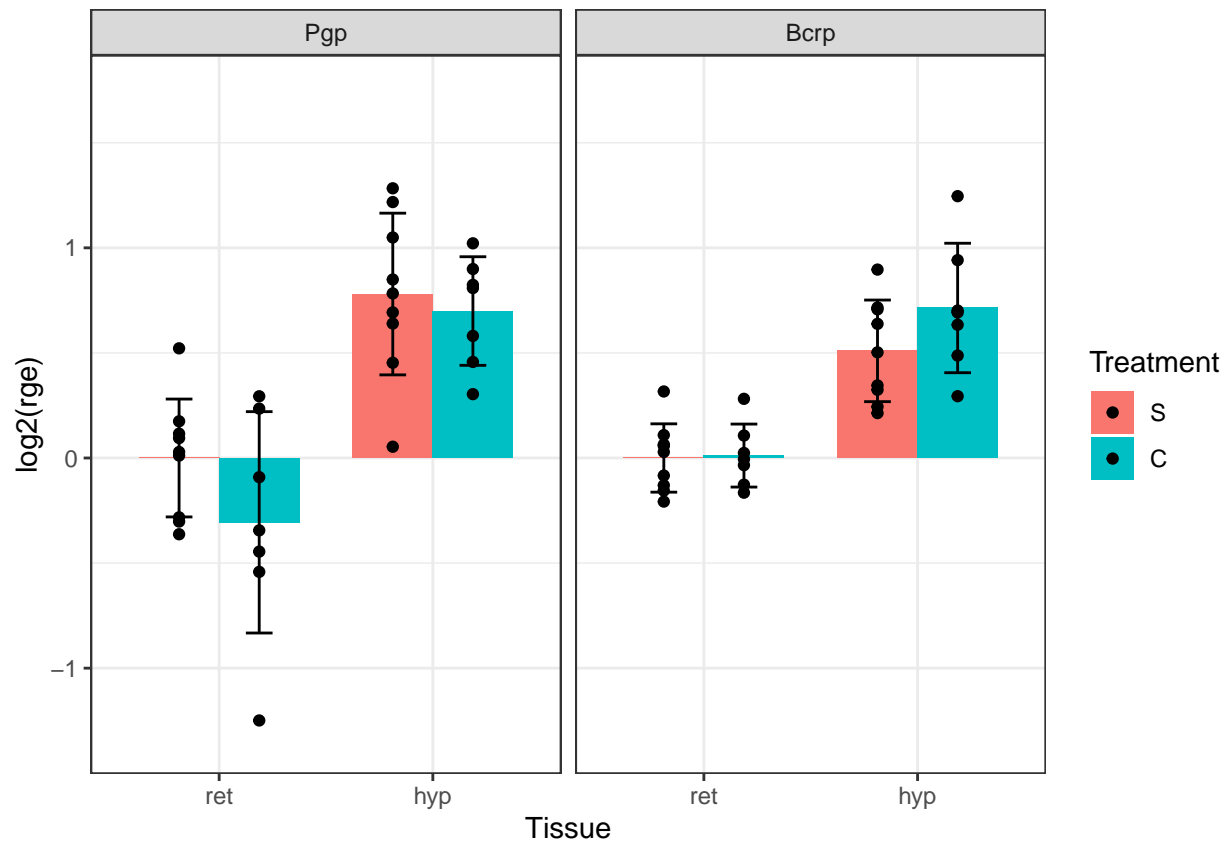
```

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)
```

RERUN STATS WITH LOG2 ON DATA ORIGINALLY PUBLISHED IN BERGUM 2022  
(p-gp chronic morphine/saline)

```
pgp <- read.csv("../data/qRT-PCR/pgp-Bergum2022a.csv", fileEncoding = 'UTF-8-BOM')
pgp <- pgp %>% mutate(Tissue = fct_relevel(Tissue, c("retina", "hypothalamus")),
                     Treatment = fct_relevel(as.factor(Treatment), c("saline", "morphine")))
#removing the outlier
pgp <- pgp %>% filter(RelativeGE<3.5)
pgp
```

##	Sample	RelativeGE	Tissue	Treatment
## 1	M1	2.9955745	hypothalamus	morphine
## 2	M2	2.2145575	hypothalamus	morphine
## 3	M3	2.0521578	hypothalamus	morphine
## 4	M4	1.7128570	hypothalamus	morphine
## 5	M5	0.9969443	hypothalamus	morphine
## 6	M6	1.2801494	hypothalamus	morphine

```
## 7      M7 0.8480968 hypothalamus morphine
## 8      S1 1.7732013 hypothalamus  saline
## 9      S2 1.8987767 hypothalamus  saline
## 10     S3 1.8391614 hypothalamus  saline
## 11     S4 1.5664282 hypothalamus  saline
## 12     S5 1.5853405 hypothalamus  saline
## 13     S6 1.6699513 hypothalamus  saline
## 14     S7 3.0282725 hypothalamus  saline
## 15     S8 2.7501401 hypothalamus  saline
## 16     S9 2.8621095 hypothalamus  saline
## 17     M2 1.0775971      retina morphine
## 18     M3 0.9306124      retina morphine
## 19     M4 1.1232327      retina morphine
## 20     M5 1.0061231      retina morphine
## 21     M6 0.6900942      retina morphine
## 22     M7 0.8444983      retina morphine
## 23     S1 0.8139308      retina  saline
## 24     S2 1.0113566      retina  saline
## 25     S3 1.0402547      retina  saline
## 26     S4 1.1486002      retina  saline
## 27     S5 0.8632570      retina  saline
## 28     S6 0.9363421      retina  saline
## 29     S7 1.3498949      retina  saline
## 30     S8 0.7846937      retina  saline
## 31     S9 1.1874759      retina  saline
```

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

```
##
## Shapiro-Wilk normality test
##
## data:  pgp$RelativeGE
## W = 0.86255, p-value = 0.0009488
```

```
shapiro.test(sqrt(pgp$RelativeGE))
```

```
##
## Shapiro-Wilk normality test
##
## data:  sqrt(pgp$RelativeGE)
## W = 0.90574, p-value = 0.01005
```

```
#normal
shapiro.test(log(pgp$RelativeGE))
```

```
##
## Shapiro-Wilk normality test
##
## data:  log(pgp$RelativeGE)
## W = 0.93745, p-value = 0.07015
```

```
shapiro.test(log2(pgp$RelativeGE))
```

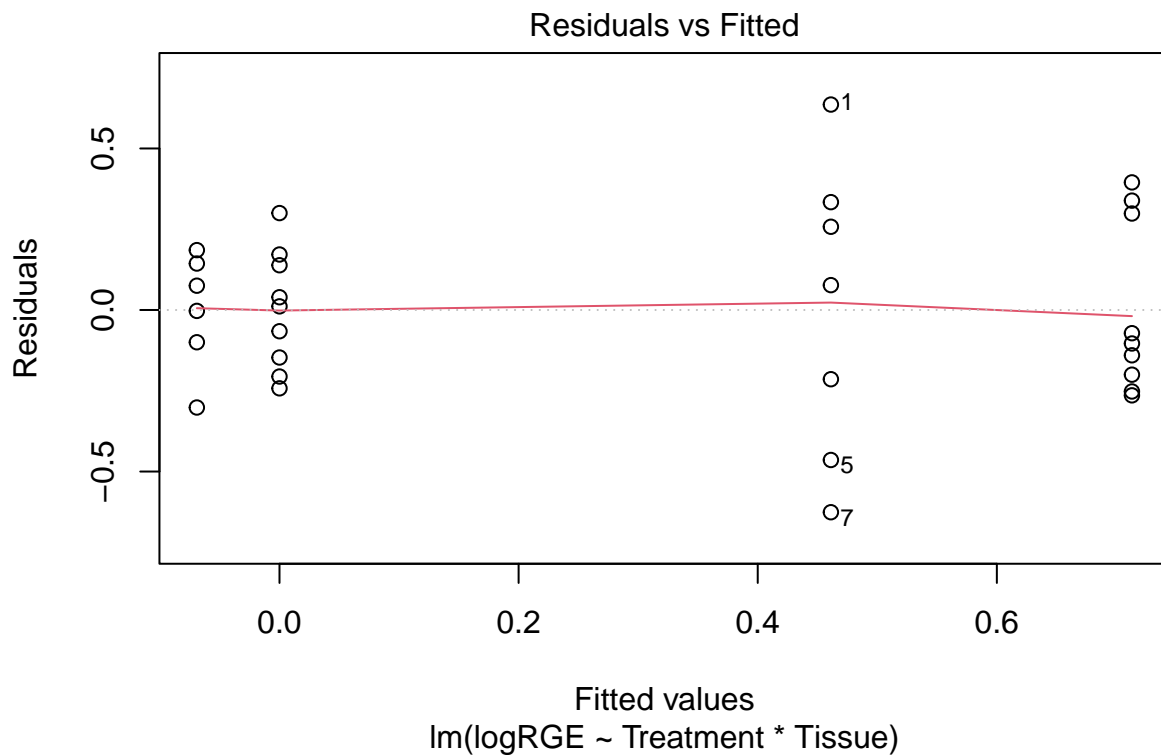
```
##
## Shapiro-Wilk normality test
##
## data: log2(pgp$RelativeGE)
## W = 0.93745, p-value = 0.07015
```

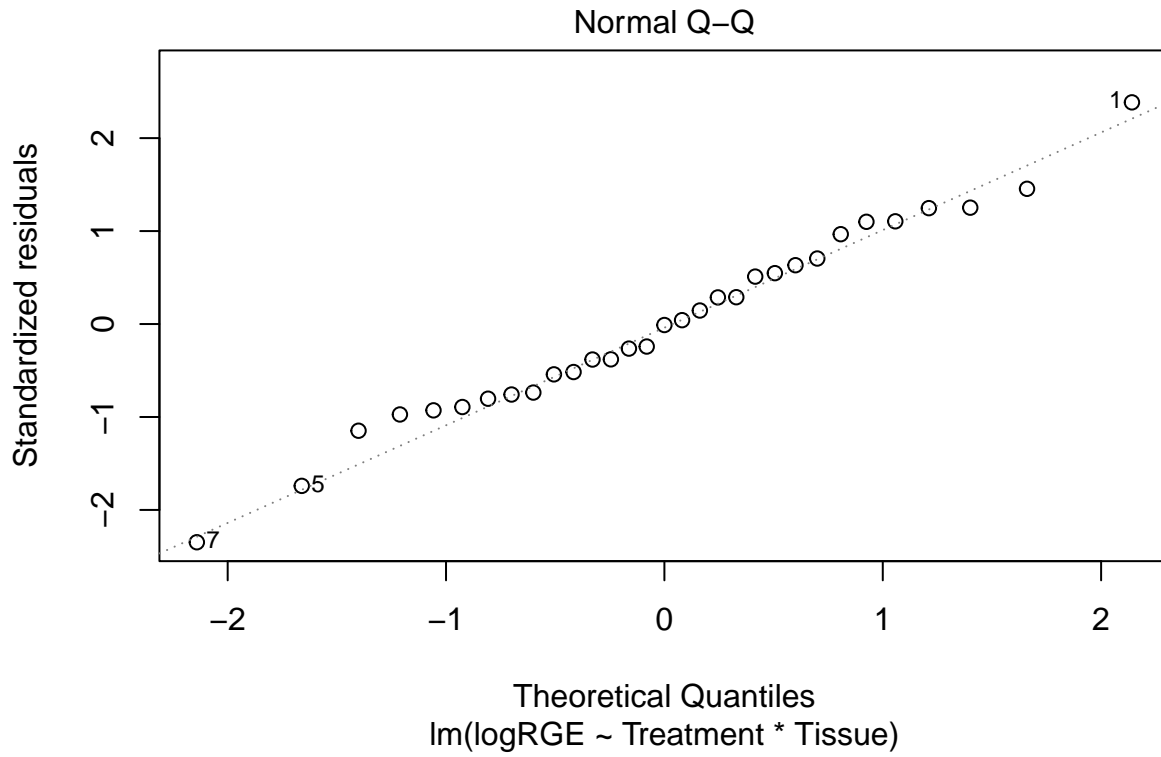
```
pgp <- pgp %>% mutate(logRGE = log(RelativeGE))
pgp <- pgp %>% mutate(log2RGE = log2(RelativeGE))
```

```
car::leveneTest(logRGE ~ Tissue * Treatment, pgp)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 3  2.6055 0.0723 .
##      27
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pgp_log_lm <- lm(logRGE ~ Treatment * Tissue, pgp)
plot(pgp_log_lm, which=c(1,2))
```





```
anova(pgp_log_lm)
```

```
## Analysis of Variance Table
##
## Response: logRGE
##          Df Sum Sq Mean Sq F value    Pr(>F)
## Treatment    1  0.14808   0.14808    1.7852   0.1927
## Tissue        1  3.13351   3.13351   37.7757 1.441e-06 ***
## Treatment:Tissue 1  0.06274   0.06274    0.7564   0.3921
## Residuals    27  2.23966   0.08295
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

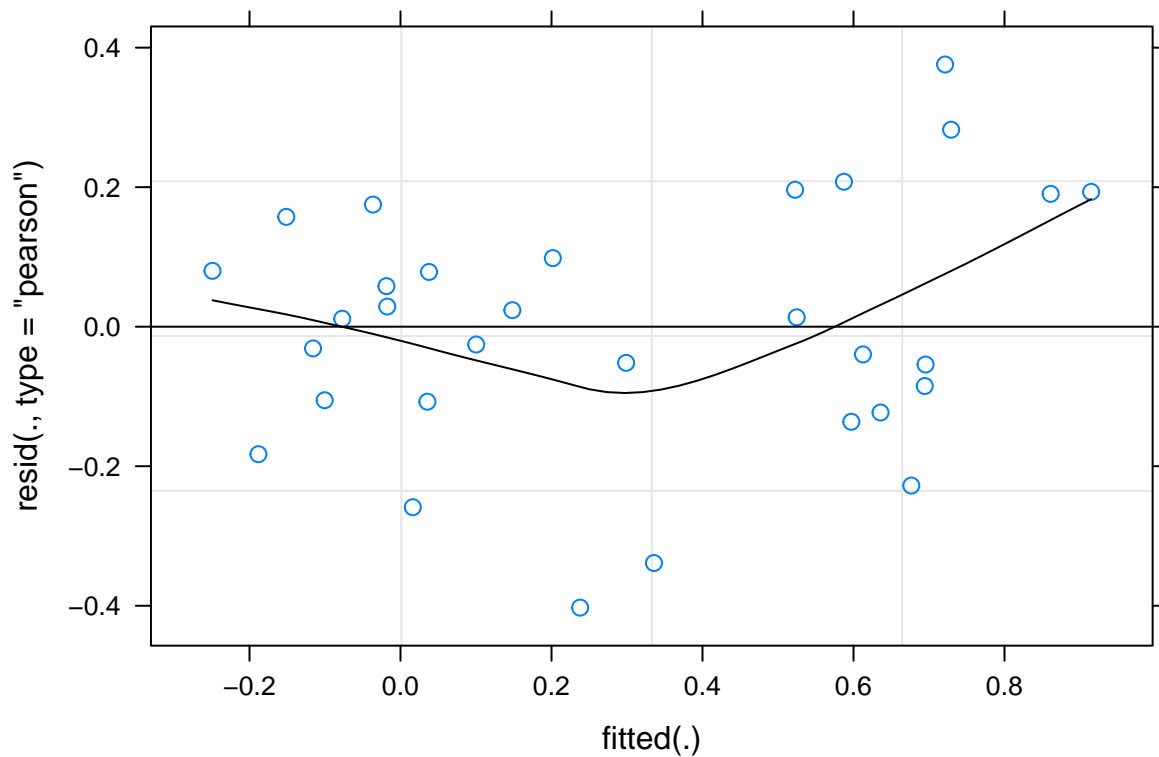
```
emmeans::emmeans(pgp_log_lm, pairwise ~ Treatment | Tissue)$contrasts
```

