

# qPCR: Transporter in 1 inj saline vs morphine males

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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)

transporters <- read.csv("../data/08022022-Pgp-Bcrp-gene-study.csv", fileEncoding = 'UTF-8-BOM')

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 = replace_na(Treatment, "sal"))

transporters <- transporters %>% mutate(Treatment = factor(Treatment, c("sal", "M")),
                                       Tissue = factor(Tissue, c("ret", "hyp")))

head(transporters)

##   Sample   Abc.RGE  Bcrp.RGE Tissue Treatment
## 1     82 1.7123557 1.0306390   hyp      sal
## 2     82 0.8463480 1.0044514   ret      sal
## 3     83 2.4618964 1.1437142   hyp      sal
## 4     83 0.9239439 0.9692421   ret      sal
## 5     84 2.1325816 1.0028340   hyp      sal
## 6     84 1.0989301 0.9950309   ret      sal

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

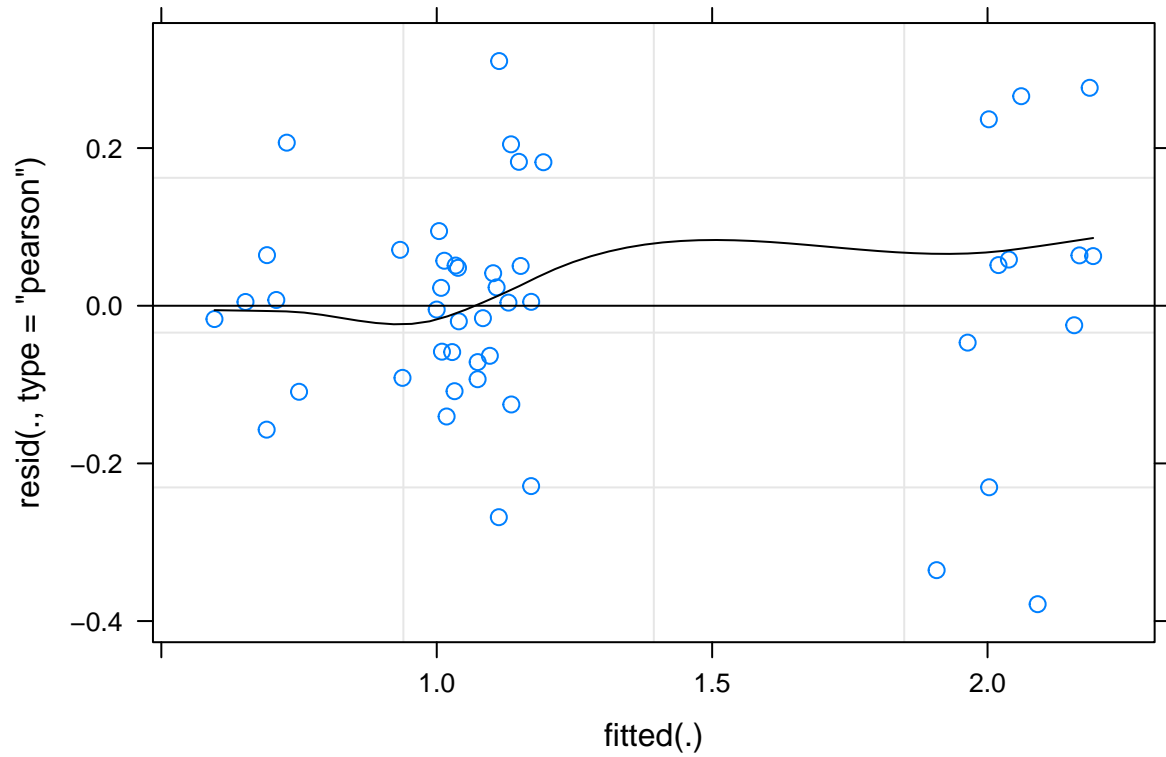
transporters_long <- transporters_long %>% mutate(gene = str_extract(gene, "[:alpha:]+(?=.)"))
transporters_long <- transporters_long %>% mutate(gene = factor(as.factor(gene), c("Abc", "Bcrp")))

head(transporters_long)

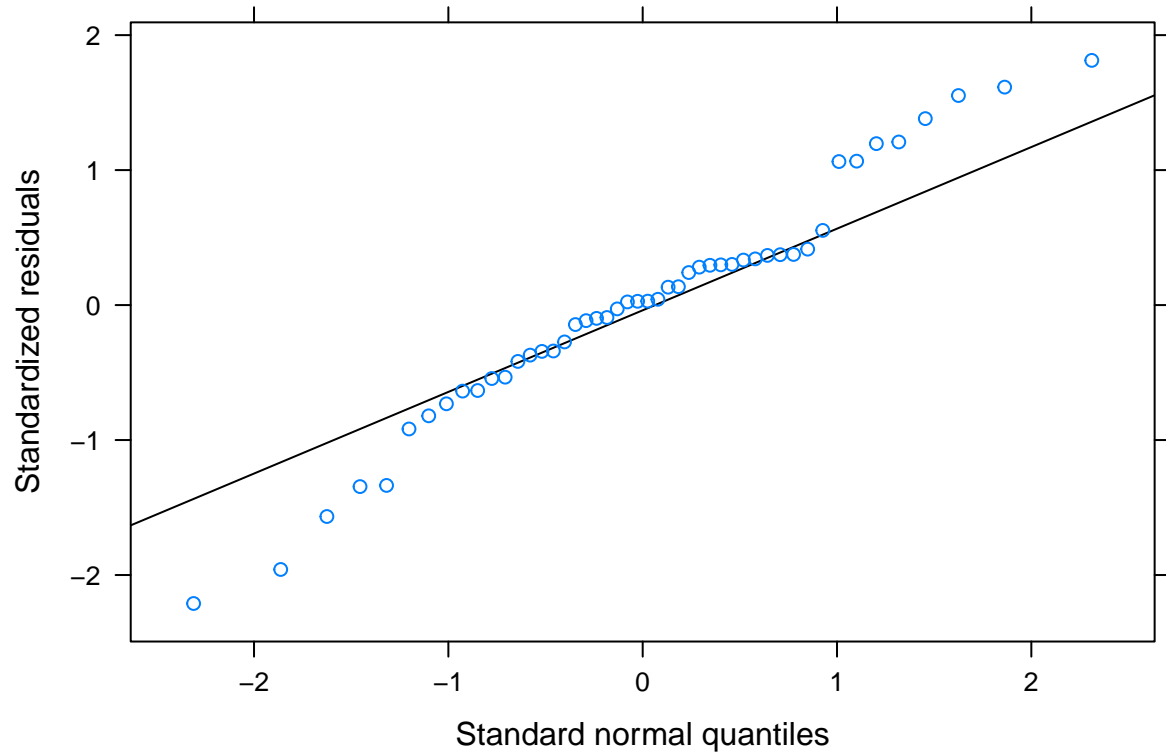
## # A tibble: 6 x 5
##   Sample Tissue Treatment gene      rge
##   <chr>   <fct>   <fct>   <fct> <dbl>
```

