

# qPCR Transporter Figures

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```
library(tidyverse)
library(ggthemes)
library(ggpubr)
library(emmeans)
library(lme4)
library(car)
library(lmerTest)
```

```
## Warning: package 'lmerTest' was built under R version 4.1.3
```

```
library(svglite)
```

## Bcrp & Mrp2 “baseline” expression (qPCR)

```
#read in the data
getwd()
```

```
## [1] "C:/Users/ctber/Documents/R/morphine_sex_differences/R/qRT-PCR"
```

```
bm_data <- read.csv("../data/qRT-PCR/Mrp-Bcrp-gene-study-subset-sal-ret-only.csv", fileEncoding = 'UTF-8')
bm_data <- bm_data %>% mutate(Sample = as.factor(Sample))
```

```
bm_data
```

```
##   Sample Bcrp.RGE Mrp2.RGE
## 1 S2-ret 0.9155193 1.0997121
## 2 S3-ret 0.8730615 0.7746964
## 3 S4-ret 1.0005778 1.2314852
## 4 S5-ret 1.0607625 1.1567199
## 5 S8-ret 1.1787417 0.8240092
```

```
#make the data so we can more easily plot both genes on the same graph
```

```
bm_data_long <- bm_data %>% pivot_longer(cols=c("Bcrp.RGE", "Mrp2.RGE"), names_to = "gene", values_to="value")
```

```
bm_data_long <- bm_data_long %>% mutate(gene = fct_recode(as.factor(gene), Bcrp = "Bcrp.RGE", Mrp2 = "Mrp2.RGE"))
```

```
#get some summary statistics
```

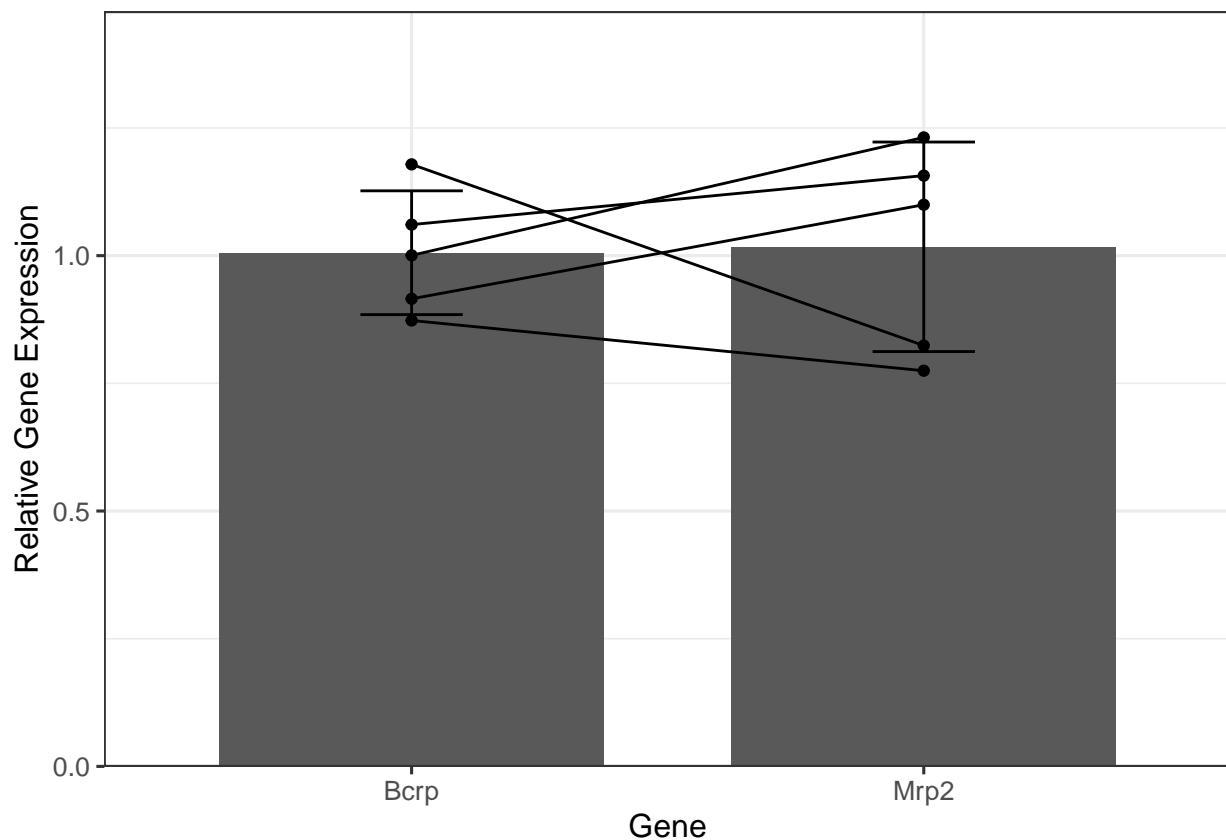
```
bm_sumstats <- bm_data_long %>% group_by(gene) %>% summarise(n = n(),
                                                             mean = mean(rge),
                                                             sd = sd(rge))
```

