

# SimpleViolins

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## Data sources

We evaluate genotype and sex differences in mouse models of Alzheimer's disease risk, based on APOE alleles: APOE2 is protective, APOE3 is control, and APOE4 represents genetic risk for AD. One allele increases risk by 3 times, and two APOE4 alleles increase risk by 10 times (ref) in relation to APOE3 . We use multiple data sources:

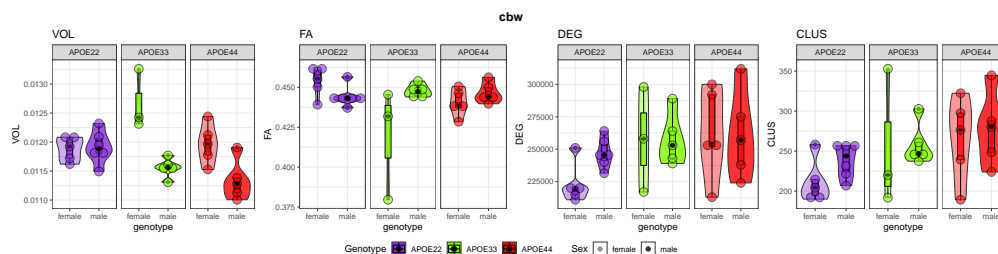
Morris Water Maze - testing spatial navigation memory Segmentation: regional volumes Microstructural properties: FA Tractography Based Connectivity: Degree, and Clustering Coefficient

We read the data and normalize volumes to the whole brain.

## Plots for cbw

We embed plots for day d8 probe trials:

```
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth`.
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth`.
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth`.
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth`.
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth`.
```



```
print('cbw')
```

```
## [1] "cbw"
```

```
lm_cbw_VOL <- lm(cbw ~ Genotype*Sex, geno_combined_VOL)
summary(lm_cbw_VOL)
```

```
##
## Call:
## lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -4.403e-04 -2.135e-04  6.100e-07  1.781e-04  5.964e-04
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      1.188e-02  1.379e-04  86.181  < 2e-16 ***
## GenotypeAPOE33      7.817e-04  2.252e-04   3.471  0.00207 **
## GenotypeAPOE44      8.164e-05  1.950e-04   0.419  0.67938
## Sexmale            2.835e-05  1.867e-04   0.152  0.88067
## GenotypeAPOE33:Sexmale -1.138e-03  2.925e-04  -3.889  0.00074 ***
## GenotypeAPOE44:Sexmale -6.545e-04  2.700e-04  -2.424  0.02362 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.0003084 on 23 degrees of freedom
## Multiple R-squared:  0.6359, Adjusted R-squared:  0.5567
## F-statistic: 8.033 on 5 and 23 DF,  p-value: 0.0001672

lm_cbw_VOL_f <- lm(cbw ~ Genotype, combo_f_FA)
#summary(lm_cbw_VOL_f)
lm_cbw_VOL_m <- lm(cbw ~ Genotype, combo_m_FA)
#summary(lm_cbw_VOL_f)

#omnibus testing
#https://cran.r-project.org/web/packages/emmeans/vignettes/confidence-intervals.html#adjust

#33333
sink(paste(outpath, "cbw_VOL_SimpleModels.txt"))
mydata.lm <- lm(cbw ~ Genotype*Sex, data = geno_combined_VOL)
anova(mydata.lm)

## Analysis of Variance Table
##
## Response: cbw
##           Df      Sum Sq    Mean Sq F value    Pr(>F)
## Genotype    2 5.2927e-07 2.6463e-07  2.7829 0.0827220 .
## Sex          1 1.7928e-06 1.7928e-06 18.8531 0.0002401 ***
## Genotype:Sex  2 1.4975e-06 7.4875e-07  7.8740 0.0024831 **
## Residuals   23 2.1871e-06 9.5090e-08
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(glht(mydata.lm, emm(pairwise ~ Genotype|Sex, adjust="sidak")))
```

*#consider save to file*

```
## $`Sex = female`
##
## Simultaneous Tests for General Linear Hypotheses
##
```

```

## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0 -7.817e-04  2.252e-04 -3.471  0.00569 **
## APOE22 - APOE44 == 0 -8.164e-05  1.950e-04 -0.419  0.90797
## APOE33 - APOE44 == 0  7.001e-04  2.252e-04  3.109  0.01314 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
##
##
## $`Sex = male`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0 0.0003561  0.0001867  1.907  0.1593
## APOE22 - APOE44 == 0 0.0005728  0.0001867  3.068  0.0145 *
## APOE33 - APOE44 == 0 0.0002167  0.0001950  1.111  0.5168
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
summary(glht(mydata.lm, emm(pairwise ~ Sex| Genotype)))

## $`Genotype = APOE22`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0 -2.835e-05  1.867e-04 -0.152  0.881
## (Adjusted p values reported -- single-step method)
##
##
## $`Genotype = APOE33`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0 0.0011095  0.0002252  4.927  5.6e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
##
##

```

```
## $`Genotype` = APOE44`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##             Estimate Std. Error t value Pr(>|t|)
## female - male == 0 0.0006261  0.0001950   3.211  0.00388 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

```
sink()
#3333

sink(paste(outpath, "cbw_VOL_SimpleStats.txt",sep=''))
'omnibus testing'
```

```
## [1] "omnibus testing"
```

```
joint_tests(ref_grid(lm_cbw_VOL))
```

```
## model term    df1 df2 F.ratio p.value
## Genotype      2  23   4.820  0.0178
## Sex           1  23  23.578  0.0001
## Genotype:Sex   2  23   7.874  0.0025
```

```
joint_tests(ref_grid(lm_cbw_VOL), by = "Sex", adjust = "sidak")
```

```
## Sex = female:
## model term df1 df2 F.ratio p.value
## Genotype   2  23   6.748  0.0049
##
## Sex = male:
## model term df1 df2 F.ratio p.value
## Genotype   2  23   4.871  0.0172
```

```
joint_tests(ref_grid(lm_cbw_VOL), by = "Genotype",adjust = "sidak" )
```

```
## Genotype = APOE22:
## model term df1 df2 F.ratio p.value
## Sex        1  23   0.023  0.8807
##
## Genotype = APOE33:
## model term df1 df2 F.ratio p.value
## Sex        1  23  24.271  0.0001
##
## Genotype = APOE44:
## model term df1 df2 F.ratio p.value
## Sex        1  23  10.307  0.0039
```

```
'anova'
```

```
## [1] "anova"
```

```
anova(lm_cbw_VOL)
```

```
## Analysis of Variance Table
```

```
##
## Response: cbw
##           Df      Sum Sq    Mean Sq F value    Pr(>F)
## Genotype    2 5.2927e-07 2.6463e-07  2.7829 0.0827220 .
## Sex          1 1.7928e-06 1.7928e-06 18.8531 0.0002401 ***
## Genotype:Sex  2 1.4975e-06 7.4875e-07  7.8740 0.0024831 **
## Residuals    23 2.1871e-06 9.5090e-08
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#anova(lm_cbw_VOL_f)
#anova(lm_cbw_VOL_m)

#post hoc comparison
cbw_VOL.emm <- emmeans(lm_cbw_VOL, ~ Genotype | Sex, adjust = "sidak")
contrast(cbw_VOL.emm, simple = c("Genotype", "Sex")) #FDR

## contrast            estimate      SE df t.ratio p.value
## APOE22 female effect -3.23e-06 0.000127 23  -0.025  0.9799
## APOE33 female effect  7.78e-04 0.000157 23   4.967  0.0003
## APOE44 female effect  7.84e-05 0.000127 23   0.618  0.8143
## APOE22 male effect    2.51e-05 0.000118 23   0.212  0.9799
## APOE33 male effect   -3.31e-04 0.000127 23  -2.608  0.0315
## APOE44 male effect   -5.48e-04 0.000127 23  -4.315  0.0008
##
## P value adjustment: fdr method for 6 tests
contrast(cbw_VOL.emm, simple = list("Genotype", "Sex"))

