Lecture 7 – Linear Models

STAT/BIOF/GSAT 540: Statistical Methods for High Dimensional Biology

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Recall from last class...

1. show how to compare means of different groups (2 or more) using a linear regression model

- dummy variables to model the levels of a qualitative explanatory variable

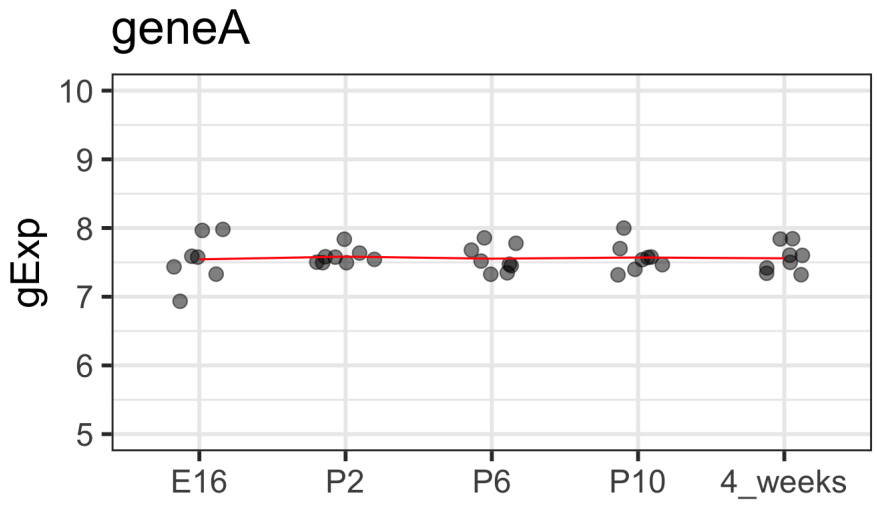
2. write a linear model using matrix notation

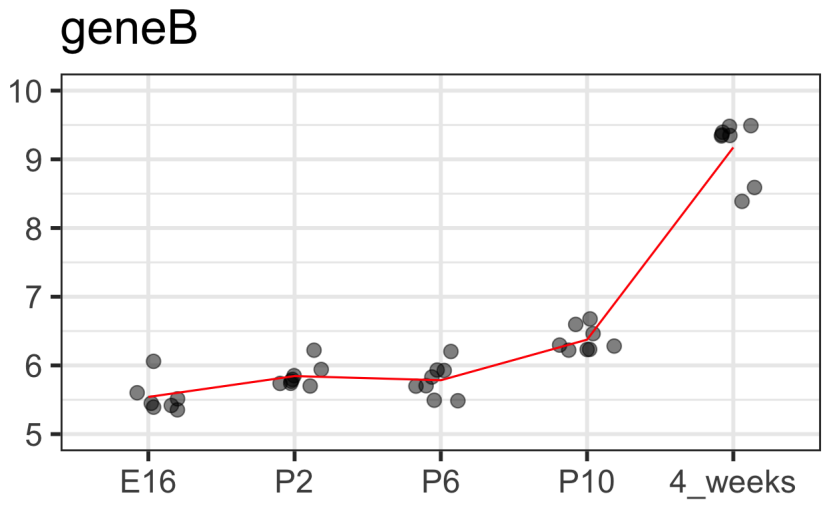
- understand which matrix is built by R

3. distinguish between single and multiple hypotheses

- t-tests vs F-tests

Do we think that the expression levels at different developmental stages are generated by distributions with different location (mean)? Or a single common distribution?





Quick review: from t-test to linear regression

2-sample t-test

$$Y \sim F;\ E[Y] = \mu_Y;\ Z \sim G;\ E[Z] = \mu_Z$$

$$H_0 : \mu_Y = \mu_Z$$

$$\downarrow ?$$

Linear regression

$$Y = X\alpha + \epsilon; \qquad H_0 : \alpha_j = 0$$

HOW? WHY?

HOW?? : Cell means model using dummy variables

$$Y \sim F;\ E[Y] = \mu_Y;\ Z \sim G;\ E[Z] = \mu_Z$$

$$\downarrow$$

$$Y_{ij} = \mu_1 x_{ij1} + \mu_2 x_{ij2} + \varepsilon_{ij};\ i = 1, \ldots, n;\ j = 1, 2$$

$$x_{ij1} = \begin{cases} 1 \text{ if } j = 1 \\ 0 \text{ otherwise} \end{cases}, \quad x_{ij2} = \begin{cases} 1 \text{ if } j = 2 \\ 0 \text{ otherwise} \end{cases}$$

$$\downarrow$$

$$\begin{aligned} E[Y_{i1}] &= \mu_1 \\ E[Y_{i2}] &= \mu_2 \end{aligned}$$

$$\begin{bmatrix} Y_{11} \\ \vdots \\ Y_{n_1 1} \\ Y_{12} \\ \vdots \\ Y_{n_2 2} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ \vdots & \vdots \\ 1 & 0 \\ 0 & 1 \\ \vdots & \vdots \\ 0 & 1 \end{bmatrix} \begin{bmatrix} \mu_1 \\ \mu_2 \end{bmatrix} + \begin{bmatrix} \varepsilon_{11} \\ \vdots \\ \varepsilon_{n_1 1} \\ \varepsilon_{12} \\ \vdots \\ \varepsilon_{n_2 2} \end{bmatrix}$$

HOW?? : Changing the parametrization to reference-treatment using dummy variables

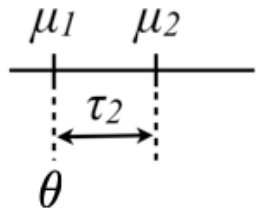
$$Y \sim F;\ E[Y] = \mu_Y;\ Z \sim G;\ E[Z] = \mu_Z$$

$$\downarrow$$

$$Y_{ij} = \theta + \tau_1 x_{ij1} + \tau_2 x_{ij2} + \varepsilon_{ij};\ i = 1, \ldots, n;\ j = 1, 2; \tau_1 = 0$$

$$x_{ij1} = \begin{cases} 1 \text{ if } j = 1 \\ 0 \text{ otherwise} \end{cases}, \quad x_{ij2} = \begin{cases} 1 \text{ if } j = 2 \\ 0 \text{ otherwise} \end{cases}$$

$$\downarrow$$



$$\begin{aligned} E[Y_{i1}] &= \theta = \mu_1 \\ E[Y_{i2}] &= \theta + \tau_2 = \mu_1 + (\mu_2 - \mu_1) = \mu_2 \end{aligned}$$

HOW?? : Changing the parametrization and using dummy variables

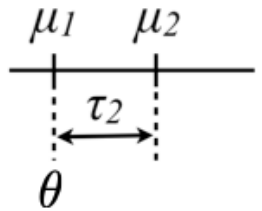
$$Y \sim F;\ E[Y] = \mu_Y;\ Z \sim G;\ E[Z] = \mu_Z$$

$$\downarrow$$

$$Y_{ij} = \theta + \tau_2 x_{ij2} + \varepsilon_{ij};\ i = 1, \ldots, n;\ j = 1, 2$$

$$x_{ij2} = \begin{cases} 1 \text{ if } j = 2 \\ 0 \text{ otherwise} \end{cases}$$

$$\downarrow$$



$$\begin{aligned} E[Y_{i1}] &= \theta = \mu_1 \\ E[Y_{i2}] &= \theta + \tau_2 = \mu_1 + (\mu_2 - \mu_1) = \mu_2 \end{aligned}$$

Using matrix notation ...

$$Y_{ij} = \theta + \tau_2 \times x_{ij2} + \varepsilon_{ij}$$

$$\begin{bmatrix} Y_{11} \\ \vdots \\ Y_{n_1 1} \\ Y_{12} \\ \vdots \\ Y_{n_2 2} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ \vdots & \vdots \\ 1 & 0 \\ 1 & 1 \\ \vdots & \vdots \\ 1 & 1 \end{bmatrix} \begin{bmatrix} \theta \\ \tau_2 \end{bmatrix} + \begin{bmatrix} \varepsilon_{11} \\ \vdots \\ \varepsilon_{n_1 1} \\ \varepsilon_{12} \\ \vdots \\ \varepsilon_{n_2 2} \end{bmatrix}$$

$$\boldsymbol{Y = X\alpha + \varepsilon}$$

- x_{ij2} is the second column of design matrix X
- $x_{112} = 0$ and $x_{122} = 1$

Red

$$Y_{11} = 1 * \theta + 0 * \tau_2 + \epsilon_{11} = \theta + \epsilon_{11}$$

Blue

$$Y_{12} = 1 * \theta + 1 * \tau_2 + \epsilon_{12} = \theta + \tau_2 + \epsilon_{12}$$