```
## Tissue = retina:
## contrast      estimate    SE df t.ratio p.value
## saline - morphine  0.0691 0.152 27   0.455  0.6524
##
## Tissue = hypothalamus:
## contrast      estimate    SE df t.ratio p.value
## saline - morphine  0.2518 0.145 27   1.735  0.0942
```

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

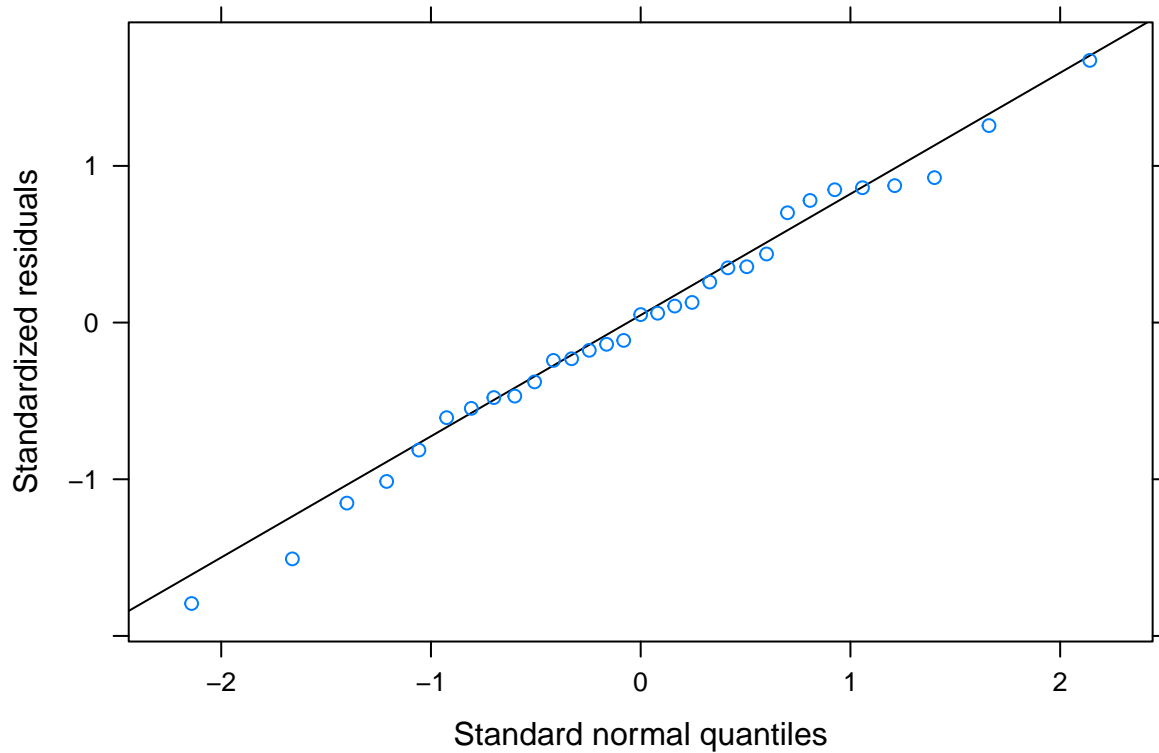
```
## Treatment = saline:
## contrast      estimate      SE df t.ratio p.value
## retina - hypothalamus -0.713 0.136 27  -5.251  <.0001
##
## Treatment = morphine:
## contrast      estimate      SE df t.ratio p.value
## retina - hypothalamus -0.530 0.160 27  -3.310  0.0027
```

```
pgp_log_lmer <- lmer(log(RelativeGE) ~ Tissue * Treatment + (1|Sample), data =pgp)
plot(pgp_log_lmer, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(pgp_log_lmer)
```





```
anova(pgp_log_lmer)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##           Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## Tissue      2.65924  2.65924      1 11.304  52.7214 1.395e-05 ***
## Treatment    0.06191  0.06191      1 11.739   1.2274   0.2901
## Tissue:Treatment 0.09433  0.09433      1 11.304   1.8701   0.1980
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(pgp_log_lmer, pairwise ~ Treatment | Tissue)
```

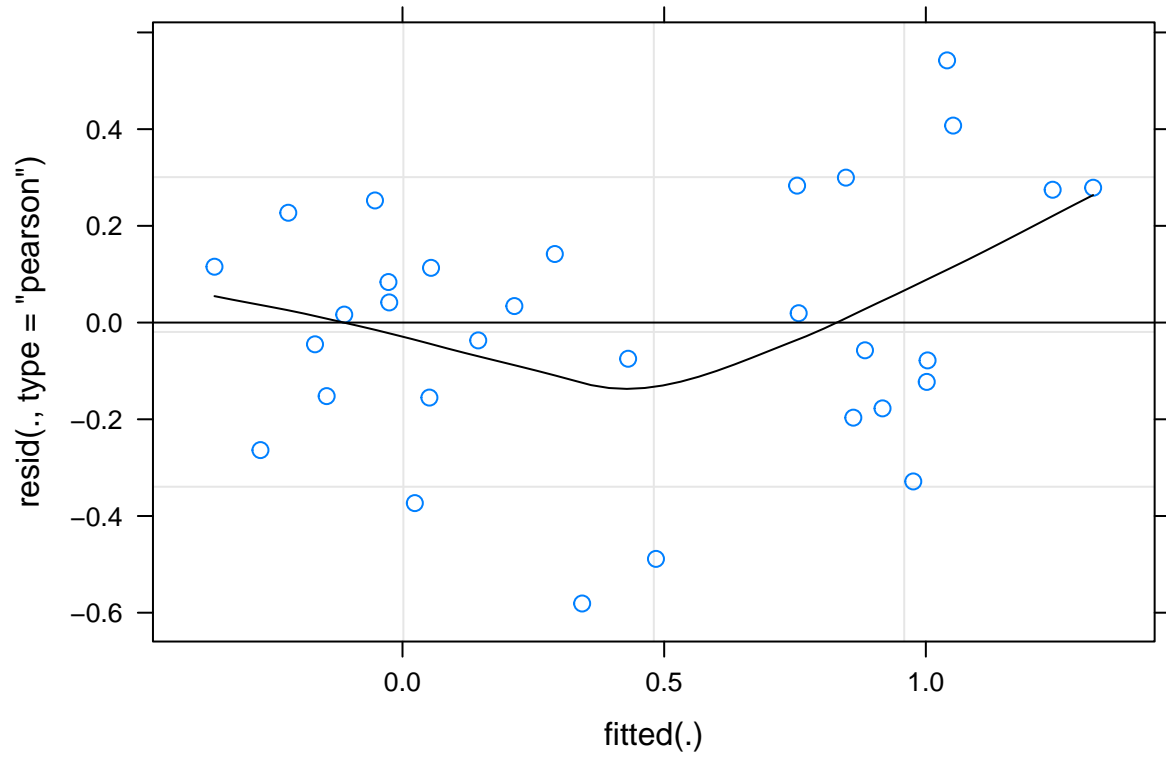
```
## $emmeans
## Tissue = retina:
## Treatment emmean      SE  df lower.CL upper.CL
## saline    0.0000 0.0974 23.3  -0.201    0.201
## morphine  -0.0258 0.1187 24.8  -0.270    0.219
##
## Tissue = hypothalamus:
## Treatment emmean      SE  df lower.CL upper.CL
## saline    0.7130 0.0974 23.3   0.512    0.914
## morphine   0.4612 0.1104 23.3   0.233    0.689
##
## Degrees-of-freedom method: kenward-roger
```

```
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## Tissue = retina:
## contrast      estimate      SE    df t.ratio p.value
## saline - morphine  0.0258 0.154 24.2   0.168  0.8680
##
## Tissue = hypothalamus:
## contrast      estimate      SE    df t.ratio p.value
## saline - morphine  0.2518 0.147 23.3   1.710  0.1005
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
```

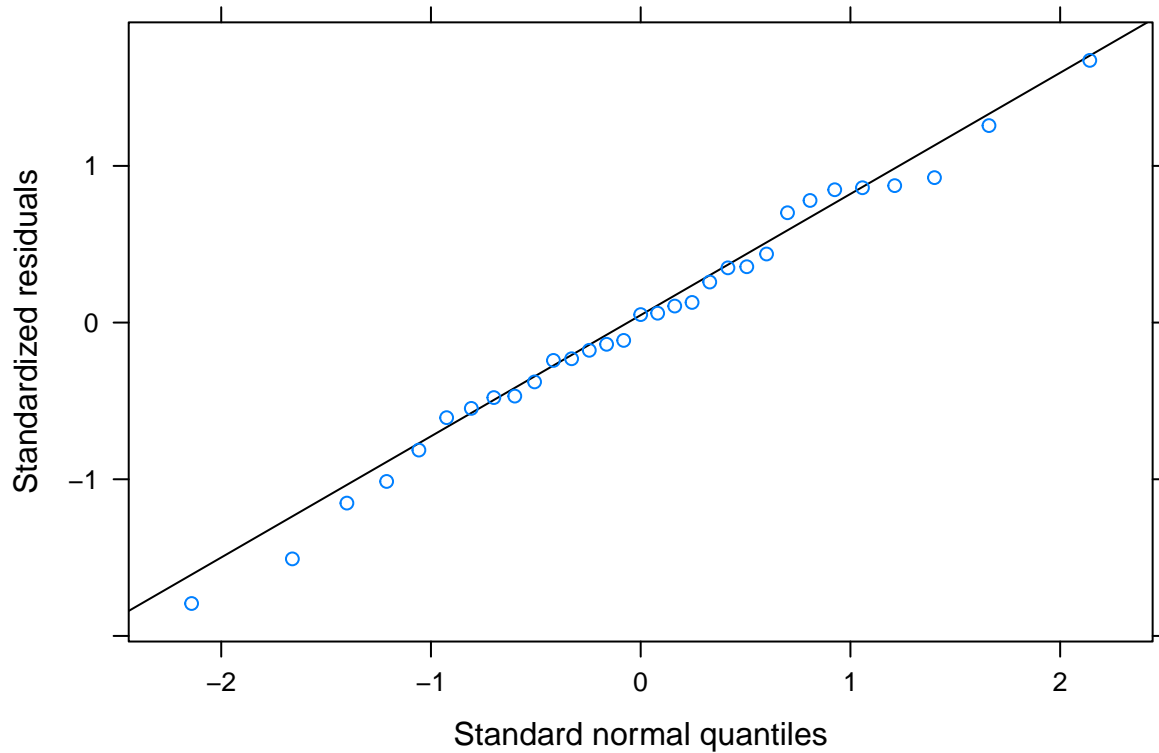
```
emmeans::emmeans(pgp_log_lmer, pairwise ~ Tissue | Treatment)
```

