Lecture 7 – Linear Models

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

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Recall from last class...

- 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

- write a linear model using matrix notation

  - understand which matrix is built by R

- distinguish between single and multiple hypotheses

  - t-tests vs F-tests

Quick review: from t-test to linear regression

HOW??

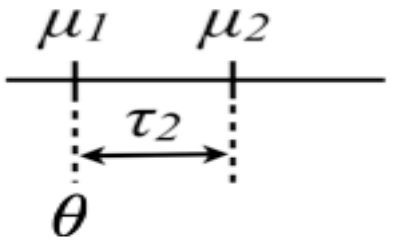
Changing the parametrization and using dummy variables

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

$$\downarrow$$

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

$$\downarrow$$



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

Using matrix notation ...

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

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

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

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

WHY??

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

This gives us a VERY FLEXIBLE framework!!

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

Parametrizations

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

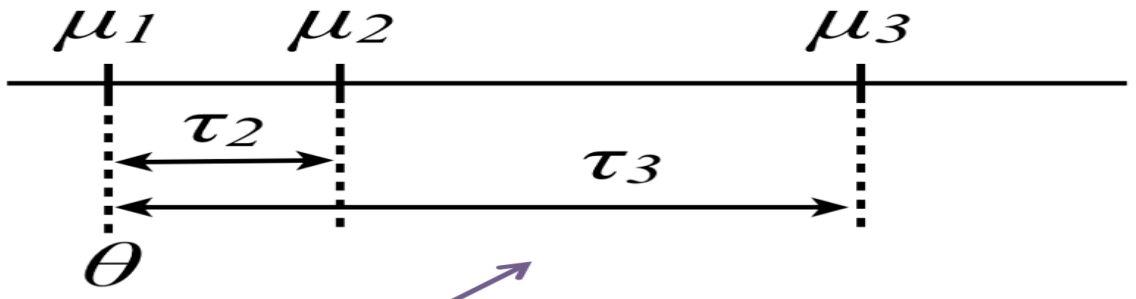
$$Y = [X\alpha] + \varepsilon$$

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!

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



These are **NOT population means**
These are **differences of means**

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

```
summary(lm(gExp~devStage,subset(devDat,gene=="theHit")))$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
## devStage4W  3.6325179  0.1398583 25.972843 5.266481e-24
```

Today... more complex models

- more than one factor with multiple levels

- how to model many categorical variables and their interaction

- distinguish between simple and main effects

- `lm` vs `anova` tests

- nested models

- t-tests vs F-tests

- continuous explanatory variables

- the regression line

Increasing the complexity of the linear model ...