```
## 1 82    hyp    sal    Abc    1.71
## 2 82    hyp    sal    Bcrp   1.03
## 3 82    ret    sal    Abc    0.846
## 4 82    ret    sal    Bcrp   1.00
## 5 83    hyp    sal    Abc    2.46
## 6 83    hyp    sal    Bcrp   1.14
```

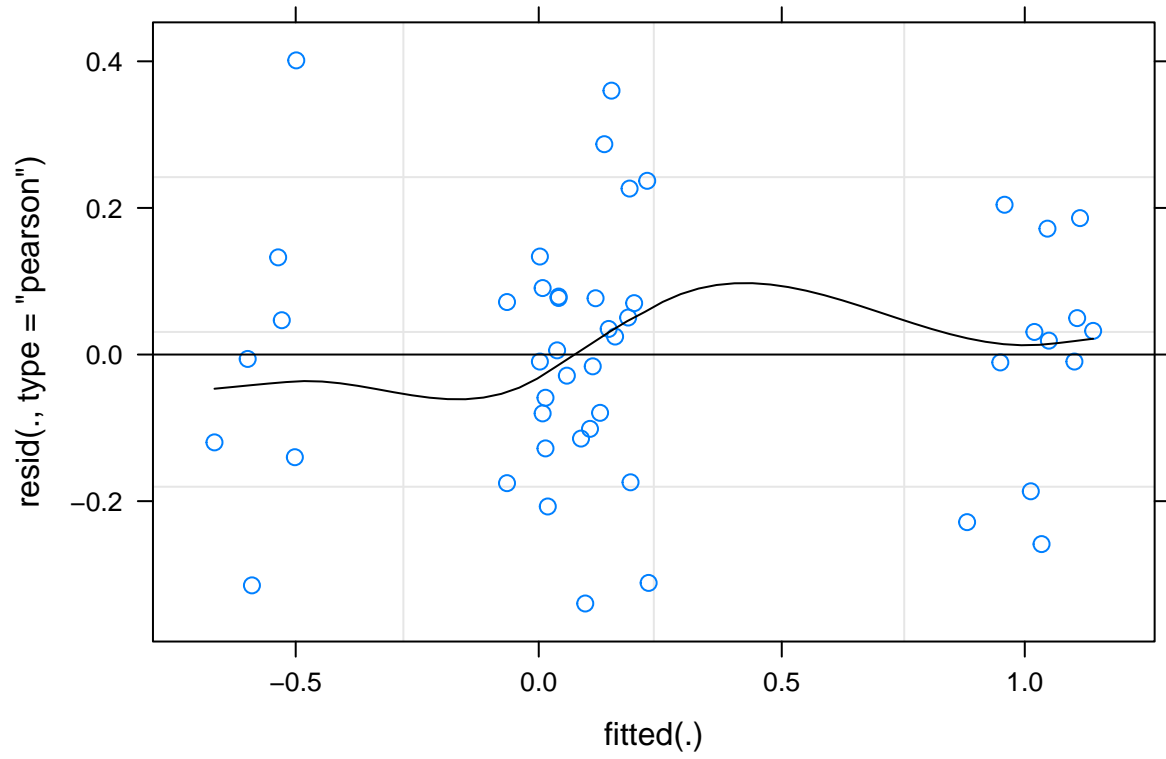
```
transporter_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=transporters_long)
plot(transporter_lm, type=c("p","smooth"), col.line=1)
```



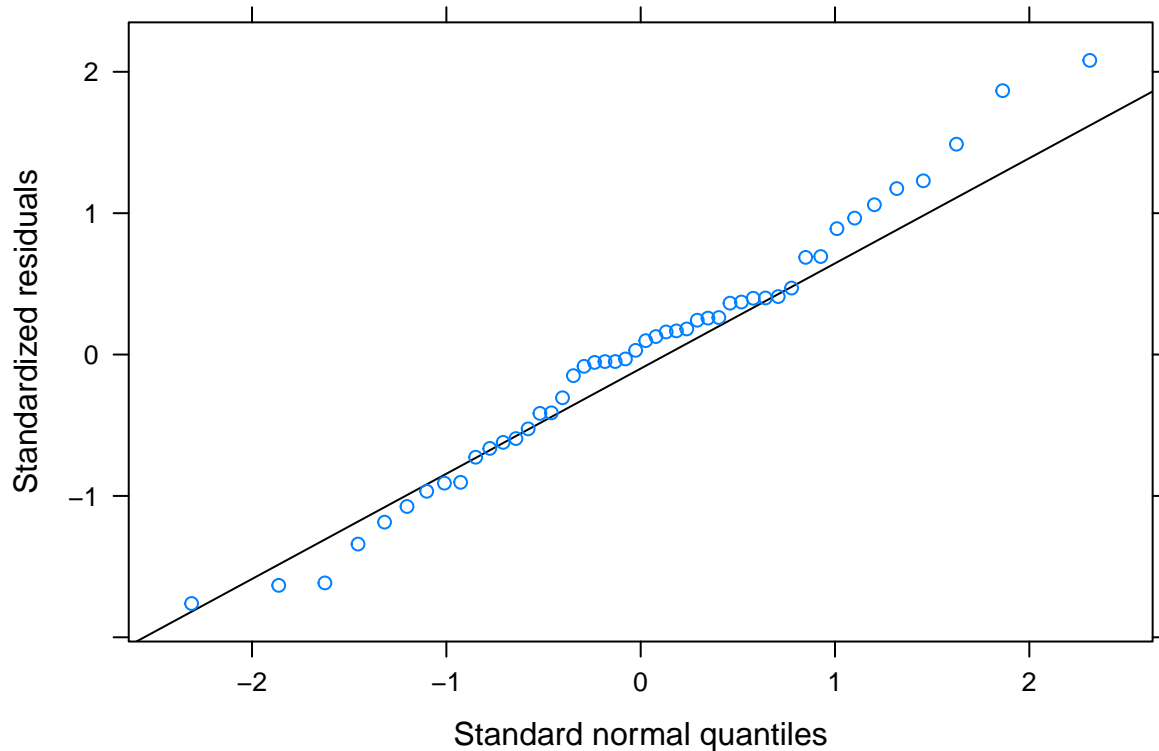
```
lattice::qqmath(transporter_lm)
```



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



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



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

```
##
##  Shapiro-Wilk normality test
##
## data:  transporters_long$rge
## W = 0.87132, p-value = 8.428e-05
```

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

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(transporters_long$rge)
## W = 0.94511, p-value = 0.02562
```

```
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	0.9384	0.9384	1	30	25.2328	2.182e-05	***
Tissue	5.6778	5.6778	1	30	152.6719	2.678e-13	***
Treatment	0.0988	0.0988	1	10	2.6563	0.1341952	
gene:Tissue	4.5790	4.5790	1	30	123.1262	3.845e-12	***
gene:Treatment	0.5380	0.5380	1	30	14.4668	0.0006533	***
Tissue:Treatment	0.1080	0.1080	1	30	2.9042	0.0986893	.
gene:Tissue:Treatment	0.1915	0.1915	1	30	5.1505	0.0305918	*