- Tip: examine design matrix in R with `model.matrix()`

... and similarly beyond 2 group comparisons (ANOVA)

WHY??

$$Y = X\alpha + \varepsilon$$

This gives us a VERY FLEXIBLE framework!!

$$\begin{bmatrix} 1 & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 0 & 1 \end{bmatrix}$$

1 categorical covariate

$$\begin{bmatrix} 1 & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ \vdots & \vdots & \vdots & \\ 1 & 0 & 1 & 0 \\ 1 & 1 & 1 & 1 \\ \vdots & \vdots & \vdots & \\ 1 & 1 & 1 & 1 \end{bmatrix}$$

2 categorical covariates

$$\begin{bmatrix} 1 & 1.22 \\ 1 & 2.02 \\ 1 & 1.42 \\ \vdots & \vdots \\ 1 & 1.89 \\ 1 & 2.01 \\ \vdots & \vdots \\ 1 & 1.56 \\ 1 & 2.17 \\ 1 & 1.51 \end{bmatrix}$$

1 continuous covariate

$$\begin{bmatrix} 1 & 0 & 1.22 & 0 \\ 1 & 0 & 2.02 & 0 \\ 1 & 0 & 1.42 & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 1 & 0 & 1.89 & 0 \\ 1 & 1 & 2.01 & 2.01 \\ \vdots & \vdots & \vdots & \vdots \\ 1 & 1 & 1.56 & 1.56 \\ 1 & 1 & 2.17 & 2.17 \\ 1 & 1 & 1.51 & 1.51 \end{bmatrix}$$

1 continuous 1 categorical

AND MANY MORE .....

Tip: ?model.matrix

Parametrizations

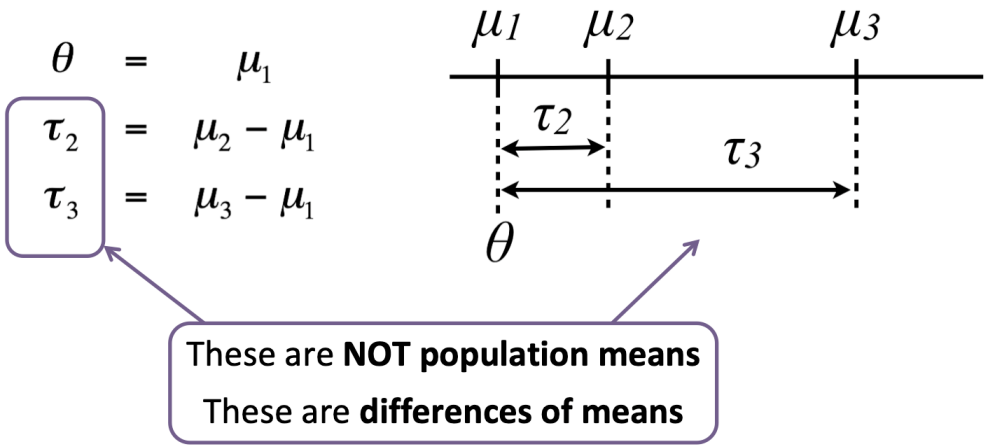
Different ways of writing this [design matrix, parameter vector] pair correspond to different parametrizations of the model

$$Y = [X\alpha] + \varepsilon$$

Understanding these concepts makes it easier ...

- to interpret fitted models
- to fit models such that comparisons you care most about are directly addressed in the inferential "report"

For example: comparisons of mean expression levels between groups!



By default, `lm` estimates group mean differences (with respect to a reference group):

```
summary(lm(gExp~devStage,subset(devDat,gene=="geneB")))$coeff
```

```
##                  Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)     5.5408571  0.1021381 54.248698 1.307554e-34
## devStageP2      0.3040179  0.1398583  2.173756 3.678022e-02
## devStageP6      0.2433929  0.1398583  1.740282 9.085489e-02
## devStageP10     0.8342679  0.1398583  5.965093 9.559065e-07
## devStage4_weeks 3.6325179  0.1398583 25.972843 5.266481e-24
```

We can tell R to use the cell-means parametrization

Write the formula as `Y ~ 0 + x` in the `lm` call to remove the intercept (θ) parameter and fit cell means parameters instead.

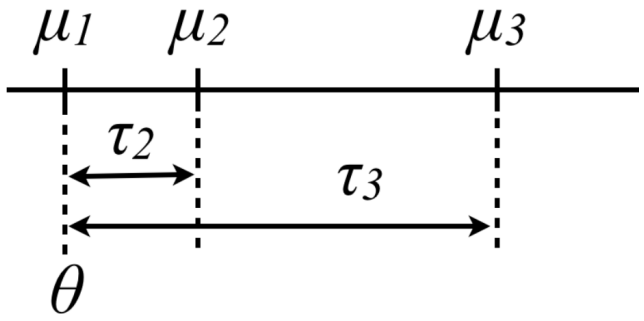
```
summary(lm(gExp~0+devStage,subset(devDat,gene=="geneB")))$coeff
```

```
##                 Estimate Std. Error  t value     Pr(>|t|)
## devStageE16     5.540857 0.10213807 54.24870 1.307554e-34
## devStageP2      5.844875 0.09554141 61.17635 2.287666e-36
## devStageP6      5.784250 0.09554141 60.54181 3.250790e-36
## devStageP10     6.375125 0.09554141 66.72630 1.223052e-37
## devStage4_weeks 9.173375 0.09554141 96.01465 5.523959e-43
```

Now, the *t*-test column of the output represents the test of whether each group mean is equal to zero

Recall that we can obtain one set of parameters from the other!

$$\mu_1 = \theta \qquad \theta = \mu_1$$
$$\mu_2 = \theta + \tau_2 \qquad \tau_2 = \mu_2 - \mu_1$$
$$\mu_3 = \theta + \tau_3 \qquad \tau_3 = \mu_3 - \mu_1$$



These are **population** means

These are **NOT population** means

These are **ref & TX** effects

```
Call:
lm(formula = gExp ~ 0 + devStage, <blah, blah>)

<snip, snip>

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
devStageE16      5.54086    0.10214   54.25   <2e-16 ***
devStageP2       5.84488    0.09554   61.18   <2e-16 ***
devStageP6       5.78425    0.09554   60.54   <2e-16 ***
devStageP10      6.37512    0.09554   66.73   <2e-16 ***
devStage4_weeks  9.17337    0.09554   96.02   <2e-16 ***
```

```
Call:
lm(formula = gExp ~ devStage, <blah, blah>)
<snip, snip>
Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      5.5409     0.1021  54.249  < 2e-16 ***
devStageP2       0.3040     0.1399   2.174   0.0368 *
devStageP6       0.2434     0.1399   1.740   0.0909 .
devStageP10      0.8343     0.1399   5.965 9.56e-07 ***
devStage4_weeks  3.6325     0.1399  25.973  < 2e-16 ***
---
<snip, snip>
F-statistic: 243.4 on 4 and 34 DF,  p-value: < 2.2e-16
```

Today... more complex models

1. more than one factor with multiple levels

- how to model many categorical variables and their interaction

2. distinguish between simple and main effects

- `lm` vs `anova` tests

3. nested models

- t-tests vs F-tests

4. continuous explanatory variables

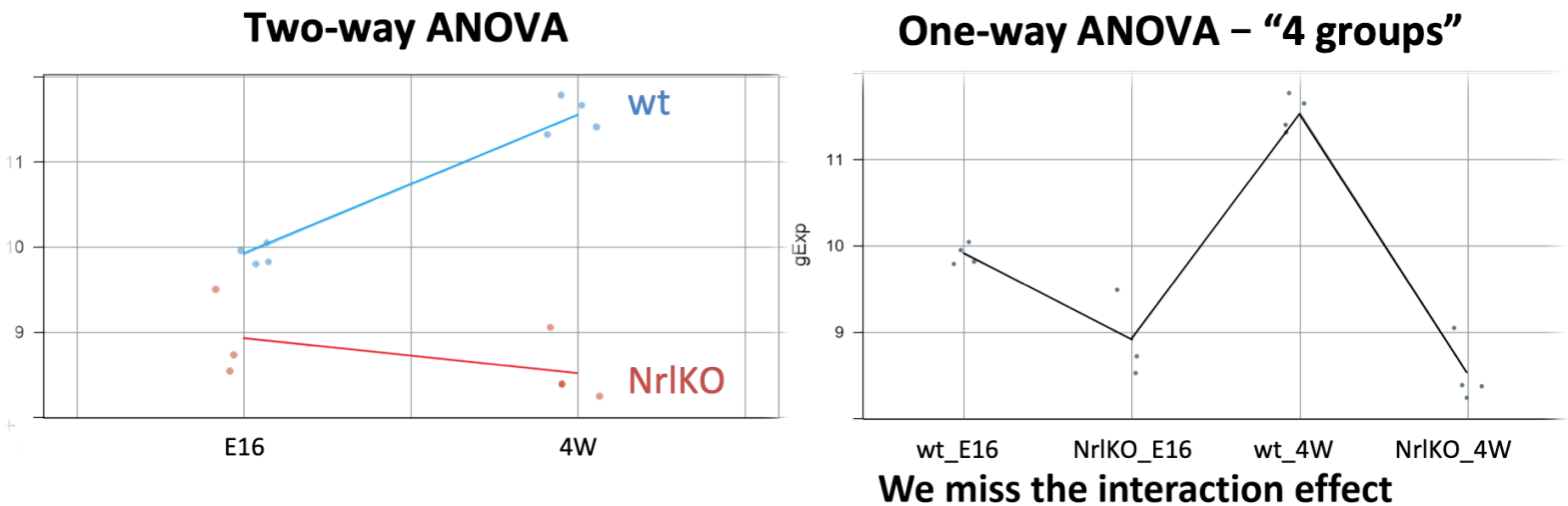
- the regression line

Increasing the complexity of the linear model ...