```
## $emmeans
## Treatment = saline:
## Tissue      emmean      SE    df lower.CL upper.CL
## retina      0.0000 0.0974 23.3   -0.201    0.201
## hypothalamus 0.7130 0.0974 23.3    0.512    0.914
##
## Treatment = morphine:
## Tissue      emmean      SE    df lower.CL upper.CL
## retina     -0.0258 0.1187 24.8   -0.270    0.219
## hypothalamus 0.4612 0.1104 23.3    0.233    0.689
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## Treatment = saline:
## contrast      estimate      SE    df t.ratio p.value
## retina - hypothalamus -0.713 0.106 13.1  -6.734  <.0001
##
## Treatment = morphine:
## contrast      estimate      SE    df t.ratio p.value
## retina - hypothalamus -0.487 0.128 13.9  -3.812  0.0019
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
```

```
pgp_log2_lmer <- lmer(log2(RelativeGE) ~ Tissue * Treatment + (1|Sample), data =pgp)
plot(pgp_log2_lmer, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(pgp_log2_lmer)
```



```
anova(pgp_log2_lmer)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##           Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
## Tissue      5.5349   5.5349     1  11.304  52.7214 1.395e-05 ***
## Treatment    0.1289   0.1289     1  11.739   1.2274   0.2901
## Tissue:Treatment 0.1963   0.1963     1  11.304   1.8701   0.1980
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(pgp_log2_lmer, pairwise ~ Treatment | Tissue)
```

```
## $emmeans
## Tissue = retina:
## Treatment emmean SE df lower.CL upper.CL
## saline    0.0000 0.140 23.3 -0.290 0.290
## morphine -0.0372 0.171 24.8 -0.390 0.316
##
## Tissue = hypothalamus:
## Treatment emmean SE df lower.CL upper.CL
## saline    1.0286 0.140 23.3 0.738 1.319
## morphine 0.6654 0.159 23.3 0.336 0.995
##
## Degrees-of-freedom method: kenward-roger
```

```
## Results are given on the log2 (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## Tissue = retina:
## contrast      estimate    SE   df t.ratio p.value
## saline - morphine  0.0372 0.222 24.2   0.168  0.8680
##
## Tissue = hypothalamus:
## contrast      estimate    SE   df t.ratio p.value
## saline - morphine  0.3633 0.212 23.3   1.710  0.1005
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

```
emmeans::emmeans(pgp_log2_lmer, pairwise ~ Tissue | Treatment)
```

```
## $emmeans
## Treatment = saline:
## Tissue      emmean    SE   df lower.CL upper.CL
## retina      0.0000 0.140 23.3  -0.290   0.290
## hypothalamus 1.0286 0.140 23.3   0.738   1.319
##
## Treatment = morphine:
## Tissue      emmean    SE   df lower.CL upper.CL
## retina     -0.0372 0.171 24.8  -0.390   0.316
## hypothalamus 0.6654 0.159 23.3   0.336   0.995
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## Treatment = saline:
## contrast      estimate    SE   df t.ratio p.value
## retina - hypothalamus -1.029 0.153 13.1  -6.734  <.0001
##
## Treatment = morphine:
## contrast      estimate    SE   df t.ratio p.value
## retina - hypothalamus -0.703 0.184 13.9  -3.812  0.0019
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

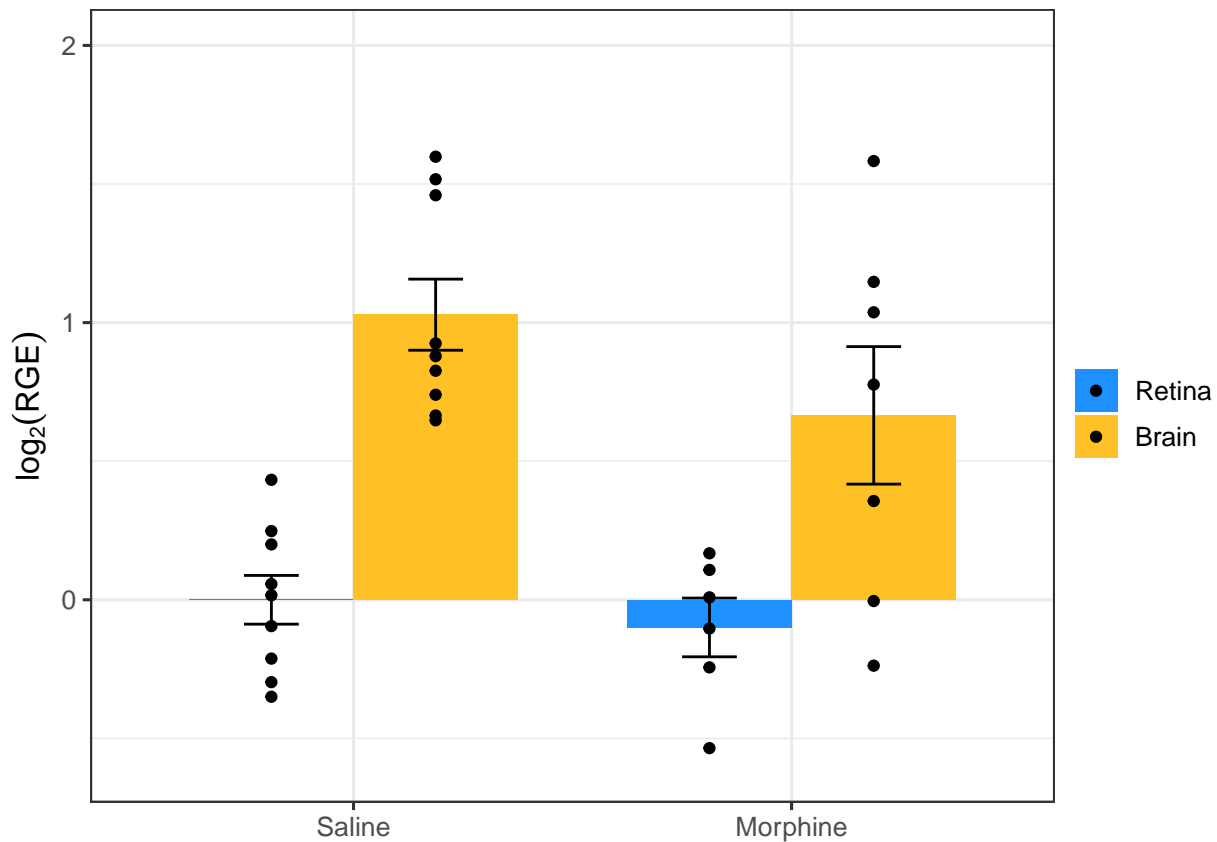
```
pgp_log2_tiss_plot <- pgp %>% ggplot(aes(x=Treatment, y=log2RGE, fill=Tissue)) +
  stat_summary(fun = 'mean', geom="bar", aes(fill=Tissue),
    width=0.75, position="dodge") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=0.25,
    position=position_dodge(width=0.75)) +
  geom_point(stat="identity", position=position_dodge(width=0.75)) +
  theme_bw(base_size = 12) %+replace%
  theme(axis.title.x=element_blank(),
    legend.title = element_blank(),
```

```

    legend.box.spacing = unit(1, "pt"),
    plot.title = element_text(hjust = 0.5, vjust=1.5)) +
  labs(y="log(Relative Gene Expression)") +
  #scale_y_continuous(expand = expansion(mult = c(0.1, 0.2))) +
  ylim(-0.6, 2) +
  scale_fill_manual(values = c("dodgerblue", "goldenrod1"),
                    labels = c("Retina", "Brain")) +
  scale_x_discrete(labels=c("saline" = "Saline", "morphine" = "Morphine")) +
  ylab(expression(log2(RGE)))

```

pgp\_log2\_tiss\_plot



```

#ggsave(filename = "../figures/pgp_log2_tiss_plot.png", plot = pgp_log2_tiss_plot, height=4, width=4)

```

```

pgp_log2_trt_plot <- pgp %>% ggplot(aes(x=Tissue, y=log2RGE, fill=Treatment)) +
  stat_summary(fun = 'mean', geom="bar", aes(fill=Treatment),
              width=0.75, position="dodge") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=0.25,
              position=position_dodge(width=0.75)) +
  geom_point(stat="identity", position=position_dodge(width=0.75)) +
  theme_bw(base_size = 12) %+replace%
  theme(axis.title.x=element_blank(),
        legend.title = element_blank(),
        legend.box.spacing = unit(1, "pt"),

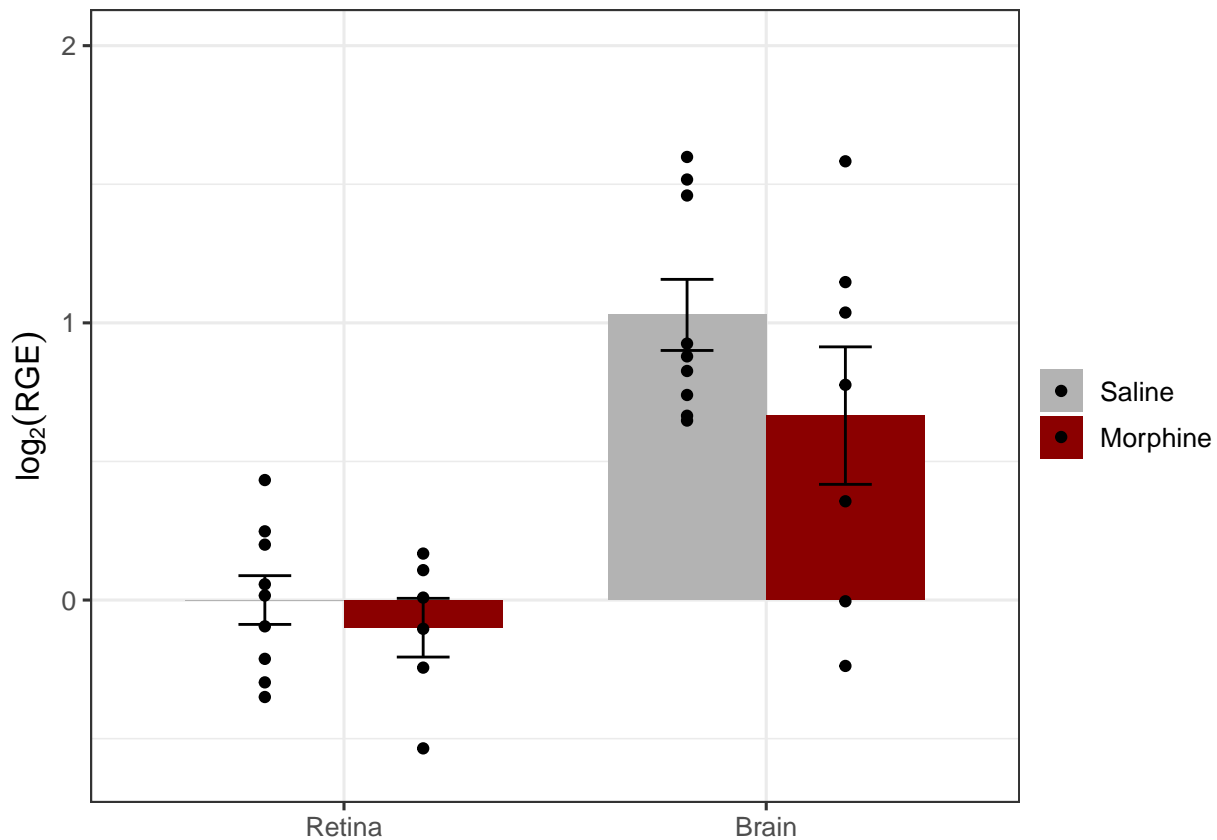
```

```

    plot.title = element_text(hjust = 0.5, vjust=1.5)) +
  labs(y="log(Relative Gene Expression)") +
  #scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  ylim(-0.6, 2) +
  scale_fill_manual(values = c("gray70", "red4"),
                    labels=c("Saline", "Morphine")) +
  scale_x_discrete(labels=c("retina" = "Retina", "hypothalamus" = "Brain")) +
  ylab(expression(log2(RGE)))

```

pgp\_log2\_trt\_plot