```
## ---
```

```

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

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

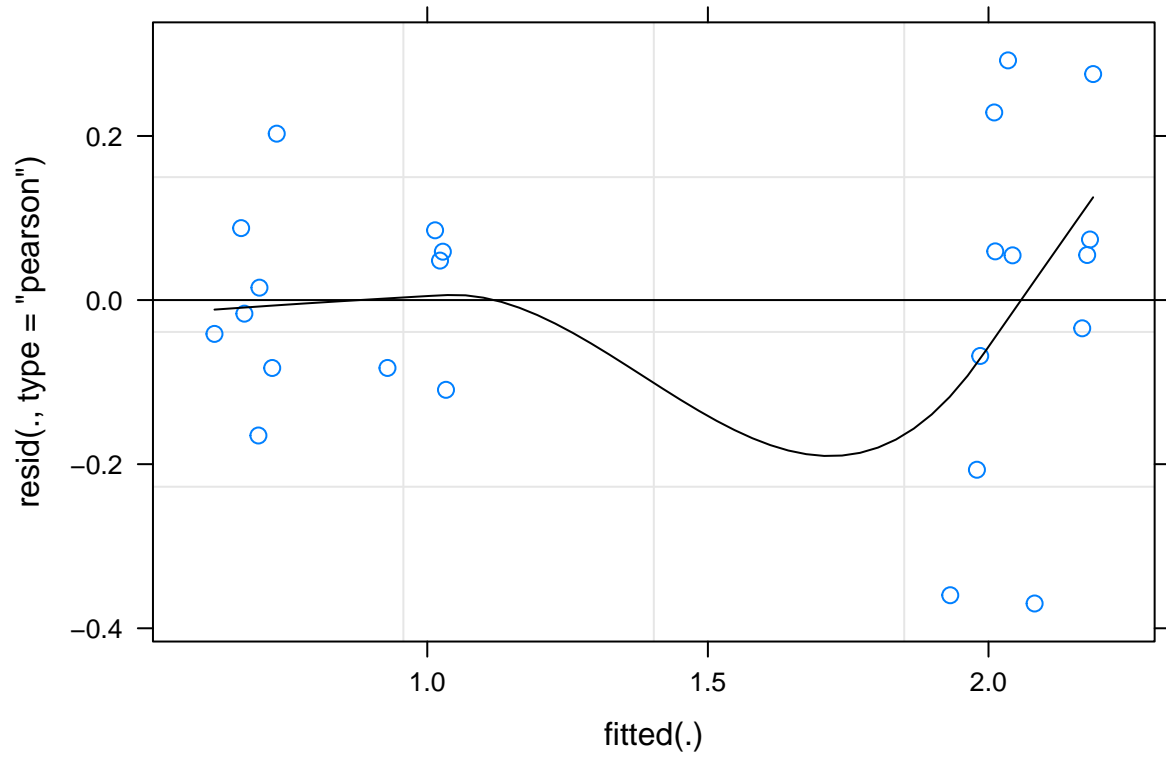
## Tissue = ret, gene = Abc:
## contrast estimate      SE    df t.ratio p.value
## sal - M      0.5602 0.123 37.3   4.561  0.0001
##
## Tissue = hyp, gene = Abc:
## contrast estimate      SE    df t.ratio p.value
## sal - M      0.1115 0.123 37.3   0.908  0.3698
##
## Tissue = ret, gene = Bcrp:
## contrast estimate      SE    df t.ratio p.value
## sal - M     -0.1256 0.123 37.3  -1.022  0.3132
##
## Tissue = hyp, gene = Bcrp:
## contrast estimate      SE    df t.ratio p.value
## sal - M     -0.0617 0.123 37.3  -0.503  0.6182
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.

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

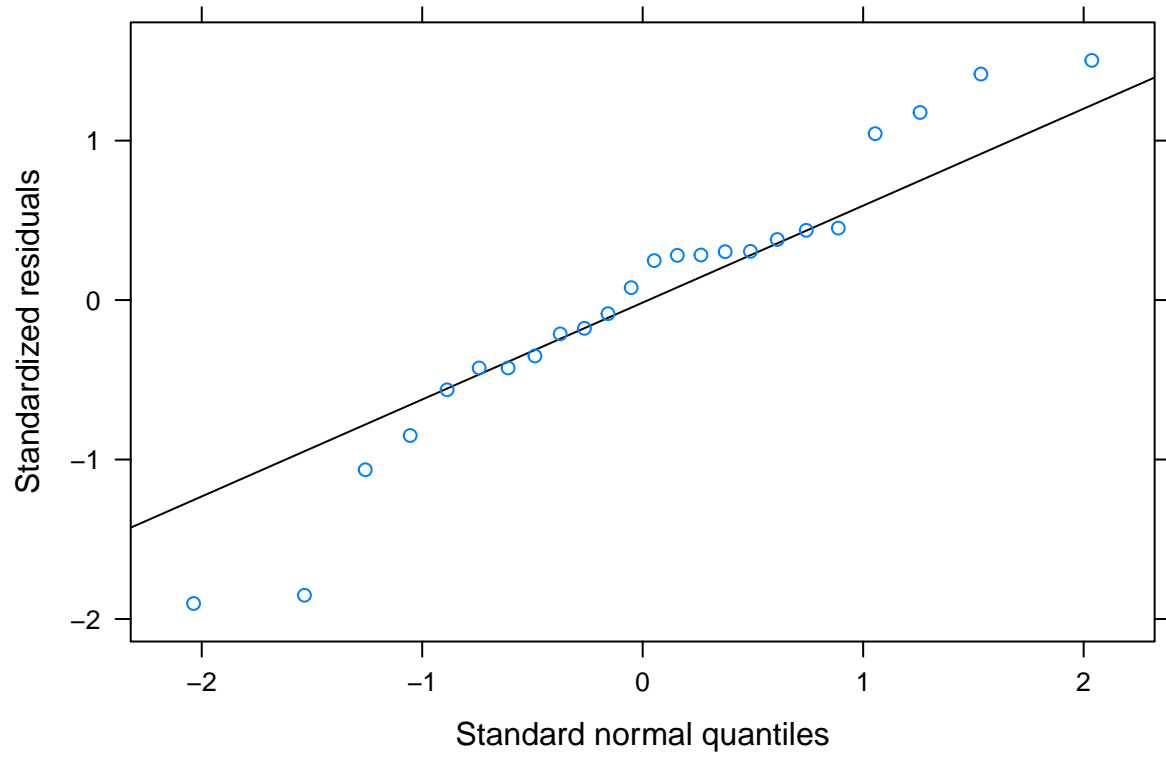
## Treatment = sal, gene = Abc:
## contrast estimate      SE df t.ratio p.value
## ret - hyp  -1.0998 0.122 30  -9.017  <.0001
##
## Treatment = M, gene = Abc:
## contrast estimate      SE df t.ratio p.value
## ret - hyp  -1.5485 0.103 30 -15.022  <.0001
##
## Treatment = sal, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp  -0.1030 0.122 30  -0.845  0.4049
##
## Treatment = M, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp  -0.0392 0.103 30  -0.380  0.7063
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.