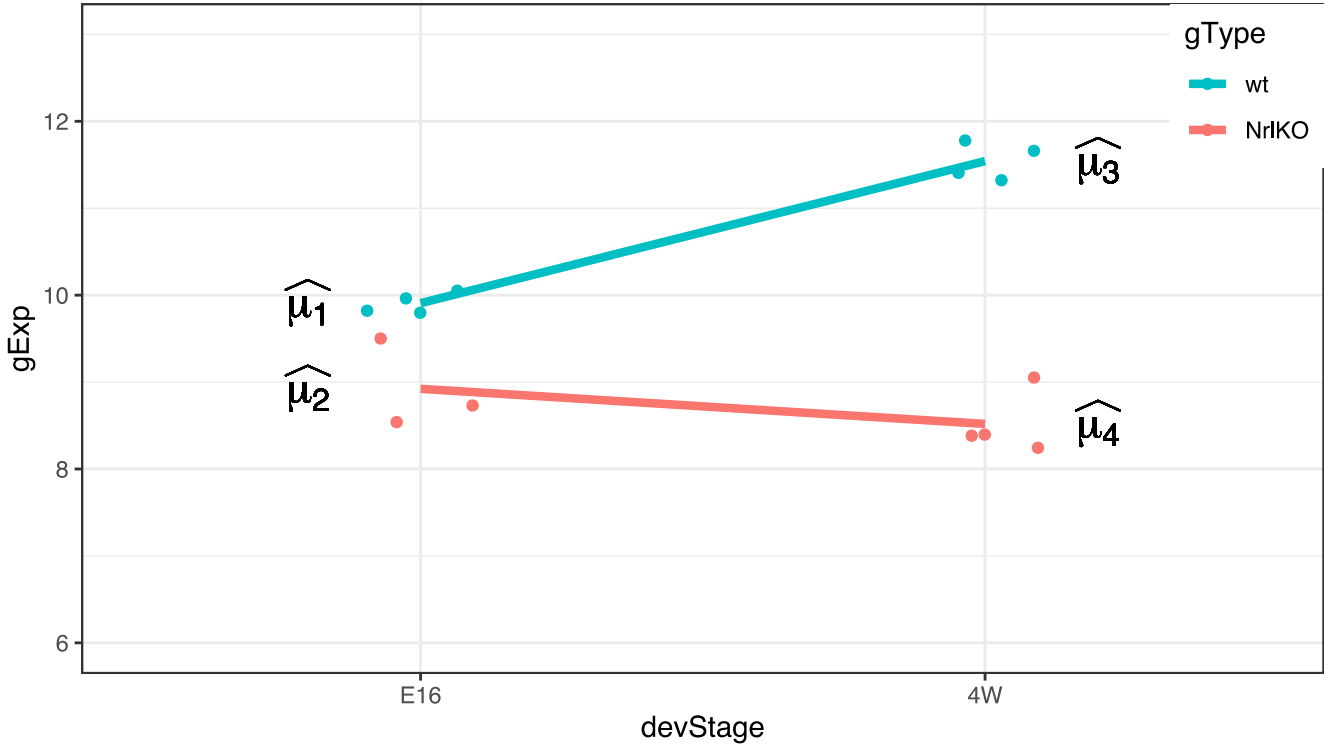
What if you have two categorical variables?

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)

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

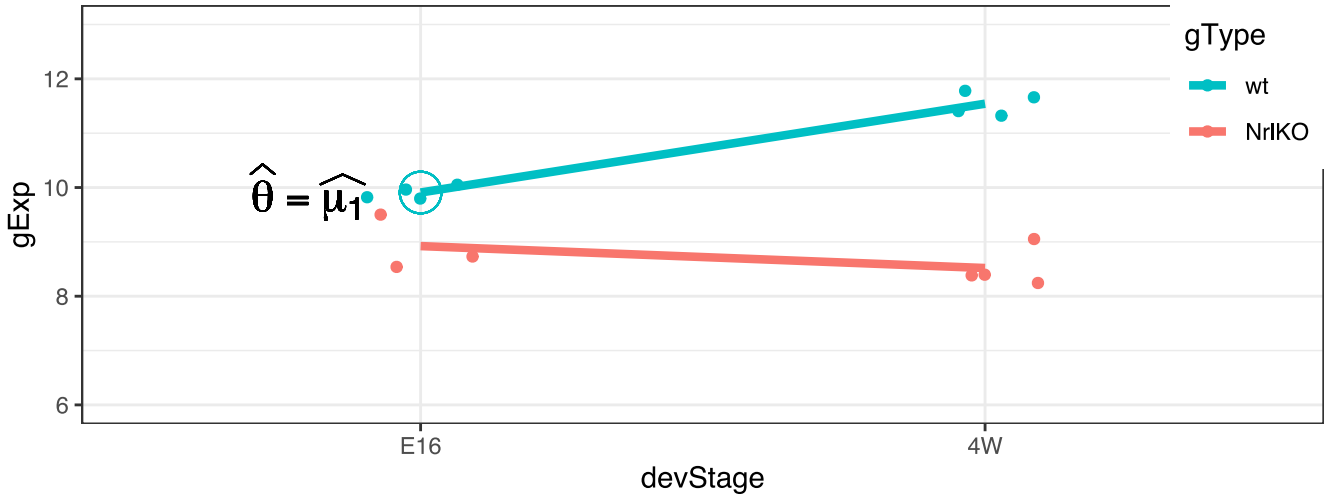
```
##                          Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)             9.9080000  0.1574912 62.911469 2.027211e-15
## gTypeNrlKO             -0.9856667  0.2405717 -4.097184 1.767824e-03
## devStage4W              1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4W -2.0380833  0.3278440 -6.216626 6.560671e-05
```

```
means.2Fact <- as.data.frame(twoDat %>%
          group_by(grp) %>% summarize(cellMeans=mean(gExp)))
(means.2Fact <-means.2Fact %>%
          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.4W 11.542500  1.6345000  1.6345000
```

