qpCR Transporter Figures

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

```r
transporters <- read.csv("../data/07222022-transporter-gene-study-males-only.csv", fileEncoding = 'UTF-8

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", "C")),
                                        Tissue = factor(Tissue, c("ret", "hyp")))

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
## 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     M1 3.7494898 1.5494993    hyp         M
## 16     M1 1.6644467 1.8381502    ret         M
## 17     M2 4.1667488 1.8663785    hyp         M
## 18     M2 1.5507018 1.3745568    ret         M
## 19     M4 4.5129157 1.7196764    hyp         M
```

```
## 20      M4 2.1962788 1.6418123      ret        M
## 21      M5 4.2766858 1.4737923      hyp        M
## 22      M5 1.8408330 1.4889148      ret        M
## 23      M6 3.8036335 1.8005443      hyp        M
## 24      M6 1.3404700 1.3168085      ret        M
## 25      M7 3.6765257 1.7050723      hyp        M
## 26      M7 1.6115485 1.7268291      ret        M
## 27      M8 4.8779551 1.8686984      hyp        M
## 28      M8 1.2074056 1.2232894      ret        M
## 29      M9 4.1925835 2.1081379      hyp        M
## 30      M9 1.1843069 1.4496997      ret        M
## 31      S1 1.8016888 1.5565636      hyp        S
## 32      S1 1.0200728 1.0407493      ret        S
## 33      S2 1.3689686 1.8615469      hyp        S
## 34      S2 0.8222355 1.0784274      ret        S
## 35      S3 1.5582238 1.1842034      hyp        S
## 36      S3 1.0081232 0.8660578      ret        S
## 37      S4 1.0379047 1.2523247      hyp        S
## 38      S4 0.7776565 1.0200962      ret        S
## 39      S5 1.6163239 1.2704658      hyp        S
## 40      S5 0.8109336 0.9139269      ret        S
## 41      S6 1.7206882 1.1597168      hyp        S
## 42      S6 1.0835666 0.9436934      ret        S
## 43      S7 2.3260412 1.6427723      hyp        S
## 44      S7 1.4357874 1.2453351      ret        S
## 45      S8 2.0696005 1.6327948      hyp        S
## 46      S8 1.0679296 1.0456424      ret        S
## 47      S9 2.4339414 1.4168757      hyp        S
## 48      S9 1.1287493 0.8979728      ret        S
```

