Statistical Methods for High Dimensional Biology

STAT/BIOF/GSAT 540

Lecture 9 – Linear Models Part III

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Based on slides byDr. Jennifer Bryan

outline

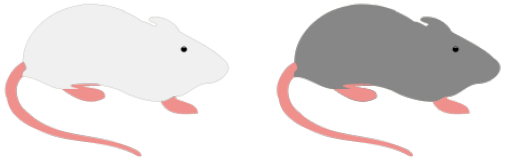
- Review of previous lecture

Developing mouse retina – time course for the experiment

So sample collections:

4 developmental stages
2 genotypes: wild-type , Nrl KO

NrlKO WT



Experimental design

devStage	wt	NrlKO
E16	4	3
P2	4	4
P6	4	4
P10	4	4
4_weeks	4	4

1) What are the genes whose expression levels differ between the two genotypes?
2) What are the genes whose expression levels differ across developmental stages?
3) What are the genes whose expression *trajectories* across development differs between the two genotypes?

Two-way ANOVA

Genotype effect

Interaction effect

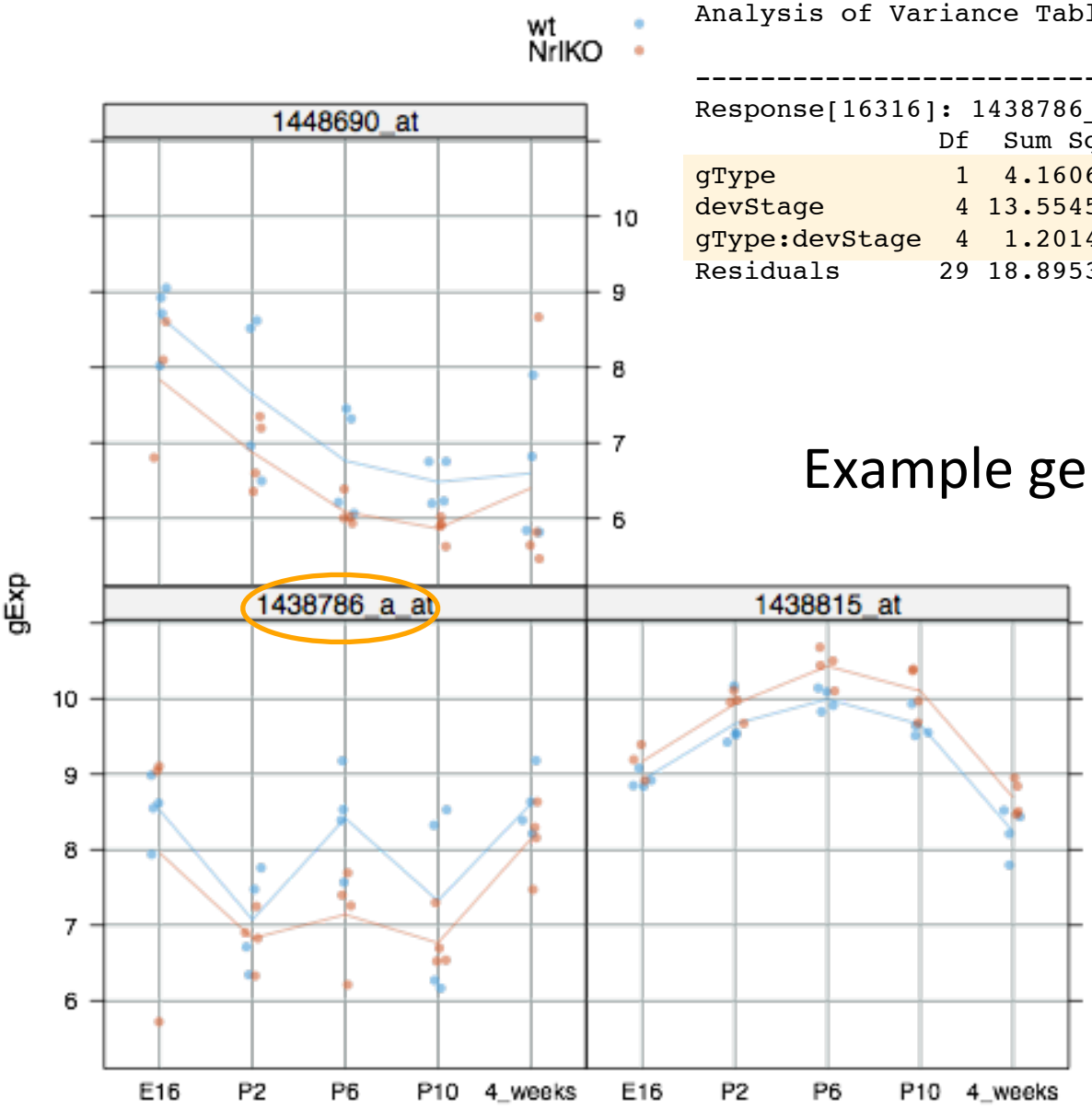
Gene i
Genotype j
Dev Stage k

$$y_{ijk} = \theta + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk}$$

Reference (i.e., intercept)

"devStage" effect

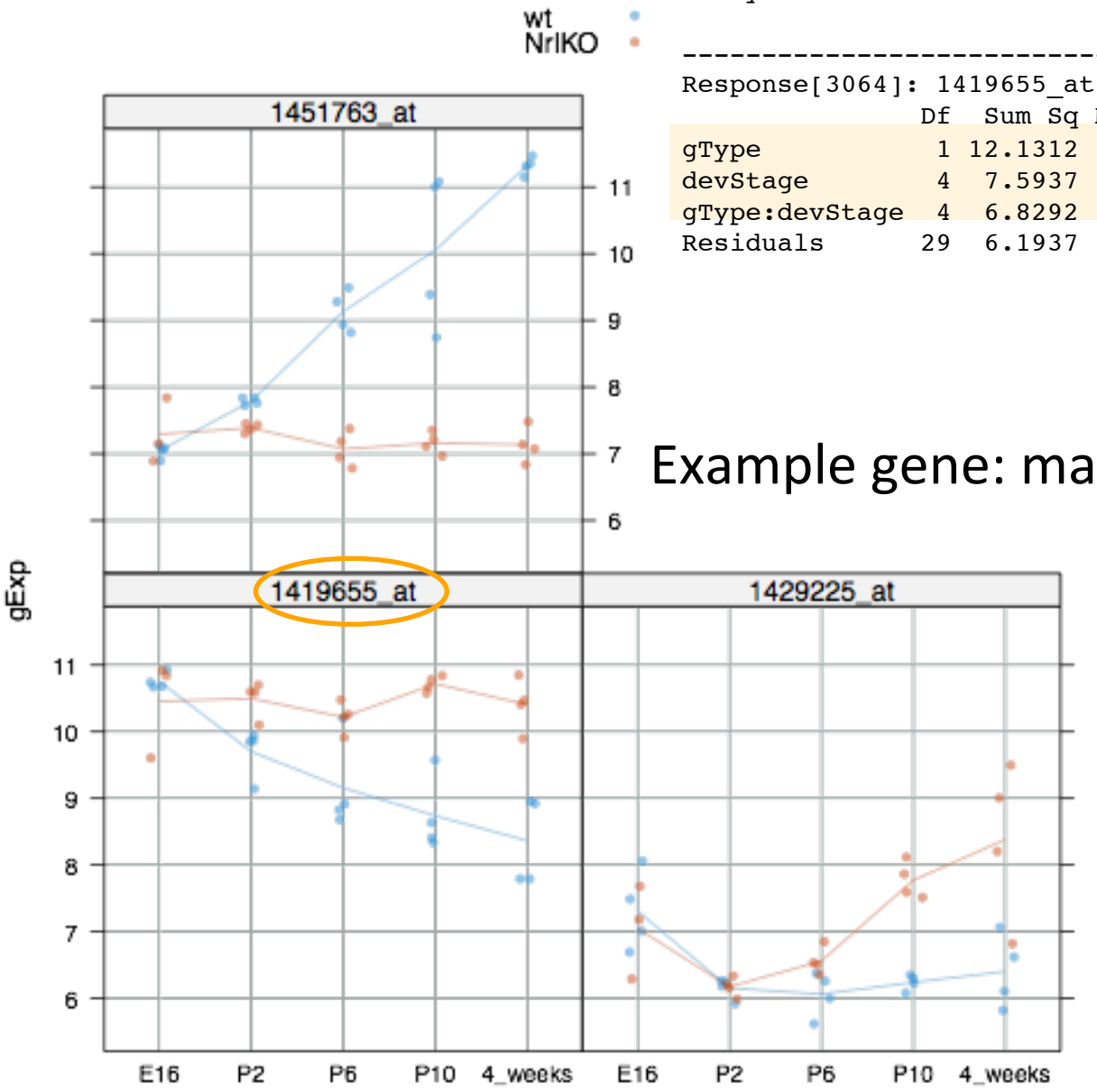
devStage / gType	E16	P2	P6	P10	4_weeks
wt	θ	β_{P2}	β_{P6}	β_{P10}	β_{4_weeks}
NrlKO	τ_{NrlKO}	$(\tau\beta)_{NrlKO,P2}$	$(\tau\beta)_{NrlKO,P6}$	$(\tau\beta)_{NrlKO,P10}$	$(\tau\beta)_{NrlKO,4\_weeks}$




```
Analysis of Variance Table

----------------------------
Response[16316]: 1438786_a_at
                Df  Sum Sq Mean Sq F value   Pr(>F)
gType            1  4.1606  4.1606  6.3855 0.017216 *
devStage         4 13.5545  3.3886  5.2008 0.002774 **
gType:devStage   4  1.2014  0.3003  0.4610 0.763712
Residuals       29 18.8953  0.6516
```

Example gene: only main effects




```
Analysis of Variance Table

-----------------------------
Response[3064]: 1419655_at
                 Df  Sum Sq Mean Sq F value    Pr(>F)
gType             1 12.1312 12.1312 56.8008 2.623e-08 ***
devStage          4  7.5937  1.8984  8.8888 8.210e-05 ***
gType:devStage    4  6.8292  1.7073  7.9939 0.0001798 ***
Residuals        29  6.1937  0.2136
```

Example gene: main and interaction effects

F tests in regression

small model is nested within big -- it's a special case where some parameters are equal to zero

model	example	# params = DF	RSS
small	lm(y ~ gType + devStage)	$p_{small} = 6$	RSS_{small}
big	lm(y ~ gType * devStage)	$p_{big} = 10$	RSS_{big}

$$y_{ijk} = \theta + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk} \quad \text{“big”}$$

$$y_{ijk} = \theta + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk} \quad \text{“small”}$$

F tests in regression

small model is nested within big -- it's a special case where some parameters are equal to zero

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$$y_{ijk} = \theta + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk} \quad \text{“small”}$$

by definition:

$p_{small} < p_{big}$

$RSS_{small} \geq RSS_{big}$

$$F = \frac{\left(\frac{RSS_{small} - RSS_{big}}{p_{big} - p_{small}} \right)}{\frac{RSS_{big}}{n - p_{big}}} \sim_{H_0} F_{(p_{big} - p_{small}, n - p_{big})}$$

Plugging the observed F into the null distribution for F with given DOF, gives us the tail probability under the null

Let’s talk about linear regression as a very general framework, how do we get the parameters?