The reference: 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
## devStage4W              1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4W -2.0380833  0.3278440 -6.216626 6.560671e-05
```

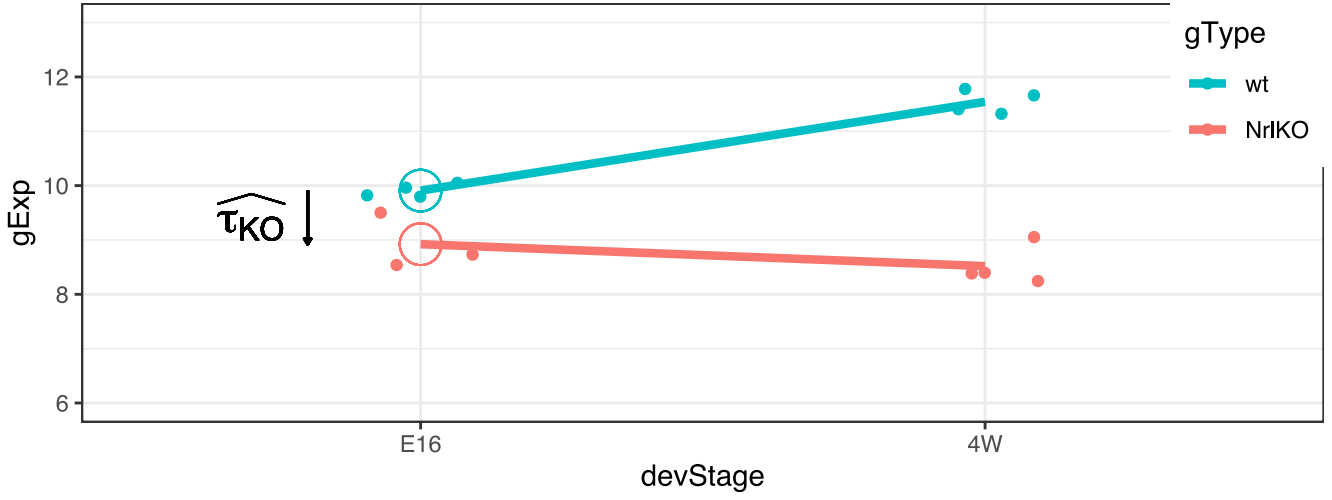
```
##         grp cellMeans  txEffects      lmEst
## 1    wt.E16  9.908000  0.0000000  9.9080000
## 2 NrlKO.E16  8.922333 -0.9856667 -0.9856667
## 3     wt.4W 11.542500  1.6345000  1.6345000
## 4  NrlKO.4W  8.518750 -1.3892500 -2.0380833
```

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)

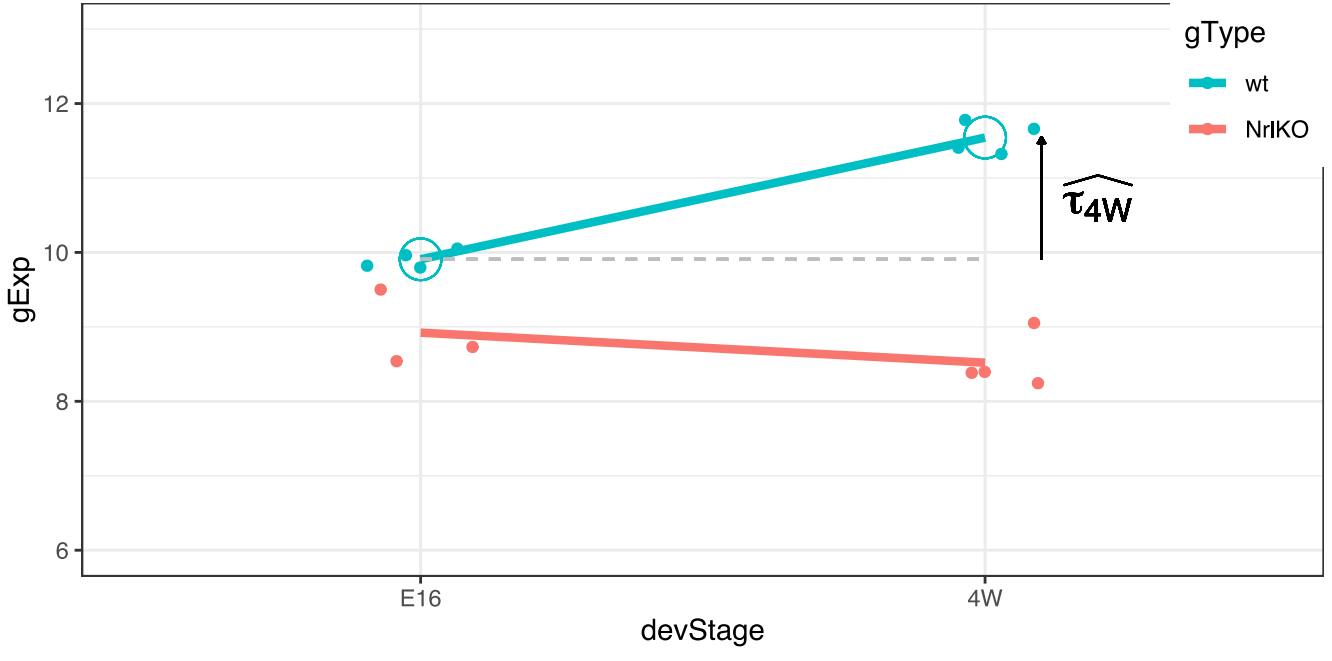
```
##                          Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)             9.9080000  0.1574912 62.911469 2.027211e-15
## gTypeNrlKO             -0.9856667  0.2405717 -4.097184 1.767824e-03
## devStage4W              1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4W -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.4W 11.542500  1.6345000  1.6345000
## 4  NrlKO.4W  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 at wt