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

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 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 86 more rows
## # i Use `print(n = ...)` to see more rows
```
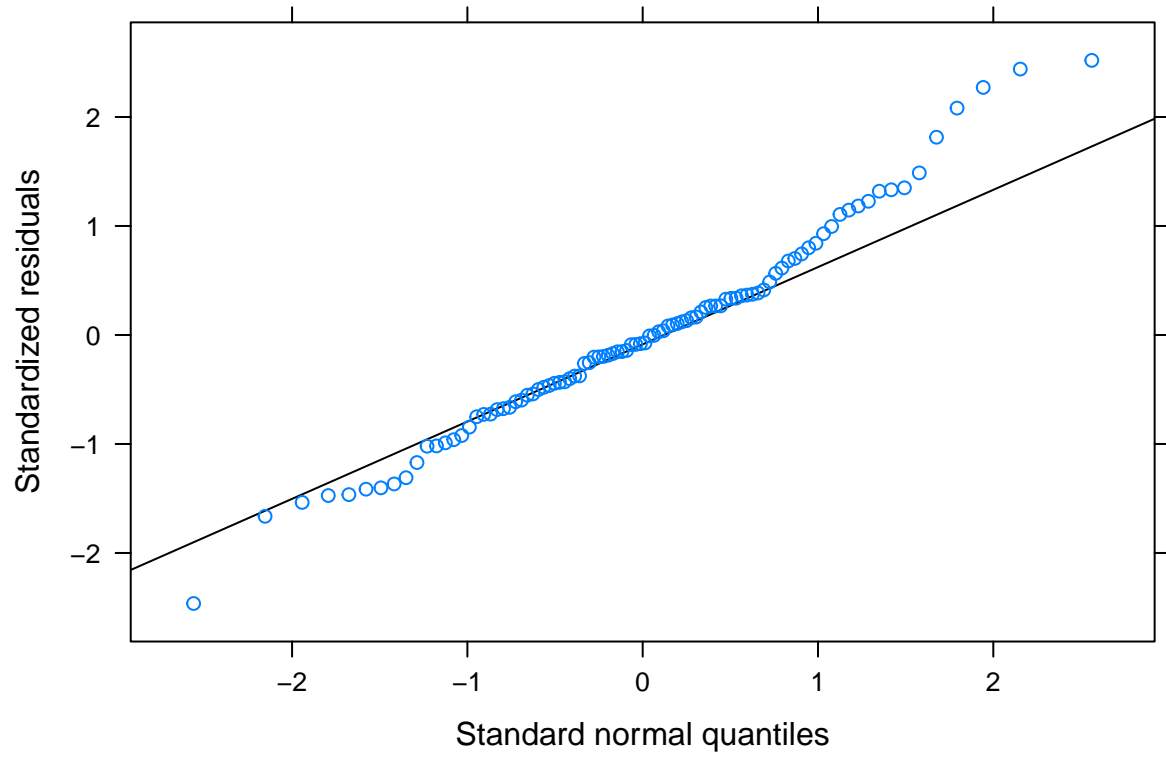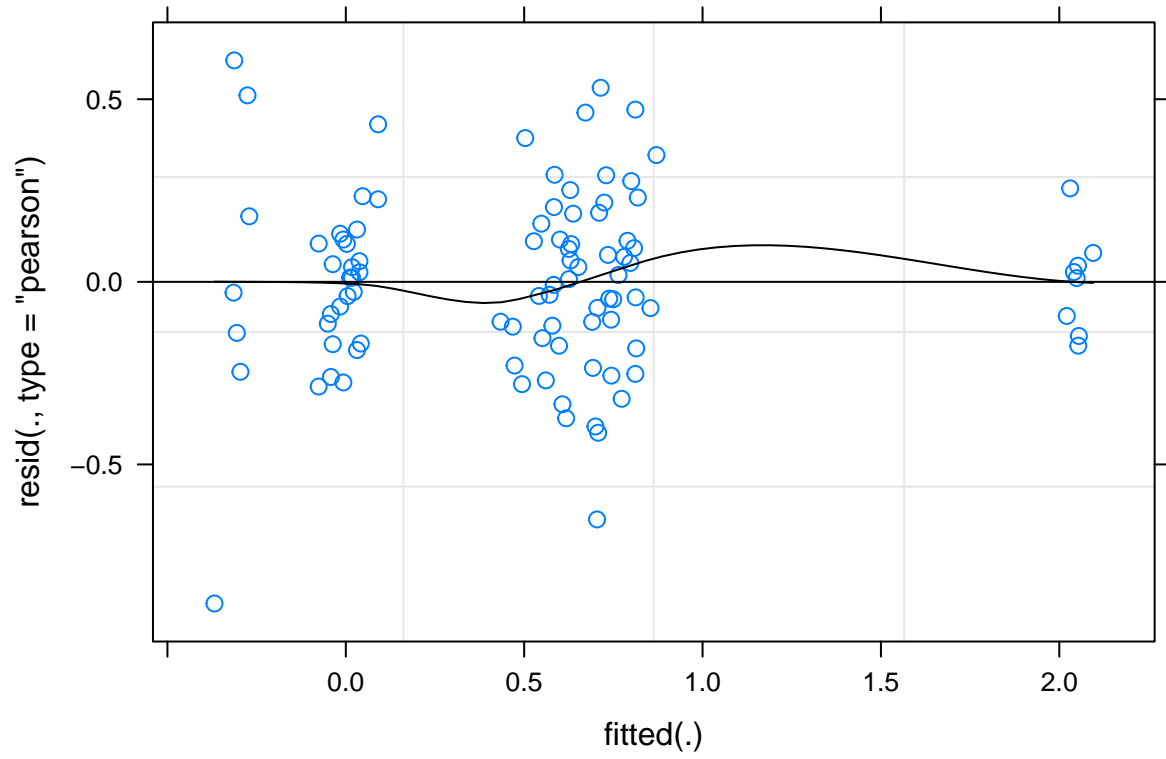
```r