```

#ggsave(filename = "../figures/pgp_log2_trt_plot.png", plot = pgp_log2_trt_plot, height=4, width=4)

```

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", "Fhi")),
                                       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 = "rge")

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

```

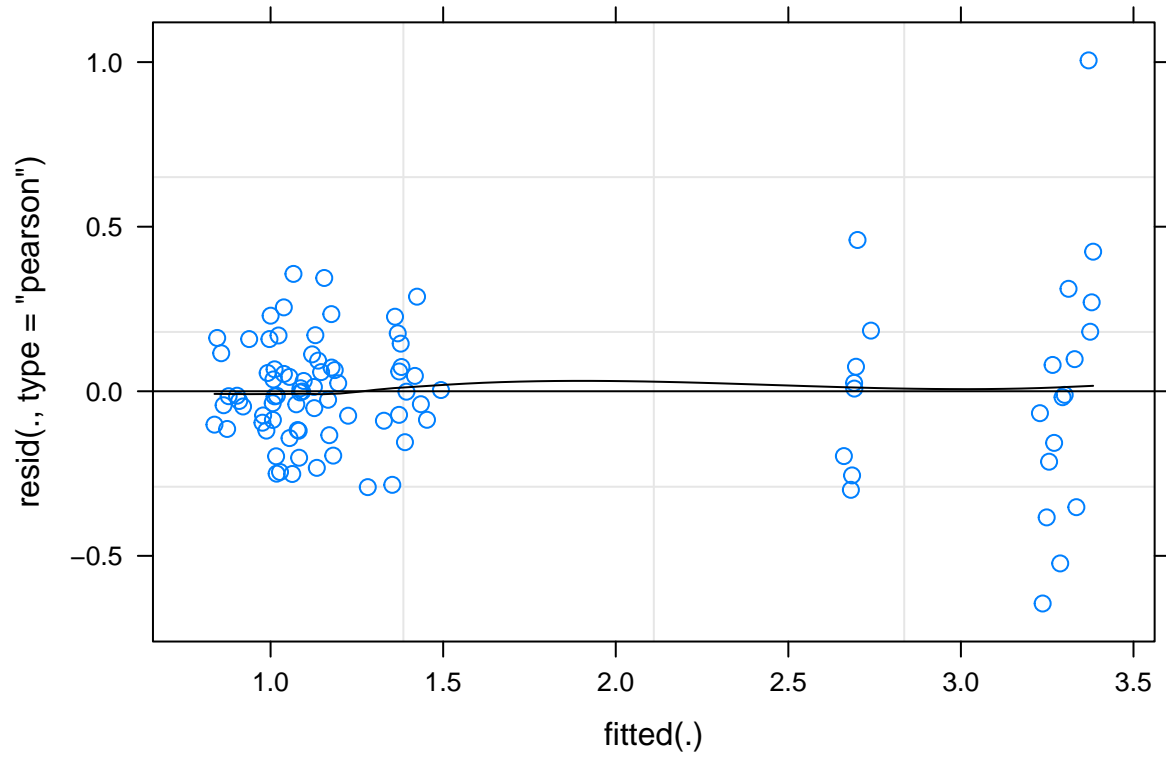
## Stats

```

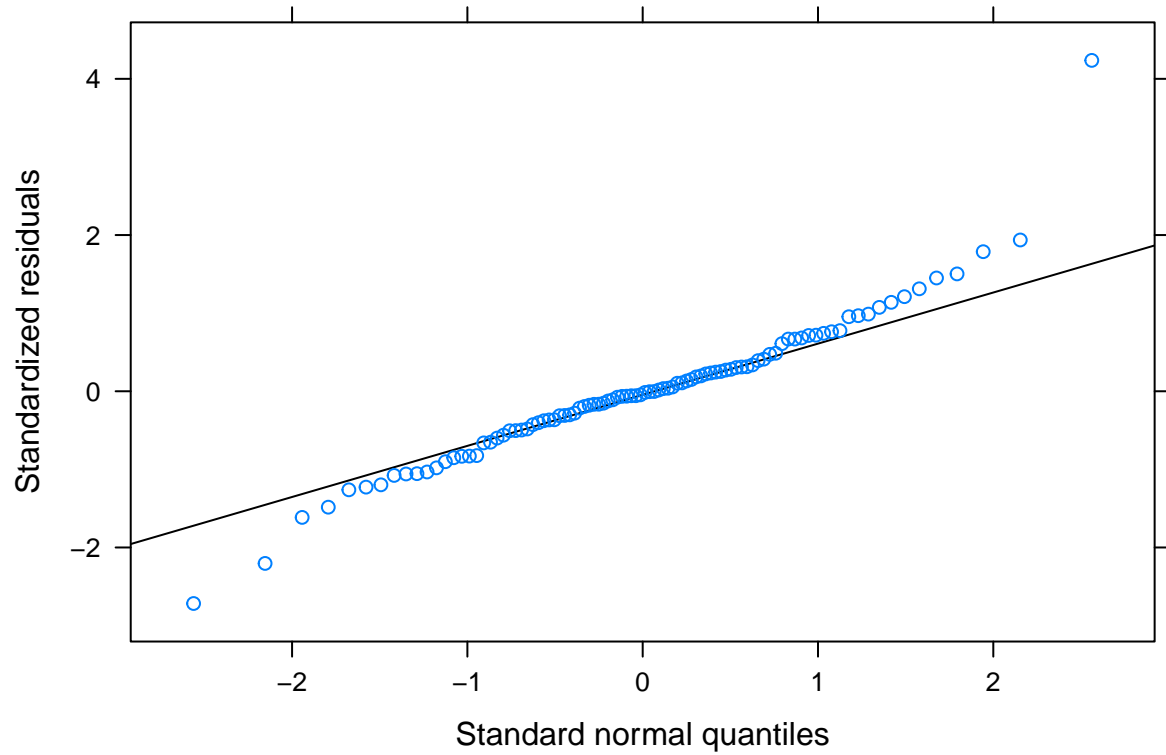
transporter_lm <- lmer(rge ~ gene * Tissue * Group + (1|Sample), data=transporters_long)
plot(transporter_lm, type=c("p","smooth"), col.line=1)

```

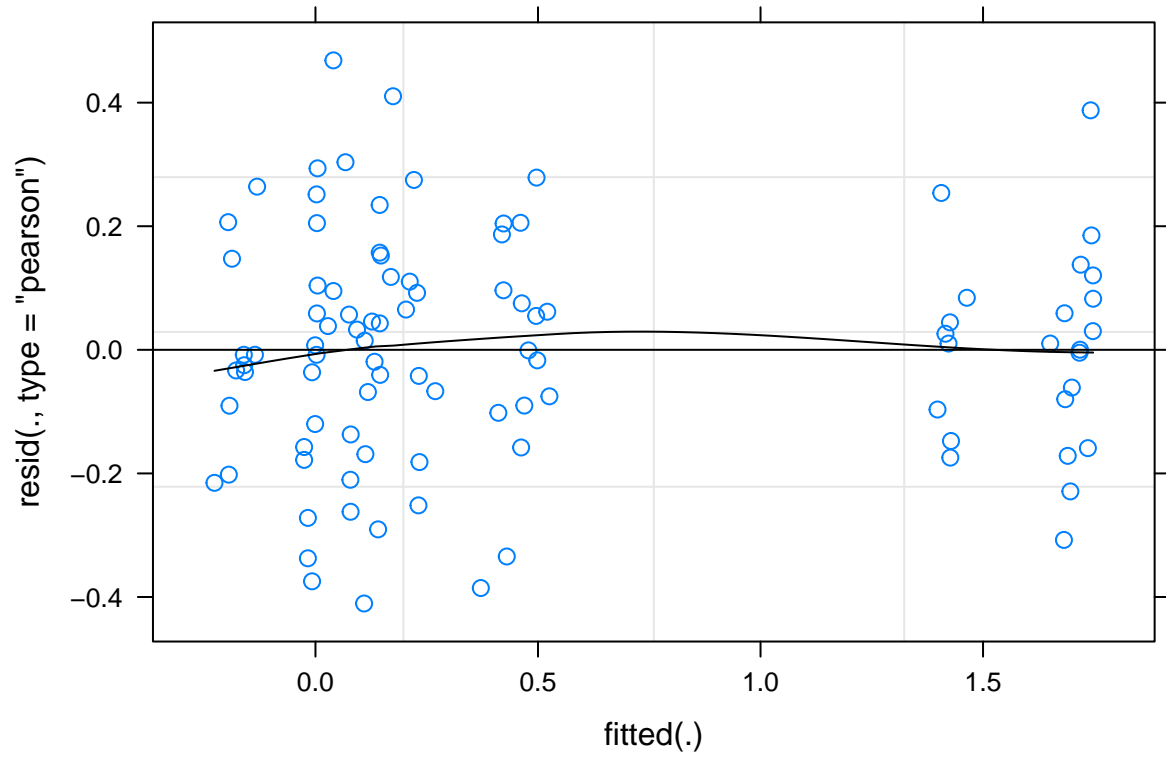




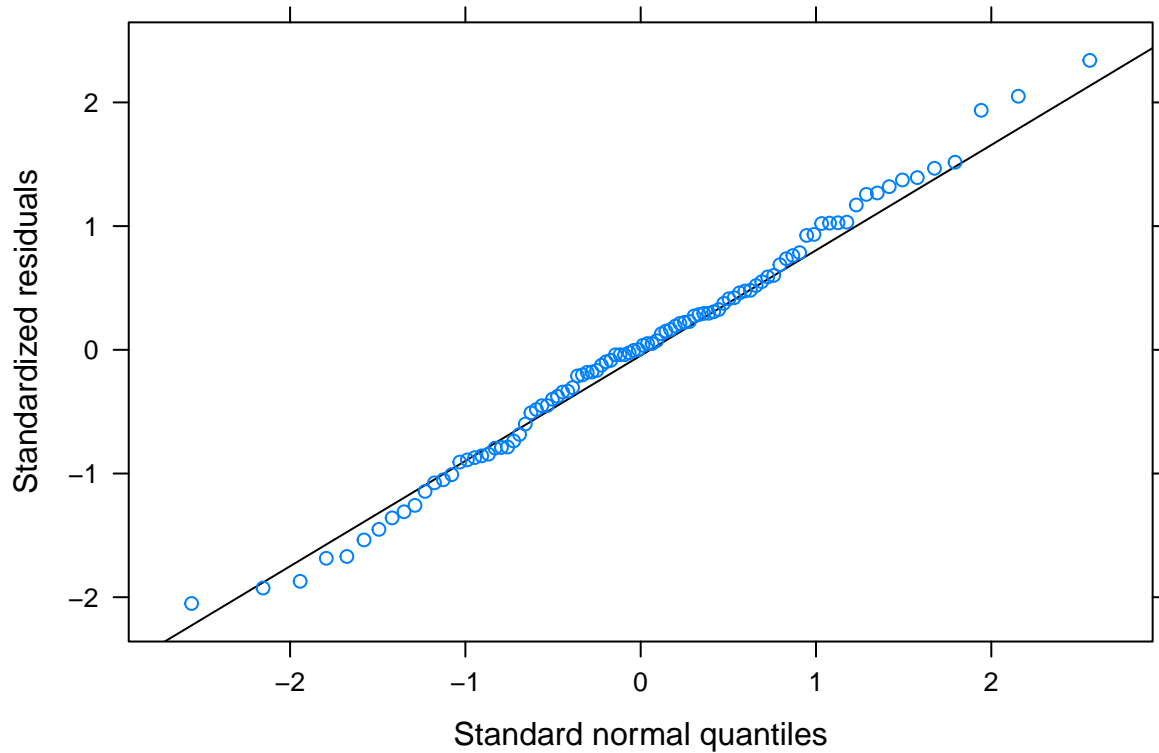
```
lattice::qqmath(transporter_lm)
```



```
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
```