Similarly, for the other factor:



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

Effect of development at 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)

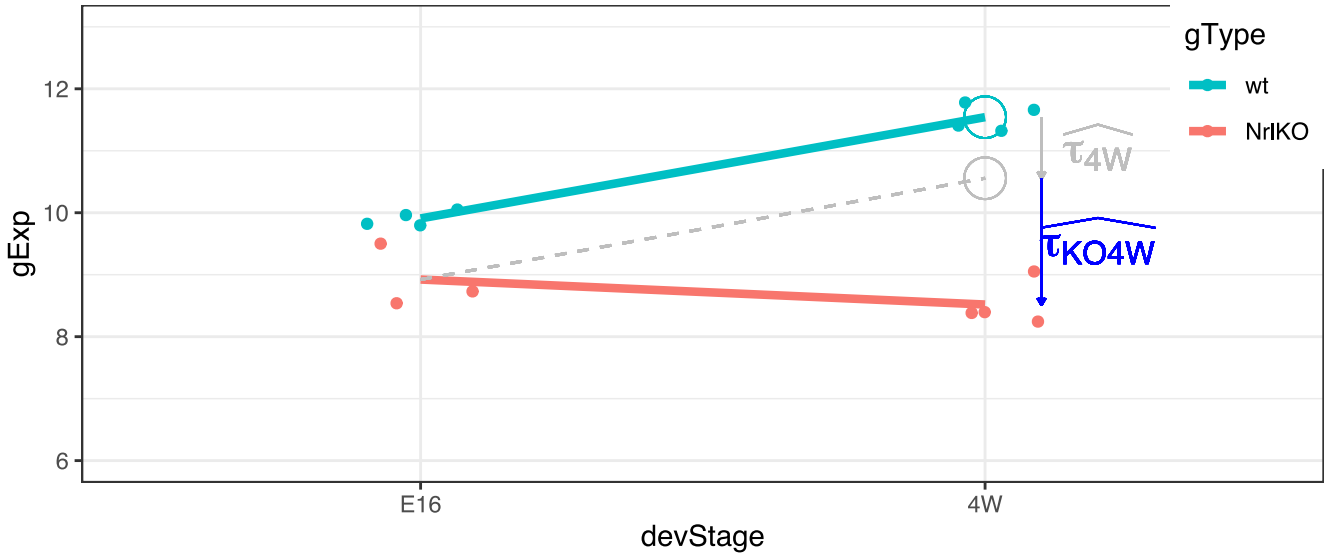
```
##                          Estimate Std. Error   t value     Pr(>|t|)
## (Intercept)             9.9080000  0.1574912 62.911469 2.027211e-15
## gTypeNrlKO             -0.9856667  0.2405717 -4.097184 1.767824e-03
## devStage4W              1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4W -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.4W 11.542500  1.6345000  1.6345000
## 4  NrlKO.4W  8.518750 -1.3892500 -2.0380833
```