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

$$\begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix} = \begin{bmatrix} 1 & x_1 \\ 1 & x_2 \\ \vdots & \vdots \\ 1 & x_n \end{bmatrix} \begin{bmatrix} \alpha_0 \\ \alpha_1 \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{bmatrix} = \begin{bmatrix} \alpha_0 \cdot 1 + \alpha_1 \cdot x_1 \\ \alpha_0 \cdot 1 + \alpha_1 \cdot x_2 \\ \vdots \\ \alpha_0 \cdot 1 + \alpha_1 \cdot x_n \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{bmatrix} = \begin{bmatrix} \alpha_0 + \alpha_1 x_1 + \varepsilon_1 \\ \alpha_0 + \alpha_1 x_2 + \varepsilon_2 \\ \vdots \\ \alpha_0 + \alpha_1 x_n + \varepsilon_n \end{bmatrix}$$

$$y_i = \alpha_0 + \alpha_1 x_i + \varepsilon_i$$

Here we are just fitting a line but using matrix notation to handle all *n* observations at once, more elegantly.

Big pay-offs ensue .....

Estimation of the parameter α

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

Two viewpoints:

- maximum likelihood estimation, assuming ε_i are iid $N(0, \sigma^2)$
- "ordinary least squares" (OLS), i.e. minimizing the sum of the squared residuals

both lead to the same estimator of α:

$$\hat{\alpha} = (X^T X)^{-1} X^T y = \min^{-1} \sum (y_i - x_i \alpha)^2$$

Linear regression: maximum likelihood estimation

$$Y = X\alpha + \varepsilon \qquad \varepsilon_i \sim N(0, \sigma^2)$$

$$P(Y \mid \theta) = N(Y \mid X\alpha, \sigma^2) = \frac{1}{2\sqrt{\pi}\sigma} \mathrm{e}^{(-\frac{(X\alpha - Y)^T (X\alpha - Y)}{2\sigma})}$$

Need to maximize the log of the likelihood function to solve for the parameter(s)

Linear regression: least squares formulation

- Minimize the *squared error* in prediction:

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

$$\underset{a}{\text{minimize}} \quad (Y - X\alpha)^T (Y - X\alpha)$$

Greatest Hits of Regression Results (normal iid errors)

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

$\hat{\alpha} = (X^T X)^{-1} X^T Y$ the MLE and OLS estimator of α

$\hat{Y} = X\hat{\alpha}$ the fitted or predicted values

$\hat{Y} = X(X^T X)^{-1} X^T Y = HY$ where $H = X(X^T X)^{-1} X^T$ is called the "hat matrix"

$\hat{\varepsilon} = Y - \hat{Y} = Y - X\hat{\alpha}$ the residuals (note NOT the same as the errors ε)

$\hat{\sigma}^2 = \frac{1}{n-p} \hat{\varepsilon}^T \hat{\varepsilon}$ the estimated error variance (p is the dimension of α)

$\hat{V}(\hat{\alpha}) = \hat{\sigma}^2 (X^T X)^{-1}$ the estimated covariance matrix of $\hat{\alpha}$

estimated standard errors for the estimated regression coefficients -- $\widehat{se}(\hat{\alpha}_j)$ -- are obtained by taking the square root of the diagonal elements of $\hat{V}(\hat{\alpha})$

Testing the significance of the parameter estimates in linear regression

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

How test $H_0 : \alpha_j = 0$?

With a t-statistic. Under H_0, we have (at least approximately) that:

$$\frac{\hat{\alpha}_j}{\widehat{se}(\hat{\alpha}_j)} \sim t_{n-p}$$

so a p-value is obtained by computing a tail probability for the observed value of $\hat{\alpha}_j$ from a t_{n-p} distribution.

$\hat{\sigma}^2 = \frac{1}{n-p}\hat{\varepsilon}^T\hat{\varepsilon}$ the estimated error variance (p is the dimension of α)

$\hat{V}(\hat{\alpha}) = \hat{\sigma}^2(X^TX)^{-1}$ the estimated covariance matrix of $\hat{\alpha}$

estimated standard errors for the estimated regression coefficients -- $\widehat{se}(\hat{\alpha}_j)$ --

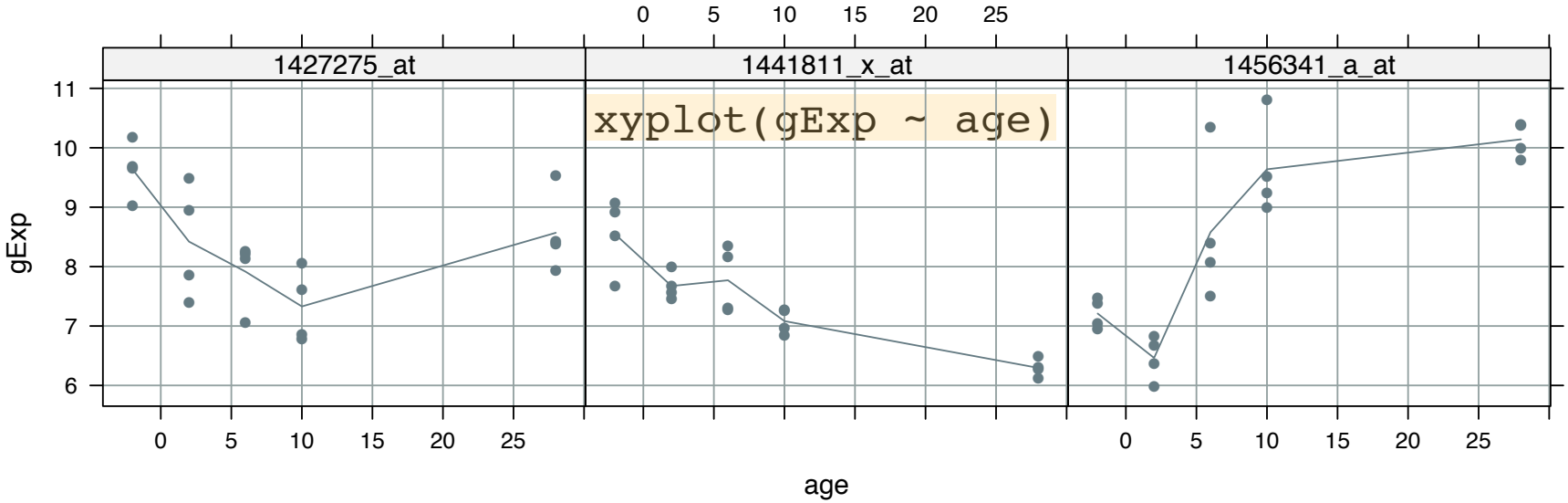
are obtained by taking the square root of the diagonal elements of $\hat{V}(\hat{\alpha})$

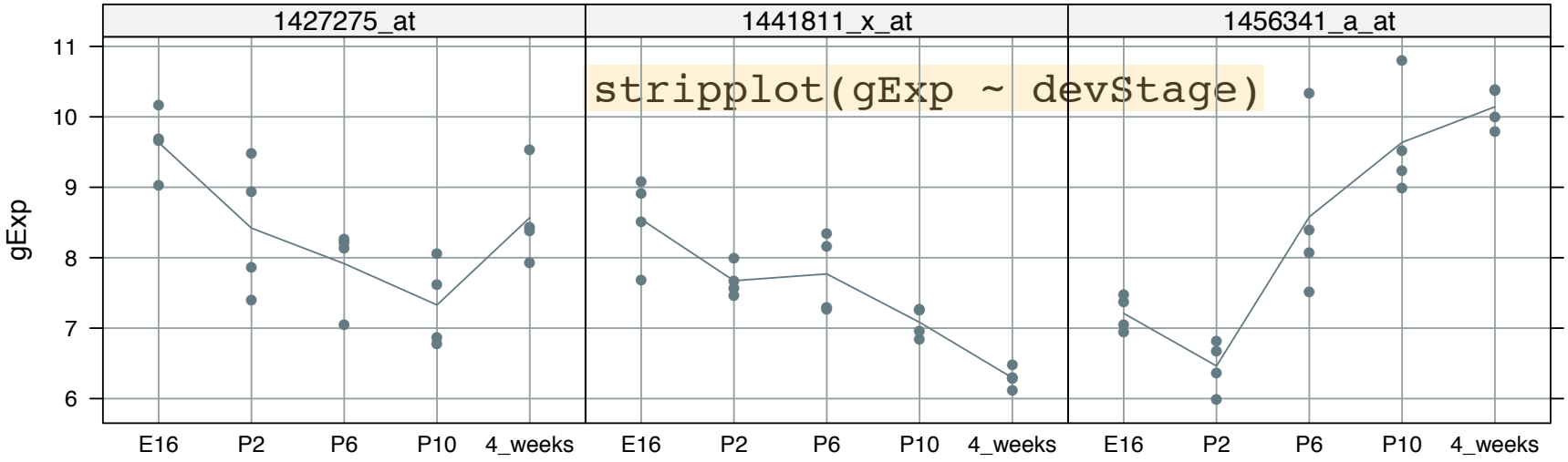
Investigating a *quantitative* covariate in our example dataset