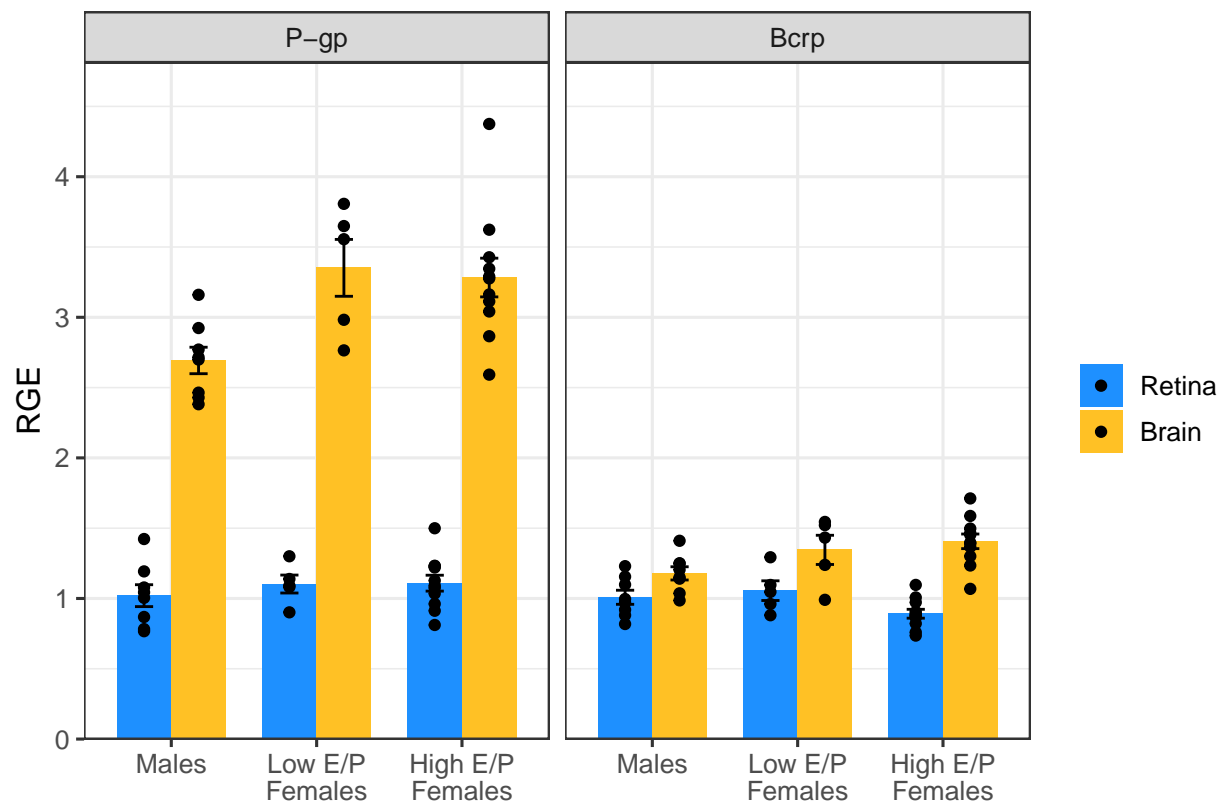
## Visualizing hyp vs ret within each treatment/gene

```
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
```



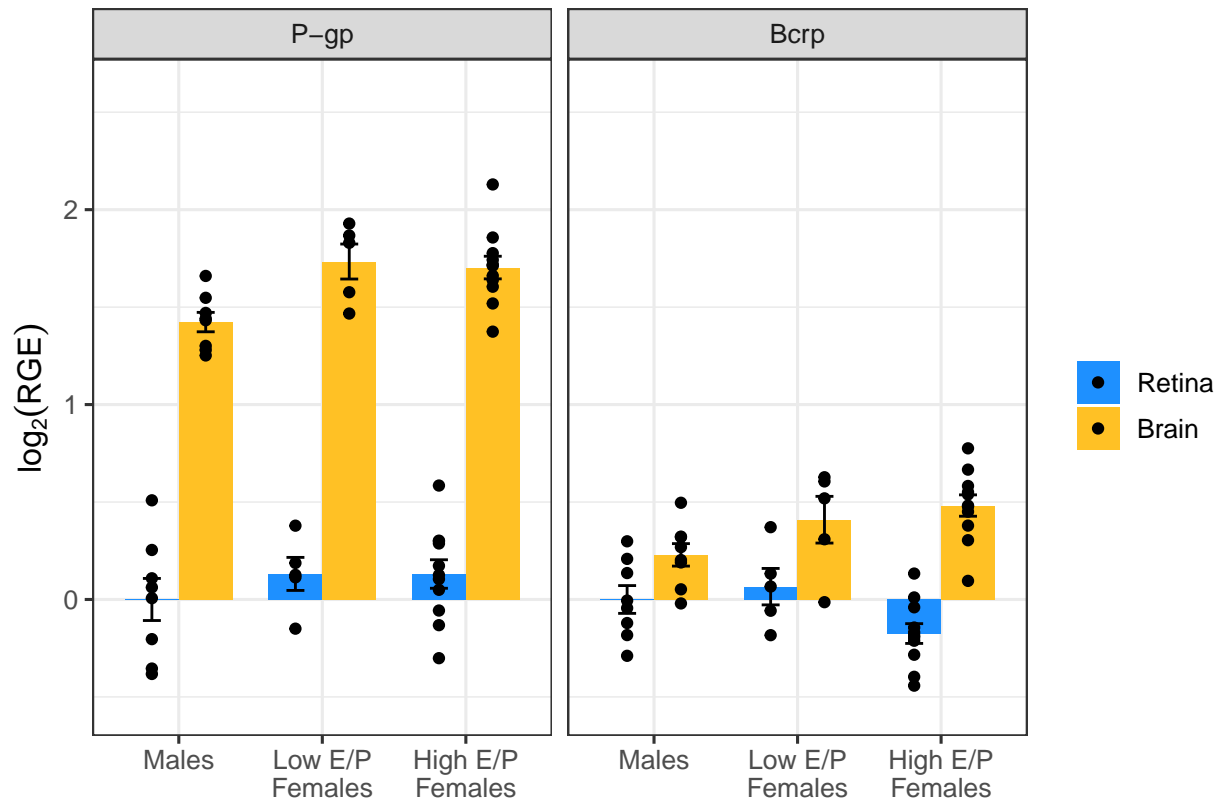
```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_1inj.png", plot=trans_hyp_ret_plot, height=4, wid
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_1inj.svg", plot=trans_hyp_ret_plot, height=4, wid
```

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

## 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"),
```

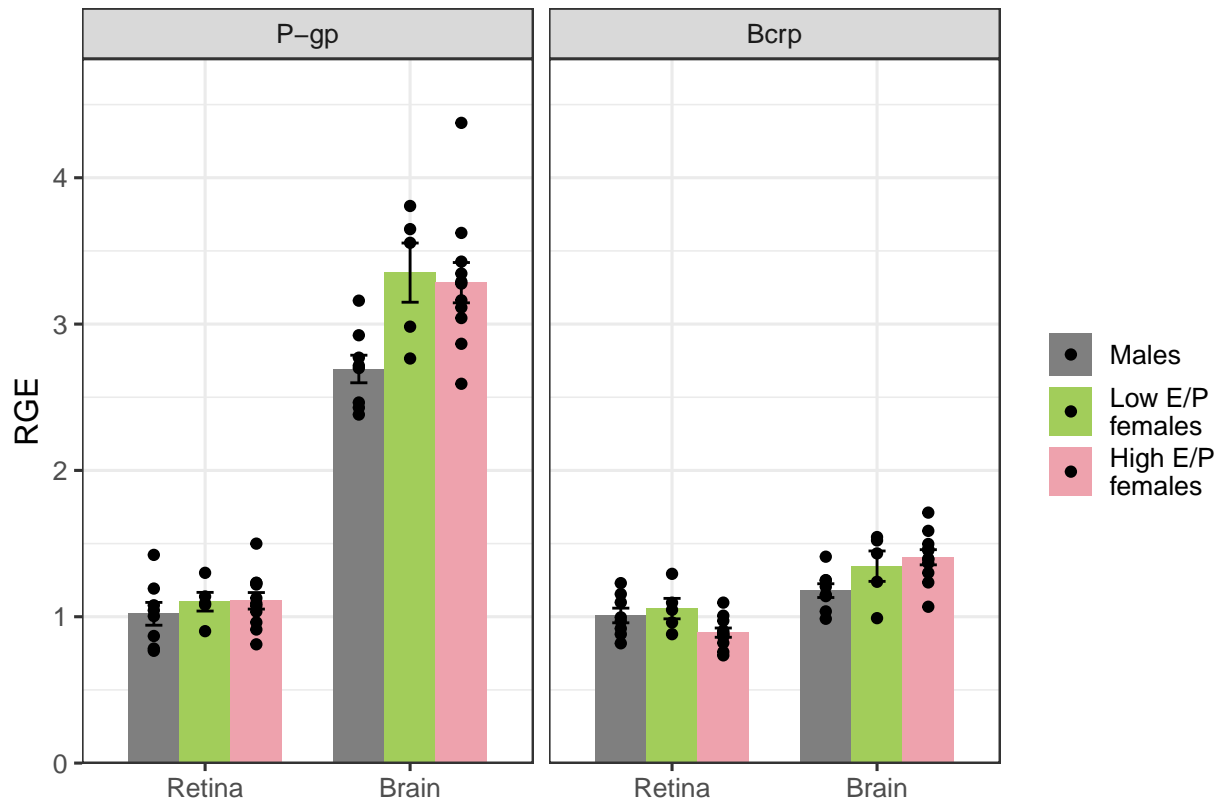
```

    "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%

```

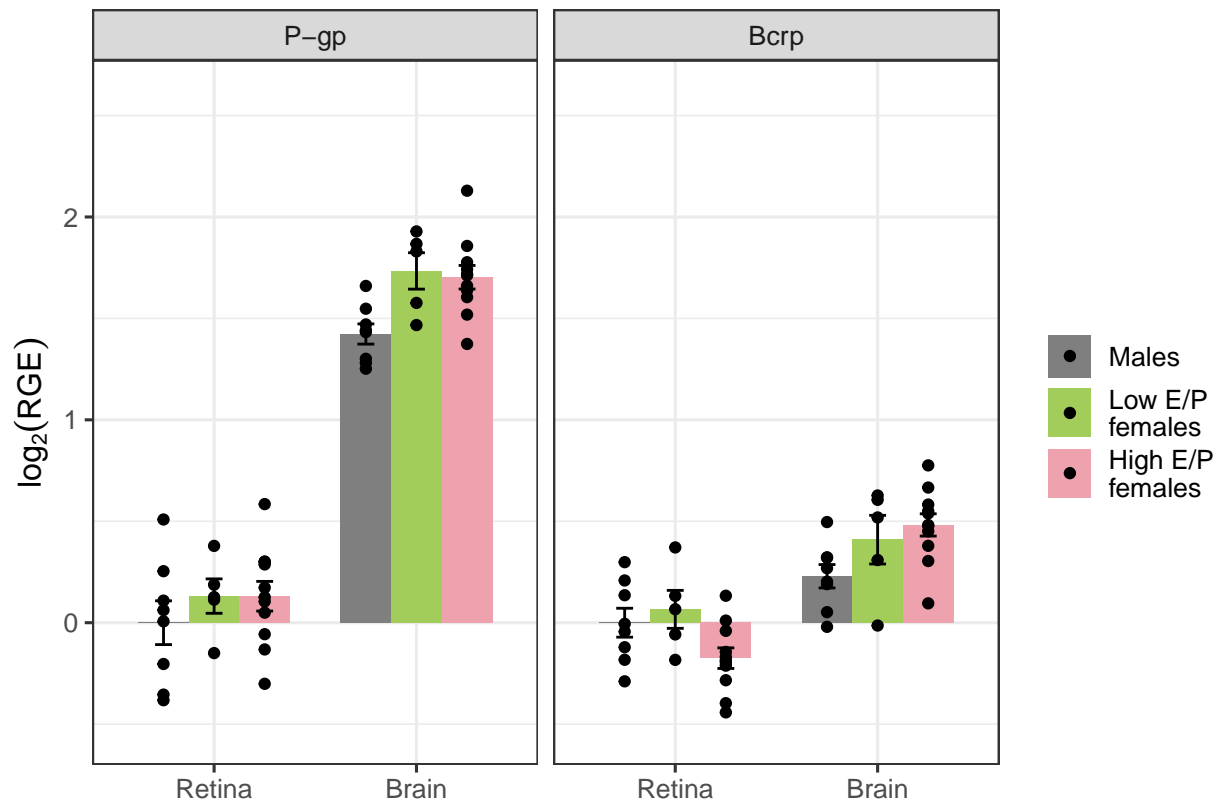
```

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(log2[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, wid
#ggsave(filename="../figures/qRT-PCR/trans_trt_log_1inj.svg", plot=trans_trt_log_plot, height=4, wid

```

## Visualizing P-gp vs Bcrp differences