transporter_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=transporters_long)
plot(transporter_lm, type=c("p","smooth"), col.line=1)
```
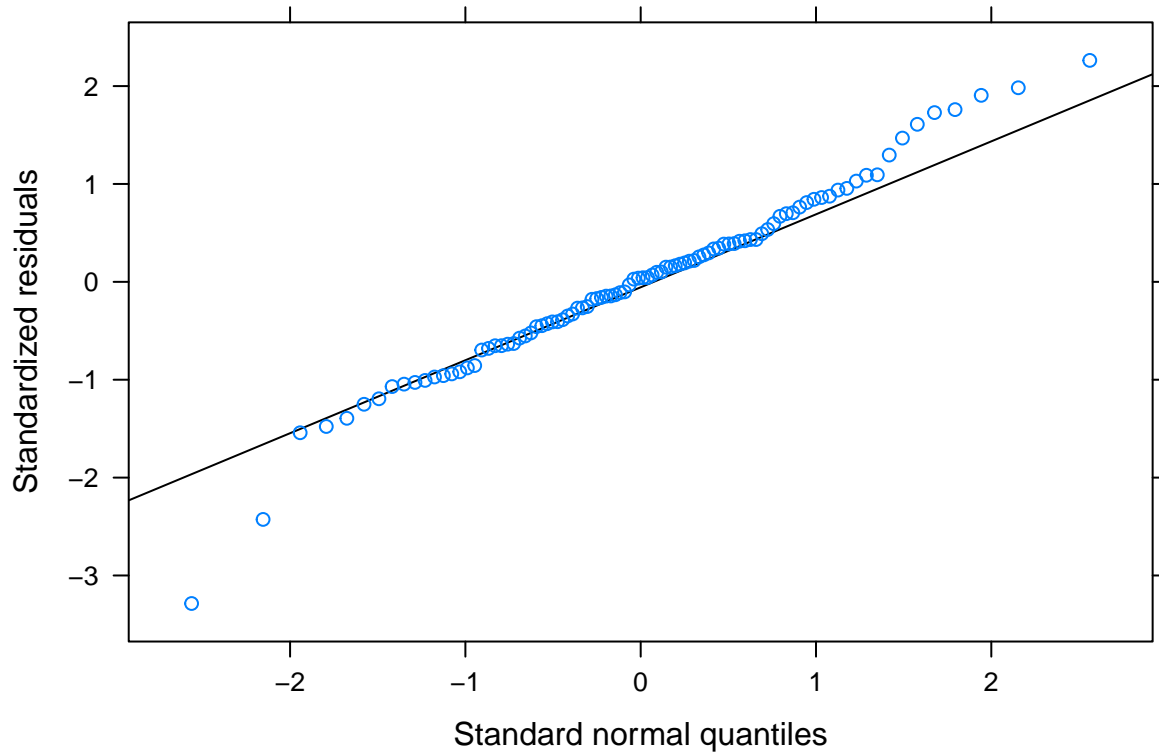
2
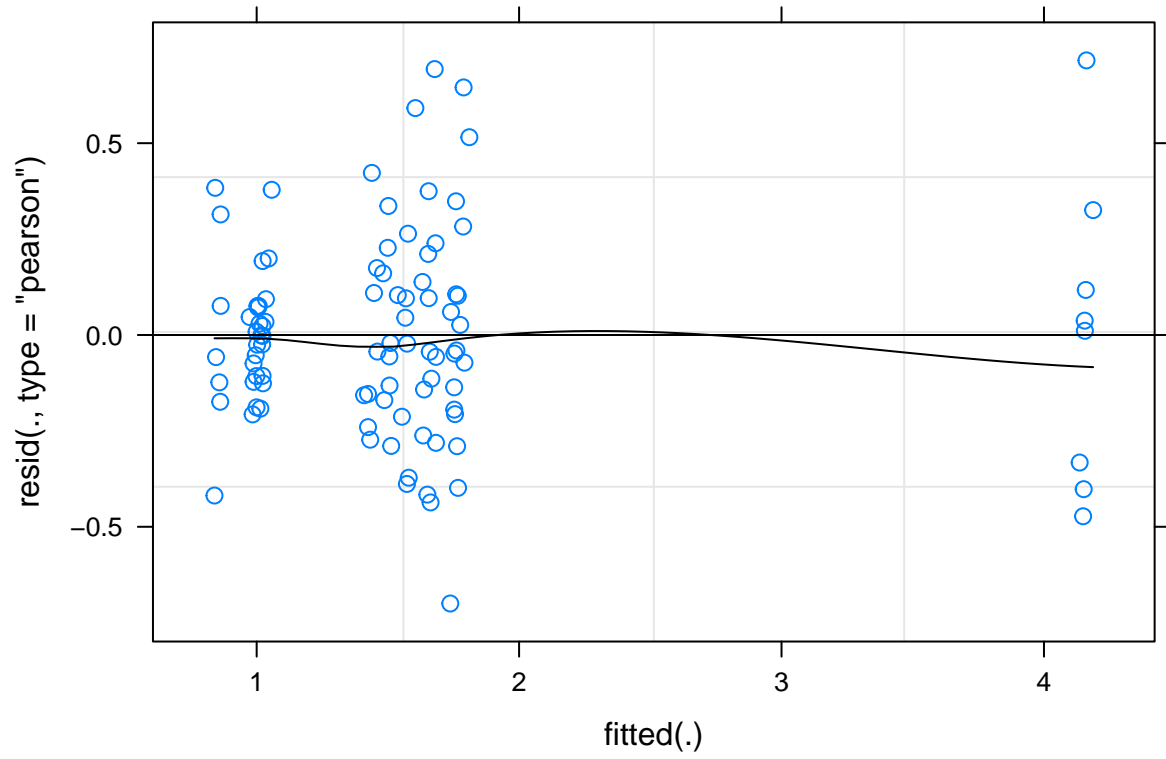
```
lattice::qqmath(transporter_lm)
```

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

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