## $`simple contrasts for Genotype`
## Sex = female:
## contrast            estimate      SE df t.ratio p.value
## APOE22 effect -2.88e-04 0.000119 23  -2.425  0.0354
## APOE33 effect  4.94e-04 0.000135 23   3.650  0.0040
## APOE44 effect -2.06e-04 0.000119 23  -1.737  0.0958
##
## Sex = male:
## contrast            estimate      SE df t.ratio p.value
## APOE22 effect  3.10e-04 0.000106 23   2.917  0.0233
## APOE33 effect -4.65e-05 0.000111 23  -0.418  0.6795
## APOE44 effect -2.63e-04 0.000111 23  -2.371  0.0398
##
## P value adjustment: fdr method for 3 tests
##
## $`simple contrasts for Sex`
## Genotype = APOE22:
## contrast            estimate      SE df t.ratio p.value
## female effect -1.42e-05 9.34e-05 23  -0.152  0.8807
## male effect    1.42e-05 9.34e-05 23   0.152  0.8807
##
## Genotype = APOE33:
## contrast            estimate      SE df t.ratio p.value
## female effect  5.55e-04 1.13e-04 23   4.927  0.0001
## male effect   -5.55e-04 1.13e-04 23  -4.927  0.0001
##
```

```

## Genotype = APOE44:
## contrast      estimate      SE df t.ratio p.value
## female effect  3.13e-04 9.75e-05 23   3.211  0.0039
## male effect   -3.13e-04 9.75e-05 23  -3.211  0.0039
##
## P value adjustment: fdr method for 2 tests

#post hoc tests
summary(glht(lm_cbw_VOL, emm(pairwise ~ Genotype*Sex, contr="sidak"))) #consider save to file

##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##
## Estimate Std. Error t value Pr(>|t|)
## APOE22 female - APOE33 female == 0 -7.817e-04 2.252e-04 -3.471 0.0220 *
## APOE22 female - APOE44 female == 0 -8.164e-05 1.950e-04 -0.419 0.9981
## APOE22 female - APOE22 male == 0 -2.835e-05 1.867e-04 -0.152 1.0000
## APOE22 female - APOE33 male == 0 3.278e-04 1.950e-04 1.681 0.5553
## APOE22 female - APOE44 male == 0 5.445e-04 1.950e-04 2.792 0.0940 .
## APOE33 female - APOE44 female == 0 7.001e-04 2.252e-04 3.109 0.0490 *
## APOE33 female - APOE22 male == 0 7.533e-04 2.180e-04 3.455 0.0229 *
## APOE33 female - APOE33 male == 0 1.109e-03 2.252e-04 4.927 <0.001 ***
## APOE33 female - APOE44 male == 0 1.326e-03 2.252e-04 5.889 <0.001 ***
## APOE44 female - APOE22 male == 0 5.330e-05 1.867e-04 0.285 0.9997
## APOE44 female - APOE33 male == 0 4.094e-04 1.950e-04 2.099 0.3205
## APOE44 female - APOE44 male == 0 6.262e-04 1.950e-04 3.211 0.0393 *
## APOE22 male - APOE33 male == 0 3.561e-04 1.867e-04 1.907 0.4212
## APOE22 male - APOE44 male == 0 5.728e-04 1.867e-04 3.068 0.0536 .
## APOE33 male - APOE44 male == 0 2.167e-04 1.950e-04 1.111 0.8708
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)

res<-summary(glht(lm_cbw_VOL, emm(pairwise ~ Genotype*Sex))) #consider save to file
#summary(as.glht(pairs(lm_cbw_VOL.emm), by = NULL))

summary(glht(lm_cbw_VOL, emm(pairwise ~ Genotype | Sex)))

## $`Sex = female`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##
## Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0 -7.817e-04 2.252e-04 -3.471 0.00552 **
## APOE22 - APOE44 == 0 -8.164e-05 1.950e-04 -0.419 0.90800
## APOE33 - APOE44 == 0 7.001e-04 2.252e-04 3.109 0.01307 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)

```

```

##
##
## $`Sex = male`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0 0.0003561  0.0001867   1.907   0.1593
## APOE22 - APOE44 == 0 0.0005728  0.0001867   3.068   0.0143 *
## APOE33 - APOE44 == 0 0.0002167  0.0001950   1.111   0.5168
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
summary(glht(lm_cbw_VOL, emm(pairwise ~ Sex | Genotype)))

## $`Genotype = APOE22`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0 -2.835e-05  1.867e-04  -0.152   0.881
## (Adjusted p values reported -- single-step method)
##
##
## $`Genotype = APOE33`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0 0.0011095  0.0002252   4.927 5.6e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
##
##
## $`Genotype = APOE44`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_VOL)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0 0.0006261  0.0001950   3.211 0.00388 **
## ---

```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)

mypairs<-pairs(cbw_VOL.emm) #_consider save to file
pairs(cbw_VOL.emm, by="Genotype")

## Genotype = APOE22:
## contrast      estimate      SE df t.ratio p.value
## female - male -2.83e-05 0.000187 23  -0.152  0.8807
##
## Genotype = APOE33:
## contrast      estimate      SE df t.ratio p.value
## female - male  1.11e-03 0.000225 23   4.927  0.0001
##
## Genotype = APOE44:
## contrast      estimate      SE df t.ratio p.value
## female - male  6.26e-04 0.000195 23   3.211  0.0039

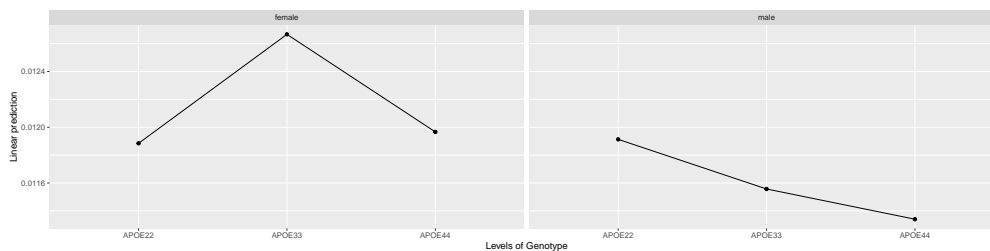
pairs(cbw_VOL.emm, by="Sex")

## Sex = female:
## contrast      estimate      SE df t.ratio p.value
## APOE22 - APOE33 -7.82e-04 0.000225 23  -3.471  0.0056
## APOE22 - APOE44 -8.16e-05 0.000195 23  -0.419  0.9083
## APOE33 - APOE44  7.00e-04 0.000225 23   3.109  0.0132
##
## Sex = male:
## contrast      estimate      SE df t.ratio p.value
## APOE22 - APOE33  3.56e-04 0.000187 23   1.907  0.1593
## APOE22 - APOE44  5.73e-04 0.000187 23   3.068  0.0145
## APOE33 - APOE44  2.17e-04 0.000195 23   1.111  0.5170
##
## P value adjustment: tukey method for comparing a family of 3 estimates

print('cbw VOL')

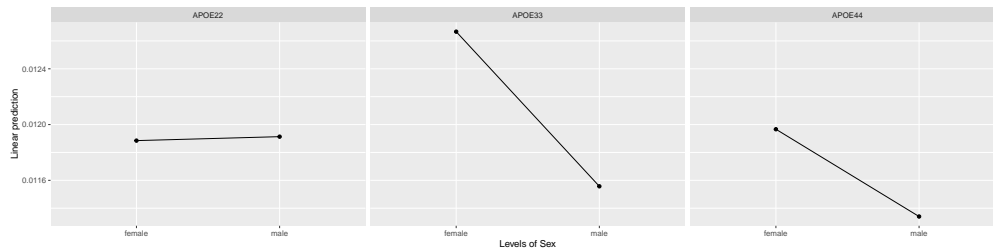
## [1] "cbw VOL"

emmip(lm_cbw_VOL, ~ Genotype | Sex )
```

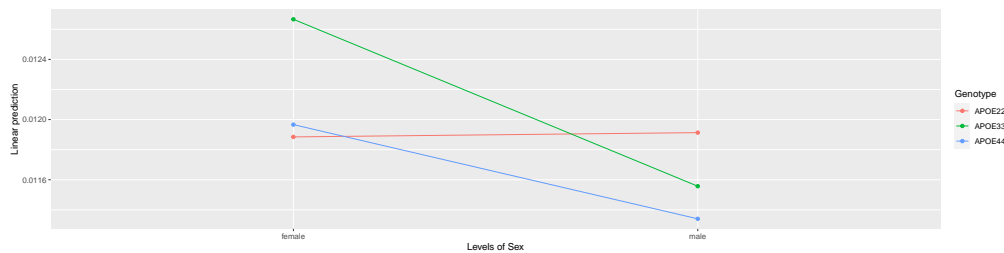


```
emmip(lm_cbw_VOL, ~ Sex | Genotype )
```