What if you have *two* categorical variables?

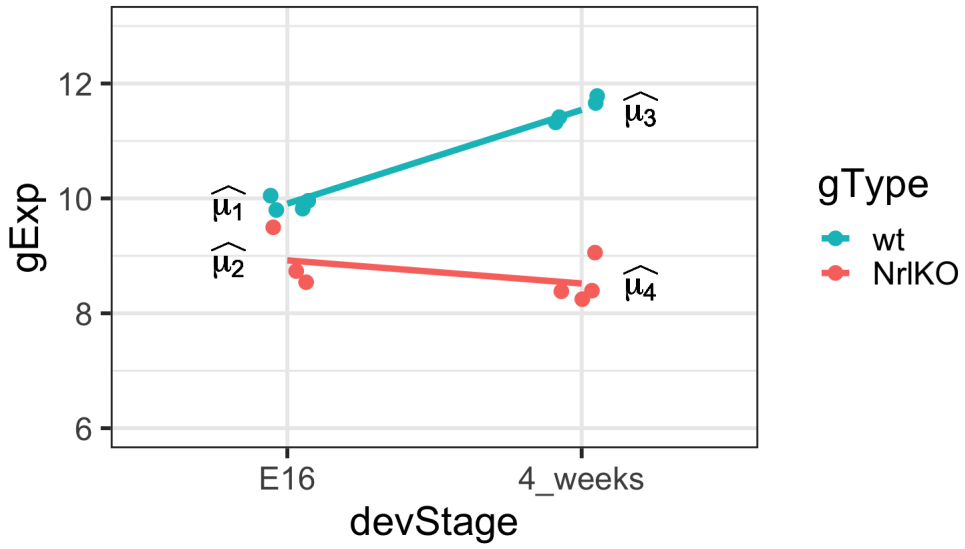
e.g., `gType` and `devStage` (for simplicity, let's consider only E16 and 4W)

- ANOVA is usually used to study models with one or more categorical variables (factors)
- Can we combine levels into 4 groups to simplify the analysis??



Two-way ANOVA or a linear model with interaction

Which group means are we comparing in a model with 2 factors?



$$\mu_1 = E[Y_{(wt,E16)}],\ \mu_2 = E[Y_{(NrlKO,E16)}],\ \mu_3 = E[Y_{(wt,4W)}],\ \mu_4 = E[Y_{(NrlKO,4W)}]$$

Reference-treatment effect parametrization

- By default, `lm` assumes a **reference-treatment effect** parametrization
- Mathematically, we need *more* dummy variables, see math handout for more details

```
twoFactFit <- lm(gExp ~ gType * devStage, twoDat)
```

```
##                                Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)                   9.9080000  0.1574912 62.911469 2.027211e-15
## gTypeNrlKO                   -0.9856667  0.2405717 -4.097184 1.767824e-03
## devStage4_weeks               1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4_weeks -2.0380833  0.3278440 -6.216626 6.560671e-05
```

Cell-means and treatment effects in the two-way model - why do we need more dummy variables?

```
table(twoDat$grp)
```

```
##
##         wt.E16      NrlKO.E16     wt.4_weeks NrlKO.4_weeks
##              4              3              4              4
```

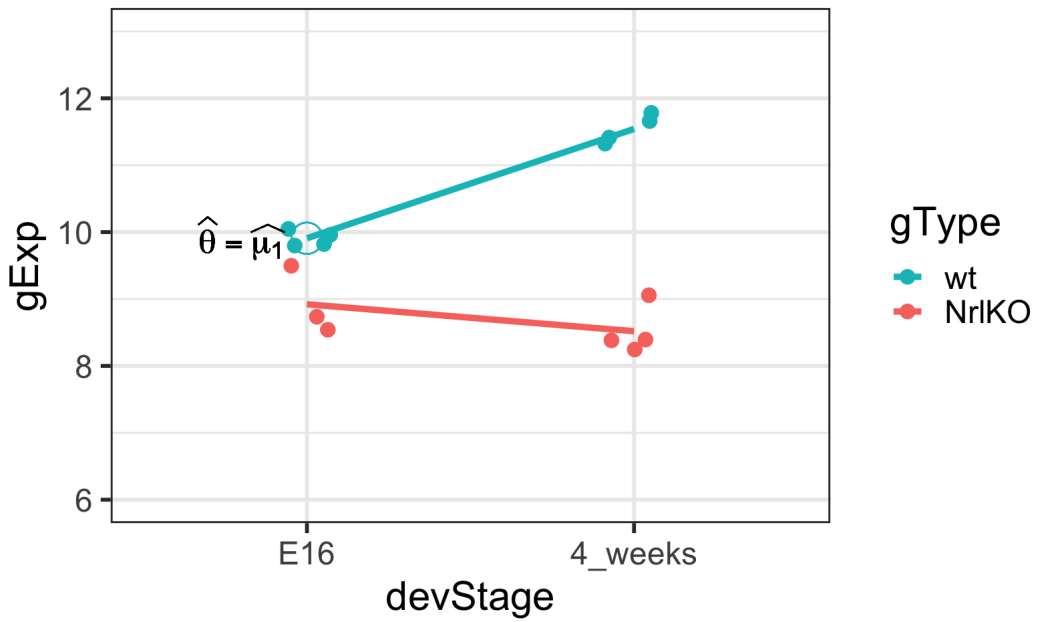
```
(means.2Fact <- as.data.frame(twoDat %>%
          group_by(grp) %>%
          summarize(cellMeans=mean(gExp))) %>%
          mutate(txEffects=cellMeans-cellMeans[1],
                 lmEst=summary(twoFactFit)$coeff[,1]))
```

```
##             grp cellMeans  txEffects      lmEst
## 1         wt.E16  9.908000  0.0000000  9.9080000
## 2      NrlKO.E16  8.922333 -0.9856667 -0.9856667
## 3     wt.4_weeks 11.542500  1.6345000  1.6345000
## 4 NrlKO.4_weeks  8.518750 -1.3892500 -2.0380833
```

What is the reference group here?

wt & E16

As before, comparisons are relative to a reference but in this case there is a reference level *in each factor*: wt and E16



The reference: wt & E16

Mean of reference group: $\theta = E[Y_{wt,E16}]$

`lm` estimate: $\hat{\theta}$ is the sample mean of the group

```
##                                Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)                   9.9080000  0.1574912 62.911469 2.027211e-15
## gTypeNrlKO                   -0.9856667  0.2405717 -4.097184 1.767824e-03
## devStage4_weeks               1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4_weeks -2.0380833  0.3278440 -6.216626 6.560671e-05
```