```
anova(transporter_lm)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                        Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## gene                   4.5108  4.5108     1    63  55.872 2.961e-10 ***
## Tissue                19.8049 19.8049     1    63 245.312 < 2.2e-16 ***
## Treatment             16.9592  8.4796     2    21 105.032 1.159e-11 ***
## gene:Tissue            5.0893  5.0893     1    63  63.039 4.549e-11 ***
## gene:Treatment         7.6589  3.8295     2    63  47.433 2.711e-13 ***
## Tissue:Treatment       3.1968  1.5984     2    63  19.799 2.131e-07 ***
## gene:Tissue:Treatment  5.8721  2.9361     2    63  36.367 3.158e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

```
## Tissue = ret, gene = Pgp:
##  contrast estimate    SE   df t.ratio p.value
##  S - M    -0.55727 0.140 83.8  -3.973  0.0004
##  S - C     0.16420 0.145 83.8   1.129  0.4991
##  M - C     0.72147 0.149 83.8   4.830  <.0001
##
## Tissue = hyp, gene = Pgp:
##  contrast estimate    SE   df t.ratio p.value
##  S - M    -2.38669 0.140 83.8 -17.017  <.0001
##  S - C     0.12468 0.145 83.8   0.857  0.6686
##  M - C     2.51137 0.149 83.8  16.811  <.0001
```

```
## 
## Tissue = ret, gene = Bcrp:
##  contrast estimate    SE   df t.ratio p.value
##  S - M    -0.50174 0.140 83.8  -3.577  0.0017
##  S - C    -0.00704 0.145 83.8  -0.048  0.9987
##  M - C     0.49470 0.149 83.8   3.312  0.0039
## 
## Tissue = hyp, gene = Bcrp:
##  contrast estimate    SE   df t.ratio p.value
##  S - M    -0.31956 0.140 83.8  -2.278  0.0644
##  S - C    -0.23160 0.145 83.8  -1.592  0.2547
##  M - C     0.08796 0.149 83.8   0.589  0.8265
## 
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

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