```
bm_sumstats
```

```
## # A tibble: 2 x 4
##   gene      n mean  sd
##   <fct> <int> <dbl> <dbl>
## 1 Bcrp      5  1.01 0.121
## 2 Mrp2      5  1.02 0.205
```

```
#plot
bm_plot <- ggplot() +
  stat_summary(fun = 'mean', geom="bar", width=0.75, data=bm_data_long, aes(x=gene, y=rge)) +
  #stat_summary(fun.data = mean_se, geom = "errorbar", width=.2) +
  geom_errorbar(width=0.2, data=bm_sumstats, aes(x=gene, ymin=mean-sd, ymax=mean+sd)) +
  geom_point(aes(x=gene, y=rge), data=bm_data_long) +
  geom_line(data=bm_data_long, aes(x=gene, y=rge, group=Sample)) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.2))) +
  theme_bw(12) +
  labs(x="Gene", y="Relative Gene Expression")
```

```
bm_plot
```



```
ggsave("../figures/qRT-PCR/bcrp_mrp2_plot.png", plot=bm_plot, height=4, width=4)
ggsave("../figures/qRT-PCR/bcrp_mrp2_plot.svg", plot=bm_plot, height=4, width=4)
```

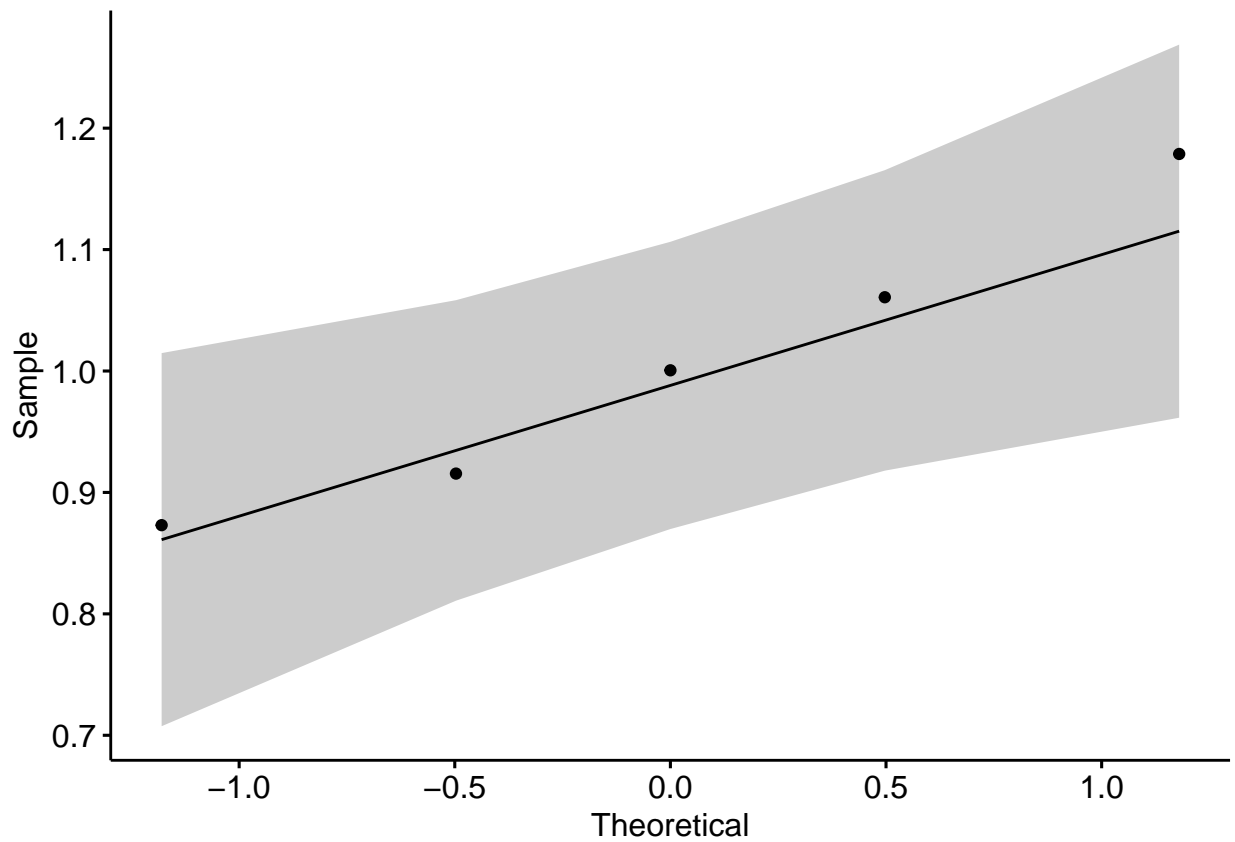
```
#checking assumptions of t-test
#normality
shapiro.test(bm_data$Bcrp.RGE)
```