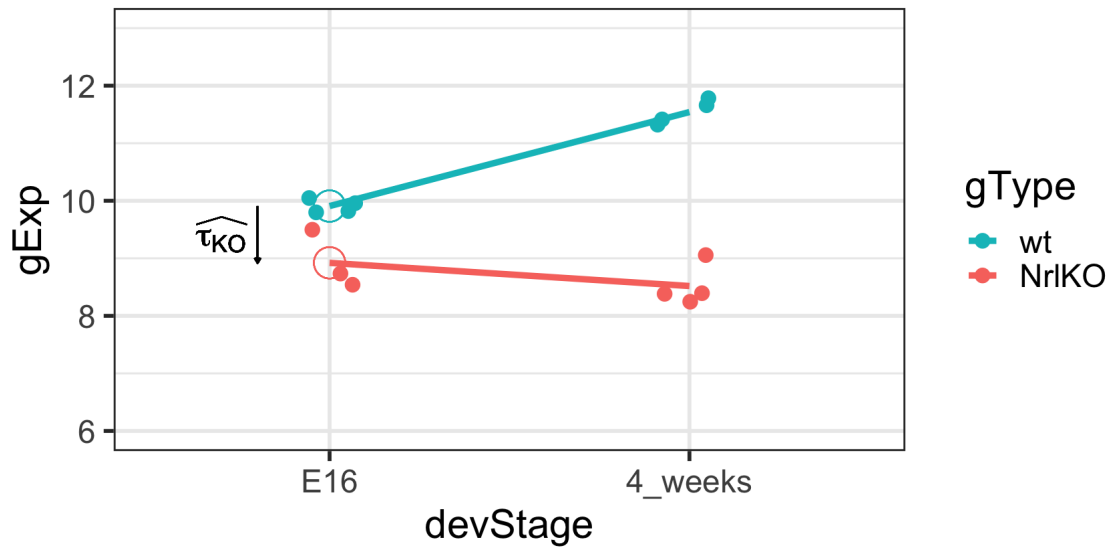
```
##             grp cellMeans  txEffects      lmEst
## 1        wt.E16  9.908000  0.0000000  9.9080000
## 2     NrlKO.E16  8.922333 -0.9856667 -0.9856667
## 3    wt.4_weeks 11.542500  1.6345000  1.6345000
## 4 NrlKO.4_weeks  8.518750 -1.3892500 -2.0380833
```

In general, one is not interested in: $H_0 : \theta = 0$

Simple genotype effect: wt *vs* NrlKO at E16

And now the "treatment effects"...

> **Important**: effects are not marginal but *conditional* effects (at a given level of the other factor, e.g., at E16), usually called **simple effects**



Simple genotype effect: wt *vs* NrlKO at E16

Effect of genotype at E16: $\tau_{KO} = E[Y_{NrlKO,E16}] - E[Y_{wt,E16}]$

`lm` estimate: $\hat{\tau}_{KO}$ is the *difference* of sample respective means (check below)

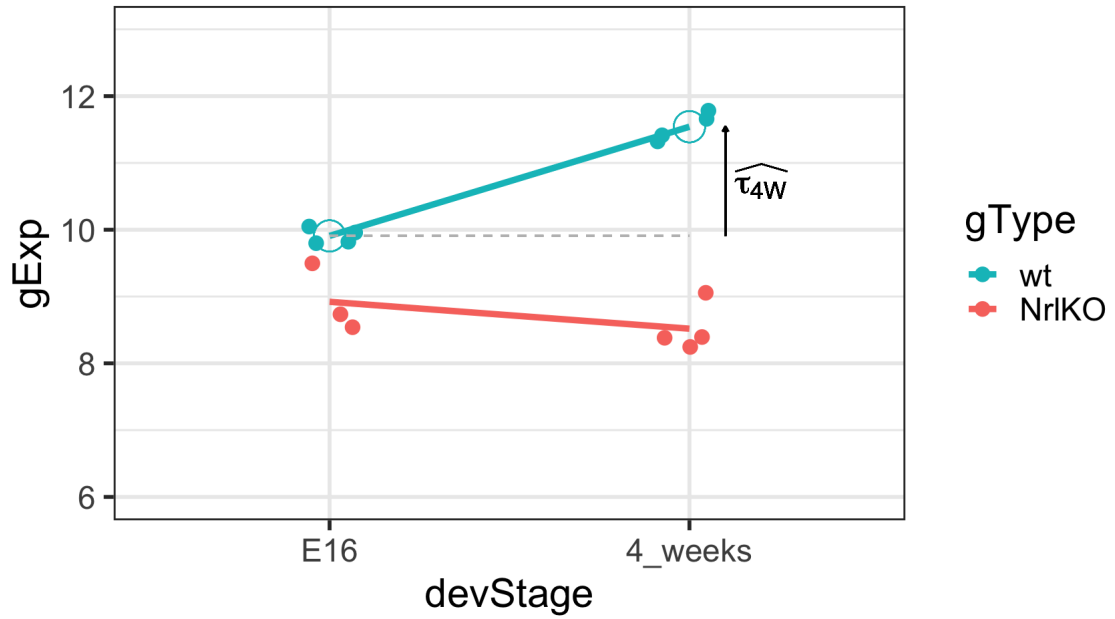
```
##                              Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)                 9.9080000  0.1574912 62.911469 2.027211e-15
## gTypeNrlKO                 -0.9856667  0.2405717 -4.097184 1.767824e-03
## devStage4_weeks             1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4_weeks -2.0380833  0.3278440 -6.216626 6.560671e-05
```

```
##             grp cellMeans  txEffects      lmEst
## 1        wt.E16  9.908000  0.0000000  9.9080000
## 2     NrlKO.E16  8.922333 -0.9856667 -0.9856667
## 3    wt.4_weeks 11.542500  1.6345000  1.6345000
## 4 NrlKO.4_weeks  8.518750 -1.3892500 -2.0380833
```

But, do you want to test the *conditional* effect at E16: $H_0 : \tau_{KO} = 0$??

Simple developmental effect: E16 *vs* 4W in wt

Similarly, for the other factor:



Simple developmental effect: E16 *vs* 4W at wt

Effect of development in wt: $\tau_{4W} = E[Y_{wt,4W}] - E[Y_{wt,E16}]$

`lm` estimate: $\hat{\tau}_{4W}$ is the *difference* of respective sample means (check below)

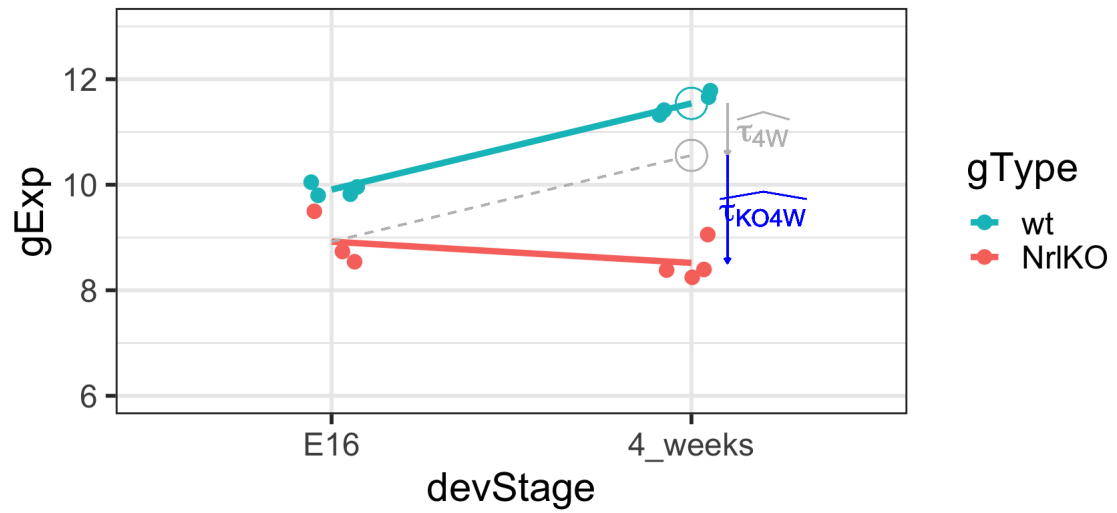
```
##                              Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)                 9.9080000  0.1574912 62.911469 2.027211e-15
## gTypeNrlKO                 -0.9856667  0.2405717 -4.097184 1.767824e-03
## devStage4_weeks             1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4_weeks -2.0380833  0.3278440 -6.216626 6.560671e-05

##             grp cellMeans  txEffects      lmEst
## 1        wt.E16  9.908000  0.0000000  9.9080000
## 2     NrlKO.E16  8.922333 -0.9856667 -0.9856667
## 3    wt.4_weeks 11.542500  1.6345000  1.6345000
## 4 NrlKO.4_weeks  8.518750 -1.3892500 -2.0380833
```

Interaction effect

Is the effect of genotype the same at different developmental stages? (or is the development effect the same for both genotypes?)

Yes if, there's no interaction effect, i.e., $\tau_{KO4W} = 0$



The genotype effect at E16 is τ_{KO}. However, τ_{KO} does not seem to be the effect at 4W. The difference is the interaction effect!