pgp <- transporters_long %>% filter(gene == "Abc")
pgp_lm <- lmer(rge ~ Tissue * Treatment + (1|Sample), data=pgp)
plot(pgp_lm, type=c("p", "smooth"), col.line=1)

```

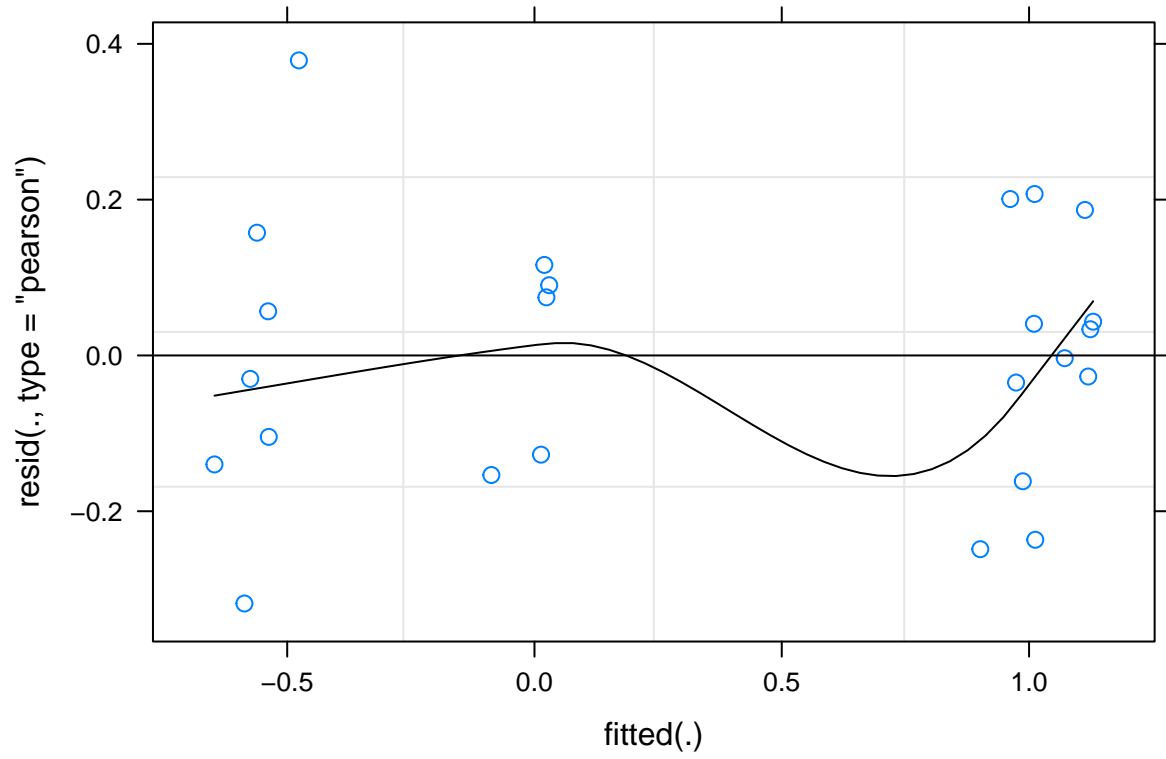


```
lattice::qqmath(pgp_lm)
```

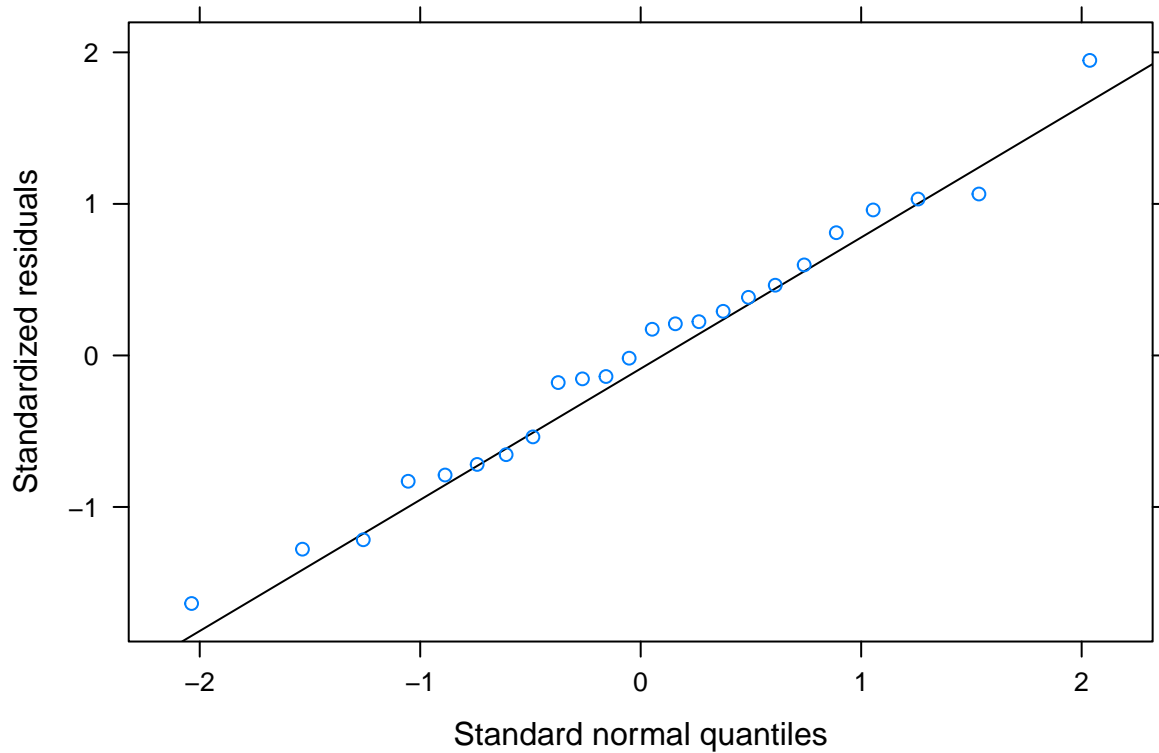


```
pgp_log2_lm <- lmer(log2(rge) ~ Tissue * Treatment + (1|Sample), data=pgp)
plot(pgp_log2_lm, type=c("p", "smooth"), col.line=1)
```





```
lattice::qqmath(pgp_log2_lm)
```



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