Interaction effect

Is the effect of genotype the same at different developmental stages? (or does hte effect of development depend on genotype?)

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



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
## devStage4W              1.6345000  0.2227261  7.338609 1.469261e-05
## gTypeNrlKO:devStage4W -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.4W 11.542500  1.6345000  1.6345000
## 4  NrlKO.4W  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

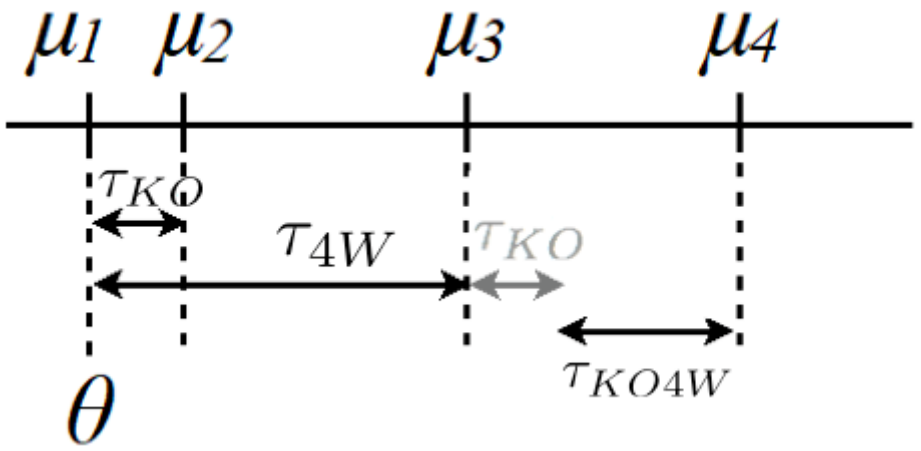
model parameter	R estimate	stats	interpretation
θ	(Intercept)	$E[Y_{wt,E16}]$	reference
τ_{KO}	gTypeNrlKO	$E[Y_{NrlKO,E16}] - E[Y_{wt,E16}]$	*conditional* effect of NrlKO *at* E16
τ_{4W}	devStage4_weeks	$E[Y_{wt,4W}] - E[Y_{wt,E16}]$	*conditional* effect of 4W *at* wt
τ_{KO4W}	gTypeNrlKO: devStage4_weeks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

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 *

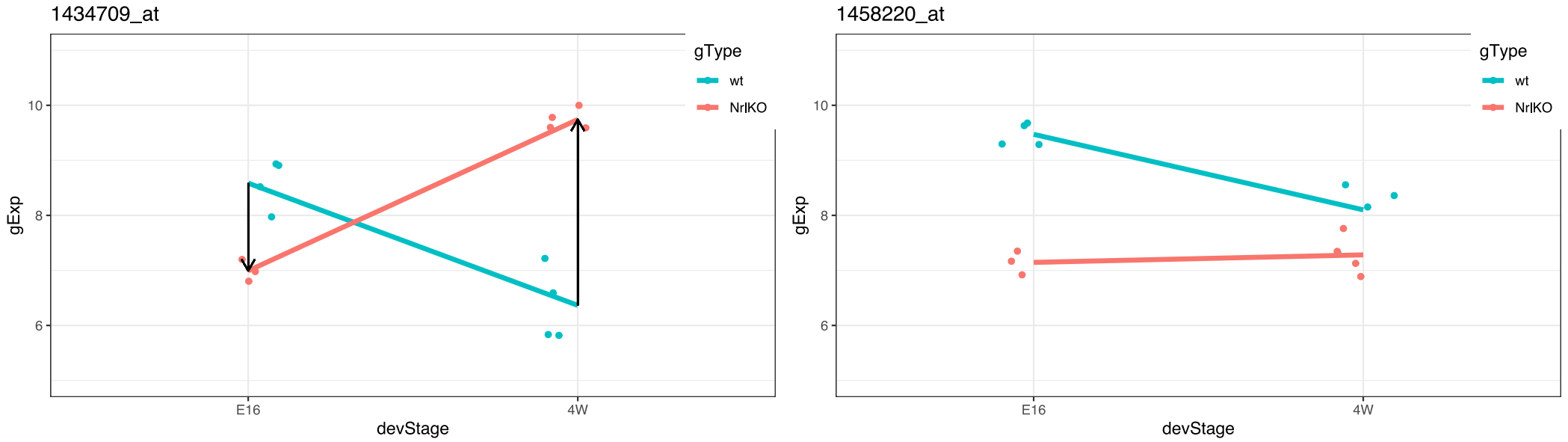
```
egDat<-prDat %>% subset(row.names(prDat) %in%
       c("1442080_at","1448243_at")) %>%
       tibble::rownames_to_column(var = "gene")%>%
       gather(sidChar, gExp,-gene) %>%
       inner_join(prDes,by="sidChar")
```