```
##
## Shapiro-Wilk normality test
##
## data:  bm_data$Bcrp.RGE
## W = 0.96634, p-value = 0.8513
```

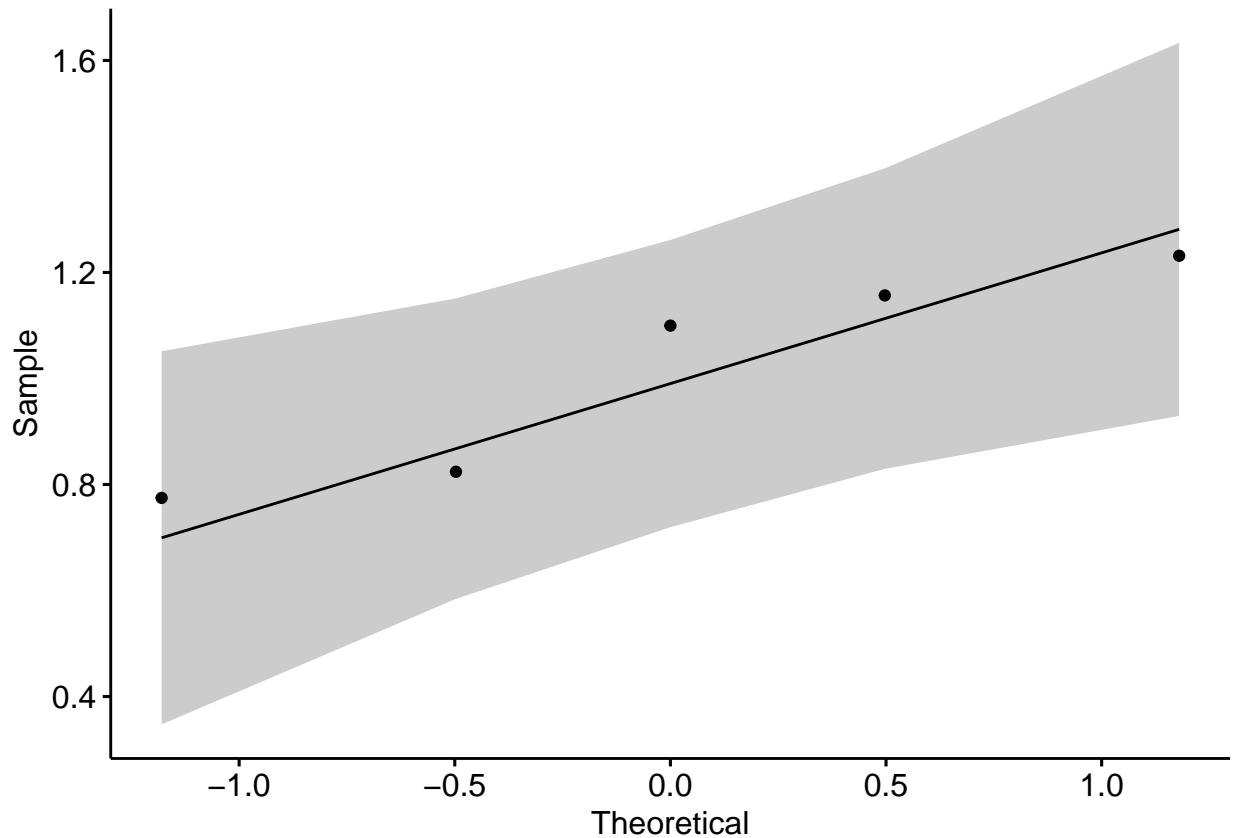
```
shapiro.test(bm_data$Mrp2.RGE)
```

```
##
## Shapiro-Wilk normality test
##
## data:  bm_data$Mrp2.RGE
## W = 0.87559, p-value = 0.2898
```

```
ggqqplot(bm_data, x = "Bcrp.RGE")
```



```
ggqqplot(bm_data, x = "Mrp2.RGE")
```



```
#no sig outliers in the difference between the groups
bm_data_stats <- bm_data %>% mutate(differences = Bcrp.RGE - Mrp2.RGE)
rstatix::identify_outliers(data=bm_data_stats, differences)
```

```
## [1] Sample      Bcrp.RGE    Mrp2.RGE    differences is.outlier is.extreme
## <0 rows> (or 0-length row.names)
```

```
t.test(rge ~ gene, data=bm_data_long, paired=TRUE)
```

```
##
## Paired t-test
##
## data:  rge by gene
## t = -0.10779, df = 4, p-value = 0.9193
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.3101677 0.2869837
## sample estimates:
## mean of the differences
## -0.01159201
```