```
##
##  Shapiro-Wilk normality test
##
## data:  pgp$rge
## W = 0.8814, p-value = 0.008868
```

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

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(pgp$rge)
## W = 0.89041, p-value = 0.01355
```

```
anova(pgp_log2_lm)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF DenDF  F value    Pr(>F)
## Tissue        10.2274  10.2274     1    10 270.2616 1.445e-08 ***
## Treatment         0.4548   0.4548     1    10  12.0170  0.006056 **
## Tissue:Treatment  0.2936   0.2936     1    10   7.7587  0.019270 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

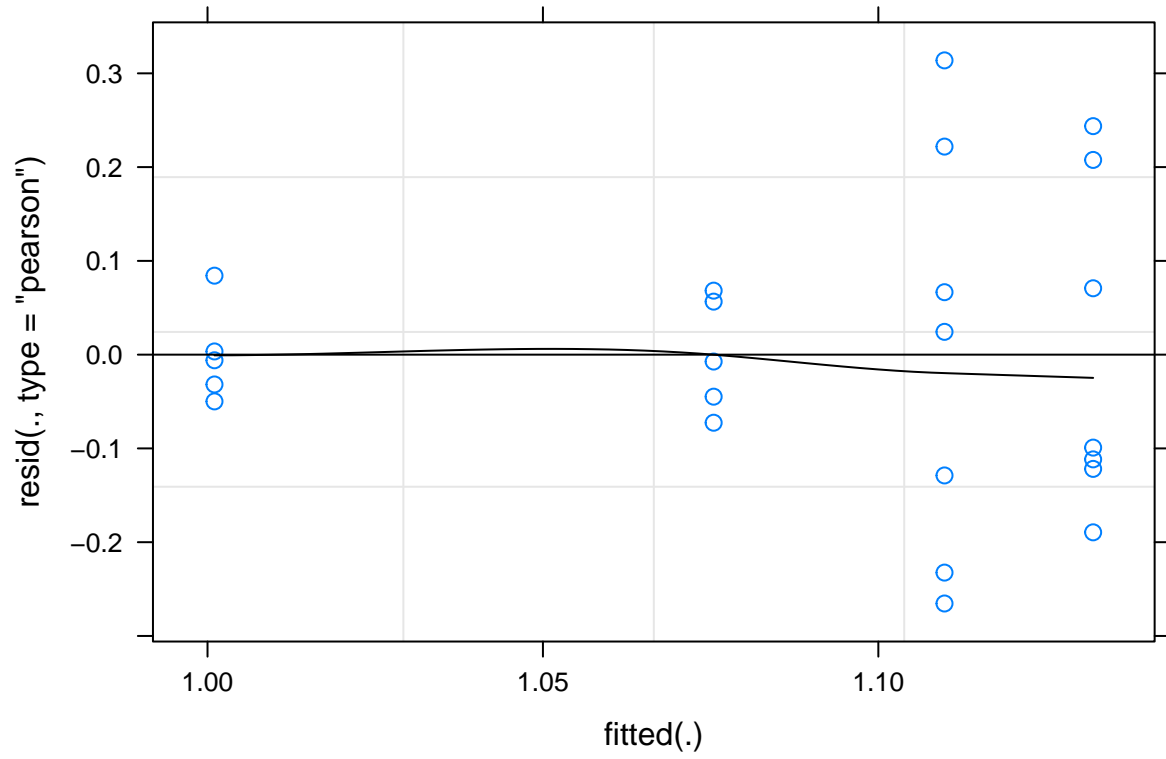
```
emmeans::emmeans(pgp_log2_lm, pairwise ~ Treatment | Tissue)$contrasts
```

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

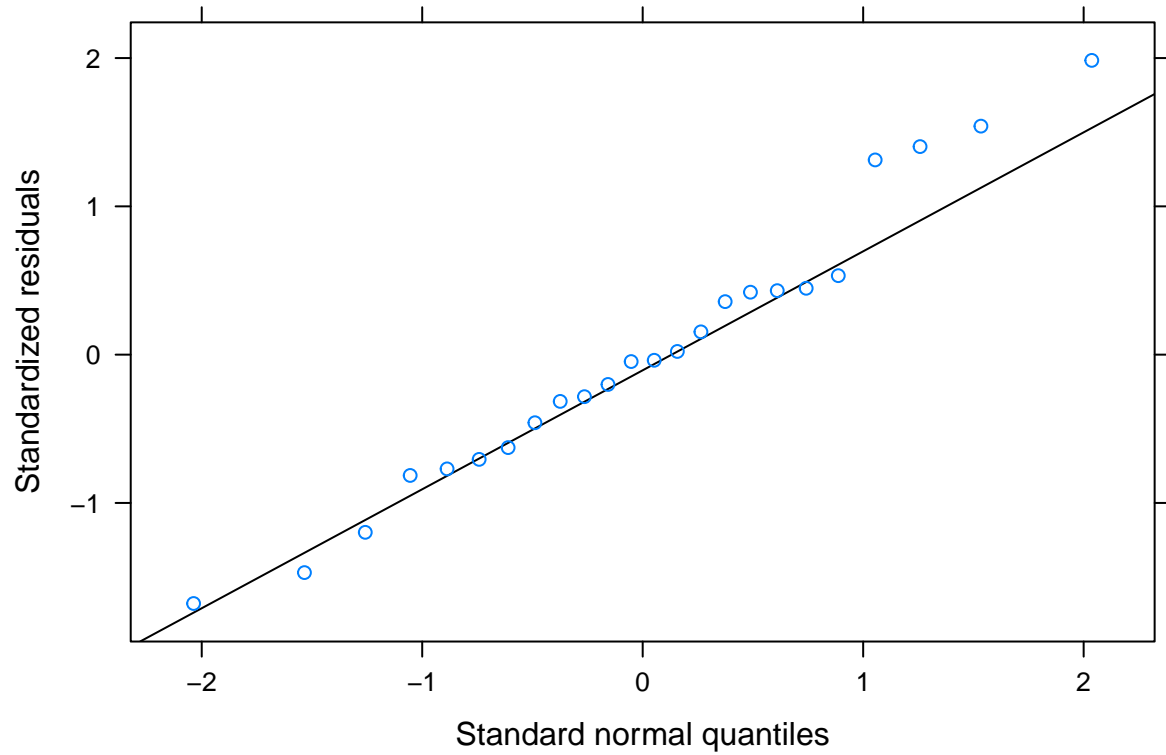
```
## contrast estimate    SE    df t.ratio p.value
## sal - M      0.560 0.126 19.4   4.446  0.0003
##
## Tissue = hyp:
## contrast estimate    SE    df t.ratio p.value
## sal - M      0.111 0.126 19.4   0.885  0.3870
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
emmeans::emmeans(pgp_log2_lm, pairwise ~ Tissue | Treatment)$contrasts

## Treatment = sal:
## contrast estimate    SE df t.ratio p.value
## ret - hyp     -1.10 0.123 10  -8.939  <.0001
##
## Treatment = M:
## contrast estimate    SE df t.ratio p.value
## ret - hyp     -1.55 0.104 10 -14.892  <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
bcrp <- transporters_long %>% filter(gene == "Bcrp")
bcrp_lm <- lmer(rge ~ Tissue * Treatment + (1|Sample), data=bcrp)

## boundary (singular) fit: see help('isSingular')
plot(bcrp_lm, type=c("p","smooth"), col.line=1)
```

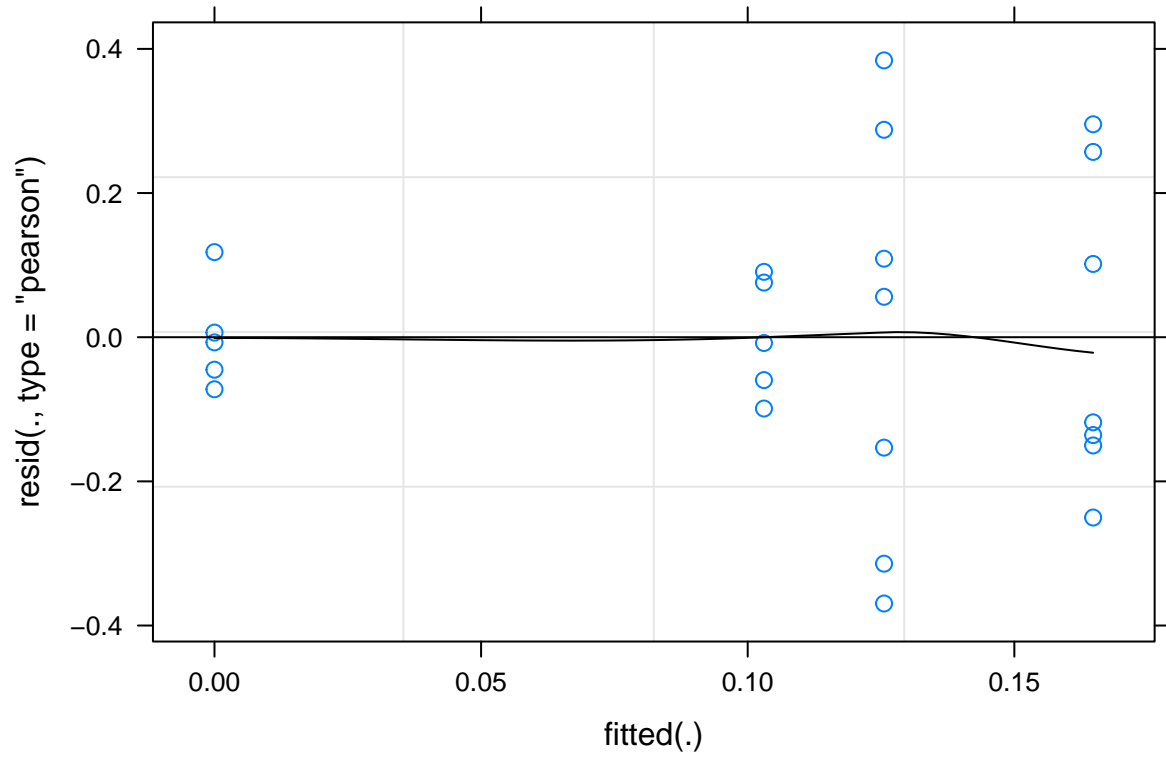