Interaction effect

$$\tau_{KO4W} = (E[Y_{NrlKO,4W}] - E[Y_{wt,4W}]) - (E[Y_{NrlKO,E16}] - E[Y_{wt,E16}])$$

```
##                              Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)                 9.9080000  0.1574912 62.911469 2.027211e-15
## gTypeNrlKO                 -0.9856667  0.2405717 -4.097184 1.767824e-03
## devStage4_weeks             1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4_weeks -2.0380833  0.3278440 -6.216626 6.560671e-05
```

```
means.2Fact
```

```
##             grp cellMeans  txEffects      lmEst
## 1        wt.E16  9.908000  0.0000000  9.9080000
## 2     NrlKO.E16  8.922333 -0.9856667 -0.9856667
## 3    wt.4_weeks 11.542500  1.6345000  1.6345000
## 4 NrlKO.4_weeks  8.518750 -1.3892500 -2.0380833
```

```
((means.2Fact$cellMeans[4]-means.2Fact$cellMeans[3])-
    (means.2Fact$cellMeans[2]-means.2Fact$cellMeans[1]))
```

```
## [1] -2.038083
```

Summary of model parameters: with interaction

model parameter	R estimate	stats	interpretation
θ	(Intercept)	$E[Y_{wt,E16}]$	reference
τ_{KO}	gTypeNrlKO	$E[Y_{NrlKO,E16}] - E[Y_{wt,E16}]$	*conditional* effect of NrlKO *at* E16
τ_{4W}	devStage4_weeks	$E[Y_{wt,4W}] - E[Y_{wt,E16}]$	*conditional* effect of 4W *at* wt
τ_{KO4W}	gTypeNrlKO: devStage4_weeks	$E[Y_{NrlKO,4W}] - E[Y_{wt,4W}] - \tau_{KO}$	*interaction* effect of NrlKO and 4W

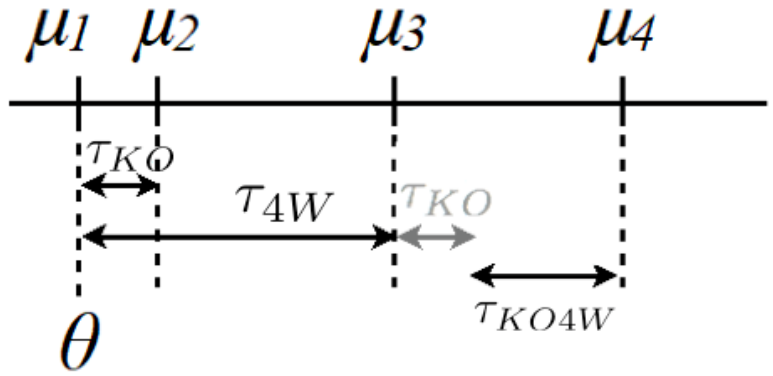
It is *important* to remember that `lm` reports *simple, not main* effects!! why?? because of the parametrization used!! (see see math handout)

It can also be shown that $\tau_{KO4W} = E[Y_{NrlKO,4W}] - \tau_{4W} - \tau_{KO} - \theta$ (see previous slide)

Let's examine these parameters closer and some examples

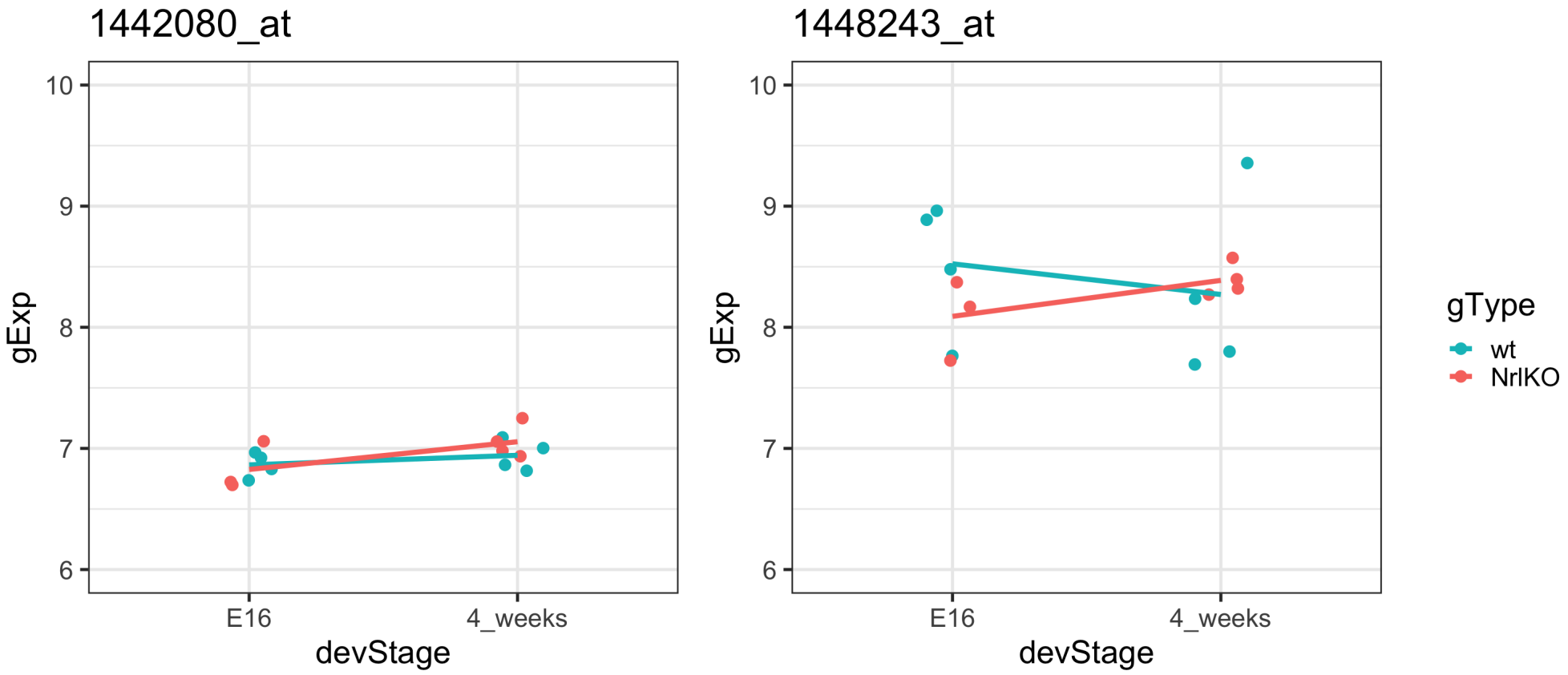
For our model, `lm` tests 4 hypotheses:

> $H_0 : \theta = 0$
>
> $H_0 : \tau_{KO} = 0$
>
> $H_0 : \tau_{4W} = 0$
>
> $H_0 : \tau_{KO4W} = 0$



We may not be interested in these hypotheses, e.g., τ_{KO} and τ_{4W} are *conditional* effects *at* a given level of a factor (*simple effects*)

Example 1: nothing is statistically significant, very flat genes



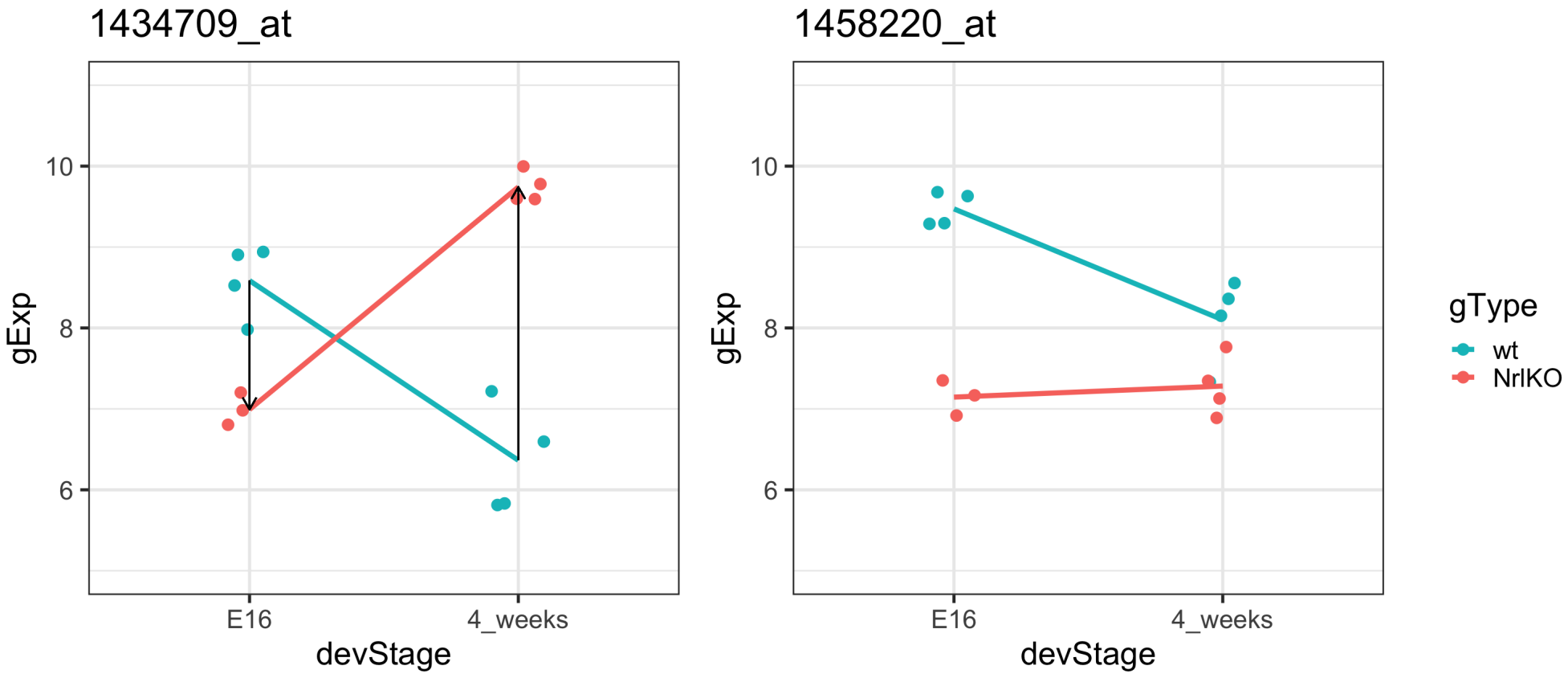
Example 1: nothing is statistically significant, very flat genes