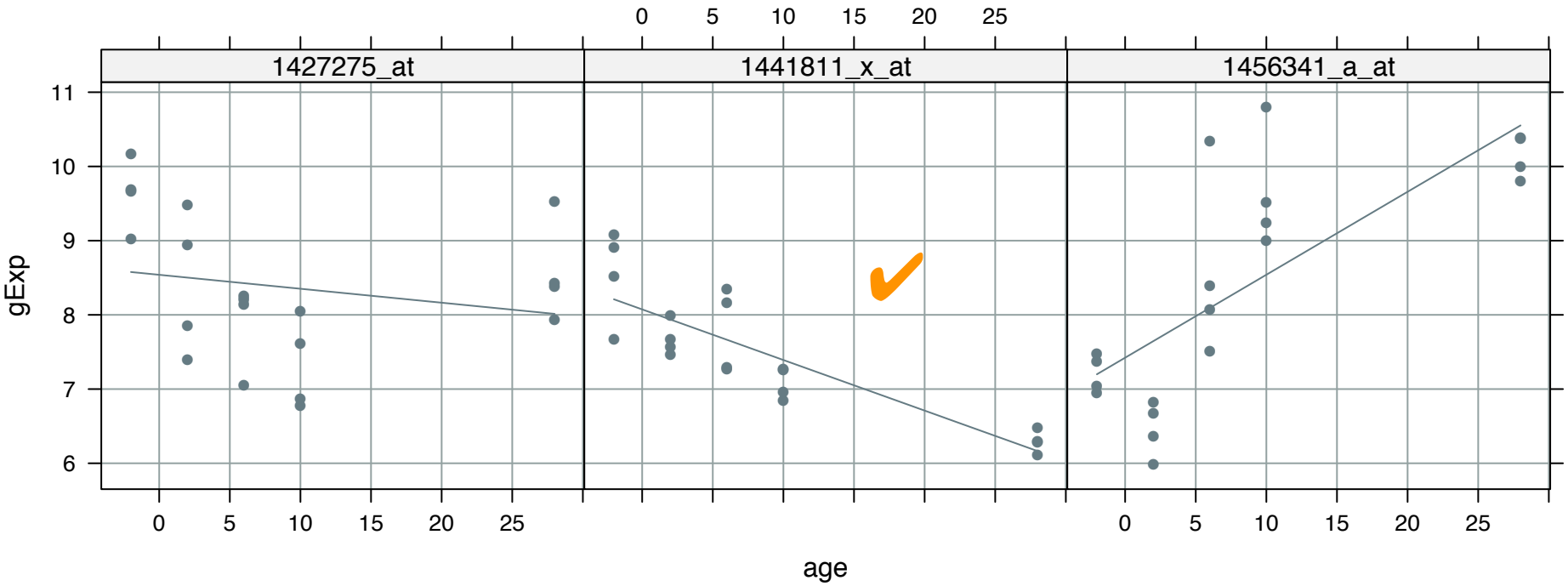
```
> ## recode() is from add-on package 'car'

> prDes$age <-
+     recode(prDes$devStage,
+            "'E16'=-2; 'P2'=2; 'P6'=6; 'P10'=10; '4_weeks'=28",
+            as.factor.result = FALSE)

> peek(prDes)
          sample devStage gType age
Sample_22     22      E16    wt  -2
Sample_16     16      E16 NrlKO  -2
Sample_5       5       P2 NrlKO   2
Sample_31     31       P6    wt   6
Sample_15     15      P10 NrlKO  10
Sample_36     36  4_weeks    wt  28
Sample_2       2  4_weeks NrlKO  28

> str(prDes)
'data.frame':  39 obs. of  4 variables:
 $ sample  : num  20 21 22 23 16 17 6 24 25 26 ...
 $ devStage: Factor w/ 5 levels "E16","P2","P6",..: 1 1 1 1 1 1 1 2 2 2 ...
 $ gType   : Factor w/ 2 levels "wt","NrlKO": 1 1 1 1 2 2 2 1 1 1 ...
 $ age     : num  -2 -2 -2 -2 -2 -2 -2 2 2 2 ...
```

meet our new quantitative covariate or predictor ...
age, which is a new version of the factor devStage

for starters, let's just work with wild type data for 3 example probesets







linear looks reasonable for 1, but not the other two

Remember: $Y=f(x)= a_0 + b\ x$

- The nature of the regression function $f(x; \alpha)$ is one of the defining characteristics of a regression model
  - f linear in $\alpha \Rightarrow$ linear model
  - f not linear in $\alpha \Rightarrow$ nonlinear model

nonlinear parametric regression

$$Y = \frac{1}{1 + e^{(\phi - x)/\xi}} + \varepsilon$$

simple linear regression (a linear model)

$$Y = \alpha_0 + \alpha_1 x + \varepsilon$$

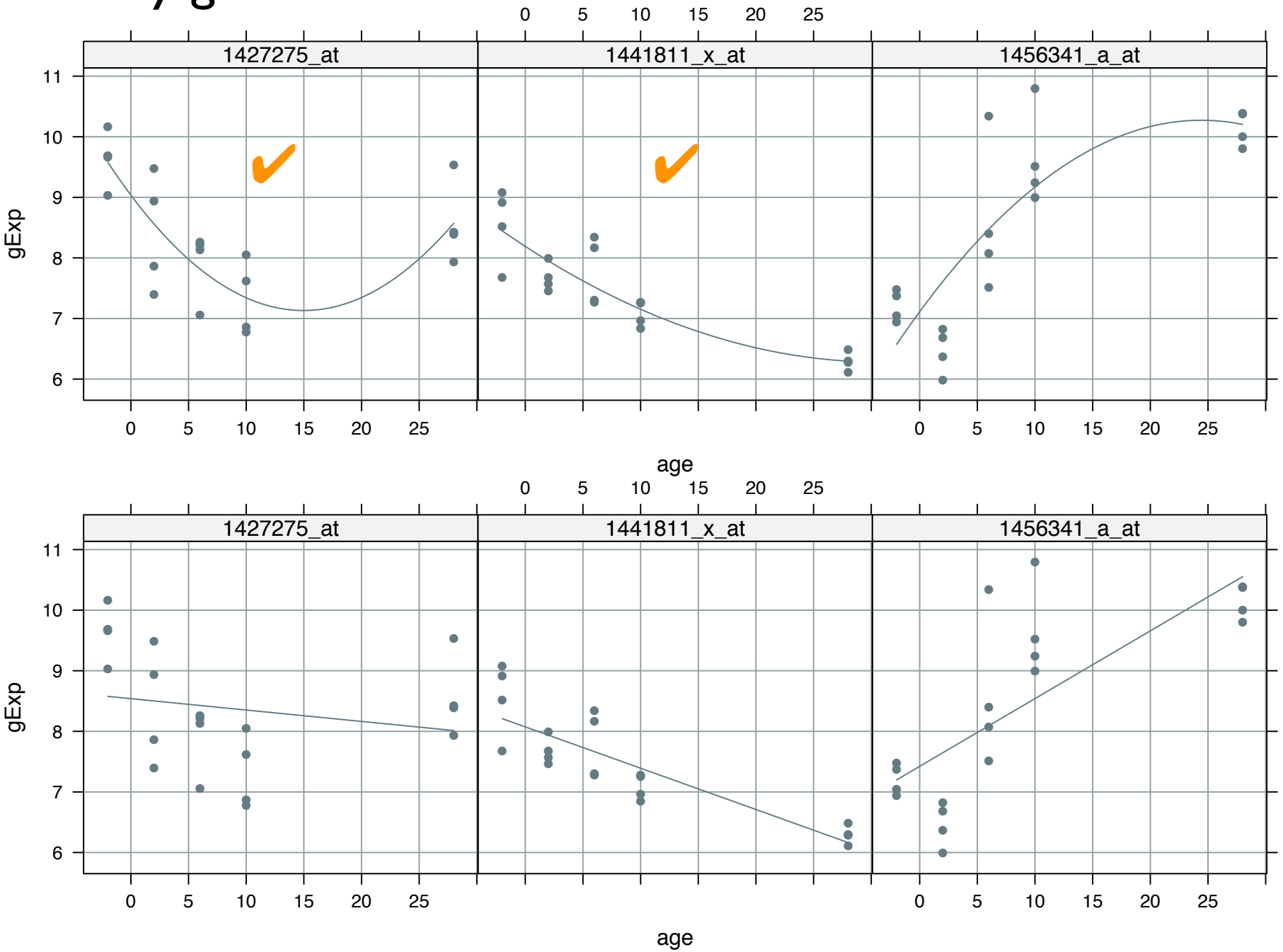
What we just did.

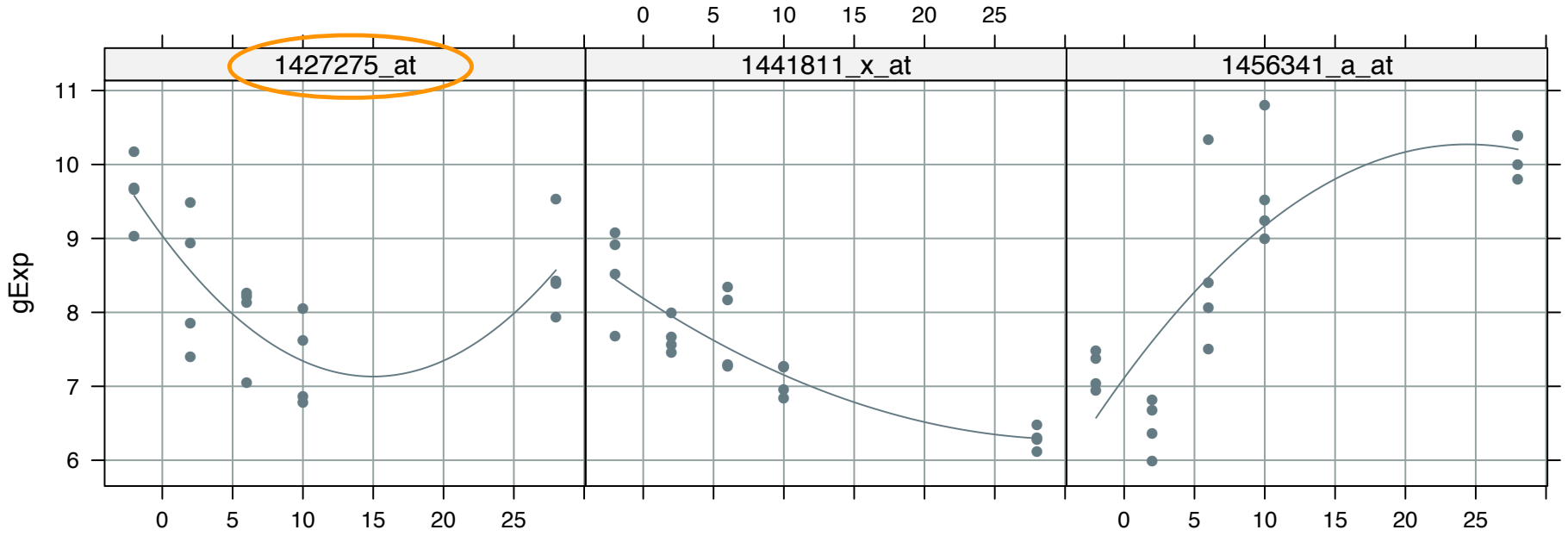
polynomial regression (also a linear model)