```
lattice::qqmath(bcrp_lm)
```

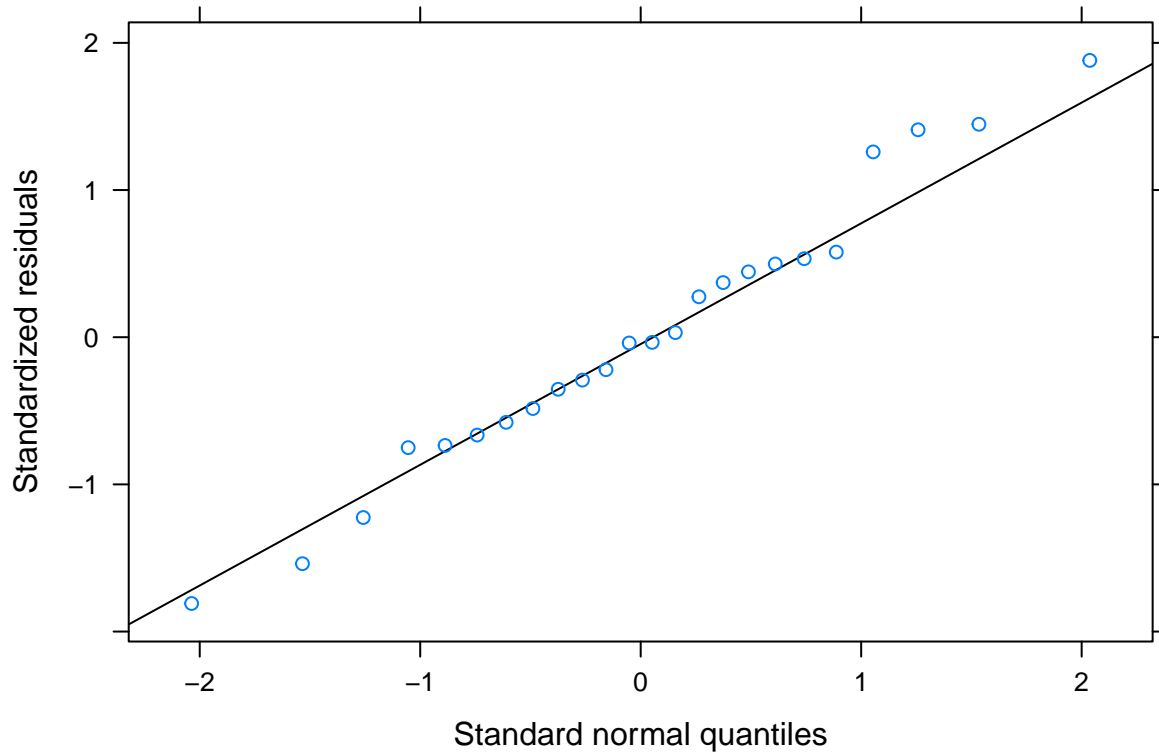


```
bcrp_log2_lm <- lmer(log2(rge) ~ Tissue * Treatment + (1|Sample), data=bcrp)

## boundary (singular) fit: see help('isSingular')
plot(bcrp_log2_lm, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(bcrp_log2_lm)
```



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

```
##
##  Shapiro-Wilk normality test
##
## data:  bcrp$rge
## W = 0.9244, p-value = 0.07312
```

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

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(bcrp$rge)
## W = 0.94978, p-value = 0.2679
```

```
anova(bcrp_lm)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq  Mean Sq NumDF DenDF  F value Pr(>F)
## Tissue          0.013601 0.013601     1    20   0.5438 0.4694
## Treatment        0.039895 0.039895     1    20   1.5952 0.2211
## Tissue:Treatment 0.003983 0.003983     1    20   0.1592 0.6941
```

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

```
## Tissue = ret:
## contrast estimate      SE df t.ratio p.value
## sal - M    -0.1088 0.0926 20  -1.175  0.2537
```

```
##
## Tissue = hyp:
## contrast estimate      SE df t.ratio p.value
## sal - M    -0.0566 0.0926 20   -0.611  0.5481
##
## Degrees-of-freedom method: kenward-roger

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

## Treatment = sal:
## contrast estimate      SE df t.ratio p.value
## ret - hyp   -0.0744 0.1000 10   -0.744  0.4740
##
## Treatment = M:
## contrast estimate      SE df t.ratio p.value
## ret - hyp   -0.0222 0.0845 10   -0.262  0.7986
##
## 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: 8 x 8
## # Groups:   Treatment, Tissue [4]
##   Treatment Tissue gene      n mean      sd log2_mean log2_sd
##   <fct>      <fct> <fct> <int> <dbl> <dbl> <dbl> <dbl>
## 1 sal      ret    Abc      5 1.01  0.114  1.62e-10  0.169
## 2 sal      ret    Bcrp     5 1.00  0.0516 -1.59e-10  0.0729
## 3 sal      hyp    Abc      5 2.16  0.277  1.10e+ 0  0.196
## 4 sal      hyp    Bcrp     5 1.08  0.0616  1.03e- 1  0.0827
## 5 M        ret    Abc      7 0.688 0.132  -5.60e- 1  0.266
## 6 M        ret    Bcrp     7 1.11  0.221  1.26e- 1  0.290
## 7 M        hyp    Abc      7 2.00  0.265  9.88e- 1  0.198
## 8 M        hyp    Bcrp     7 1.13  0.173  1.65e- 1  0.216
```