Summary of `lm` for the gene in the left plot on previous slide:

```
multFit <- lm(gExp ~ gType * devStage, subset(egDat,gene=="1448243_at"))
summary(multFit)$coeff
```

```
##                               Estimate Std. Error    t value     Pr(>|t|)
## (Intercept)                  8.5240000  0.2561305 33.2799114 2.154028e-12
## gTypeNrlKO                  -0.4336667  0.3912458 -1.1084251 2.913251e-01
## devStage4_weeks             -0.2532500  0.3622232 -0.6991545 4.989723e-01
## gTypeNrlKO:devStage4_weeks   0.5504167  0.5331781  1.0323317 3.240804e-01
```

Example 2: statistically significant interaction effect: non-parallel



Example 2: statistically significant interaction effect: non-parallel

Summary of `lm` for the gene in the left plot on previous slide:

```
multFit <- lm(gExp ~ gType * devStage, subset(egDat,gene=="1434709_at"))
summary(multFit)$coeff
```

```
##                              Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)                   8.58550  0.2215161 38.757897 4.083165e-13
## gTypeNrlKO                   -1.59250  0.3383715 -4.706366 6.434783e-04
## devStage4_weeks              -2.22075  0.3132711 -7.088907 2.021975e-05
## gTypeNrlKO:devStage4_weeks    4.96975  0.4611226 10.777502 3.481389e-07
```

When the interaction effect is significant, the *simple* effects may not agree: compare the genotype effect @E16 with that @4W!

Main effects (overall): does genotype have an effect on gene expression? we can't answer this question! it depends (on the level of devStage)! (more later)

Example 3: BALANCED & only genotype @E16 is statistically significant

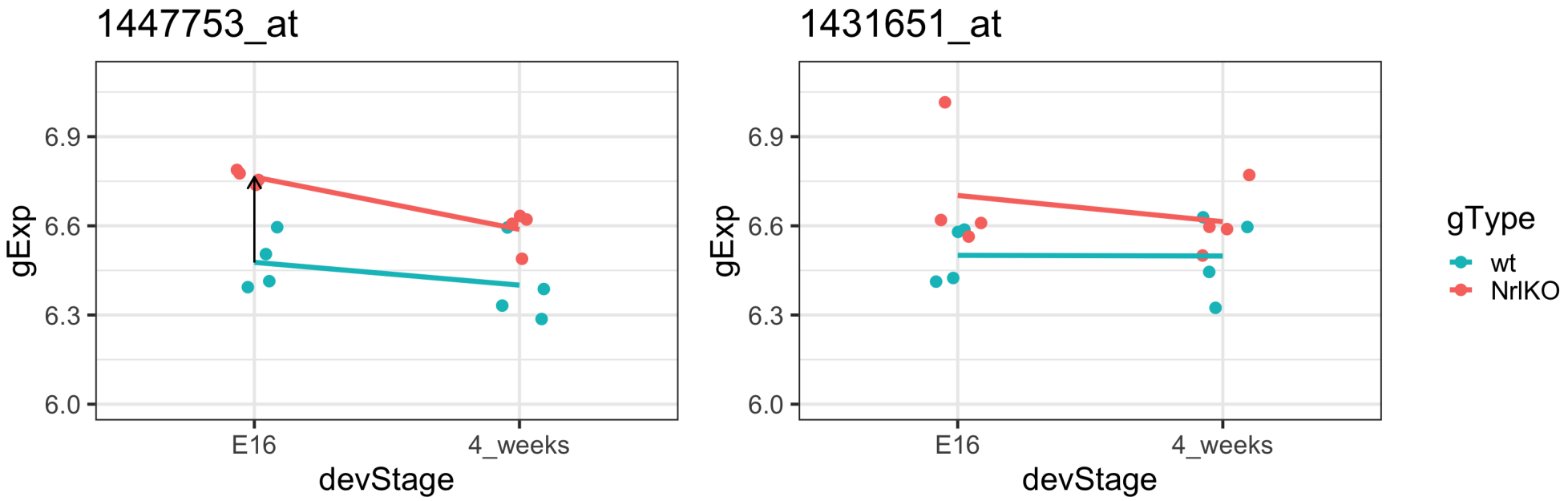
For simplicity here, I've added a random observation in the NrlKO.E16 group (close to its mean) to have a *balanced* design

> In *unbalanced* designs the *main* effects are a *weighted* average of the simple effects, and the weights are not easy to interpret (beyond the scope of this course but worth noting the issue!)

```
egDat<-prDat %>% subset(row.names(prDat) %in%
       c("1447753_at","1431651_at")) %>%
       tibble::rownames_to_column(var = "gene")%>%
       gather(sidChar, gExp,-gene) %>%
       inner_join(prDes,by="sidChar") %>%
       mutate(grp=interaction(gType, devStage))

#duplicate sample 6 and add noise to gExp of genes
set.seed(123)
egDat <-egDat %>% subset(sidChar=="Sample_6") %>%
       mutate(sidChar="Sample_r",sidNum="r",
       gExp=gExp+rnorm(2,0,.1)) %>% rbind(egDat) %>%
       arrange(grp)
```

Example 3: only genotype @E16 is statistically significant: parallel and flat



- The interaction effect is not significant (almost parallel pattern).
- The effect of developmental stage is not significant for WT (almost flat pattern).

Example 3: only genotype @E16 is statistically significant: parallel

```
multFit <- lm(gExp ~ gType * devStage, subset(egDat,gene=="1447753_at"))
summary(multFit)$coeff
```

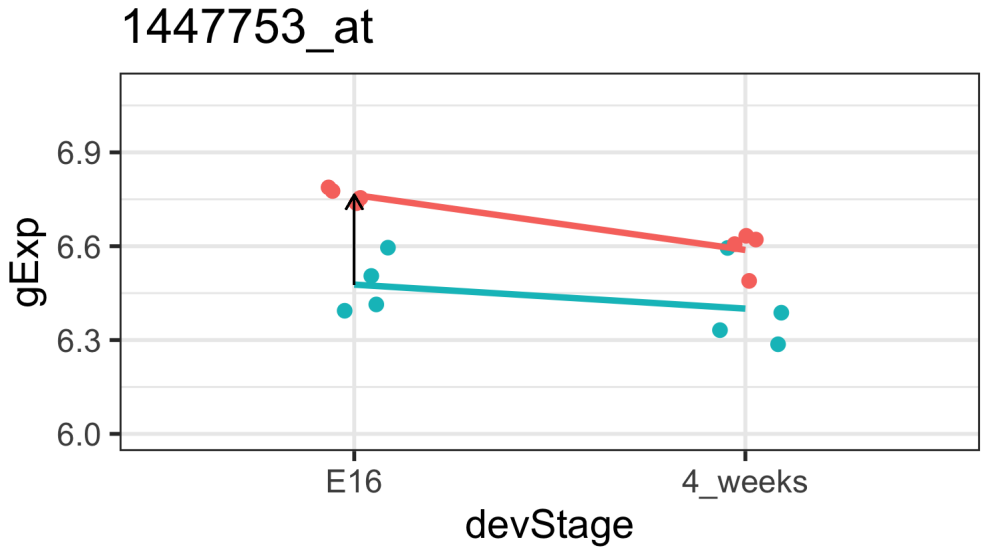
```
##                                Estimate Std. Error    t value     Pr(>|t|)
## (Intercept)                 6.47725000 0.04513333 143.513681 8.795702e-21
## gTypeNrlKO                  0.28699556 0.06382816   4.496378 7.312501e-04
## devStage4_weeks            -0.07675000 0.06382816  -1.202447 2.523805e-01
## gTypeNrlKO:devStage4_weeks -0.09949556 0.09026666  -1.102240 2.919732e-01
```

- There is a genotype effect at E16
- There may be a genotype effect *regardless* of the developmental stage (*main* effect). However, that hypothesis is *not* tested here!!
- How do we test a *main effect*??