$$Y = \alpha_0 + \alpha_1 x + \alpha_2 x^2 + \varepsilon$$

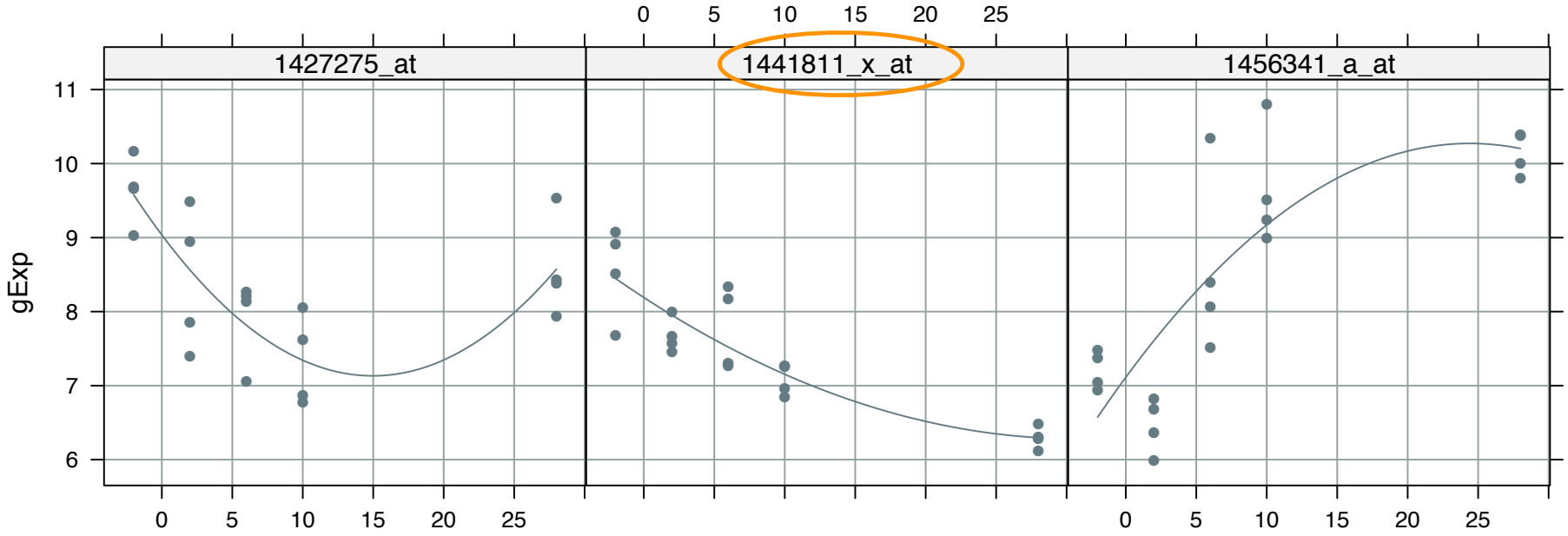
What we're about to do.

fairly good fit for 2 of 3 now!

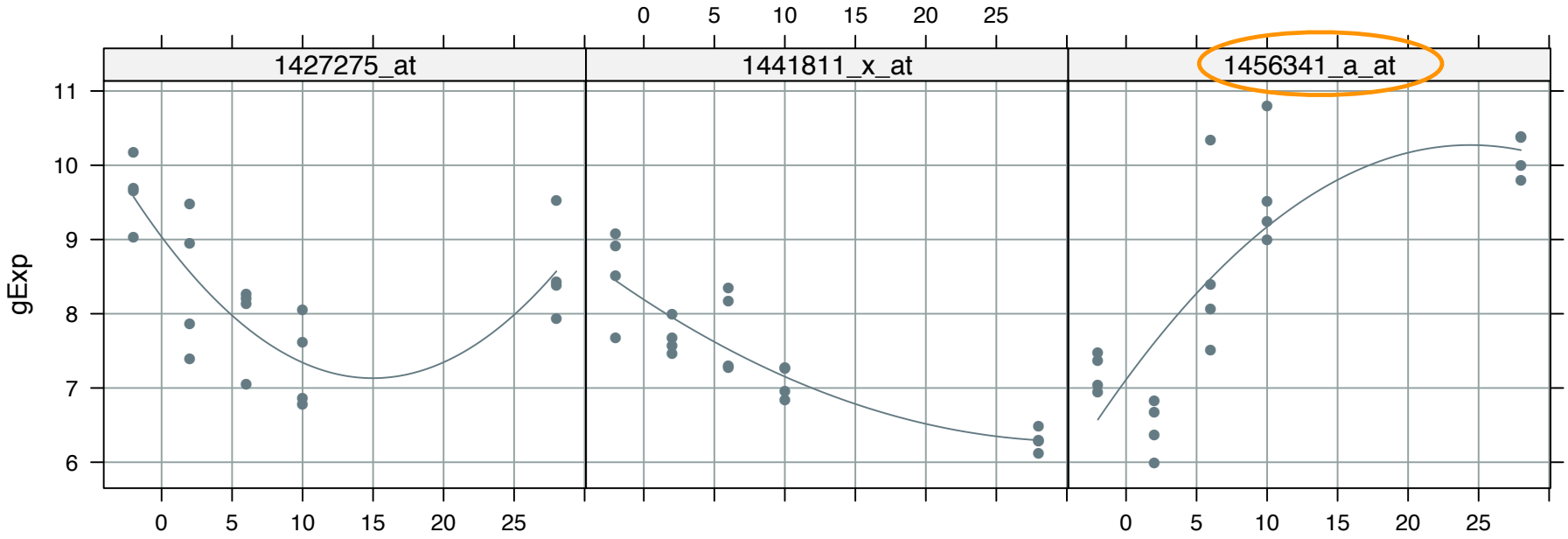





```
> summary(quadFits[["1427275_at"]])

Call:
lm(formula = gExp ~ age + I(age^2), data = z)

Residuals:
     Min       1Q   Median       3Q      Max
-1.16275 -0.55506  0.09503  0.40804  0.95803

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  9.036401   0.212313  42.562  < 2e-16 ***
age         -0.254305   0.048125  -5.284 6.07e-05 ***
I(age^2)     0.008490   0.001661   5.110 8.71e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.6444 on 17 degrees of freedom
Multiple R-squared: 0.6218,    Adjusted R-squared: 0.5773
F-statistic: 13.98 on 2 and 17 DF,  p-value: 0.0002572
```




```
> summary(quadFits[["1441811_x_at"]])

Call:
lm(formula = gExp ~ age + I(age^2), data = z)

Residuals:
     Min       1Q   Median       3Q      Max
-0.76946 -0.25477 -0.00589  0.13662  0.82202

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  8.190766   0.140969  58.103  < 2e-16 ***
age         -0.123836   0.031953  -3.876  0.00121 **
I(age^2)     0.002006   0.001103   1.819  0.08660 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4279 on 17 degrees of freedom
Multiple R-squared: 0.774,     Adjusted R-squared: 0.7475
F-statistic: 29.12 on 2 and 17 DF,  p-value: 3.23e-06
```




```
> summary(quadFits[["1456341_a_at"]])

Call:
lm(formula = gExp ~ age + I(age^2), data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-1.6211 -0.5010 -0.0050  0.3955  1.8651

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  7.112481   0.310922  22.875  3.3e-14 ***
age          0.258892   0.070477   3.673  0.00188 **
I(age^2)    -0.005303   0.002433  -2.180  0.04363 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9437 on 17 degrees of freedom
Multiple R-squared: 0.6737,    Adjusted R-squared: 0.6353
F-statistic: 17.55 on 2 and 17 DF,  p-value: 7.337e-05
```

F tests in regression

Remember this?

small model is nested within big, e.g., it's a special case where some parameters are equal to zero

model	example	# params = DF	RSS
small	lm(y ~ gType + devStage)	$p_{small} = 6$	RSS_{small}
big	lm(y ~ gType * devStage)	$p_{big} = 10$	RSS_{big}

$$y_{ijk} = \theta + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk} \text{ "big"}$$

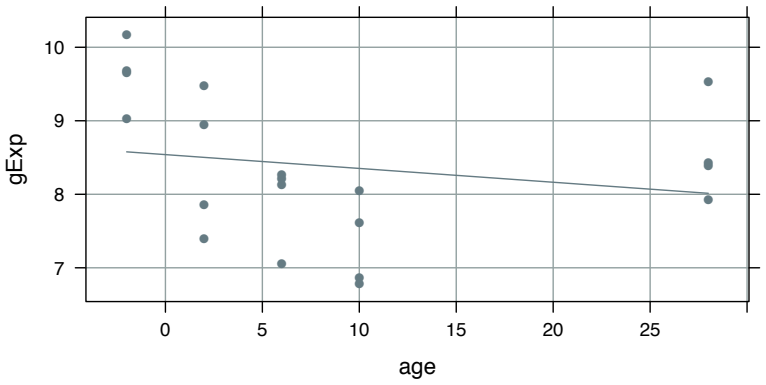
$$y_{ijk} = \theta + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk} \text{ "small"}$$

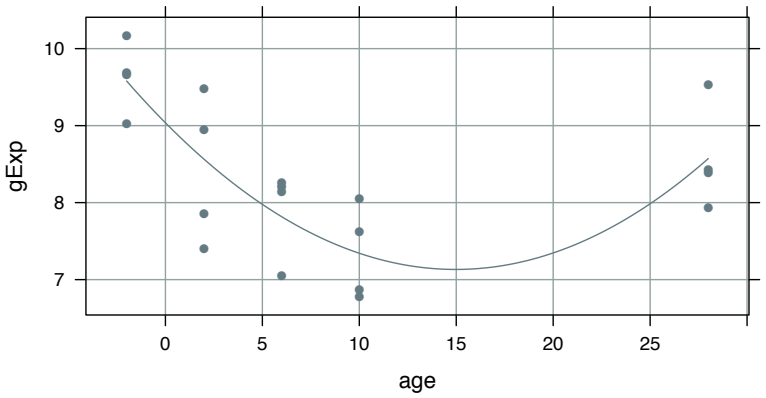
by definition:

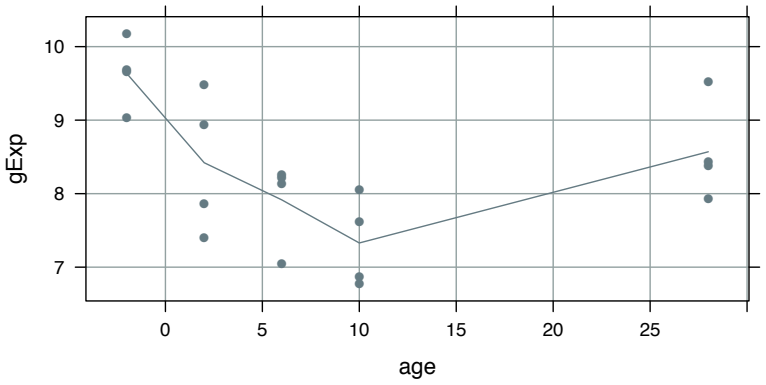
$p_{small} < p_{big}$

$RSS_{small} \geq RSS_{big}$

$$F = \frac{\left(\frac{RSS_{small} - RSS_{big}}{p_{big} - p_{small}} \right)}{\frac{RSS_{big}}{n - p_{big}}} \sim_{H_0} F_{(p_{big} - p_{small}, n - p_{big})}$$








```
> (jGene <- luckyGenes[1])
[1] "1427275_at"
```

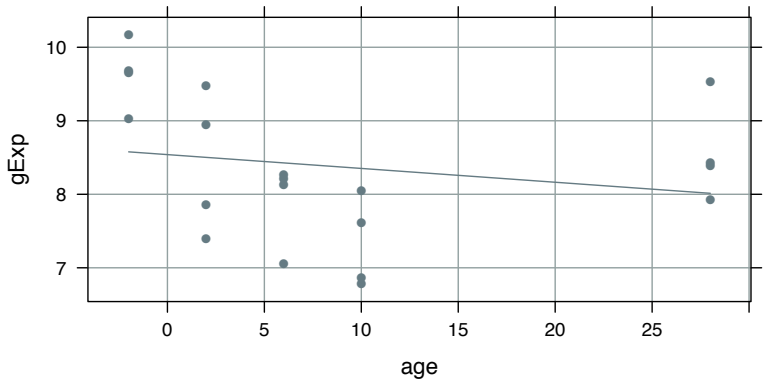
small big

```
> anova(linFits[[jGene]], quadFits[[jGene]])

Analysis of Variance Table

Model 1: gExp ~ age
Model 2: gExp ~ age + I(age^2)
  Res.Df     RSS Df Sum of Sq      F   Pr(>F)
1     18 17.9021
2     17  7.0591  1    10.843 26.113 8.71e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> AIC(linFits[[jGene]], quadFits[[jGene]], factFits[[jGene]])
                   df      AIC
linFits[[jGene]]    3 60.54129
quadFits[[jGene]]   4 43.92930
factFits[[jGene]]   6 47.54810
```

it's "worth it" to go from linear to quadratic here

but hard to justify going from quadratic to one-way ANOVA

possible links to read more about using AIC to compare non-nested models: stackexchange and Wikipedia

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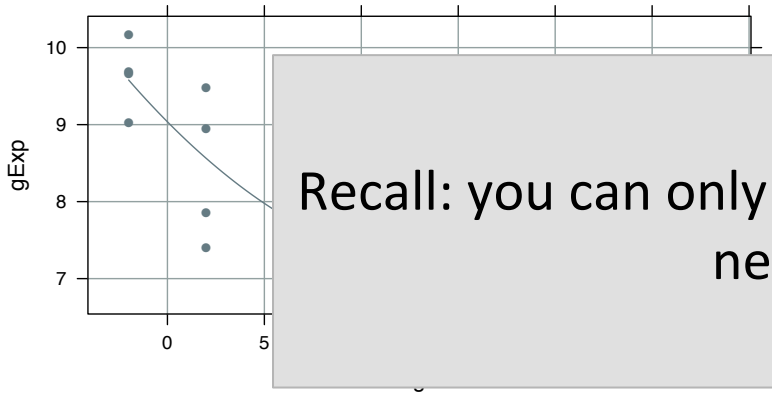
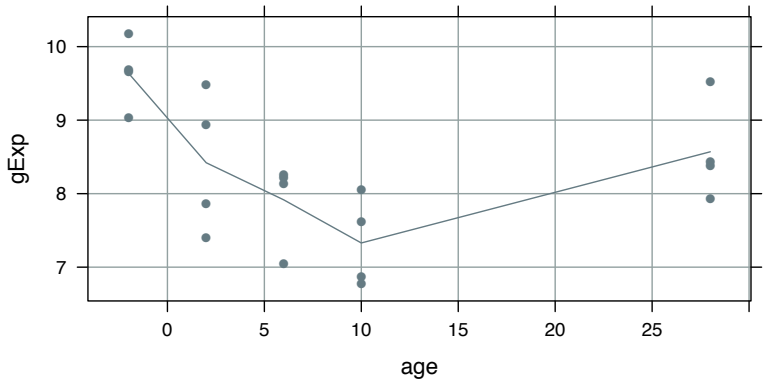
1     18 17.9021
```

```
e-05 ***
' 0.05 '.' 0.1 ' ' 1
 factFits[[jGene]])
```



Recall: you can only use the F test when you have nested models



it's "worth it" to go from linear to quadratic here

but hard to justify going from quadratic to one-way ANOVA

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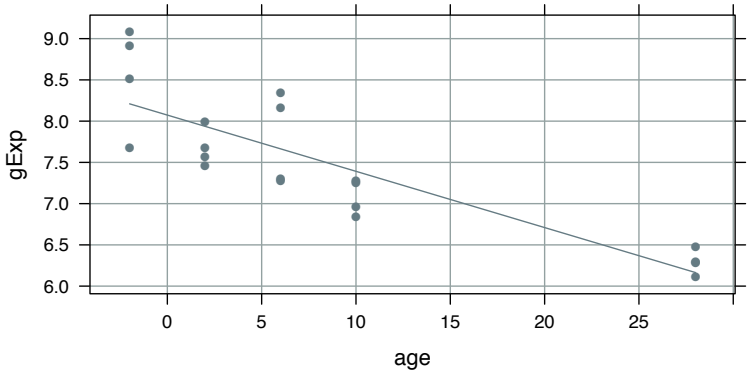
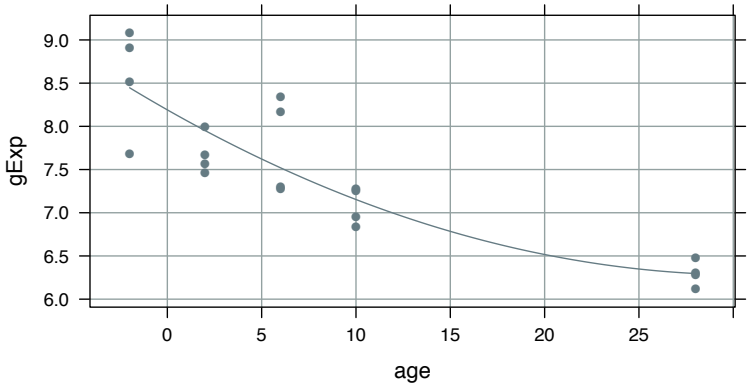
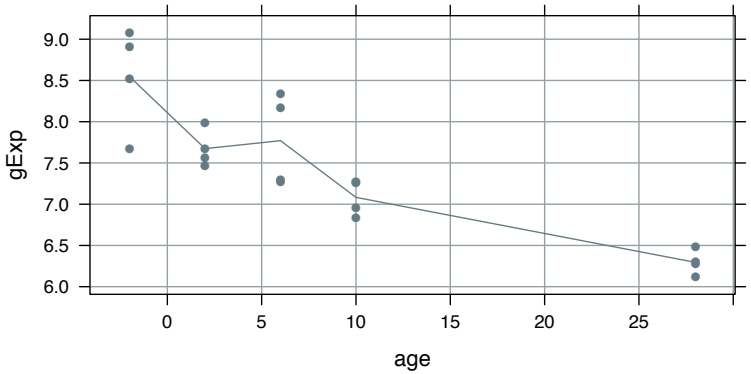







```
> (jGene <- luckyGenes[3])
[1] "1441811_x_at"
```

small big

```
> anova(linFits[[jGene]], quadFits[[jGene]])

Analysis of Variance Table

Model 1: gExp ~ age
Model 2: gExp ~ age + I(age^2)
  Res.Df    RSS Df Sum of Sq      F Pr(>F)
1     18 3.7176
2     17 3.1120  1   0.60559 3.3081 0.0866 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> AIC(linFits[[jGene]], quadFits[[jGene]], factFits[[jGene]])
                  df      AIC
linFits[[jGene]]   3 29.10466
quadFits[[jGene]]  4 27.54851
factFits[[jGene]]  6 27.12587
```

meh

not clear it's "worth it" to go from linear to quadratic here

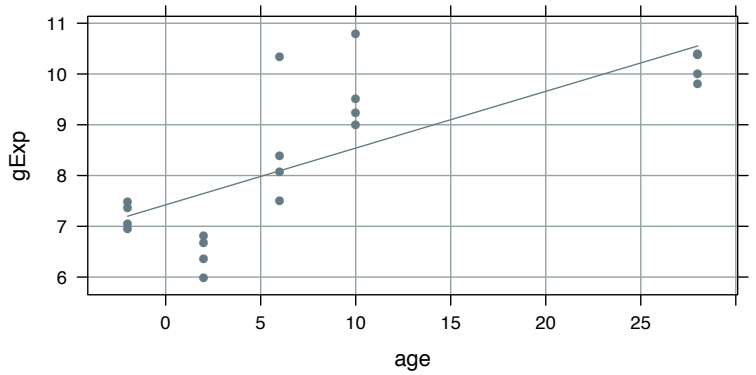
even less payoff to go from quadratic to one-way ANOVA

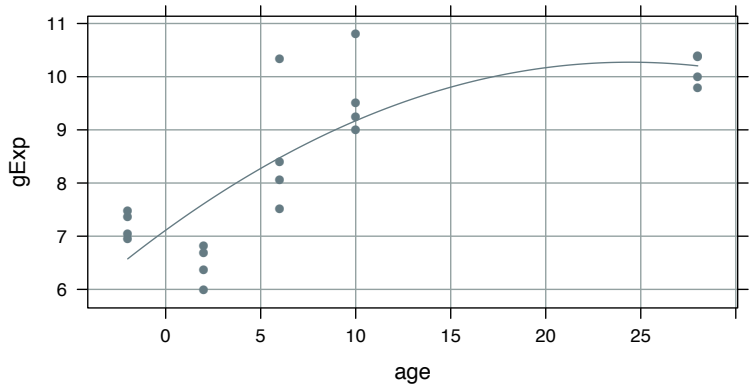
Occam's Razor and the KISS principle → stick w/ simple linear model

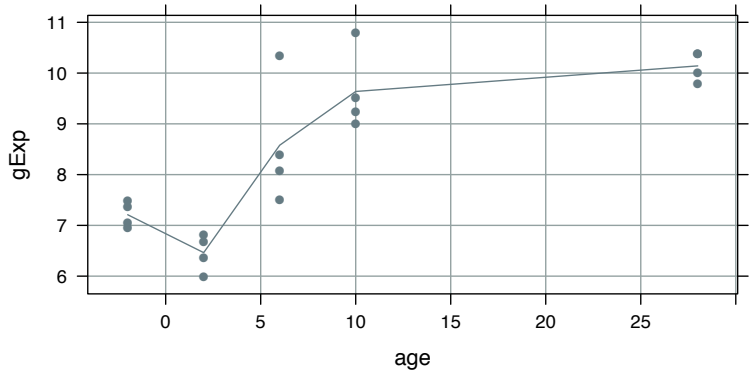
Occam's razer

- Principle of parsimony: states that between **competing hypotheses**, the one with the fewest assumptions.
  - Roughly speaking: do not make things more complicated than needs to be

*Alan Baker (2010) [2004]. "Simplicity". Stanford Encyclopedia of Philosophy. California: Stanford University.