## Comparing hyp vs ret within each treatment/gene

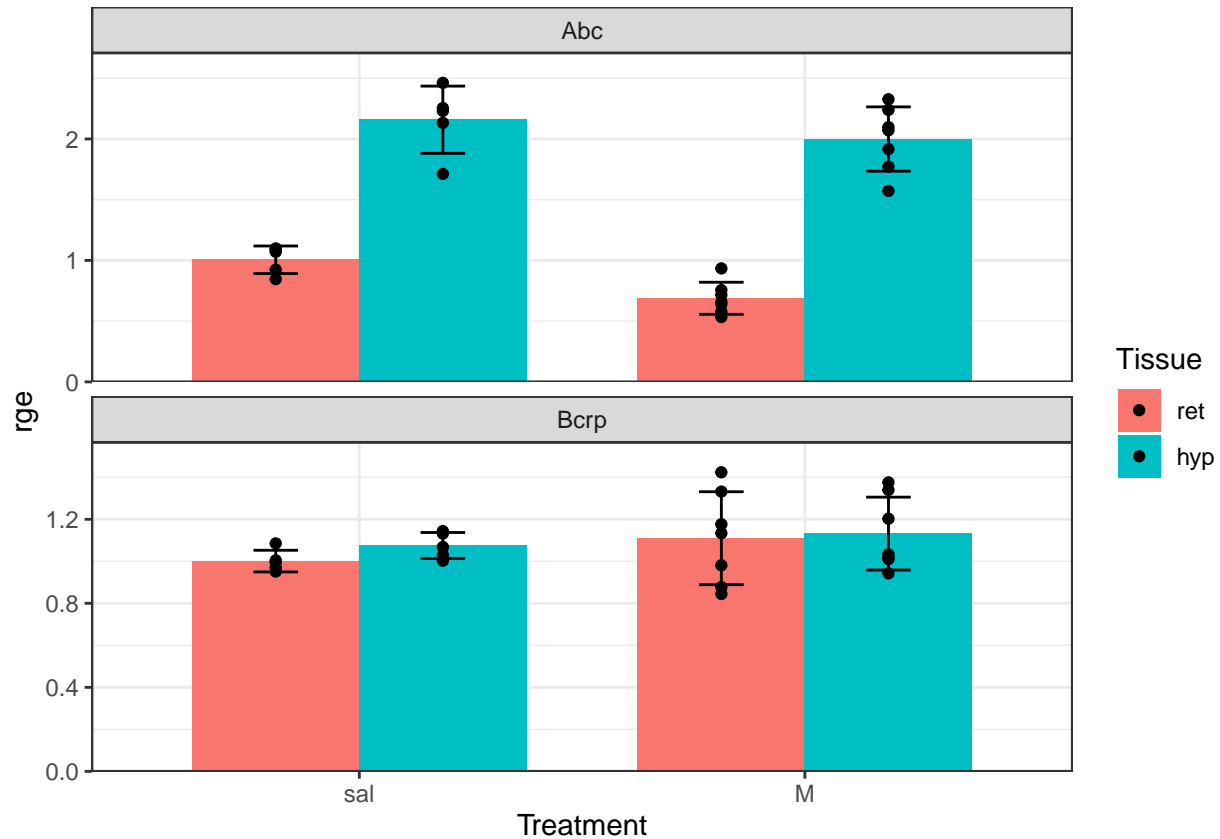
```
trans_hyp_ret_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=mean-sd, ymax=mean+sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75))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

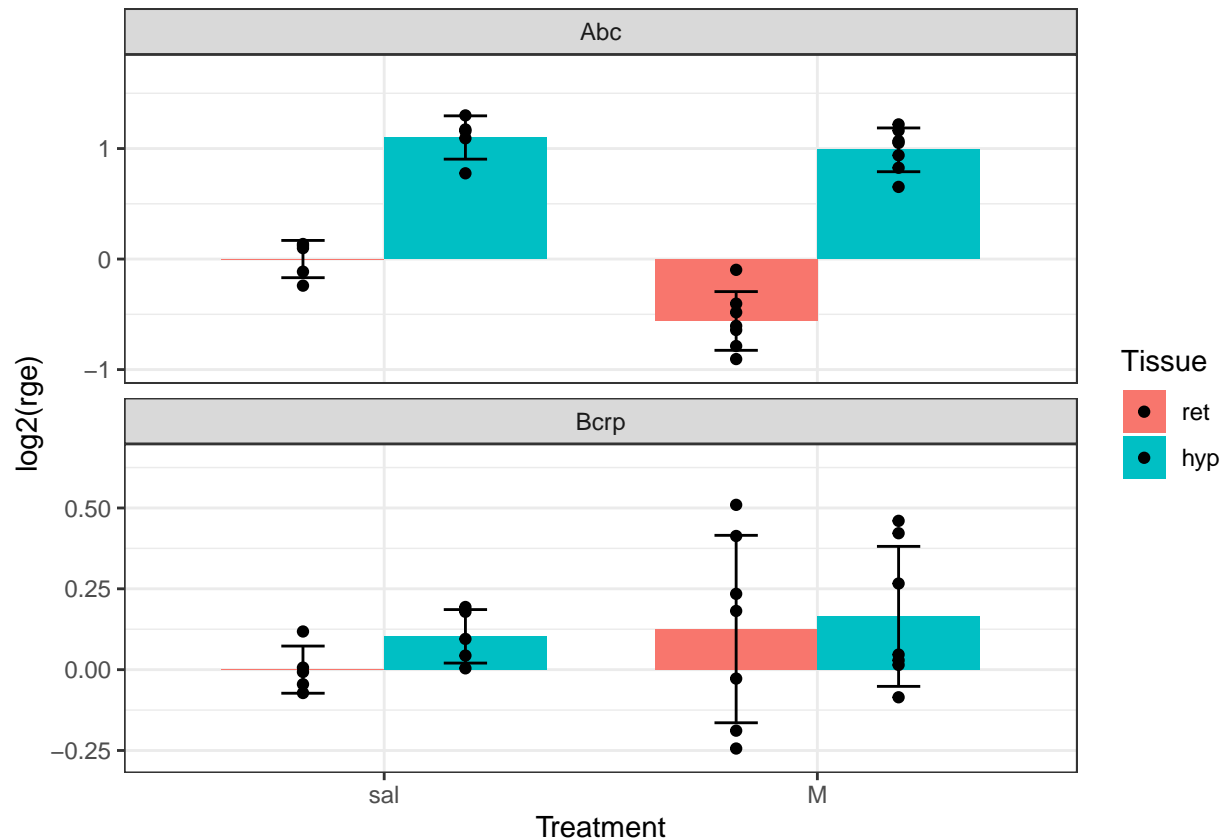


```
#ggsave(filename="//Mac/Home/Documents/R_coding/figures/trans_hyp_ret_1inj.png", plot=trans_hyp_ret_plo
#ggsave(filename="//Mac/Home/Documents/R_coding/figures/trans_hyp_ret_1inj.svg", plot=trans_hyp_ret_plo
```

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

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