```

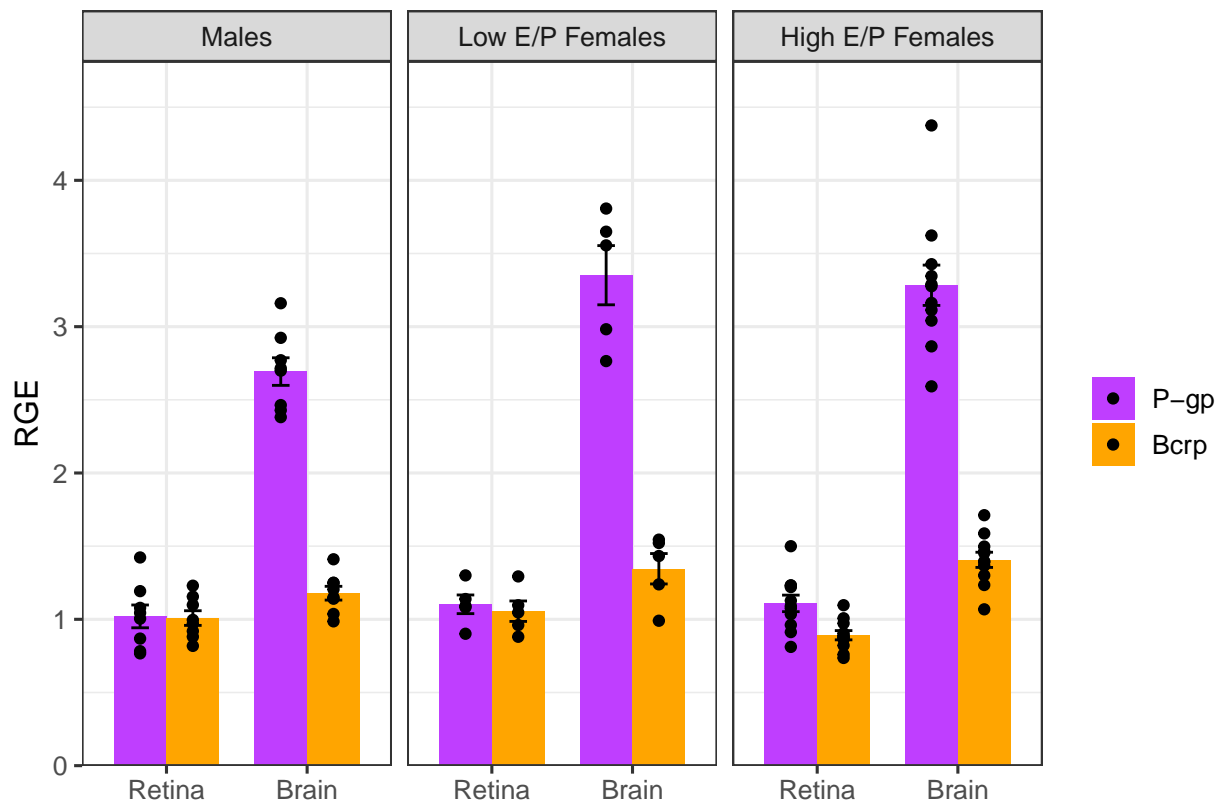
group.labs <- c("Males", "Low E/P Females", "High E/P Females")
names(group.labs) <- c("M", "Flo", "Phi")

```

```
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

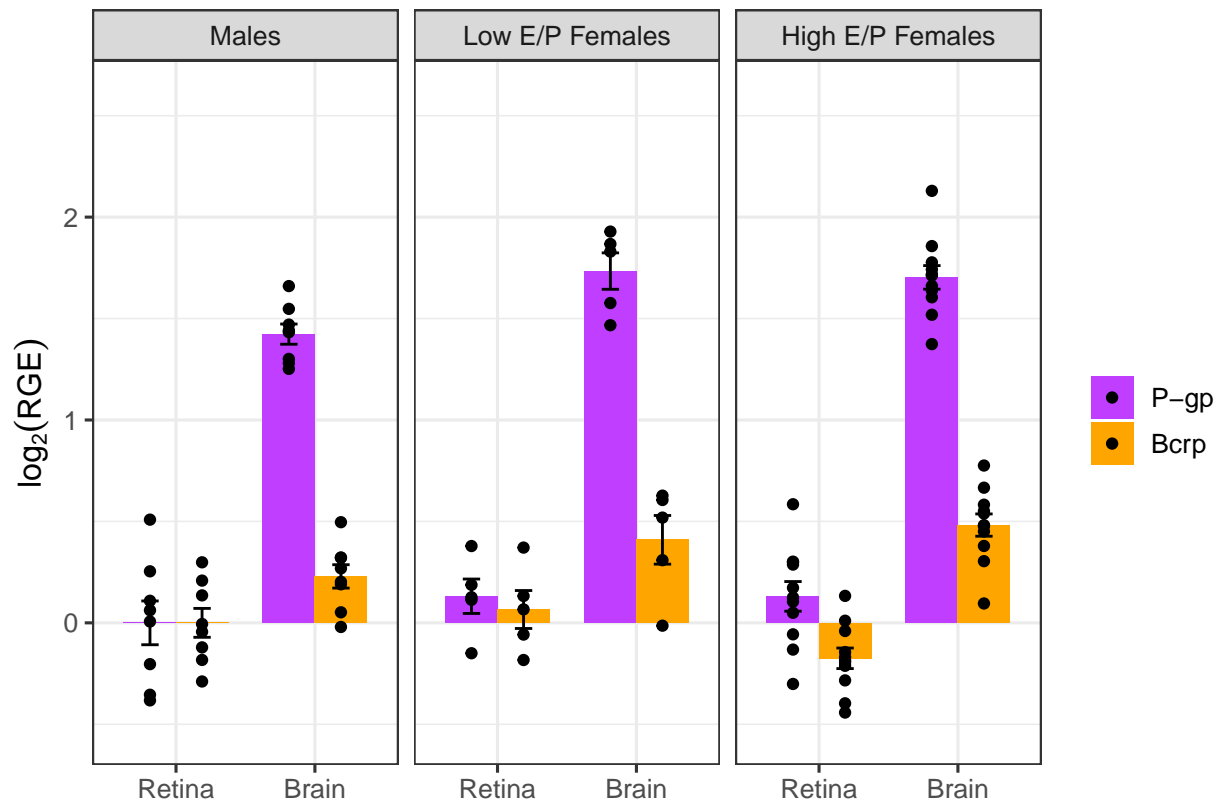


```
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_1inj.png", plot=trans_g2g_plot, height=4, width=7)
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_1inj.svg", plot=trans_g2g_plot, height=4, width=7)
```

```
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
```

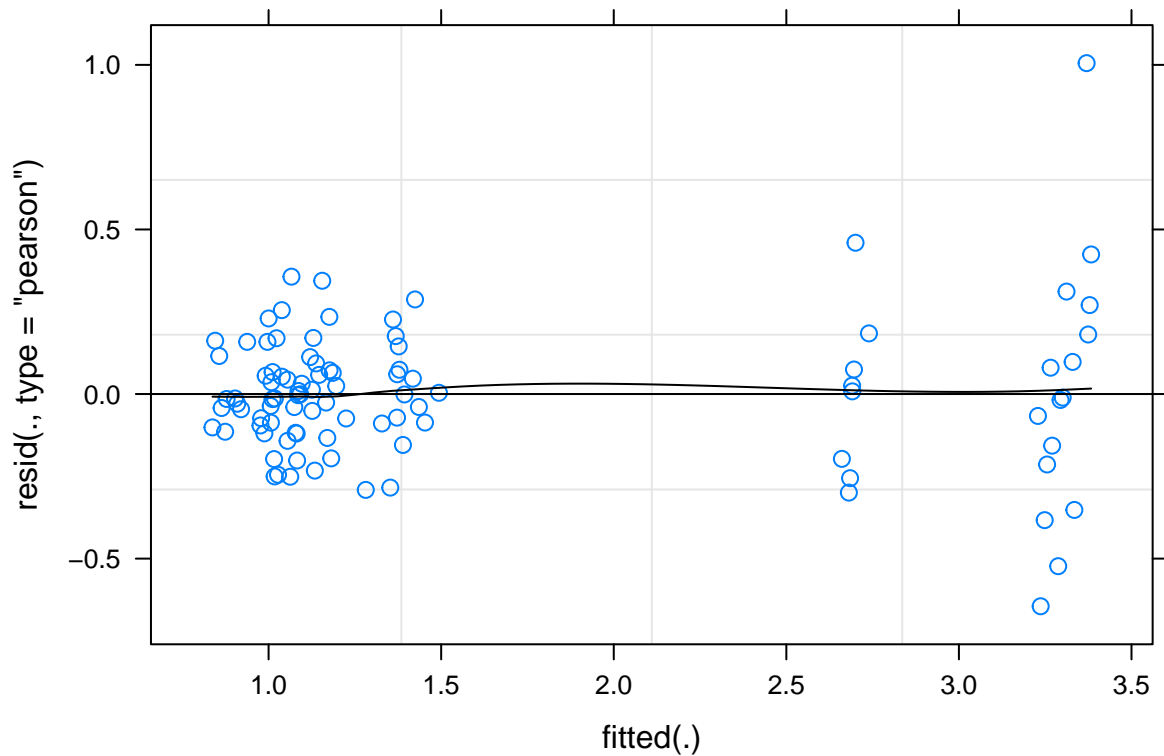


```
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_log_1inj.png", plot=trans_g2g_log_plot, height=4, wid
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_log_1inj.svg", plot=trans_g2g_log_plot, height=4, wid
```

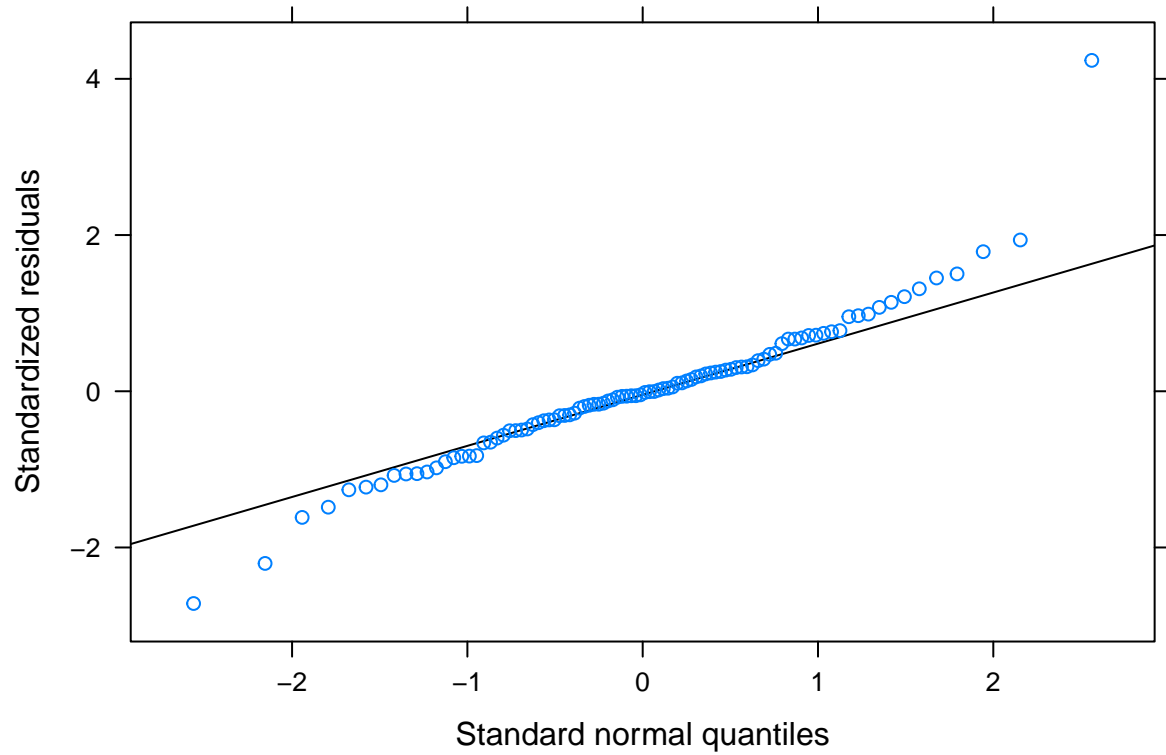
**To bottom: Full analysis (all 5 groups - M chronic saline, M chronic morphine,**

**#M acute morphine, Flo & Fhi acute morphine) - not used!!**

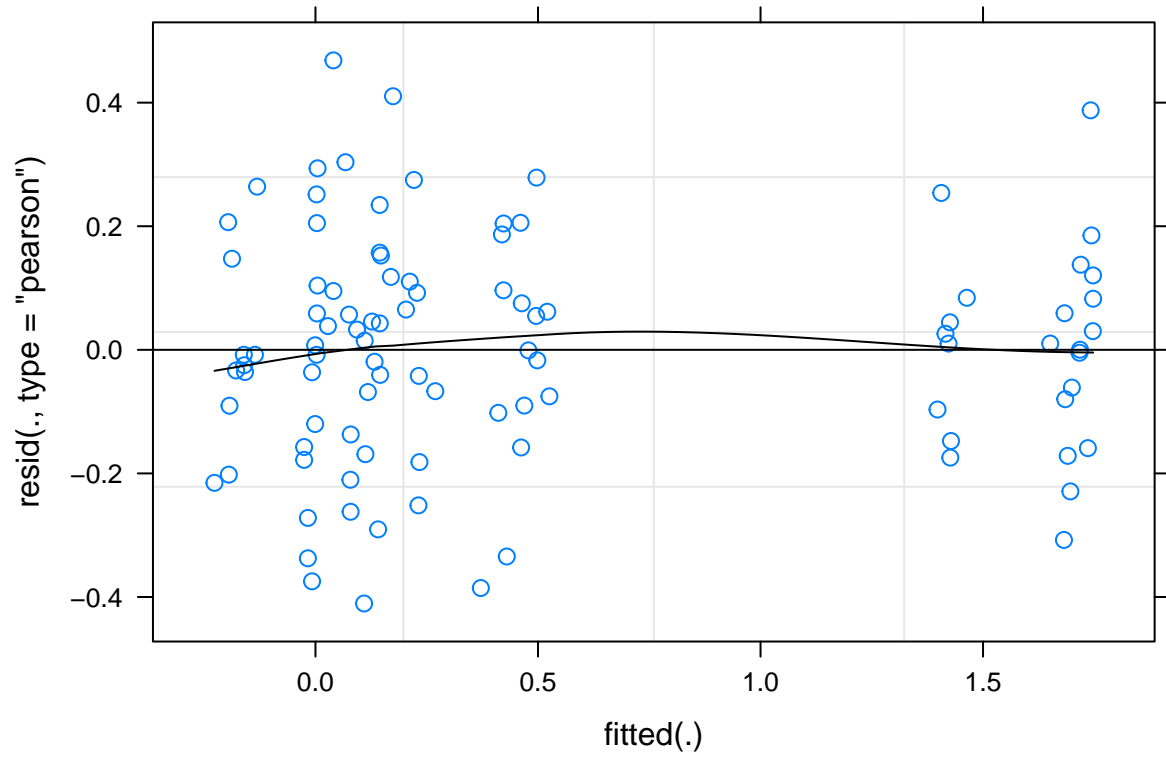
```
transporter_lm <- lmer(rge ~ gene * Tissue * Group + (1|Sample), data=transporters_long)
plot(transporter_lm, type=c("p","smooth"), col.line=1)
```