* Here and in next slides, summary of `lm` shown for the gene in the left plot

```
##                          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
## devStage4W             -0.2532500  0.3622232 -0.6991545 4.989723e-01
## gTypeNrlKO:devStage4W   0.5504167  0.5331781  1.0323317 3.240804e-01
```

Example 2: statistically significant interaction effect: non-parallel

```
##                        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
## devStage4W             -2.22075  0.3132711 -7.088907 2.021975e-05
## gTypeNrlKO:devStage4W   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!

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

To simplify future explanations, 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

The interaction effect is not significant (almost parallel pattern).

Thus, there may be a genotype effect *regardless* of the developmental stage (*main* effect). However, that hypothesis is *not* tested here!!

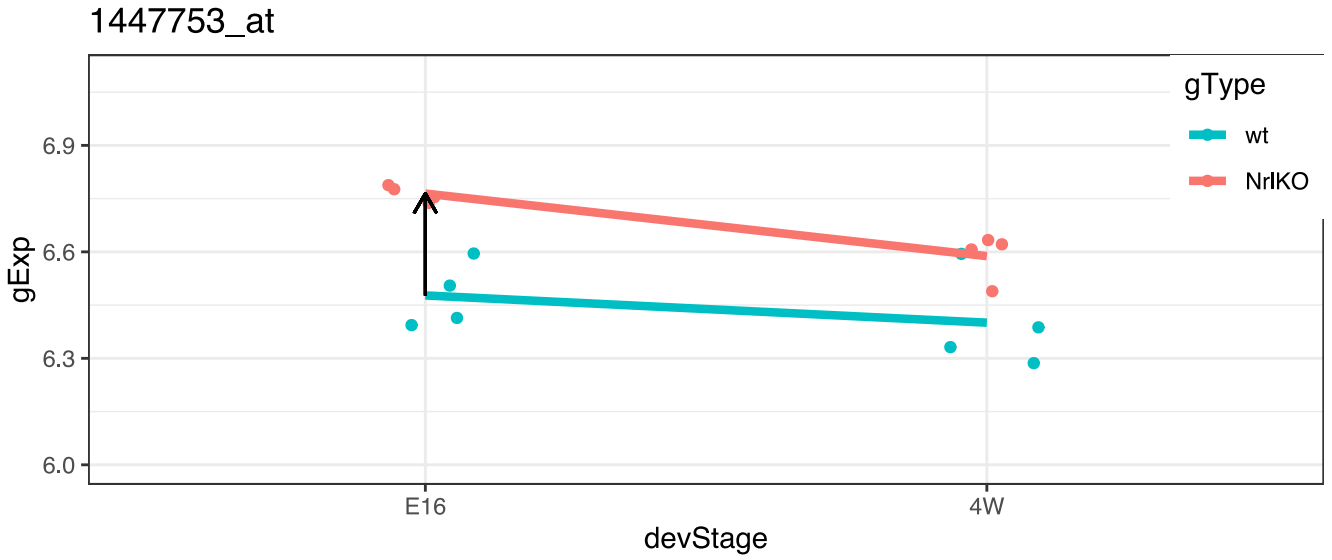
How do we test a *main effect*??