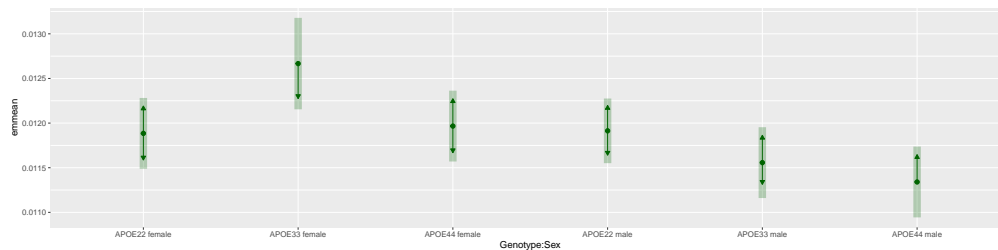


```
emmip(lm_cbw_VOL, Genotype~ Sex )
```



```
sink()
```

```
plot(cbw_VOL.emm, by = NULL, comparisons = TRUE, adjust = "mvt",
horizontal = FALSE, colors = "darkgreen", main ="cbw VOL")
```



```
ggsave(paste(outpath,'cbw_VOL_emmeans.pdf',sep=''), plot = last_plot(), device='pdf', scale=1, width=16
```

```
'simple stats'
```

```
## [1] "simple stats"
```

```
print('cbw')
```

```
## [1] "cbw"
```

```
lm_cbw_FA <- lm(cbw ~ Genotype*Sex, geno_combined_FA)
summary(lm_cbw_FA)
```

```
##
```

```
## Call:
```

```
## lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
```

```
##
```

```
## Residuals:
```

```
##      Min       1Q   Median       3Q      Max
## -0.039395 -0.003338 -0.000471  0.006009  0.026497
```

```
##
```

```
## Coefficients:
```

```
##              Estimate Std. Error t value Pr(>|t|)
```

```

## (Intercept)          0.453619   0.005526  82.087 < 2e-16 ***
## GenotypeAPOE33      -0.034649   0.009024  -3.840 0.000837 ***
## GenotypeAPOE44      -0.013226   0.007815  -1.692 0.104073
## Sexmale             -0.009249   0.007482  -1.236 0.228907
## GenotypeAPOE33:Sexmale 0.038259   0.011723   3.264 0.003415 **
## GenotypeAPOE44:Sexmale 0.015581   0.010819   1.440 0.163311
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01236 on 23 degrees of freedom
## Multiple R-squared:  0.4179, Adjusted R-squared:  0.2914
## F-statistic: 3.303 on 5 and 23 DF,  p-value: 0.02155

lm_cbw_FA_f <- lm(cbw ~ Genotype, combo_f_FA)
#summary(lm_cbw_FA_f)
lm_cbw_FA_m <- lm(cbw ~ Genotype, combo_m_FA)
#summary(lm_cbw_FA_m)

#33333
sink(paste(outpath, "cbw_FA_SimpleModels.txt"))
mydata.lm <- lm(cbw ~ Genotype*Sex, data = geno_combined_FA)
anova(mydata.lm)

## Analysis of Variance Table
##
## Response: cbw
##           Df    Sum Sq   Mean Sq F value    Pr(>F)
## Genotype    2 0.0006099 0.00030494   1.9972 0.15857
## Sex          1 0.0002851 0.00028514   1.8675 0.18497
## Genotype:Sex  2 0.0016264 0.00081320   5.3259 0.01257 *
## Residuals   23 0.0035118 0.00015269
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(glht(mydata.lm, emm(pairwise ~ Genotype|Sex, adjust="sidak")))) #consider save to file

## $`Sex = female`
##
##      Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
##              Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0  0.034649   0.009024   3.840 0.00246 **
## APOE22 - APOE44 == 0  0.013226   0.007815   1.692 0.22867
## APOE33 - APOE44 == 0 -0.021423   0.009024  -2.374 0.06539 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
##
##
## $`Sex = male`
##

```

```

## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0 -0.003610  0.007482  -0.482    0.880
## APOE22 - APOE44 == 0 -0.002355  0.007482  -0.315    0.947
## APOE33 - APOE44 == 0  0.001255  0.007815   0.161    0.986
## (Adjusted p values reported -- single-step method)
summary(glht(mydata.lm, emm(pairwise ~ Sex| Genotype)))

## $`Genotype = APOE22`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0 0.009249  0.007482   1.236    0.229
## (Adjusted p values reported -- single-step method)
##
## $`Genotype = APOE33`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0 -0.029010  0.009024  -3.215  0.00384 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
##
## $`Genotype = APOE44`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0 -0.006332  0.007815  -0.81    0.426
## (Adjusted p values reported -- single-step method)

sink()
#3333

#omnibus testing
#https://cran.r-project.org/web/packages/emmeans/vignettes/confidence-intervals.html#adjust

```

```

sink(paste(outpath, "cbw_FA_SimpleStats.txt",sep=''))
'omnibus testing'

## [1] "omnibus testing"
joint_tests(ref_grid(lm_cbw_FA))

## model term      df1 df2 F.ratio p.value
## Genotype        2  23   3.522  0.0463
## Sex              1  23   3.430  0.0769
## Genotype:Sex     2  23   5.326  0.0126

joint_tests(ref_grid(lm_cbw_FA), by = "Sex", adjust = "sidak")

## Sex = female:
## model term df1 df2 F.ratio p.value
## Genotype    2  23   7.372  0.0034
##
## Sex = male:
## model term df1 df2 F.ratio p.value
## Genotype    2  23   0.122  0.8856

joint_tests(ref_grid(lm_cbw_FA), by = "Genotype",adjust = "sidak" )

## Genotype = APOE22:
## model term df1 df2 F.ratio p.value
## Sex        1  23   1.528  0.2289
##
## Genotype = APOE33:
## model term df1 df2 F.ratio p.value
## Sex        1  23  10.335  0.0038
##
## Genotype = APOE44:
## model term df1 df2 F.ratio p.value
## Sex        1  23   0.657  0.4261

'anova'

## [1] "anova"
anova(lm_cbw_FA)

## Analysis of Variance Table
##
## Response: cbw
##           Df      Sum Sq    Mean Sq F value    Pr(>F)
## Genotype    2  0.0006099  0.00030494   1.9972  0.15857
## Sex          1  0.0002851  0.00028514   1.8675  0.18497
## Genotype:Sex  2  0.0016264  0.00081320   5.3259  0.01257 *
## Residuals   23  0.0035118  0.00015269
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#anova(lm_cbw_FA_f)
#anova(lm_cbw_FA_m)

#post hoc comparison

```

```

cbw_FA.emm <- emmeans(lm_cbw_FA , ~ Genotype | Sex, adjust = "sidak")
contrast(cbw_FA.emm, simple = c("Genotype", "Sex")) #FDR

## contrast            estimate      SE df t.ratio p.value
## APOE22 female effect  0.01161 0.00509 23   2.282  0.0961
## APOE33 female effect -0.02304 0.00628 23  -3.668  0.0077
## APOE44 female effect -0.00162 0.00509 23  -0.318  0.7535
## APOE22 male effect    0.00236 0.00474 23   0.498  0.7479
## APOE33 male effect    0.00597 0.00509 23   1.174  0.5050
## APOE44 male effect    0.00472 0.00509 23   0.927  0.5452
##
## P value adjustment: fdr method for 6 tests

contrast(cbw_FA.emm, simple = list("Genotype", "Sex"))

## $`simple contrasts for Genotype`
## Sex = female:
## contrast            estimate      SE df t.ratio p.value
## APOE22 effect      0.015959 0.00476 23   3.355  0.0041
## APOE33 effect     -0.018691 0.00542 23  -3.447  0.0041
## APOE44 effect      0.002732 0.00476 23   0.574  0.5712
##
## Sex = male:
## contrast            estimate      SE df t.ratio p.value
## APOE22 effect     -0.001988 0.00425 23  -0.467  0.9350
## APOE33 effect      0.001622 0.00445 23   0.364  0.9350
## APOE44 effect      0.000367 0.00445 23   0.082  0.9350
##
## P value adjustment: fdr method for 3 tests
##
## $`simple contrasts for Sex`
## Genotype = APOE22:
## contrast            estimate      SE df t.ratio p.value
## female effect      0.00462 0.00374 23   1.236  0.2289
## male effect       -0.00462 0.00374 23  -1.236  0.2289
##
## Genotype = APOE33:
## contrast            estimate      SE df t.ratio p.value
## female effect     -0.01451 0.00451 23  -3.215  0.0038
## male effect       0.01451 0.00451 23   3.215  0.0038
##
## Genotype = APOE44:
## contrast            estimate      SE df t.ratio p.value
## female effect     -0.00317 0.00391 23  -0.810  0.4261
## male effect       0.00317 0.00391 23   0.810  0.4261
##
## P value adjustment: fdr method for 2 tests

#post hoc tests
summary(glht(lm_cbw_FA, emm(pairwise ~ Genotype*Sex, contr="sidak"))) #consider save to file

##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)