How do we test the *main* effects?

The main effect measures the *overall* association between the response and a factor. They are the (weighted) average of an effect over the levels of the other factor

> **Note**: a significant interaction means that the effect of a factor depends on the level of the other one. Thus, main effects may mask interesting results!



Main effects

`anova()` can be used to test the main effects:

$$H_0 : ((E[Y_{KO,E16}] - E[Y_{wt,E16}]) + (E[Y_{KO,4W}] - E[Y_{wt,4W}]))/2 = 0$$

> for unbalanced experiments $H_0 : w_1 \text{effect}_{E16} + w_2 \text{effect}_{4W} = 0$

Main effects

```
tidy(anova(lm(gExp ~ gType * devStage, plot1Dat)))
```

```
## # A tibble: 4 x 6
##   term              df   sumsq  meansq statistic   p.value
##   <chr>          <int>   <dbl>   <dbl>     <dbl>     <dbl>
## 1 gType              1 0.225   0.225       27.6   0.000202
## 2 devStage           1 0.0640  0.0640       7.86  0.0160
## 3 gType:devStage     1 0.00990 0.00990      1.21  0.292
## 4 Residuals         12 0.0978  0.00815     NA    NA
```

As we suspected in slide #35, there is a significant genotype effect for this gene (1447753_at), i.e., its mean expression changes in NrlKO group (compared to wt), on average over developmental stages.

* Note: `anova` uses type II sums of squares, which follows the principle of marginality, thus order matters in unbalanced designs!

Main & interaction effects: important notes

- A **significant interaction effect** means that the effect of one factor depends on the levels of the other one.
    - e.g., the effect of genotype depends on developmental stage
- **Main effects**: are the (weighted) average of an effect over the levels of the other factor.
- A **non-significant main effect** means that, on average, there's no evidence of a factor's effect
    - e.g, no evidence of a genotype effect, on average over both developmental stages
- **Note of caution**: if the interaction is significant, it is possible that one or both simple effects are significant but the average effect (i.e., the main effect) is not. This is because the effect of a factor *depends on* the level of the other one!

Additive models

- In some applications, we need to/want to test the interaction term
- However, additive models are easier and smaller
- If there are no statistical or theoretical grounds to include the interaction term, additive models are preferred
- Additive effects: $E[Y_{NrlKO,4W}] - E[Y_{wt,E16}] = \tau_{KO} + \tau_{4W}$

```
addFit <- summary(lm(formula = gExp ~ gType +devStage,plot1Dat))$coeff
addFit
```

```
##                   Estimate Std. Error    t value     Pr(>|t|)
## (Intercept)      6.5021239  0.0394084 164.993346 5.609641e-23
## gTypeNrlKO       0.2372478  0.0455049   5.213675 1.670701e-04
## devStage4_weeks -0.1264978  0.0455049  -2.779872 1.561988e-02
```

* gene=1447753_at in table

Additive models and balanced designs

- In an additive model, the parameters are **average effects**, over the levels of the other factor. Now, same as in anova()!!

> Note the agreement!! This is gone in unbalanced designs since weights are computed differently! try!!

- TypeIII sum of squares are required for agreement in unbalanced designs (use `car::Anova`), beyond our scope

Parameters in additive models represent main effects

```
summary(lm(formula = gExp ~ gType + devStage, plot1Dat))$coeff
```

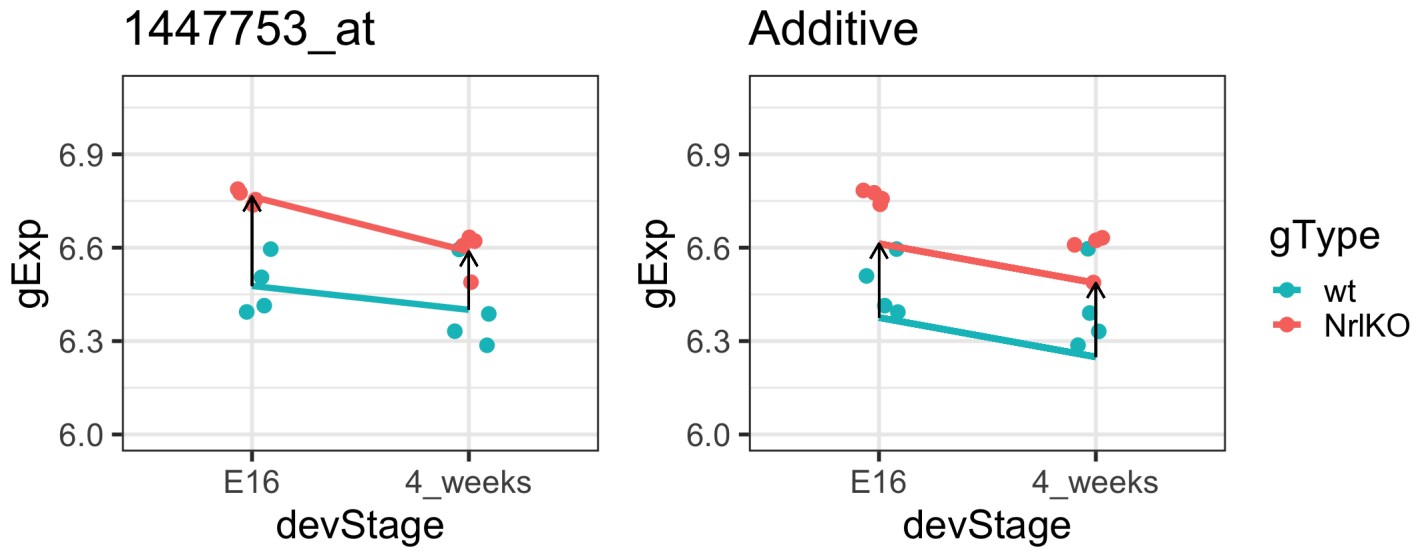
```
##                   Estimate Std. Error    t value     Pr(>|t|)
## (Intercept)      6.5021239  0.0394084 164.993346 5.609641e-23
## gTypeNrlKO       0.2372478  0.0455049   5.213675 1.670701e-04
## devStage4_weeks -0.1264978  0.0455049  -2.779872 1.561988e-02
```

```
summary(lm(formula = gExp ~ gType + devStage, plot1Dat))$coeff[2,3]^2
```

```
## [1] 27.18241
```

```
tidy(anova(lm(gExp ~ gType + devStage, plot1Dat)))
```

```
## # A tibble: 3 x 6
##   term         df  sumsq  meansq statistic   p.value
##   <chr>     <int>  <dbl>   <dbl>     <dbl>     <dbl>
## 1 gType         1 0.225  0.225       27.2   0.000167
## 2 devStage      1 0.0640 0.0640       7.73  0.0156
## 3 Residuals    13 0.108  0.00828     NA    NA
```




```
multEst
```

```
##                 (Intercept)                   gTypeNrlKO
##                  6.47725000                   0.28699556
##             devStage4_weeks gTypeNrlKO:devStage4_weeks
##                 -0.07675000                  -0.09949556
```

```
addEst
```

```
##     (Intercept)      gTypeNrlKO devStage4_weeks
##       6.5021239       0.2372478      -0.1264978
```

Factors with multiple levels

We can generalize the regression model to factors with more levels (e.g., E16, P2, P10 and 4W): we just add more dummy variables (and parameters)!

> With interaction

```
##                              Estimate Std. Error    t value     Pr(>|t|)
## (Intercept)                 5.43325000  0.1240289 43.8063081 4.740219e-28
## gTypeNrlKO                  0.25108333  0.1894573  1.3252764 1.954265e-01
## devStageP2                  0.39900000  0.1754034  2.2747562 3.049627e-02
## devStageP6                  0.19525000  0.1754034  1.1131483 2.747868e-01
## devStageP10                 0.92000000  0.1754034  5.2450520 1.283680e-05
## devStage4_weeks             3.96125000  0.1754034 22.5836544 5.952464e-20
## gTypeNrlKO:devStageP2      -0.22583333  0.2581868 -0.8746896 3.889296e-01
## gTypeNrlKO:devStageP6       0.06041667  0.2581868  0.2340037 8.166263e-01
## gTypeNrlKO:devStageP10     -0.20733333  0.2581868 -0.8030361 4.284868e-01
## gTypeNrlKO:devStage4_weeks -0.69333333  0.2581868 -2.6853939 1.185648e-02
```

Note that all the `devStage` parameters are still *simple* effects, but we now have more: one for each level compared to the reference

Factors with multiple levels (cont.)