```
trans_hyp_ret_log_plot
```



```
#ggsave(filename="//Mac/Home/Documents/R_coding/figures/trans_hyp_ret_log_1inj.png", plot=trans_hyp_ret_log_plot)
#ggsave(filename="//Mac/Home/Documents/R_coding/figures/trans_hyp_ret_log_1inj.svg", plot=trans_hyp_ret_log_plot)
```

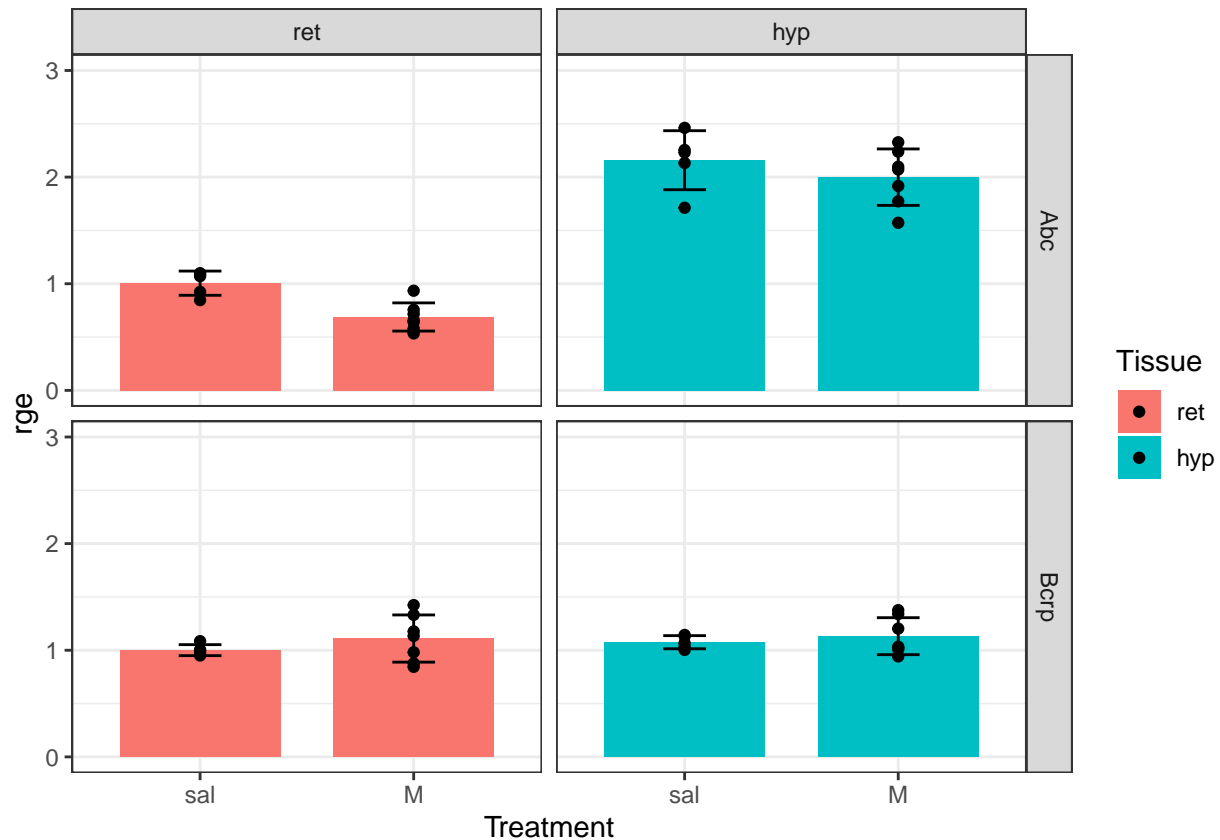
## Comparing treatments within each tissue/gene

```
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() +
  ylim(0,3)
```

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

```
## Scale for 'y' is already present. Adding another scale for 'y', which will
## replace the existing scale.
```

```
trans_trt_plot
```

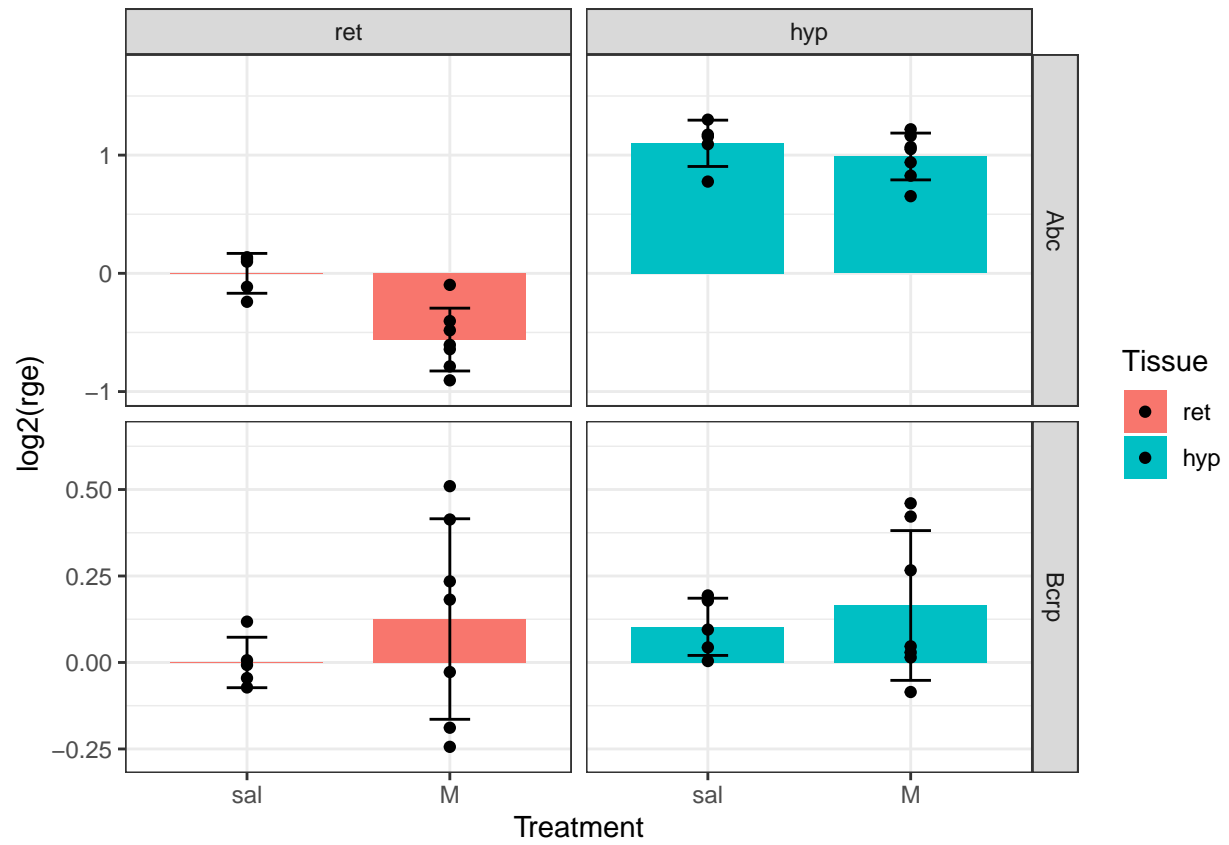


```
#ggsave(filename="//Mac/Home/Documents/R_coding/figures/trans_trt_1inj.png", plot=trans_trt_plot, height=10)
#ggsave(filename="//Mac/Home/Documents/R_coding/figures/trans_trt_1inj.svg", plot=trans_trt_plot, height=10)
```

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

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

```
trans_trt_log_plot
```



```
#ggsave(filename="//Mac/Home/Documents/R_coding/figures/trans_trt_log_1inj.png", plot=trans_trt_log_plo
#ggsave(filename="//Mac/Home/Documents/R_coding/figures/trans_trt_log_1inj.svg", plot=trans_trt_log_plo
```