```

```
##
## Linear Hypotheses:
##
## Estimate Std. Error t value Pr(>|t|)
## APOE22 female - APOE33 female == 0 0.034649 0.009024 3.840 0.00939 **
## APOE22 female - APOE44 female == 0 0.013226 0.007815 1.692 0.54804
## APOE22 female - APOE22 male == 0 0.009249 0.007482 1.236 0.81367
## APOE22 female - APOE33 male == 0 0.005639 0.007815 0.722 0.97705
## APOE22 female - APOE44 male == 0 0.006894 0.007815 0.882 0.94659
## APOE33 female - APOE44 female == 0 -0.021423 0.009024 -2.374 0.20541
## APOE33 female - APOE22 male == 0 -0.025401 0.008737 -2.907 0.07442 .
## APOE33 female - APOE33 male == 0 -0.029010 0.009024 -3.215 0.03895 *
## APOE33 female - APOE44 male == 0 -0.027756 0.009024 -3.076 0.05259 .
## APOE44 female - APOE22 male == 0 -0.003978 0.007482 -0.532 0.99419
## APOE44 female - APOE33 male == 0 -0.007587 0.007815 -0.971 0.92192
## APOE44 female - APOE44 male == 0 -0.006332 0.007815 -0.810 0.96237
## APOE22 male - APOE33 male == 0 -0.003610 0.007482 -0.482 0.99630
## APOE22 male - APOE44 male == 0 -0.002355 0.007482 -0.315 0.99952
## APOE33 male - APOE44 male == 0 0.001255 0.007815 0.161 0.99998
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)

res<-summary(glht(lm_cbw_FA, emm(pairwise ~ Genotype*Sex))) #consider save to file
#summary(as.glht(pairs(lm_cbw_FA.emm), by = NULL))

summary(glht(lm_cbw_FA, emm(pairwise ~ Genotype | Sex)))

## $`Sex = female`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
## Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0 0.034649 0.009024 3.840 0.00238 **
## APOE22 - APOE44 == 0 0.013226 0.007815 1.692 0.22869
## APOE33 - APOE44 == 0 -0.021423 0.009024 -2.374 0.06510 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
##
## $`Sex = male`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
## Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0 -0.003610 0.007482 -0.482 0.880
## APOE22 - APOE44 == 0 -0.002355 0.007482 -0.315 0.947
## APOE33 - APOE44 == 0 0.001255 0.007815 0.161 0.986
## (Adjusted p values reported -- single-step method)
```

```
summary(glht(lm_cbw_FA, emm(pairwise ~ Sex | Genotype)))

## $`Genotype = APOE22`
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0 0.009249   0.007482   1.236   0.229
## (Adjusted p values reported -- single-step method)
##
## $`Genotype = APOE33`
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0 -0.029010   0.009024  -3.215  0.00384 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
##
## $`Genotype = APOE44`
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_FA)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0 -0.006332   0.007815  -0.81   0.426
## (Adjusted p values reported -- single-step method)

mypairs<-pairs(cbw_FA.emm) #_consider save to file
pairs(cbw_FA.emm, by="Genotype")

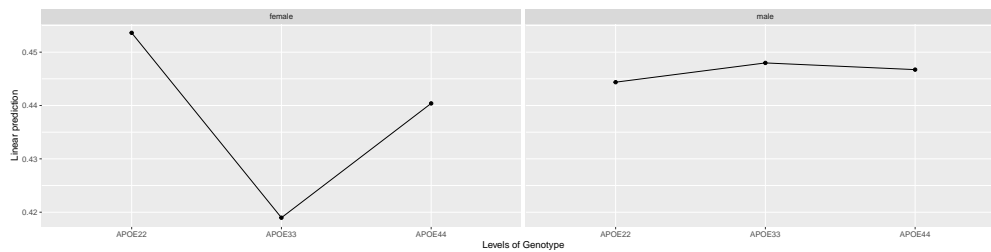
## Genotype = APOE22:
##   contrast      estimate      SE df t.ratio p.value
## female - male  0.00925 0.00748 23   1.236  0.2289
##
## Genotype = APOE33:
##   contrast      estimate      SE df t.ratio p.value
## female - male -0.02901 0.00902 23  -3.215  0.0038
##
## Genotype = APOE44:
##   contrast      estimate      SE df t.ratio p.value
## female - male -0.00633 0.00782 23  -0.810  0.4261
```

```
pairs(cbw_FA.emm, by="Sex")
```

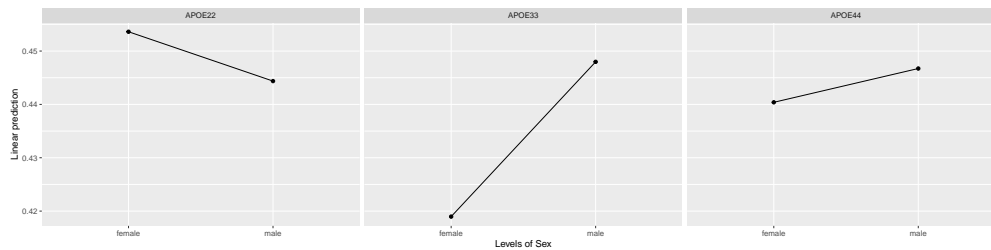
```
## Sex = female:
## contrast      estimate      SE df t.ratio p.value
## APOE22 - APOE33  0.03465  0.00902 23   3.840  0.0023
## APOE22 - APOE44  0.01323  0.00782 23   1.692  0.2295
## APOE33 - APOE44 -0.02142  0.00902 23  -2.374  0.0654
##
## Sex = male:
## contrast      estimate      SE df t.ratio p.value
## APOE22 - APOE33 -0.00361  0.00748 23  -0.482  0.8803
## APOE22 - APOE44 -0.00235  0.00748 23  -0.315  0.9470
## APOE33 - APOE44  0.00125  0.00782 23   0.161  0.9859
##
## P value adjustment: tukey method for comparing a family of 3 estimates
print('cbw FA')
```

```
## [1] "cbw FA"
```

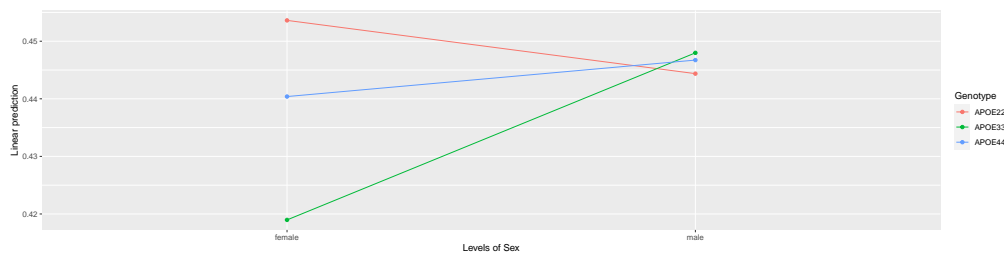
```
emmip(lm_cbw_FA, ~ Genotype | Sex )
```



```
emmip(lm_cbw_FA, ~ Sex | Genotype )
```