```
## Treatment = S, gene = Pgp:
##  contrast  estimate    SE df t.ratio p.value
##  ret - hyp   -0.753 0.134 63  -5.623  <.0001
## 
## Treatment = M, gene = Pgp:
##  contrast  estimate    SE df t.ratio p.value
##  ret - hyp   -2.583 0.142 63 -18.178  <.0001
## 
## Treatment = C, gene = Pgp:
##  contrast  estimate    SE df t.ratio p.value
##  ret - hyp   -0.793 0.152 63  -5.219  <.0001
## 
## Treatment = S, gene = Bcrp:
##  contrast  estimate    SE df t.ratio p.value
##  ret - hyp   -0.436 0.134 63  -3.256  0.0018
## 
## Treatment = M, gene = Bcrp:
##  contrast  estimate    SE df t.ratio p.value
##  ret - hyp   -0.254 0.142 63  -1.788  0.0786
## 
## Treatment = C, gene = Bcrp:
##  contrast  estimate    SE df t.ratio p.value
##  ret - hyp   -0.661 0.152 63  -4.350  0.0001
## 
## Degrees-of-freedom method: kenward-roger
```

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

```
## Treatment = S, Tissue = ret:
##  contrast   estimate    SE df t.ratio p.value
##  Pgp - Bcrp   0.0115 0.134 63   0.086  0.9321
## 
## Treatment = M, Tissue = ret:
##  contrast   estimate    SE df t.ratio p.value
##  Pgp - Bcrp   0.0670 0.142 63   0.472  0.6389
## 
## Treatment = C, Tissue = ret:
```

```
## contrast   estimate    SE df t.ratio p.value
## Pgp - Bcrp  -0.1598 0.152 63  -1.052  0.2968
##
## Treatment = S, Tissue = hyp:
## contrast   estimate    SE df t.ratio p.value
## Pgp - Bcrp   0.3285 0.134 63   2.452  0.0170
##
## Treatment = M, Tissue = hyp:
## contrast   estimate    SE df t.ratio p.value
## Pgp - Bcrp   2.3956 0.142 63  16.862  <.0001
##
## Treatment = C, Tissue = hyp:
## contrast   estimate    SE df t.ratio p.value
## Pgp - Bcrp  -0.0278 0.152 63  -0.183  0.8552
##
## Degrees-of-freedom method: kenward-roger
```

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

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