> Without interaction: additive

```
##                   Estimate Std. Error    t value     Pr(>|t|)
## (Intercept)     5.52731618 0.11010606 50.1999257 9.574287e-33
## gTypeNrlKO      0.03159559 0.08783425  0.3597183 7.213497e-01
## devStageP2      0.30176103 0.14182289  2.1277315 4.091897e-02
## devStageP6      0.24113603 0.14182289  1.7002617 9.848949e-02
## devStageP10     0.83201103 0.14182289  5.8665498 1.428982e-06
## devStage4_weeks 3.63026103 0.14182289 25.5971450 2.412597e-23
```

Parameters are now *main* effects (on average over the levels of the other factor) but we have more!

Is developmental stage a significant effect? We haven't tested that!!

Simultaneous hypotheses again

We generally test two types of null hypotheses:

$$H_0 : \tau_j = 0$$

vs

$$H_0 : \tau_j \neq 0$$

for each *j* individually

e.g., Is gene A differentially expressed 2 days after birth compared to E16?

$$H_0 : \tau_{P2} = 0$$

$$H_0 : \tau_j = 0$$

vs

$$H_0 : \tau_j \neq 0$$

for all *j* at the same time

e.g., Is gene A significantly affected by time (`devStage`)?

$$H_0 : \tau_{P2} = \tau_{P6} = \tau_{P10} = \tau_{4W} = 0$$

F-test and overall significance: a deja vu

- the *t*-test in linear regression allows us to test single hypotheses. Those are given in the summary of `lm`

$$H_0 : \tau_i = 0$$

$$H_A : \tau_j \neq 0$$

- but we often like to test multiple hypotheses *simultaneously*:

$$H_0 : \tau_{P2} = \tau_{P6} = \tau_{P10} = \tau_{4W} = 0 \; [\text{AND statement}]$$

$$H_A : \tau_j \neq 0 \text{ for at least one j } [\text{OR statement}]$$

  the *F*-test allows us to test such compound tests

Overall effects: compound tests

With interaction

> $H_0 : \tau_{KO} = 0$ (1 df)
> $H_0 : \tau_{P2} = \tau_{P6} = \tau_{P10} = \tau_{4W} = 0$ (at wt!, 4 df)
> $H_0 : \tau_{KOP2} = \tau_{KOP6} = \tau_{KOP10} = \tau_{KO4W} = 0$ (4 df)

```
tidy(anova(lm(gExp~gType*devStage,hitDat)))
```

```
## # A tibble: 4 x 6
##   term              df   sumsq  meansq statistic   p.value
##   <chr>          <int>   <dbl>   <dbl>     <dbl>     <dbl>
## 1 gType              1  0.0692  0.0692      1.12  2.98e- 1
## 2 devStage           4 71.0    17.8       289.    6.69e-23
## 3 gType:devStage     4  0.689   0.172       2.80  4.44e- 2
## 4 Residuals         29  1.78    0.0615     NA    NA
```

Tests of overall effects of a factor controlling for the other one

Overall effects: compound tests (cont.)

Without interaction

> $H_0 : \tau_{KO} = 0$ (1 df)
> $H_0 : \tau_{P2} = \tau_{P6} = \tau_{P10} = \tau_{4W} = 0$ (on average!, 4 df)

```
tidy(anova(lm(gExp~gType+devStage,hitDat)))
```

```
## # A tibble: 3 x 6
##   term         df   sumsq  meansq statistic   p.value
##   <chr>     <int>   <dbl>   <dbl>     <dbl>     <dbl>
## 1 gType         1  0.0692  0.0692     0.924  3.44e- 1
## 2 devStage      4 71.0    17.8      237.     8.40e-24
## 3 Residuals    33  2.47    0.0749    NA     NA
```

Tests of overall effects of a factor controlling for the other one

The t-test in `lm` and the F-test (1 df) in `anova` for gType are not equivalent here due to unbalancedness (order matters)

Nested models: These examples are just special cases of nested models

For example: does development have a significant effect on gene expression?

Compare the models with and without `devStage`!!

Model 1: gExp~gType

Model 2: gExp~gType + devStage

Mathematically:

Model 1: $Y_{ijk} = \theta + \tau_{KO} x_{KO,ijk} + \varepsilon$

Model 2:
$$Y_{ijk} = \theta + \tau_{KO} x_{KO,ijk} + \tau_{P2} x_{P2,ijk} + \tau_{P6} x_{P6,ijk} + \tau_{P10} x_{P10,ijk} + \tau_{4W} x_{4W,ijk} + \varepsilon$$

$$H_0 : \tau_{P2} = \tau_{P6} = \tau_{P10} = \tau_{4W} = 0$$

The $x_{DD,ijk}$ are dummy variables (see math handout)

More general!

F-test: selection of nested models

$$H_0 : \beta_{k+1} = \ldots = \beta_{k+p} = 0$$

$$F = \frac{\left(SS_{reduced} - SS_{full}\right)/p}{SS_{full}/(n-p-k-1)} \sim \mathcal{F}_{p,n-p-k-1}$$

Compares:

Model 1: $y_i = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_k x_{ik} + \varepsilon_i$ (reduced: **1+k** parameters)

versus

Model 2: $y_i = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_k x_{ik} + \ldots + \beta_{k+p} x_{i(k+p)} + \varepsilon_i$

(full: **1+k+p** parameters)

Nested models in R

```
addReduced <- lm(gExp ~ gType, data = hitDat)
addFull <- lm(gExp ~ gType+devStage, data = hitDat)
anova(addReduced,addFull)
```

```
## Analysis of Variance Table
##
## Model 1: gExp ~ gType
## Model 2: gExp ~ gType + devStage
##   Res.Df    RSS Df Sum of Sq      F    Pr(>F)
## 1     37 73.498
## 2     33  2.473  4    71.024 236.92 < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
tidy(anova(addFull))
```

```
## # A tibble: 3 x 6
##   term         df   sumsq  meansq statistic   p.value
##   <chr>     <int>   <dbl>   <dbl>     <dbl>     <dbl>
## 1 gType         1  0.0692  0.0692     0.924  3.44e- 1
## 2 devStage      4 71.0    17.8      237.     8.40e-24
## 3 Residuals    33  2.47    0.0749    NA     NA
```

Another special case: goodness of fit!

Compare the full *vs* the intercept-only models (compound test)!

$$H_0 : \tau_{KO} = \tau_{P2} = \tau_{P6} = \tau_{P10} = \tau_{4W} = 0, \ (5 \text{ df})$$

```
## Analysis of Variance Table
##
## Model 1: gExp ~ 1
## Model 2: gExp ~ gType + devStage
##   Res.Df    RSS Df Sum of Sq      F    Pr(>F)
## 1     38 73.567
## 2     33  2.473  5    71.094 189.72 < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(addFull)$fstatistic # also given in the summary of lm
```

```
##    value    numdf    dendf
## 189.7238   5.0000  33.0000
```

Goodness of fit also given in output of `lm`

```
summary(addFull)
```

```
## Call:
## lm(formula = gExp ~ gType + devStage, data = hitDat)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.80117 -0.12450 -0.03208  0.17062  0.50009
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)      5.52732    0.11011  50.200  < 2e-16 ***
## gTypeNrlKO       0.03160    0.08783   0.360   0.7213
## devStageP2       0.30176    0.14182   2.128   0.0409 *
## devStageP6       0.24114    0.14182   1.700   0.0985 .
## devStageP10      0.83201    0.14182   5.867 1.43e-06 ***
## devStage4_weeks  3.63026    0.14182  25.597  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2738 on 33 degrees of freedom
## Multiple R-squared:  0.9664,    Adjusted R-squared:  0.9613
## F-statistic: 189.7 on 5 and 33 DF,  p-value: < 2.2e-16
```

Summary

- ***t*-tests** can be used to test the equality of **2** population means
- **ANOVA** can be used to test the equality of **more than 2** population means simultaneously (main effects)
- **Linear regression** provides a general framework for modelling the relationship between a response and different type of explanatory variables
  - *t*-tests are used to test the significance of *simple effects* (*individual* coefficients)
  - *F*-tests are used to test the significance of *main effects* (*simultaneously* multiple coefficients)
- *F*-tests are used to compare nested models
  - e.g., *overall* effects or *goodness of fit*