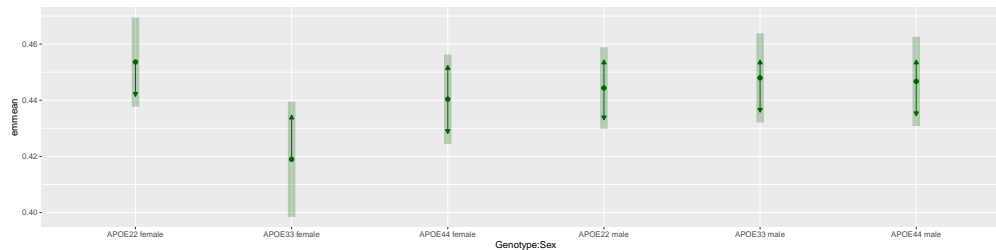
```
emmip(lm_cbw_FA, Genotype~ Sex )
```



```
sink()
```

```
plot(cbw_FA.emm, by = NULL, comparisons = TRUE, adjust = "mvt",
horizontal = FALSE, colors = "darkgreen", main ="cbw FA")
```





```
ggsave(paste(outpath, 'cbw_FA_emmeans.pdf', sep=''), plot = last_plot(), device='pdf', scale=1, width=16,
'simple stats')
```

```
## [1] "simple stats"
```

```
print('cbw')
```

```
## [1] "cbw"
```

```
lm_cbw_DEG <- lm(cbw ~ Genotype*Sex, geno_combined_DEG)
summary(lm_cbw_DEG)
```

```
##
## Call:
## lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -49200 -12400  -4200   13800   50800
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      223400      11863  18.832 1.79e-15 ***
## GenotypeAPOE33       34267      19372   1.769  0.0902 .
## GenotypeAPOE44       38800      16776   2.313  0.0300 *
## Sexmale             23683      16062   1.474  0.1539
## GenotypeAPOE33:Sexmale -23750      25164  -0.944  0.3551
## GenotypeAPOE44:Sexmale -24683      23226  -1.063  0.2989
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 26530 on 23 degrees of freedom
## Multiple R-squared:  0.2502, Adjusted R-squared:  0.08717
## F-statistic: 1.535 on 5 and 23 DF, p-value: 0.2182
```

```
lm_cbw_DEG_f <- lm(cbw ~ Genotype, combo_f_FA)
```

```
#summary(lm_cbw_DEG_f)
```

```
lm_cbw_DEG_m <- lm(cbw ~ Genotype, combo_m_FA)
```

```
#summary(lm_cbw_DEG_f)
```

```
#33333
```

```
sink(paste(outpath, "cbw_DEG_SimpleModels.txt"))
```

```
mydata.lm <- lm(cbw ~ Genotype*Sex, data = geno_combined_DEG)
```

```
anova(mydata.lm)
```

```
## Analysis of Variance Table
```

```
##
```

```

## Response: cbw
##           Df      Sum Sq    Mean Sq F value    Pr(>F)
## Genotype    2 3.8671e+09 1933530525  2.7480 0.08508 .
## Sex          1 5.4064e+08  540640164  0.7684 0.38978
## Genotype:Sex  2 9.9160e+08  495798100  0.7047 0.50464
## Residuals    23 1.6183e+10  703603261
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(glht(mydata.lm, emm(pairwise ~ Genotype|Sex, adjust="sidak")))
```

*#consider save to file*

```

## `$Sex = female`
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0   -34267    19372  -1.769   0.2014
## APOE22 - APOE44 == 0   -38800    16776  -2.313   0.0735 .
## APOE33 - APOE44 == 0    -4533    19372  -0.234   0.9702
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
##
##
## `$Sex = male`
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0  -10517    16062  -0.655   0.791
## APOE22 - APOE44 == 0  -14117    16062  -0.879   0.659
## APOE33 - APOE44 == 0   -3600    16776  -0.215   0.975
## (Adjusted p values reported -- single-step method)

summary(glht(mydata.lm, emm(pairwise ~ Sex| Genotype)))

## `$Genotype = APOE22`
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##           Estimate Std. Error t value Pr(>|t|)
## female - male == 0  -23683    16062  -1.474   0.154
## (Adjusted p values reported -- single-step method)
##
##
## `$Genotype = APOE33`

```

```

##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##             Estimate Std. Error t value Pr(>|t|)
## female - male == 0    66.67   19371.50   0.003   0.997
## (Adjusted p values reported -- single-step method)
##
##
## $`Genotype = APOE44`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##             Estimate Std. Error t value Pr(>|t|)
## female - male == 0     1000     16776   0.06   0.953
## (Adjusted p values reported -- single-step method)

sink()
#3333

#omnibus testing
#https://cran.r-project.org/web/packages/emmeans/vignettes/confidence-intervals.html#adjust

sink(paste(outpath, "cbw_DEG_SimpleStats.txt", sep=''))
'omnibus testing'

## [1] "omnibus testing"

joint_tests(ref_grid(lm_cbw_DEG))

## model term    df1 df2 F.ratio p.value
## Genotype      2  23   2.967  0.0714
## Sex            1  23   0.559  0.4622
## Genotype:Sex   2  23   0.705  0.5046

joint_tests(ref_grid(lm_cbw_DEG), by = "Sex", adjust = "sidak")

## Sex = female:
## model term df1 df2 F.ratio p.value
## Genotype   2  23   3.037  0.0675
##
## Sex = male:
## model term df1 df2 F.ratio p.value
## Genotype   2  23   0.427  0.6574

joint_tests(ref_grid(lm_cbw_DEG), by = "Genotype", adjust = "sidak" )

## Genotype = APOE22:
## model term df1 df2 F.ratio p.value
## Sex        1  23   2.174  0.1539
##
## Genotype = APOE33:

```

```
## model term df1 df2 F.ratio p.value
## Sex          1  23   0.000  0.9973
##
## Genotype = APOE44:
## model term df1 df2 F.ratio p.value
## Sex          1  23   0.004  0.9530

'anova'

## [1] "anova"
anova(lm_cbw_DEG)

## Analysis of Variance Table
##
## Response: cbw
##           Df      Sum Sq    Mean Sq F value  Pr(>F)
## Genotype    2 3.8671e+09 1933530525  2.7480 0.08508 .
## Sex          1 5.4064e+08  540640164  0.7684 0.38978
## Genotype:Sex  2 9.9160e+08  495798100  0.7047 0.50464
## Residuals   23 1.6183e+10  703603261
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#anova(lm_cbw_DEG_f)
#anova(lm_cbw_DEG_m)

#post hoc comparison
cbw_DEG.emm <- emmeans(lm_cbw_DEG , ~ Genotype | Sex, adjust = "sidak")
contrast(cbw_DEG.emm, simple = c("Genotype", "Sex")) #FDR

## contrast          estimate      SE df t.ratio p.value
## APOE22 female effect -28125 10919 23  -2.576  0.1014
## APOE33 female effect   6142 13482 23   0.456  0.6666
## APOE44 female effect  10675 10919 23   0.978  0.6666
## APOE22 male effect  -4442 10178 23  -0.436  0.6666
## APOE33 male effect   6075 10919 23   0.556  0.6666
## APOE44 male effect   9675 10919 23   0.886  0.6666
##
## P value adjustment: fdr method for 6 tests
contrast(cbw_DEG.emm, simple = list("Genotype", "Sex"))

## `$`simple contrasts for Genotype`
## Sex = female:
## contrast          estimate      SE df t.ratio p.value
## APOE22 effect  -24356 10210 23  -2.386  0.0770
## APOE33 effect   9911 11641 23   0.851  0.4033
## APOE44 effect  14444 10210 23   1.415  0.2558
##
## Sex = male:
## contrast          estimate      SE df t.ratio p.value
## APOE22 effect  -8211  9132 23  -0.899  0.8114
## APOE33 effect   2306  9550 23   0.241  0.8114
## APOE44 effect   5906  9550 23   0.618  0.8114
##
## P value adjustment: fdr method for 3 tests
```

```
##
## $`simple contrasts for Sex`
## Genotype = APOE22:
## contrast      estimate    SE df t.ratio p.value
## female effect -11841.7 8031 23  -1.474  0.1539
## male effect   11841.7 8031 23   1.474  0.1539
##
## Genotype = APOE33:
## contrast      estimate    SE df t.ratio p.value
## female effect    33.3 9686 23   0.003  0.9973
## male effect    -33.3 9686 23  -0.003  0.9973
##
## Genotype = APOE44:
## contrast      estimate    SE df t.ratio p.value
## female effect    500.0 8388 23   0.060  0.9530
## male effect    -500.0 8388 23  -0.060  0.9530
##
## P value adjustment: fdr method for 2 tests
#post hoc tests
summary(glht(lm_cbw_DEG, emm(pairwise ~ Genotype*Sex, contr="sidak"))) #consider save to file

##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##
##               Estimate Std. Error t value Pr(>|t|)
## APOE22 female - APOE33 female == 0 -34266.67  19371.50  -1.769    0.502
## APOE22 female - APOE44 female == 0 -38800.00  16776.21  -2.313    0.228
## APOE22 female - APOE22 male == 0  -23683.33  16062.00  -1.474    0.681
## APOE22 female - APOE33 male == 0  -34200.00  16776.21  -2.039    0.351
## APOE22 female - APOE44 male == 0  -37800.00  16776.21  -2.253    0.252
## APOE33 female - APOE44 female == 0  -4533.33  19371.50  -0.234    1.000
## APOE33 female - APOE22 male == 0   10583.33  18756.38   0.564    0.992
## APOE33 female - APOE33 male == 0     66.67  19371.50   0.003    1.000
## APOE33 female - APOE44 male == 0  -3533.33  19371.50  -0.182    1.000
## APOE44 female - APOE22 male == 0   15116.67  16062.00   0.941    0.931
## APOE44 female - APOE33 male == 0    4600.00  16776.21   0.274    1.000
## APOE44 female - APOE44 male == 0    1000.00  16776.21   0.060    1.000
## APOE22 male - APOE33 male == 0   -10516.67  16062.00  -0.655    0.985
## APOE22 male - APOE44 male == 0   -14116.67  16062.00  -0.879    0.947
## APOE33 male - APOE44 male == 0    -3600.00  16776.21  -0.215    1.000
## (Adjusted p values reported -- single-step method)
res<-summary(glht(lm_cbw_DEG, emm(pairwise ~ Genotype*Sex))) #consider save to file
#summary(as.glht(pairs(lm_cbw_DEG.emm), by = NULL))

summary(glht(lm_cbw_DEG, emm(pairwise ~ Genotype | Sex)))

## $`Sex = female`
##
## Simultaneous Tests for General Linear Hypotheses
##
```

```

## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0   -34267      19372  -1.769   0.2015
## APOE22 - APOE44 == 0   -38800      16776  -2.313   0.0739 .
## APOE33 - APOE44 == 0    -4533      19372  -0.234   0.9702
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
##
##
## $`Sex = male`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0   -10517      16062  -0.655   0.792
## APOE22 - APOE44 == 0   -14117      16062  -0.879   0.659
## APOE33 - APOE44 == 0    -3600      16776  -0.215   0.975
## (Adjusted p values reported -- single-step method)
summary(glht(lm_cbw_DEG, emm(pairwise ~ Sex | Genotype)))

## $`Genotype = APOE22`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0   -23683      16062  -1.474   0.154
## (Adjusted p values reported -- single-step method)
##
##
## $`Genotype = APOE33`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0    66.67   19371.50   0.003   0.997
## (Adjusted p values reported -- single-step method)
##
##
## $`Genotype = APOE44`
##
## Simultaneous Tests for General Linear Hypotheses
##

```

```
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_DEG)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0      1000      16776    0.06    0.953
## (Adjusted p values reported -- single-step method)

mypairs<-pairs(cbw_DEG.emm) #_consider save to file
pairs(cbw_DEG.emm, by="Genotype")

## Genotype = APOE22:
## contrast      estimate      SE df t.ratio p.value
## female - male -23683.3 16062 23  -1.474  0.1539
##
## Genotype = APOE33:
## contrast      estimate      SE df t.ratio p.value
## female - male   66.7 19372 23   0.003  0.9973
##
## Genotype = APOE44:
## contrast      estimate      SE df t.ratio p.value
## female - male  1000.0 16776 23   0.060  0.9530

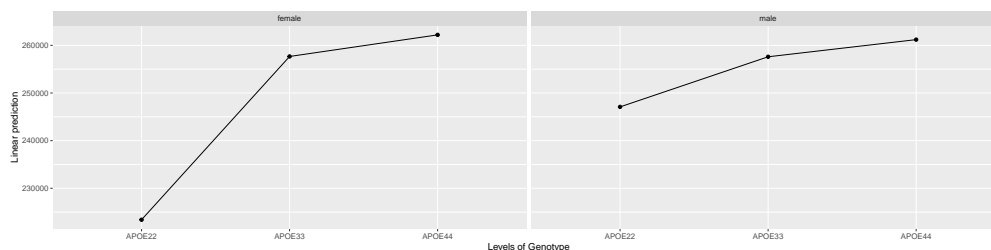
pairs(cbw_DEG.emm, by="Sex")

## Sex = female:
## contrast      estimate      SE df t.ratio p.value
## APOE22 - APOE33  -34267 19372 23  -1.769  0.2022
## APOE22 - APOE44  -38800 16776 23  -2.313  0.0740
## APOE33 - APOE44   -4533 19372 23  -0.234  0.9703
##
## Sex = male:
## contrast      estimate      SE df t.ratio p.value
## APOE22 - APOE33  -10517 16062 23  -0.655  0.7916
## APOE22 - APOE44  -14117 16062 23  -0.879  0.6587
## APOE33 - APOE44   -3600 16776 23  -0.215  0.9750
##
## P value adjustment: tukey method for comparing a family of 3 estimates

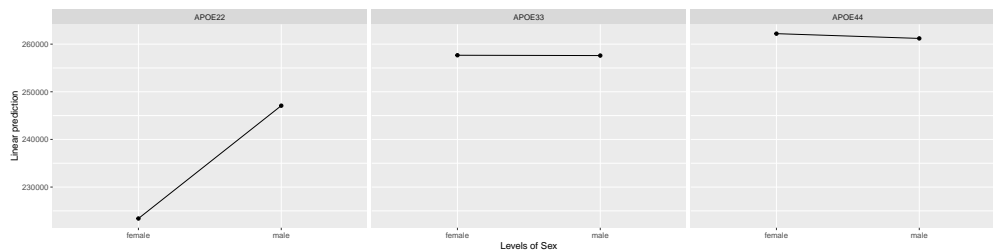
print('cbw DEG')

## [1] "cbw DEG"
```

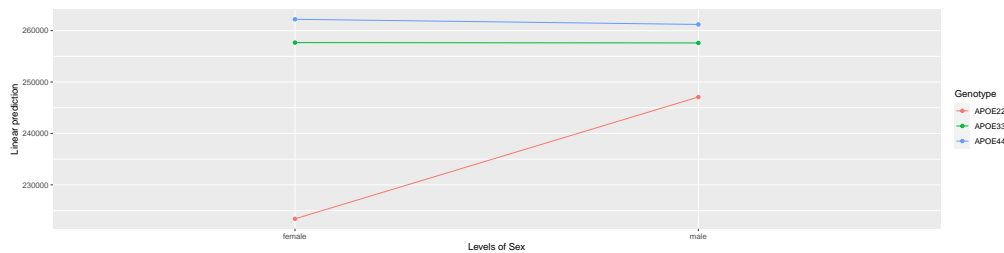
```
emmip(lm_cbw_DEG, ~ Genotype | Sex )
```



```
emmip(lm_cbw_DEG, ~ Sex | Genotype )
```