```
trans_sumstats
```

```
## # A tibble: 12 x 8
## # Groups:   Treatment, Tissue [6]
##    Treatment Tissue gene      n  mean     sd log2_mean log2_sd
##    <fct>     <fct>  <fct> <int> <dbl>  <dbl>     <dbl>   <dbl>
##  1 S         ret    Pgp       9 1.02  0.204 -1.11e-11   0.280
##  2 S         ret    Bcrp      9 1.01  0.117 -4.39e-11   0.163
##  3 S         hyp    Pgp       9 1.77  0.448  7.80e- 1   0.385
##  4 S         hyp    Bcrp      9 1.44  0.244  5.10e- 1   0.242
##  5 M         ret    Pgp       8 1.57  0.340  6.26e- 1   0.305
##  6 M         ret    Bcrp      8 1.51  0.212  5.80e- 1   0.202
##  7 M         hyp    Pgp       8 4.16  0.411  2.05e+ 0   0.141
##  8 M         hyp    Bcrp      8 1.76  0.199  8.09e- 1   0.163
##  9 C         ret    Pgp       7 0.853 0.284 -3.06e- 1   0.527
## 10 C         ret    Bcrp      7 1.01  0.109  1.15e- 2   0.150
## 11 C         hyp    Pgp       7 1.65  0.285  6.99e- 1   0.258
## 12 C         hyp    Bcrp      7 1.67  0.375  7.14e- 1   0.308
```

Comparing hyp vs ret within each treatment/gene

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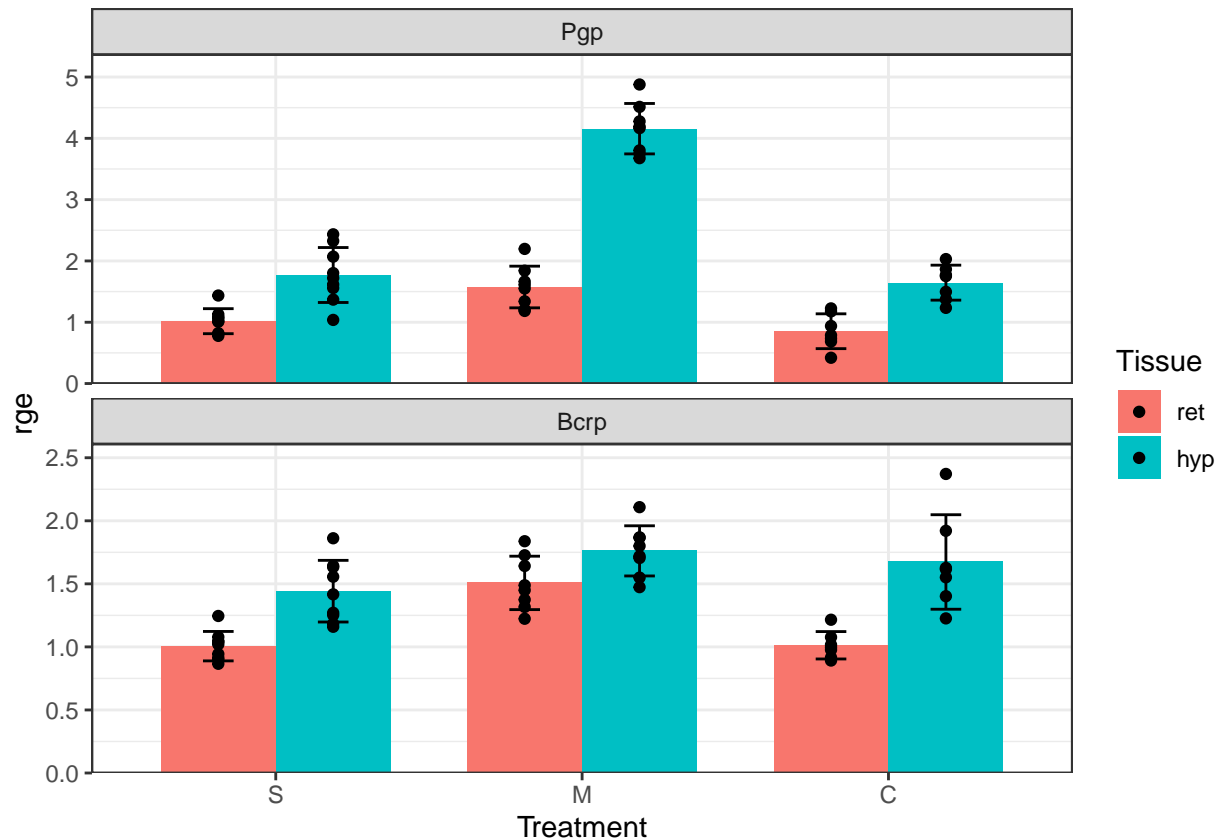
```
                stat="identity", position=position_dodge(width=0.75)) +
   facet_wrap(~gene, scales="free_y", ncol=1) +
   scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
   #scale_fill_manual(values=c("maroon1", "springgreen3")) +
   theme_bw()
```

Warning: Ignoring unknown aesthetics: fill