```
##                          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
## devStage4W            -0.07675000 0.06382816  -1.202447 2.523805e-01
## gTypeNrlKO:devStage4W -0.09949556 0.09026666  -1.102240 2.919732e-01
```

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

```
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 #26, 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 develpmental stages.

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 development
- **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

Additive models

- In some applications, we need 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
## devStage4W  -0.1264978  0.0455049  -2.779872 1.561988e-02
```

Additive models

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

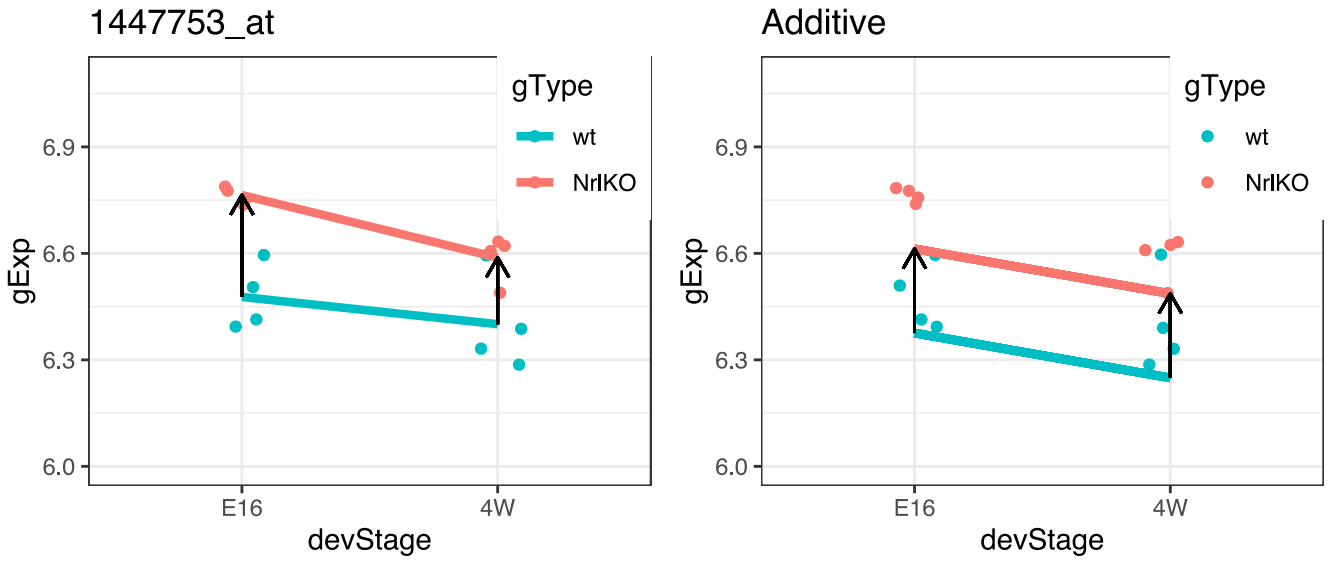
> Note the agreement!! This is gone in unbalanced designs since weights are computed differently! try!!

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

```
##               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
## devStage4W  -0.1264978  0.0455049  -2.779872 1.561988e-02
```

```
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>
```




```
multEst
```

```
##            (Intercept)             gTypeNrlKO             devStage4W
##             6.47725000             0.28699556            -0.07675000
## gTypeNrlKO:devStage4W
##            -0.09949556
```

```
addEst
```

```
## (Intercept)  gTypeNrlKO  devStage4W
##   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 additional 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
## devStage4W              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:devStage4W  -0.69333333  0.2581868 -2.6853939 1.185648e-02
```

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
## devStage4W  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 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 differencially expressed 2 days after birth?

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

$$H_0 : \tau_j = 0$$

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*:

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

$$H_A : \tau_i \neq 0 \text{ for some i [OR statement]}$$

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 due to unbalancedness

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

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

More general!

F-test: selection of nested models

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

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

Compares:

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

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
```

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

WHY??

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

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

Next class: linear models provides a general flexible framework to study the relation of a response with many variables!