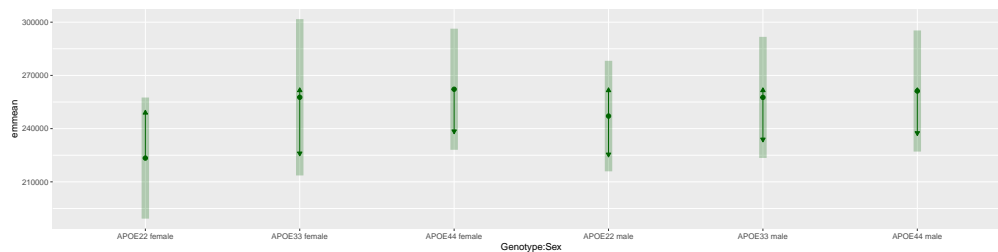


```
emmip(lm_cbw_DEG, Genotype~ Sex )
```



```
sink()
```

```
plot(cbw_DEG.emm, by = NULL, comparisons = TRUE, adjust = "mvt",
horizontal = FALSE, colors = "darkgreen", main ="cbw DEG")
```



```
ggsave(paste(outpath,'cbw_DEG_emmeans.pdf',sep=''), plot = last_plot(), device='pdf', scale=1, width=16
```

```
'simple stats'
```

```
## [1] "simple stats"
```

```
print('cbw')
```

```
## [1] "cbw"
```

```
lm_cbw_CLUS <- lm(cbw ~ Genotype*Sex, geno_combined_CLUS)
summary(lm_cbw_CLUS)
```

```
##
```

```
## Call:
```

```
## lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
```

```
##
```

```
## Residuals:
```

```
##      Min       1Q   Median       3Q      Max
## -75.792 -22.224  -6.825  18.395  97.930
```

```
##
```

```
## Coefficients:
```

```
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      212.26      19.12  11.100 1.03e-10 ***
## GenotypeAPOE33       42.70      31.23   1.367   0.1848
```



```

## GenotypeAPOE44          52.75      27.04    1.950    0.0634 .
## Sexmale                 26.06      25.89    1.006    0.3247
## GenotypeAPOE33:Sexmale -22.41      40.57   -0.552    0.5861
## GenotypeAPOE44:Sexmale -13.98      37.44   -0.373    0.7122
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 42.76 on 23 degrees of freedom
## Multiple R-squared:  0.2386, Adjusted R-squared:  0.07303
## F-statistic: 1.441 on 5 and 23 DF,  p-value: 0.2473

lm_cbw_CLUS_f <- lm(cbw ~ Genotype, combo_f_FA)
#summary(lm_cbw_CLUS_f)
lm_cbw_CLUS_m <- lm(cbw ~ Genotype, combo_m_FA)
#summary(lm_cbw_CLUS_f)

#33333
sink(paste(outpath, "cbw_CLUS_SimpleModels.txt"))
mydata.lm <- lm(cbw ~ Genotype*Sex, data = geno_combined_CLUS)
anova(mydata.lm)

## Analysis of Variance Table
##
## Response: cbw
##              Df Sum Sq Mean Sq F value   Pr(>F)
## Genotype      2  10934   5466.8   2.9898 0.07011 .
## Sex            1   1646   1646.1   0.9003 0.35258
## Genotype:Sex  2     596    298.0   0.1630 0.85060
## Residuals    23  42055   1828.5
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(glht(mydata.lm, emm(pairwise ~ Genotype|Sex, adjust="sidak")))) #consider save to file

## $`Sex = female`
##
##      Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##              Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0   -42.70      31.23  -1.367   0.373
## APOE22 - APOE44 == 0   -52.75      27.04  -1.950   0.147
## APOE33 - APOE44 == 0   -10.05      31.23  -0.322   0.944
## (Adjusted p values reported -- single-step method)
##
##
## $`Sex = male`
##
##      Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:

```

```

##               Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0   -20.29      25.89  -0.784    0.717
## APOE22 - APOE44 == 0   -38.77      25.89  -1.497    0.311
## APOE33 - APOE44 == 0   -18.47      27.04  -0.683    0.775
## (Adjusted p values reported -- single-step method)
summary(ghlt(mydata.lm, emm(pairwise ~ Sex| Genotype)))

## $`Genotype = APOE22`
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0   -26.06      25.89  -1.006    0.325
## (Adjusted p values reported -- single-step method)
##
## $`Genotype = APOE33`
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0    -3.656     31.228  -0.117    0.908
## (Adjusted p values reported -- single-step method)
##
## $`Genotype = APOE44`
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0   -12.08      27.04  -0.447    0.659
## (Adjusted p values reported -- single-step method)

sink()
#3333

#omnibus testing
#https://cran.r-project.org/web/packages/emmeans/vignettes/confidence-intervals.html#adjust

sink(paste(outpath, "cbw_CLUS_SimpleStats.txt", sep=''))
'omnibus testing'

## [1] "omnibus testing"
joint_tests(ref_grid(lm_cbw_CLUS))

```

```
## model term    df1 df2 F.ratio p.value
## Genotype      2  23   3.126  0.0629
## Sex           1  23   0.735  0.4002
## Genotype:Sex   2  23   0.163  0.8506

joint_tests(ref_grid(lm_cbw_CLUS), by = "Sex", adjust = "sidak")

## Sex = female:
## model term df1 df2 F.ratio p.value
## Genotype    2  23   2.070  0.1490
##
## Sex = male:
## model term df1 df2 F.ratio p.value
## Genotype    2  23   1.127  0.3411

joint_tests(ref_grid(lm_cbw_CLUS), by = "Genotype", adjust = "sidak" )

## Genotype = APOE22:
## model term df1 df2 F.ratio p.value
## Sex        1  23   1.013  0.3247
##
## Genotype = APOE33:
## model term df1 df2 F.ratio p.value
## Sex        1  23   0.014  0.9078
##
## Genotype = APOE44:
## model term df1 df2 F.ratio p.value
## Sex        1  23   0.199  0.6593

'anova'

## [1] "anova"

anova(lm_cbw_CLUS)

## Analysis of Variance Table
##
## Response: cbw
##              Df Sum Sq Mean Sq F value    Pr(>F)
## Genotype      2  10934   5466.8    2.9898  0.07011 .
## Sex           1   1646   1646.1    0.9003  0.35258
## Genotype:Sex   2    596    298.0    0.1630  0.85060
## Residuals    23  42055   1828.5
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#anova(lm_cbw_CLUS_f)
#anova(lm_cbw_CLUS_m)

#post hoc comparison
cbw_CLUS.emm <- emmeans(lm_cbw_CLUS , ~ Genotype | Sex, adjust = "sidak")
contrast(cbw_CLUS.emm, simple = c("Genotype", "Sex")) #FDR

## contrast            estimate    SE df t.ratio p.value
## APOE22 female effect  -38.78 17.6 23  -2.203  0.2272
## APOE33 female effect   3.92 21.7 23   0.180  0.8586
## APOE44 female effect  13.97 17.6 23   0.794  0.6691
## APOE22 male effect    -12.72 16.4 23  -0.775  0.6691
```

```

## APOE33 male effect      7.57 17.6 23    0.430  0.8053
## APOE44 male effect      26.05 17.6 23    1.480  0.4576
##
## P value adjustment: fdr method for 6 tests
contrast(cbw_CLUS.emm, simple = list("Genotype", "Sex"))

## $`simple contrasts for Genotype`
## Sex = female:
## contrast      estimate    SE df t.ratio p.value
## APOE22 effect  -31.815 16.5 23  -1.933  0.1969
## APOE33 effect   10.881 18.8 23   0.580  0.5677
## APOE44 effect   20.933 16.5 23   1.272  0.3242
##
## Sex = male:
## contrast      estimate    SE df t.ratio p.value
## APOE22 effect  -19.685 14.7 23  -1.337  0.3416
## APOE33 effect   0.606 15.4 23   0.039  0.9690
## APOE44 effect   19.080 15.4 23   1.239  0.3416
##
## P value adjustment: fdr method for 3 tests
##
## $`simple contrasts for Sex`
## Genotype = APOE22:
## contrast      estimate    SE df t.ratio p.value
## female effect  -13.03 12.9 23  -1.006  0.3247
## male effect     13.03 12.9 23   1.006  0.3247
##
## Genotype = APOE33:
## contrast      estimate    SE df t.ratio p.value
## female effect   -1.83 15.6 23  -0.117  0.9078
## male effect     1.83 15.6 23   0.117  0.9078
##
## Genotype = APOE44:
## contrast      estimate    SE df t.ratio p.value
## female effect   -6.04 13.5 23  -0.447  0.6593
## male effect     6.04 13.5 23   0.447  0.6593
##
## P value adjustment: fdr method for 2 tests
#post hoc tests
summary(glht(lm_cbw_CLUS, emm(pairwise ~ Genotype*Sex, contr="sidak"))) #consider save to file

##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##
## Estimate Std. Error t value Pr(>|t|)
## APOE22 female - APOE33 female == 0 -42.696 31.228 -1.367 0.744
## APOE22 female - APOE44 female == 0 -52.748 27.044 -1.950 0.397
## APOE22 female - APOE22 male == 0 -26.061 25.893 -1.006 0.910
## APOE22 female - APOE33 male == 0 -46.352 27.044 -1.714 0.535
## APOE22 female - APOE44 male == 0 -64.826 27.044 -2.397 0.197