```
trans_hyp_ret_plot
```



```
ggsave(filename="../figures/trans_hyp_ret_males.png", plot=trans_hyp_ret_plot, height=4, width=6)
ggsave(filename="../figures/trans_hyp_ret_males.svg", plot=trans_hyp_ret_plot, height=4, width=6)
```

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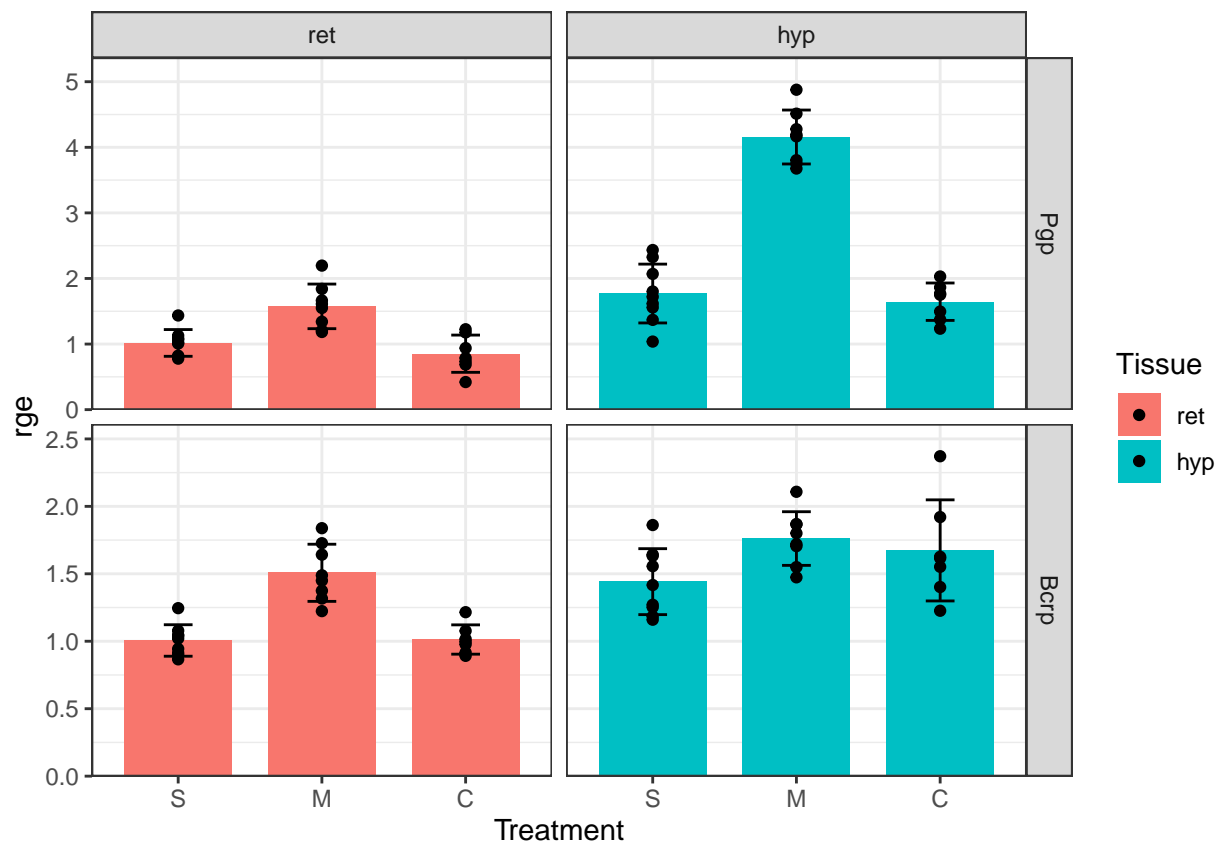
```
#trans_hyp_ret_log_plot

#ggsave(filename="../figures/trans_hyp_ret_log.png", plot=trans_hyp_ret_log_plot, height=4, width=6)
#ggsave(filename="../figures/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=Treatment, y=rge, fill=Tissue),
               fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=mean-sd, ymax=mean+sd, fill=Tissue),
               width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
             stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(gene~Tissue, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

Warning: Ignoring unknown aesthetics: fill

```
trans_trt_plot
```



```
ggsave(filename="../figures/trans_trt_males.png", plot=trans_trt_plot, height=4, width=6)
ggsave(filename="../figures/trans_trt_males.svg", plot=trans_trt_plot, height=4, width=6)
```