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

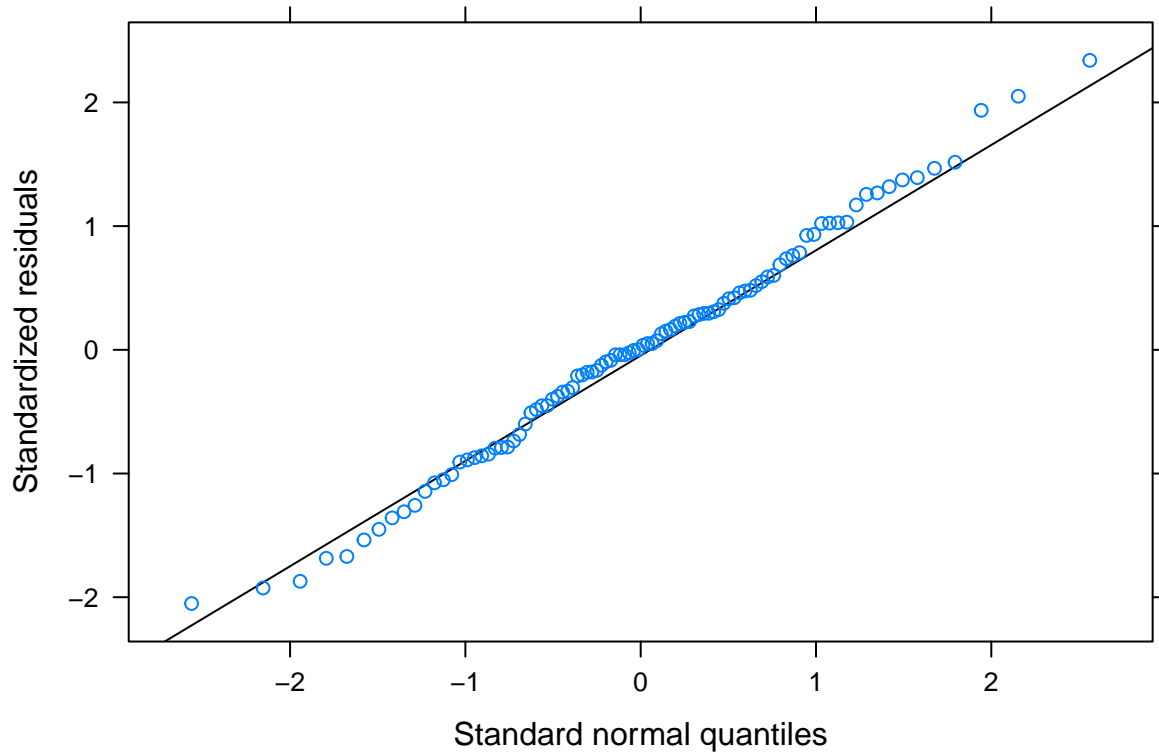


```
transporter_log2_lm <- lmer(log2(rge) ~ gene * Tissue * Group + (1|Sample), data=transporters_long)
plot(transporter_log2_lm, type=c("p","smooth"), col.line=1)
```



```
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
```

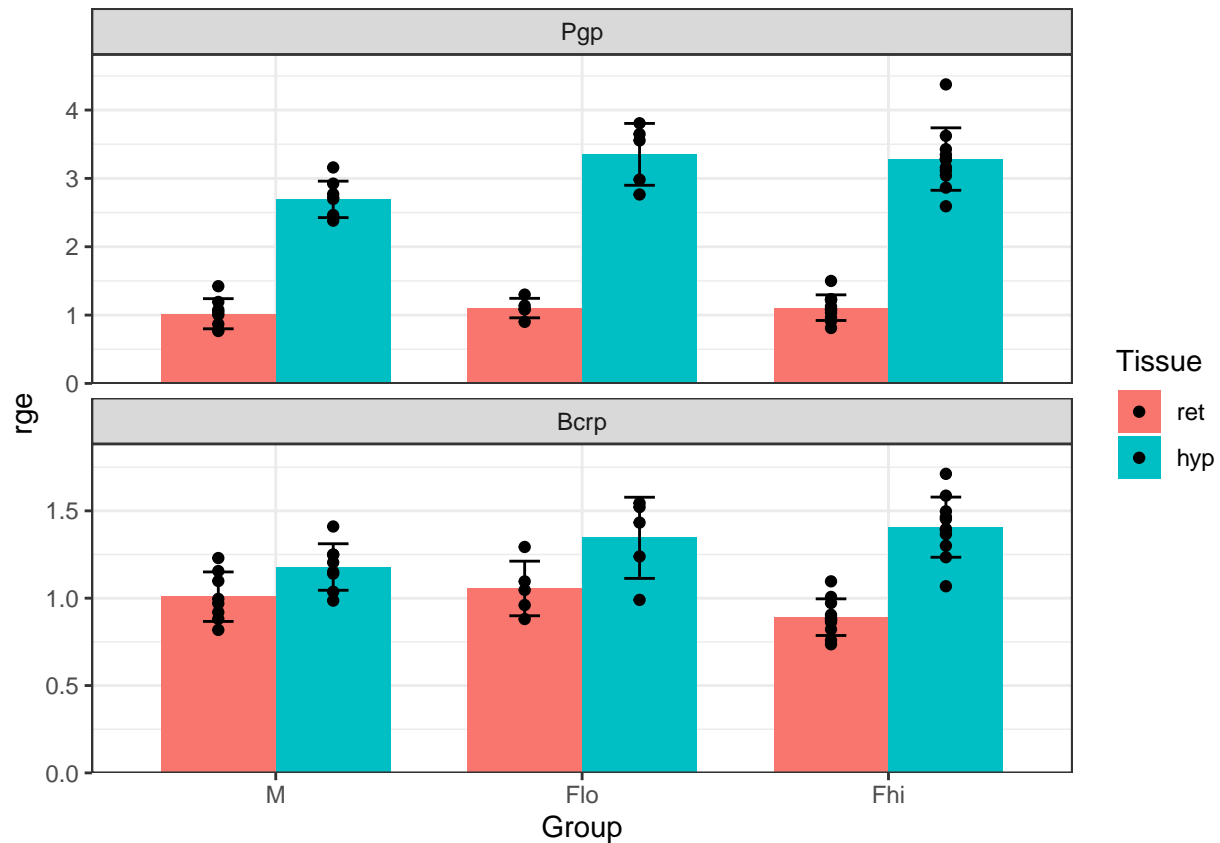
```
## # A tibble: 12 x 8
## # Groups:   Group, Tissue [6]
##   Group Tissue gene      n mean    sd log2_mean log2_sd
##   <fct> <fct> <fct> <int> <dbl> <dbl>    <dbl>    <dbl>
## 1 M     ret   Pgp      8 1.02 0.220 2.02e-10 0.305
## 2 M     ret   Bcrp      8 1.01 0.142 6.58e-11 0.202
## 3 M     hyp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

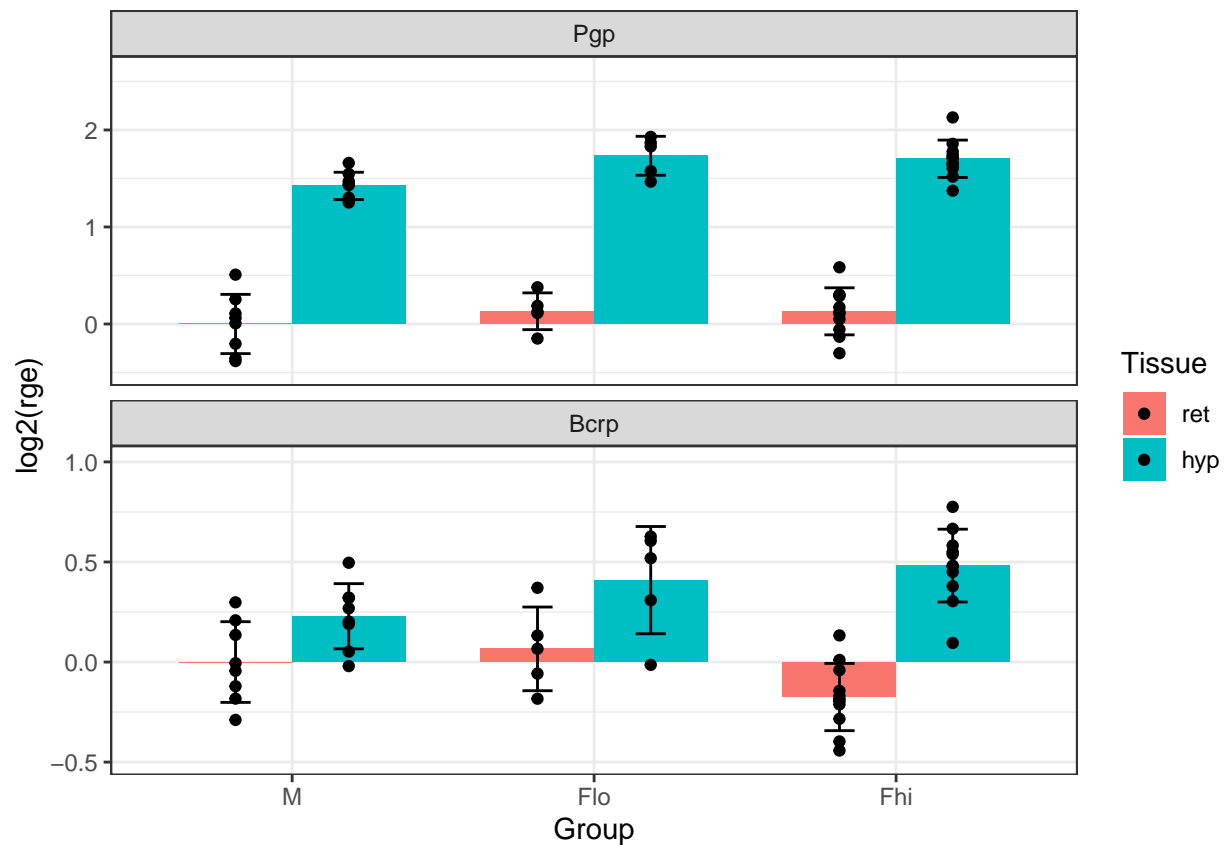


```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret.png", plot=trans_hyp_ret_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret.svg", plot=trans_hyp_ret_plot, height=4, width=6)
```