```
> (jGene <- luckyGenes[2])

[1] "1456341_a_at"
```

small big

```
> anova(linFits[[jGene]], quadFits[[jGene]])

Analysis of Variance Table

Model 1: gExp ~ age
Model 2: gExp ~ age + I(age^2)
  Res.Df    RSS Df Sum of Sq      F  Pr(>F)
1     18 19.370
2     17 15.139  1    4.2308 4.7509 0.04363 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> AIC(linFits[[jGene]], quadFits[[jGene]], factFits[[jGene]])
                   df      AIC
linFits[[jGene]]    3 62.11743
quadFits[[jGene]]   4 59.18864
factFits[[jGene]]   6 48.70210
```

it's probably "worth it" to go from linear to quadratic here (?)

going from quadratic to one-way ANOVA seems justified

Break to talk about projects

Project teams

FISCH

Studying metabolites in hyperphenylalaninemia patients using Mass Spec

GutCHECK

Studying the correlation of microbial composition and metabolic pathways in type 2 diabetes (T2D) or inflammatory bowel disease (IBD) patients of different ethnicity and geographical origin.

GLLAD

Looking at RNA microarray gene expression data in HIV/HCV co-infected population, HCV mono-infected, a healthy comparison group to explain differences in clinical outcome in co-infected and mono-infected patients

IUGRoup

Comparing DNA methylation patterns in intrauterine growth restriction (IUGR) and in healthy placenta

Project teams

JaWSPR

Looking at expression signature in mice with different alcohol tolerance

Leptin

Investigating the mechanism of leptin therapy in type 1 diabetes

META-VEG

Analyzing grape berries and their microflora by looking at RNAseq dataset of grapes throughout development

PHASAX

Characterizing the effect of the immune microenvironment on tumour evolution in high-grade serous ovarian cance

Treed

Looking at DNA methylation in male and female mice

Next steps

- Start thinking about your proposal (outline; specifics; task assignment)
- You'll get assigned a TA and an instructor
- Consult with your assigned TA/instructor when you have a good sketch of the outline/aims/tasks etc

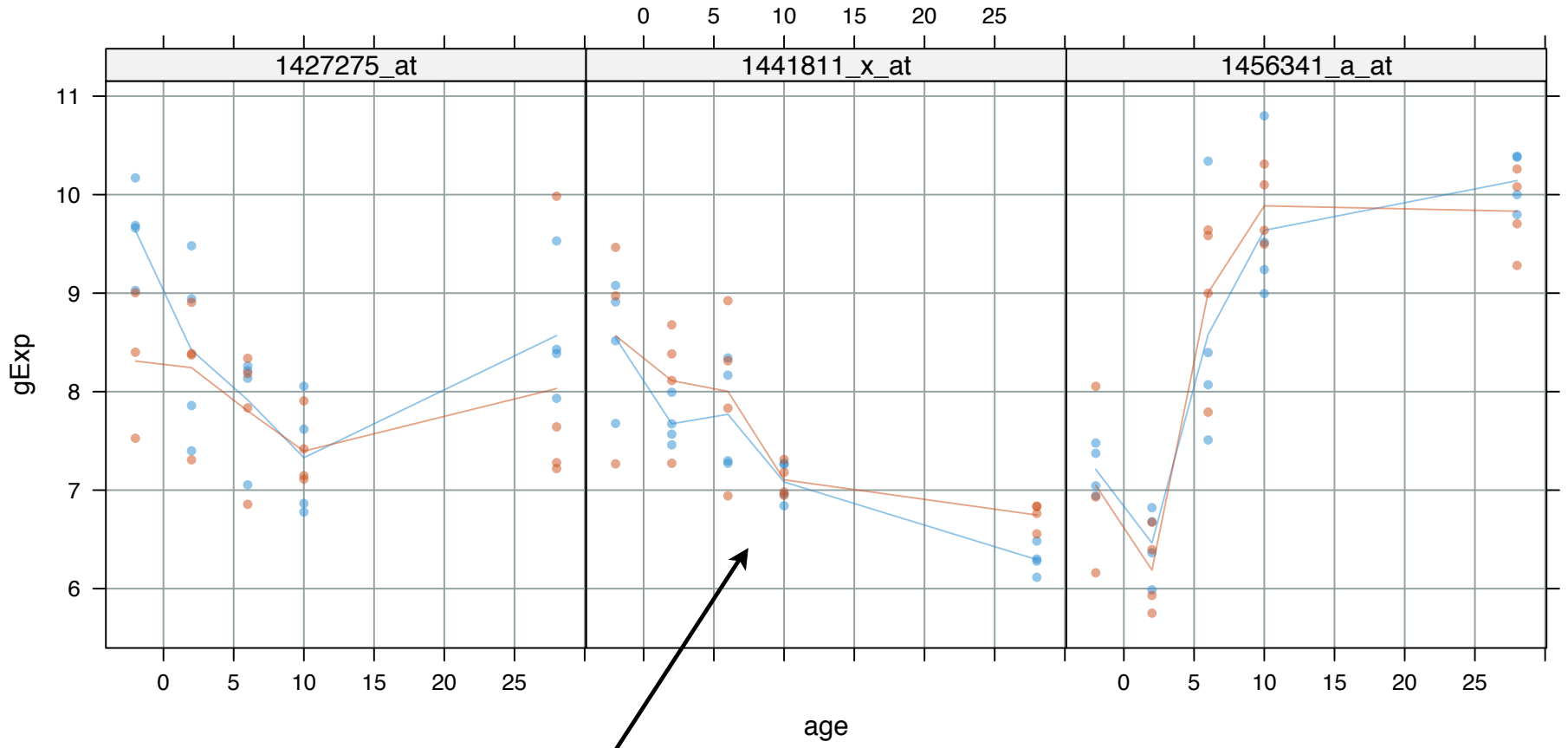
Back to lecture

increase the complexity ...

1 quantitative covariate: age

AND 1 categorical covariate:

genotype = wt vs. Nrl knockout



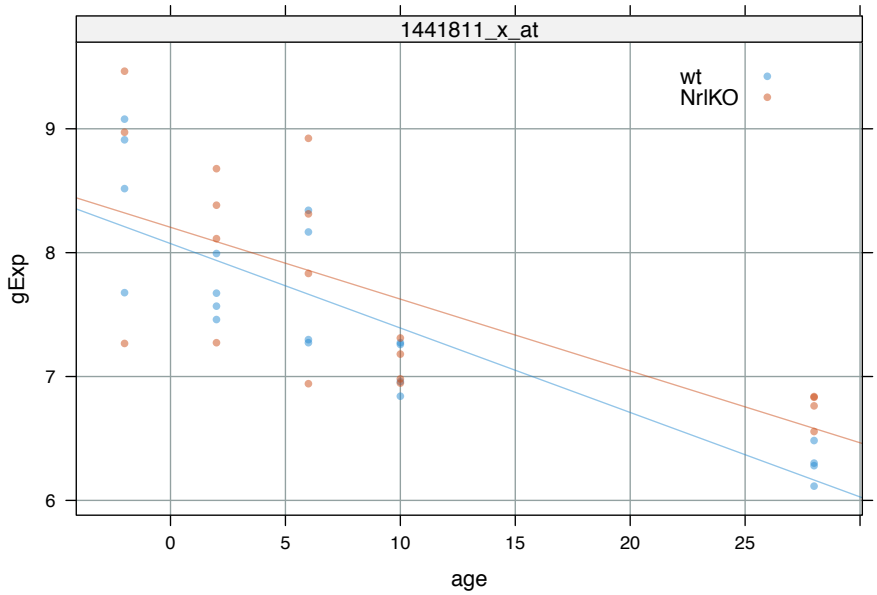
let's focus on this one for a model with just intercept and slope, possibly different for wt and NrlKO

$$y_{ij} = \alpha_{0,wt} + \tau_{0,j} + (\alpha_{1,wt} + \tau_{1,j})age_i + \varepsilon_{ij}$$

where $j \in \{wt, NrlKO\}$

$i = 1,2,...,n_j$

$\tau_{0,wt} = \tau_{1,wt} \equiv 0$




```
> jFit <- lm(gExp ~ gType * age, jDat)
> summary(jFit)

Call:
lm(formula = gExp ~ gType * age, data = jDat)

Residuals:
     Min       1Q   Median       3Q      Max
-1.05383 -0.41194 -0.02491  0.31295  1.14417