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

#trans_trt_log_plot

#ggsave(filename="../figures/trans_trt_log.png", plot=trans_trt_log_plot, height=4, width=6)
#ggsave(filename="../figures/trans_trt_log.svg", plot=trans_trt_log_plot, height=4, width=6)
```
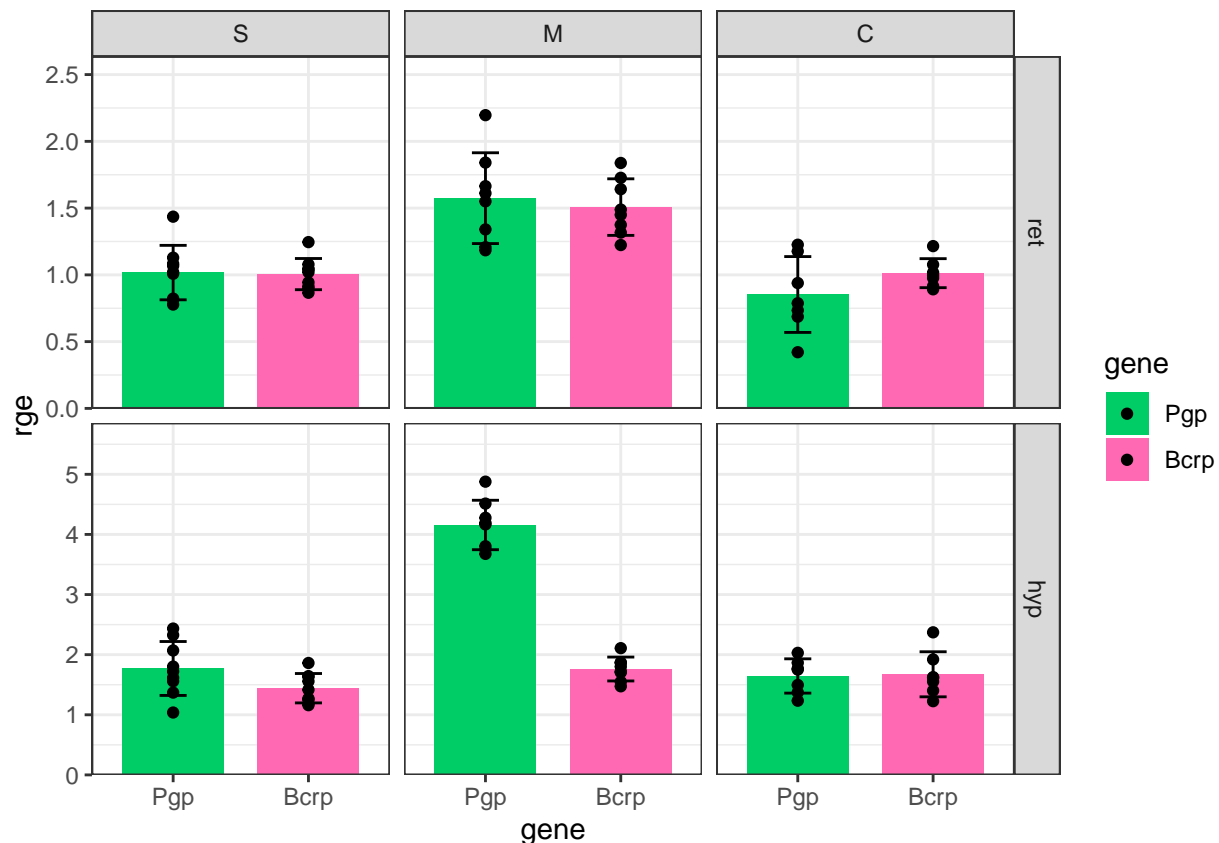
```r
trans_g2g_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=gene, y=rge, fill=gene),
              fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=gene, ymin=mean-sd,
                                    ymax=mean+sd, fill=gene),
              width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=gene, y=rge, fill=gene),
            position=position_dodge(width=0.75)) +
  facet_grid(Tissue~Treatment, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.2))) +
  scale_fill_manual(values=c("springgreen3", "hotpink")) +
  theme_bw()
```

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

```r
trans_g2g_plot
```

```
ggsave(filename="../figures/trans_gene_to_gene_males.png", plot=trans_g2g_plot, height=4, width=6)
ggsave(filename="../figures/trans_gene_to_gene_males.svg", plot=trans_g2g_plot, height=4, width=6)


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

#trans_g2g_log_plot

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