```
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
```



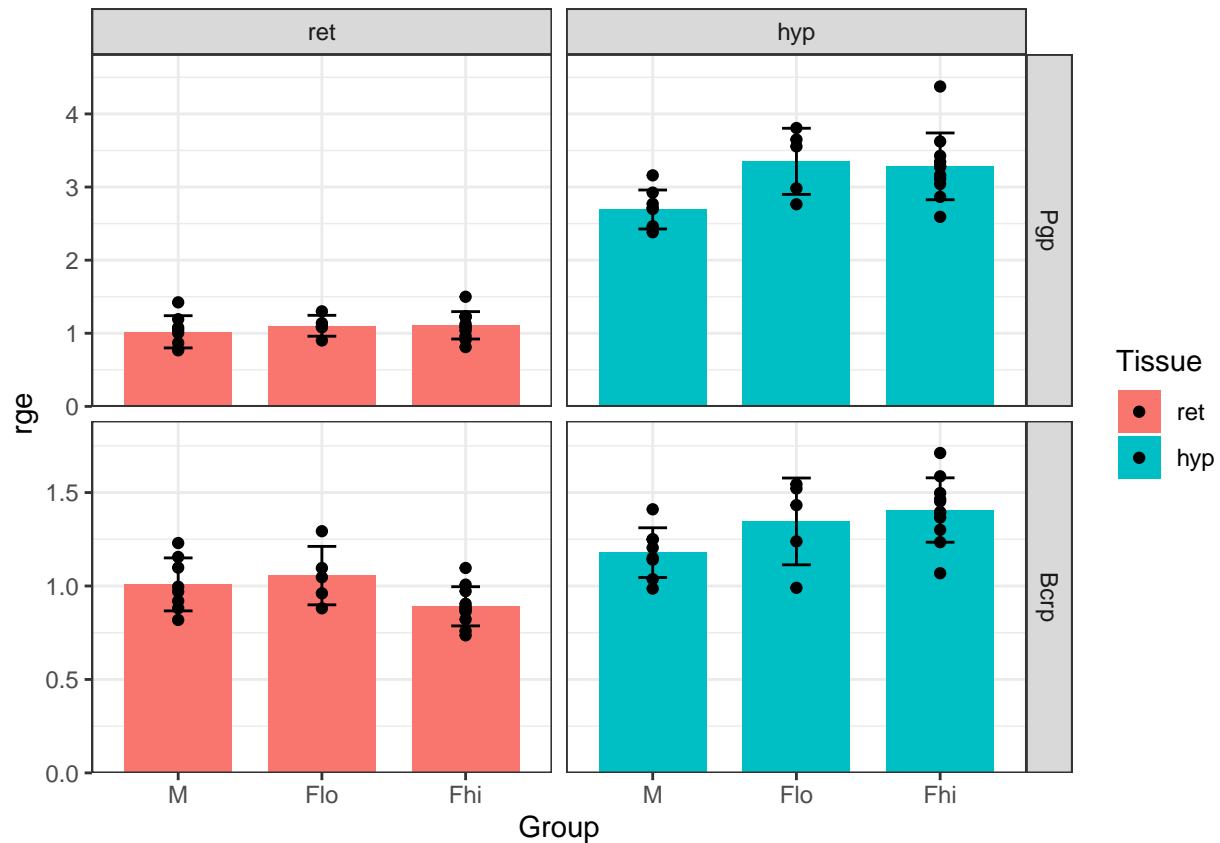
```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log.png", plot=trans_hyp_ret_log_plot, height=4, width=10)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log.svg", plot=trans_hyp_ret_log_plot, height=4, width=10)
```

Comparing treatments within each tissue/gene

```
trans_trt_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=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
```

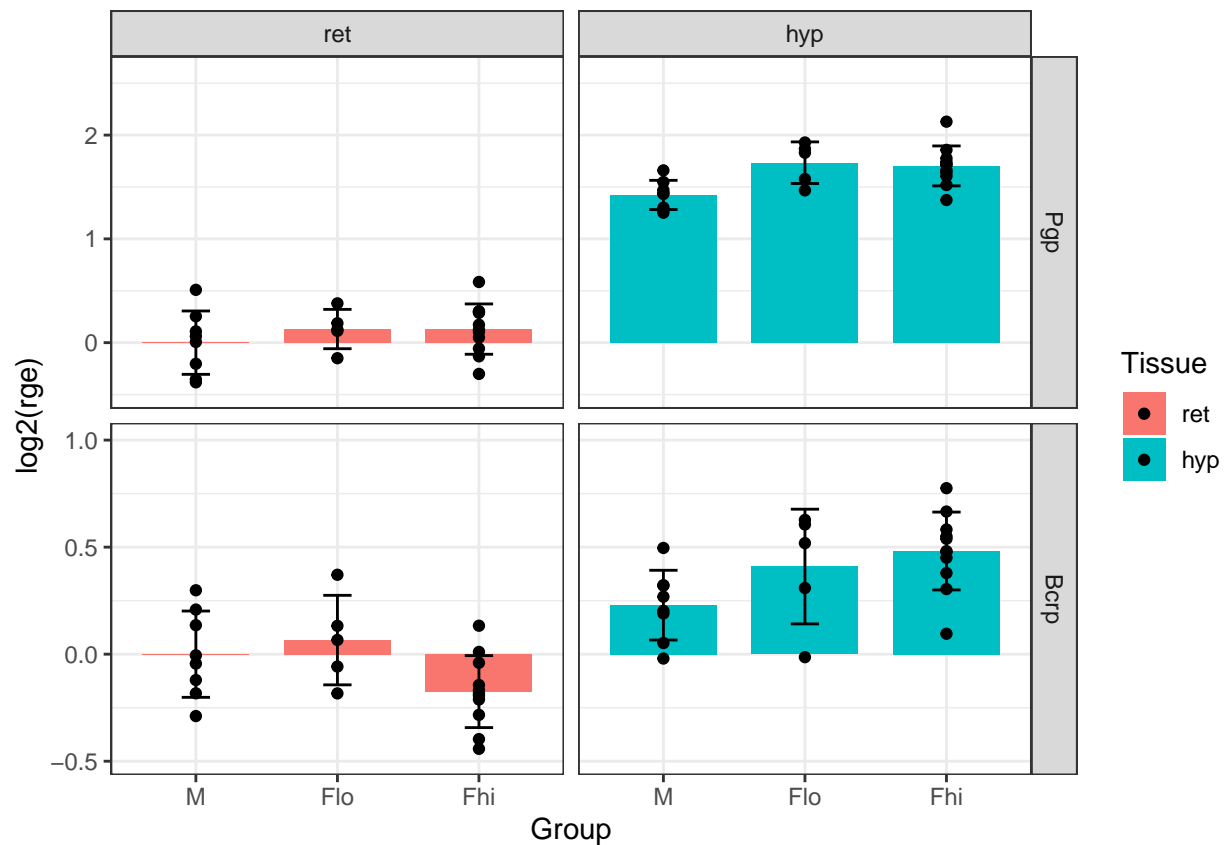


```
#ggsave(filename="../../figures/qRT-PCR/trans_trt.png", plot=trans_trt_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_trt.svg", plot=trans_trt_plot, height=4, width=6)
```

```
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)
```

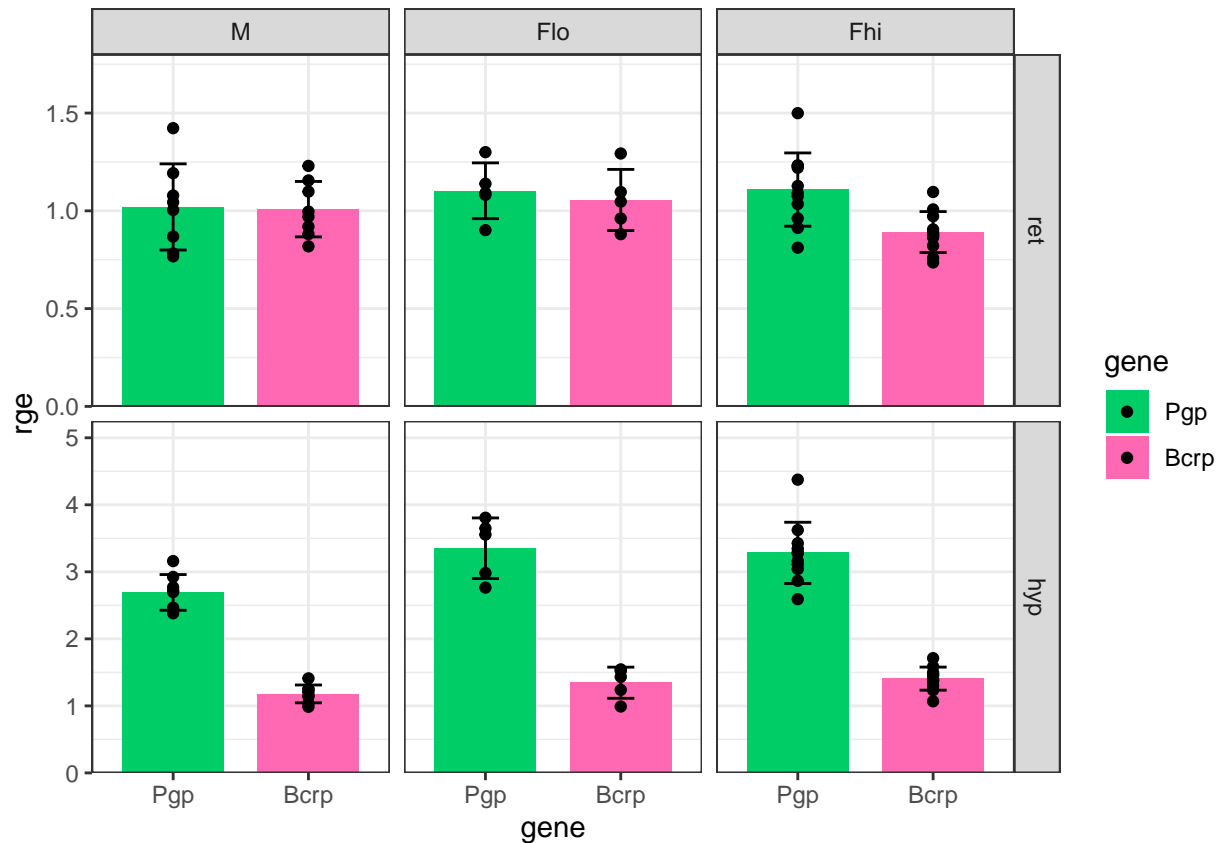
### Comparing P-gp to Bcrp

```
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
```



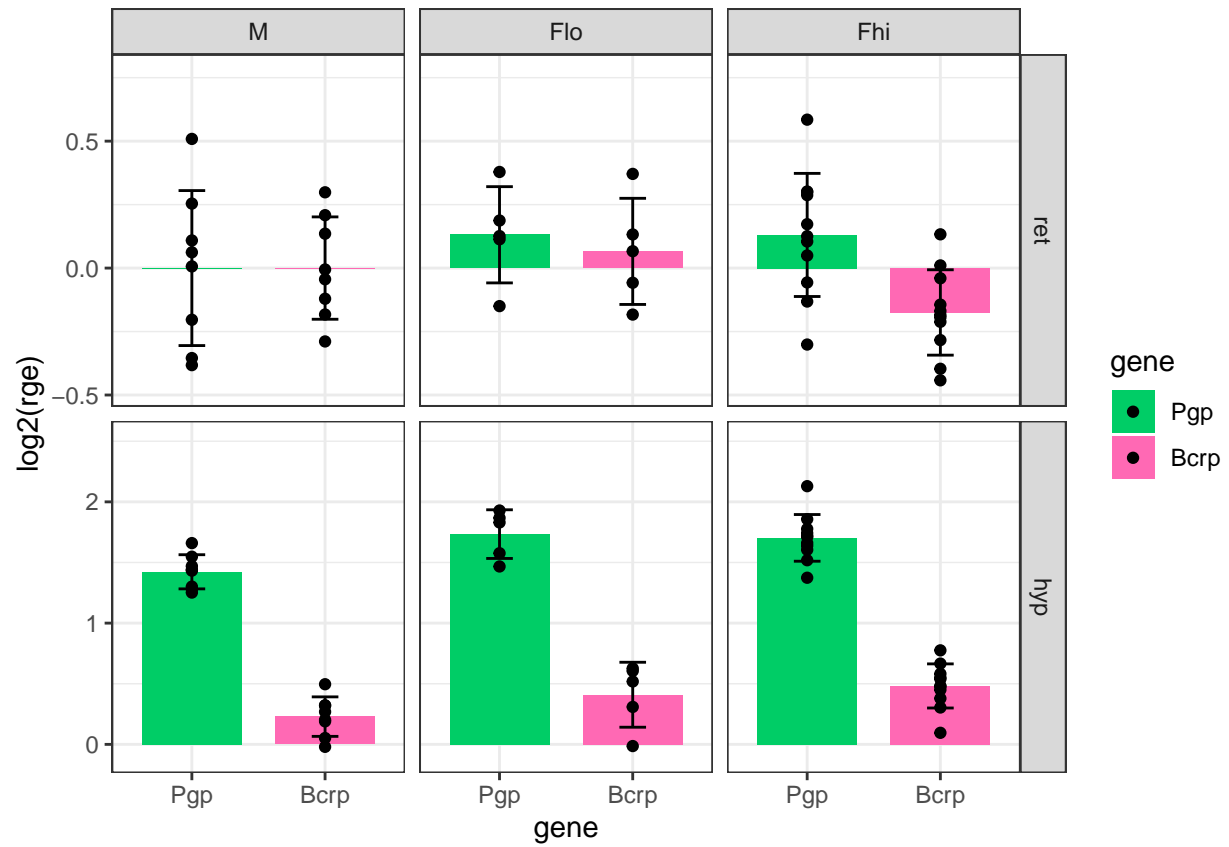


```
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene.png", plot=trans_g2g_plot, height=4, width=6)
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene.svg", plot=trans_g2g_plot, height=4, width=6)
```

```
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)
```