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   8.07337    0.16552  48.776  < 2e-16 ***
gTypeNrlKO    0.13148    0.24070   0.546    0.588
age          -0.06818    0.01215  -5.612 2.51e-06 ***
gTypeNrlKO:age  0.01019    0.01744   0.584    0.563
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.5651 on 35 degrees of freedom
Multiple R-squared: 0.607,   Adjusted R-squared: 0.5733
F-statistic: 18.02 on 3 and 35 DF,  p-value: 3.047e-07
```

The intercept for the knockouts is:

$\alpha_{0,wt} + \tau_{0,\Delta Nrl}$

and the slope for knockouts is:

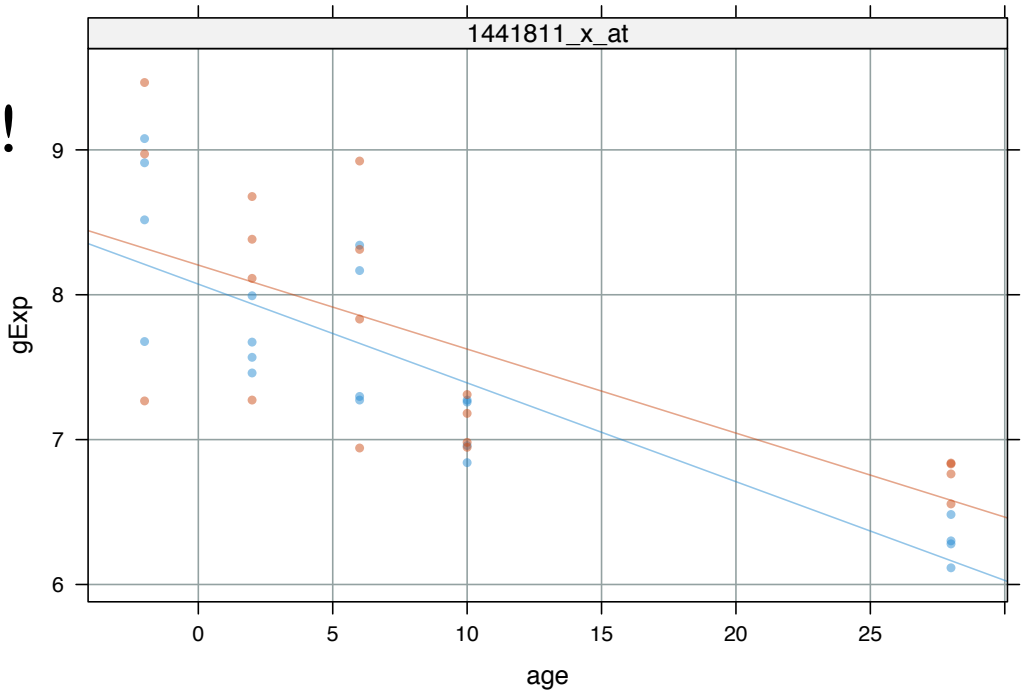
$\alpha_{1,wt} + \tau_{1,\Delta Nrl}$

as always, different parametrizations are possible!

$$y_{ij} = \alpha_{0,j} + \alpha_{1,j} age_i + \varepsilon_{ij}$$

where $j \in \{wt, NrlKO\}$

$i = 1,2,...,n_j$




```
> jFitAlt <- lm(gExp ~ gType/age - 1, jDat)
> summary(jFitAlt)

Call:
lm(formula = gExp ~ gType/age - 1, data = jDat)

Residuals:
     Min       1Q   Median       3Q      Max
-1.05383 -0.41194 -0.02491  0.31295  1.14417

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
gTypewt         8.07337    0.16552  48.776  < 2e-16 ***
gTypeNrlKO      8.20485    0.17476  46.949  < 2e-16 ***
gTypewt:age    -0.06818    0.01215  -5.612 2.51e-06 ***
gTypeNrlKO:age -0.05799    0.01251  -4.636 4.80e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.5651 on 35 degrees of freedom
Multiple R-squared: 0.9951,  Adjusted R-squared: 0.9945
F-statistic:  1761 on 4 and 35 DF,  p-value: < 2.2e-16
```

(intercept, slope) for wild type:

$(\alpha_{0,wt}, \alpha_{1,wt})$

(intercept, slope) for the knockouts:

$(\alpha_{0,\Delta Nrl}, \alpha_{1,\Delta Nrl})$

as always, you can switch between parametrizations via multiplication by an appropriate contrast matrix!

$$y_{ij} = \alpha_{0,wt} + \tau_{0,j} + (\alpha_{1,wt} + \tau_{1,j})age_i + \varepsilon_{ij}$$

where $j \in \{wt, NrlKO\}$

$i = 1,2,...,n_j$

$\tau_{0,wt} = \tau_{1,wt} \equiv 0$

$\Leftrightarrow$

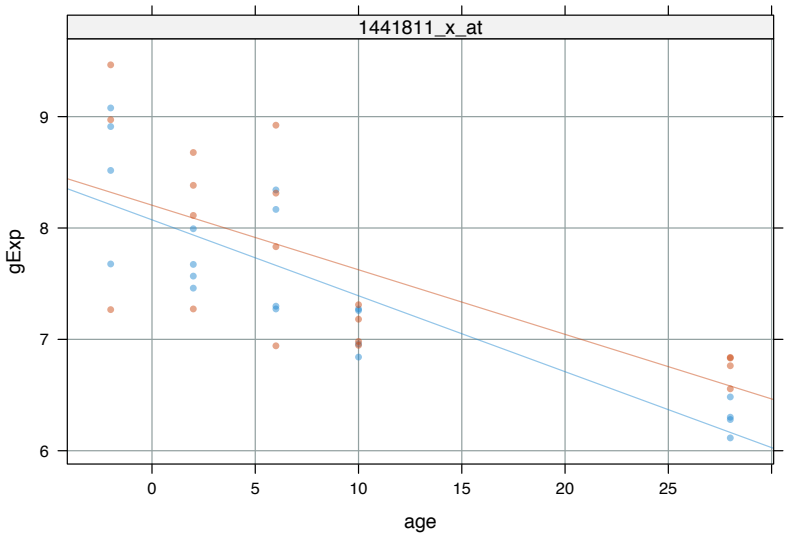
$$y_{ij} = \alpha_{0,j} + \alpha_{1,j}age_i + \varepsilon_{ij}$$

where $j \in \{wt, NrlKO\}$

$i = 1,2,...,n_j$

```
> (contMat <- rbind(c(1, 0, 0, 0),
+                   c(1, 1, 0, 0),
+                   c(0, 0, 1, 0),
+                   c(0, 0, 1, 1)))
     [,1] [,2] [,3] [,4]
[1,]    1    0    0    0
[2,]    1    1    0    0
[3,]    0    0    1    0
[4,]    0    0    1    1
```

```
> cbind(coefDefault = coef(jFit),
+       coefAlt = coef(jFitAlt),
+       matrixResult = as.vector(contMat %*% coef(jFit)))
                coefDefault     coefAlt matrixResult
(Intercept)      8.07337352  8.07337352   8.07337352
gTypeNrlKO       0.13147574  8.20484926   8.20484926
age             -0.06817881 -0.06817881  -0.06817881
gTypeNrlKO:age   0.01018928 -0.05798953  -0.05798953
```



as always, you can assess the relevance of several terms at once -- such as everything involving genotype -- with an F test

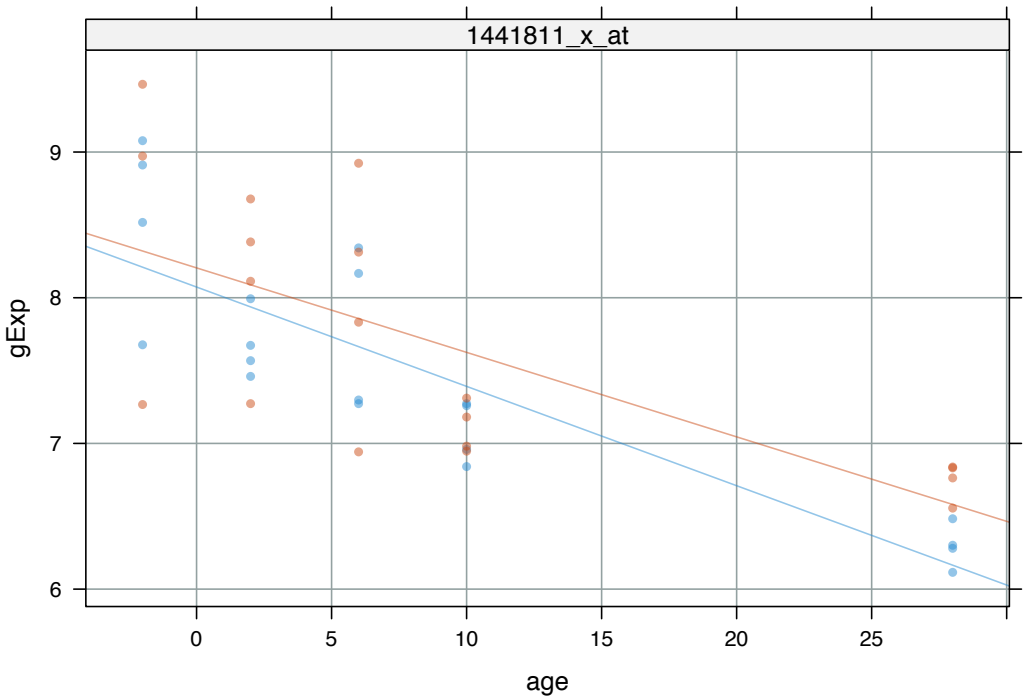
it's not clear that genotype affects the intercept or the slope

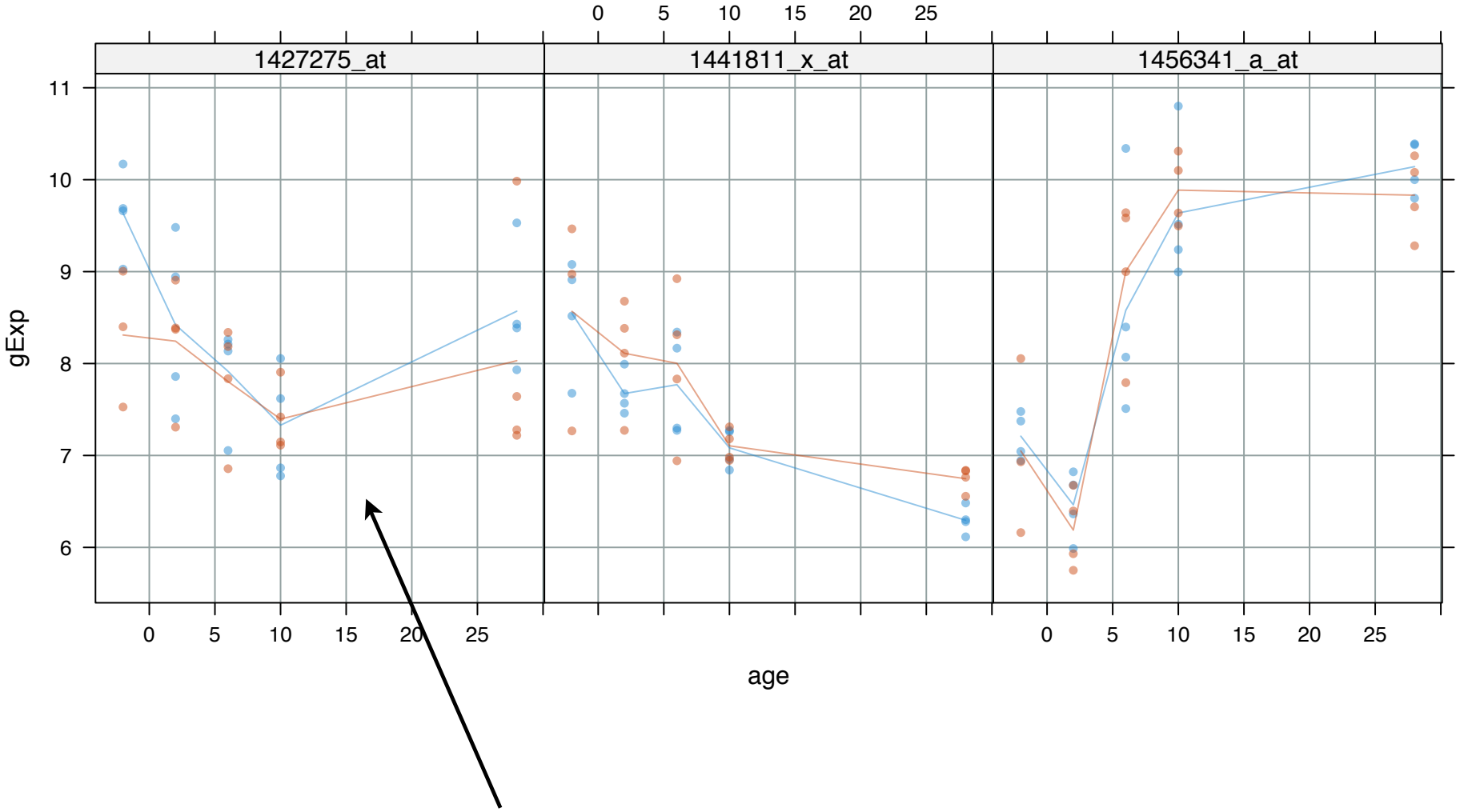
small big