```

```

## APOE33 female - APOE44 female == 0 -10.052      31.228 -0.322      0.999
## APOE33 female - APOE22 male == 0      16.635      30.236  0.550      0.993
## APOE33 female - APOE33 male == 0      -3.656      31.228 -0.117      1.000
## APOE33 female - APOE44 male == 0     -22.130      31.228 -0.709      0.979
## APOE44 female - APOE22 male == 0      26.687      25.893  1.031      0.902
## APOE44 female - APOE33 male == 0       6.396      27.044  0.237      1.000
## APOE44 female - APOE44 male == 0     -12.078      27.044 -0.447      0.997
## APOE22 male - APOE33 male == 0     -20.291      25.893 -0.784      0.967
## APOE22 male - APOE44 male == 0     -38.765      25.893 -1.497      0.668
## APOE33 male - APOE44 male == 0     -18.474      27.044 -0.683      0.982
## (Adjusted p values reported -- single-step method)

res<-summary(glht(lm_cbw_CLUS, emm(pairwise ~ Genotype*Sex))) #consider save to file
#summary(as.glht(pairs(lm_cbw_CLUS.emm), by = NULL))

summary(glht(lm_cbw_CLUS, emm(pairwise ~ Genotype | Sex)))

## $`Sex = female`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##              Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0   -42.70      31.23  -1.367   0.373
## APOE22 - APOE44 == 0   -52.75      27.04  -1.950   0.147
## APOE33 - APOE44 == 0   -10.05      31.23  -0.322   0.944
## (Adjusted p values reported -- single-step method)
##
## $`Sex = male`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##              Estimate Std. Error t value Pr(>|t|)
## APOE22 - APOE33 == 0   -20.29      25.89  -0.784   0.717
## APOE22 - APOE44 == 0   -38.77      25.89  -1.497   0.311
## APOE33 - APOE44 == 0   -18.47      27.04  -0.683   0.775
## (Adjusted p values reported -- single-step method)

summary(glht(lm_cbw_CLUS, emm(pairwise ~ Sex | Genotype)))

## $`Genotype = APOE22`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##              Estimate Std. Error t value Pr(>|t|)
## female - male == 0    -26.06      25.89  -1.006   0.325

```

```

## (Adjusted p values reported -- single-step method)
##
##
## $`Genotype = APOE33`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0   -3.656      31.228  -0.117   0.908
## (Adjusted p values reported -- single-step method)
##
##
## $`Genotype = APOE44`
##
## Simultaneous Tests for General Linear Hypotheses
##
## Fit: lm(formula = cbw ~ Genotype * Sex, data = geno_combined_CLUS)
##
## Linear Hypotheses:
##               Estimate Std. Error t value Pr(>|t|)
## female - male == 0   -12.08      27.04  -0.447   0.659
## (Adjusted p values reported -- single-step method)
mypairs<-pairs(cbw_CLUS.emm) #_consider save to file
pairs(cbw_CLUS.emm, by="Genotype")

## Genotype = APOE22:
## contrast      estimate    SE df t.ratio p.value
## female - male  -26.06 25.9 23  -1.006  0.3247
##
## Genotype = APOE33:
## contrast      estimate    SE df t.ratio p.value
## female - male   -3.66 31.2 23  -0.117  0.9078
##
## Genotype = APOE44:
## contrast      estimate    SE df t.ratio p.value
## female - male  -12.08 27.0 23  -0.447  0.6593
pairs(cbw_CLUS.emm, by="Sex")

## Sex = female:
## contrast      estimate    SE df t.ratio p.value
## APOE22 - APOE33   -42.7 31.2 23  -1.367  0.3740
## APOE22 - APOE44   -52.7 27.0 23  -1.950  0.1475
## APOE33 - APOE44   -10.1 31.2 23  -0.322  0.9446
##
## Sex = male:
## contrast      estimate    SE df t.ratio p.value
## APOE22 - APOE33   -20.3 25.9 23  -0.784  0.7166
## APOE22 - APOE44   -38.8 25.9 23  -1.497  0.3108
## APOE33 - APOE44   -18.5 27.0 23  -0.683  0.7756
##

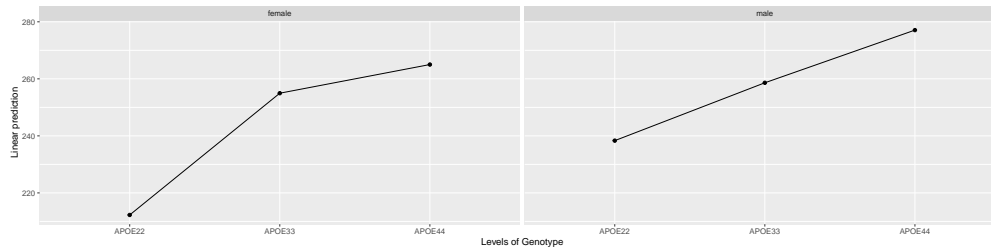
```

```
## P value adjustment: tukey method for comparing a family of 3 estimates
```

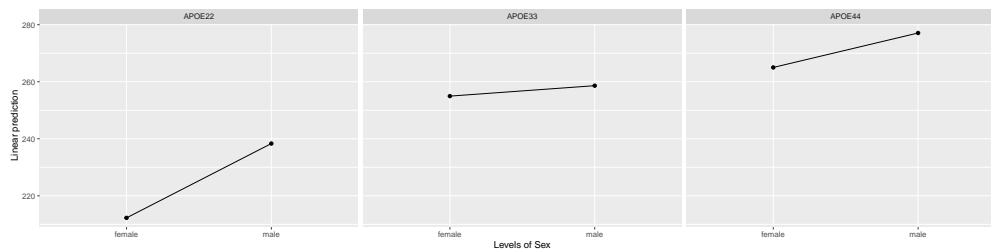
```
print('cbw CLUS')
```

```
## [1] "cbw CLUS"
```

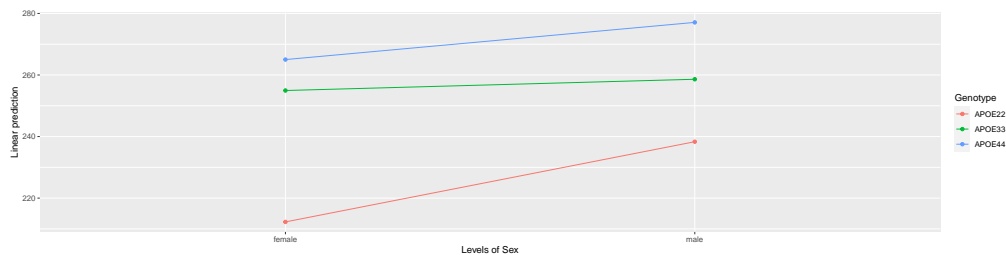
```
emmip(lm_cbw_CLUS, ~ Genotype | Sex )
```



```
emmip(lm_cbw_CLUS, ~ Sex | Genotype )
```

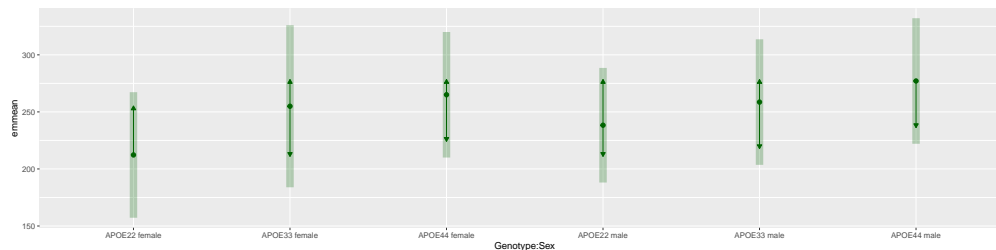


```
emmip(lm_cbw_CLUS, Genotype~ Sex )
```



```
sink()
```

```
plot(cbw_CLUS.emm, by = NULL, comparisons = TRUE, adjust = "mvt",  
horizontal = FALSE, colors = "darkgreen", main = "cbw CLUS")
```



```
ggsave(paste(outpath, 'cbw_CLUS_emmeans.pdf', sep=''), plot = last_plot(), device='pdf', scale=1, width=1000, height=1000)
```