```
> anova(lm(gExp ~ age, jDat), jFit)

Analysis of Variance Table

Model 1: gExp ~ age
Model 2: gExp ~ gType * age
  Res.Df    RSS Df Sum of Sq      F Pr(>F)
1     37 11.774
2     35 11.176  2   0.59807 0.9365 0.4016
```





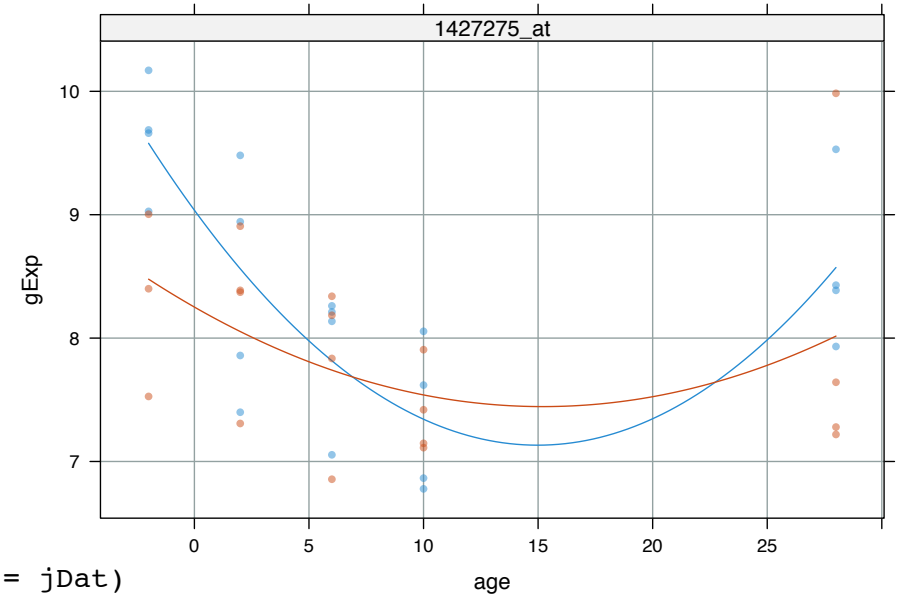
let's focus here for a model including a quadratic age term

$$y_{ij} = \alpha_{0,wt} + \tau_{0,j} + (\alpha_{1,wt} + \tau_{1,j})age_i + (\alpha_{2,wt} + \tau_{2,j})age_i^2 + \varepsilon_{ij}$$

where $j \in \{wt, NrlKO\}$

$i = 1,2,...,n_j$

$\tau_{0,wt} = \tau_{1,wt} = \tau_{2,wt} \equiv 0$




```
> summary(jFit)

Call:
lm(formula = gExp ~ gType * (age + I(age^2)), data = jDat)

Residuals:
     Min       1Q   Median       3Q      Max
-1.16275 -0.55816  0.08203  0.42020  1.96803

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)         9.036401   0.234853  38.477  < 2e-16 ***
gTypeNrlKO         -0.784969   0.350249  -2.241   0.0319 *
age                -0.254305   0.053234  -4.777 3.55e-05 ***
I(age^2)            0.008490   0.001838   4.620 5.63e-05 ***
gTypeNrlKO:age      0.148195   0.078232   1.894   0.0670 .
gTypeNrlKO:I(age^2) -0.005001   0.002673  -1.871   0.0702 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.7128 on 33 degrees of freedom
Multiple R-squared: 0.4755,  Adjusted R-squared: 0.3961
F-statistic: 5.984 on 5 and 33 DF,  p-value: 0.0004804
```

as always, you can assess the relevance of several terms at once -- such as everything involving genotype -- with an F test

borderline evidence that genotype affects something about the parabola (location or shape)

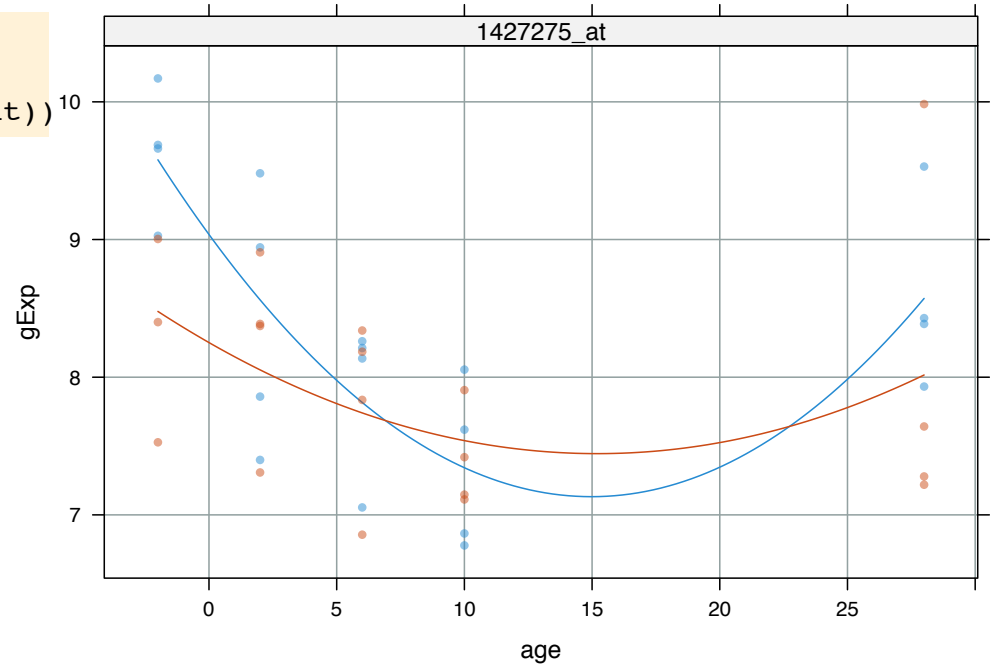
small

```
> anova(lm(gExp ~ age + I(age^2), jDat),
+       lm(gExp ~ gType * (age + I(age^2)), jDat))
```

big

```
Analysis of Variance Table

Model 1: gExp ~ age + I(age^2)
Model 2: gExp ~ gType * (age + I(age^2))
  Res.Df    RSS Df Sum of Sq      F Pr(>F)
1     36 20.081
2     33 16.767  3    3.3144 2.1744 0.1097
```



linear model framework is extremely general!

one extreme (simple): two-sample common variance t-test

another extreme (flexible): a polynomial, potentially different for each level of some factor

dichotomous variable? OK!
categorical variable? OK!
quantitative variable? OK!
various combinations of the above? OK!

don't be afraid to build models with more than 1 covariate
don't be intimidated by all the "contrast" talk

`lm(yMat ~ x)`

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

$$\begin{bmatrix} y_{11} & \cdots & y_{1G} \\ y_{21} & & y_{2G} \\ \vdots & & \\ y_{n1} & & y_{nG} \end{bmatrix} = X \begin{bmatrix} \alpha_1 & \cdots & \alpha_G \end{bmatrix} + \begin{bmatrix} \varepsilon_{11} & & \varepsilon_{1G} \\ \varepsilon_{21} & & \varepsilon_{2G} \\ \vdots & \cdots & \\ \varepsilon_{n1} & & \varepsilon_{nG} \end{bmatrix}$$

built-in function lm() can do "multivariate regression" = many dependent vars ("responses")
aka "multivariate multiple regression"

```
From lm() documentation:

If response is a matrix a linear model is fitted separately by least-squares to each
column of the matrix.

lm returns an object of class "lm" or for multiple responses of class c("mlm", "lm").
```

Industrial scale model fitting is good because things like this are not recomputed 30K times unnecessarily*

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

$\hat{\alpha} = (X^T X)^{-1} X^T Y$ the MLE and OLS estimator of α

$\hat{\sigma}^2 = \frac{1}{n-p} \hat{\varepsilon}^T \hat{\varepsilon}$ the estimated error variance

$\hat{V}(\hat{\alpha}) = \hat{\sigma}^2 (X^T X)^{-1}$ the estimated covariance matrix of $\hat{\alpha}$

How test $H_0 : \alpha_j = 0$?

With a t-statistic. Under H_0, we have (at least approximately) that:

$$\frac{\hat{\alpha}_j}{\widehat{se}(\hat{\alpha}_j)} \sim t_{n-p}$$

so a p-value is obtained by computing a tail probability for the observed value of $\hat{\alpha}_j$ from a t_{n-p} distribution.

* under the hood, lm() is doing something more clever and numerically stable than this

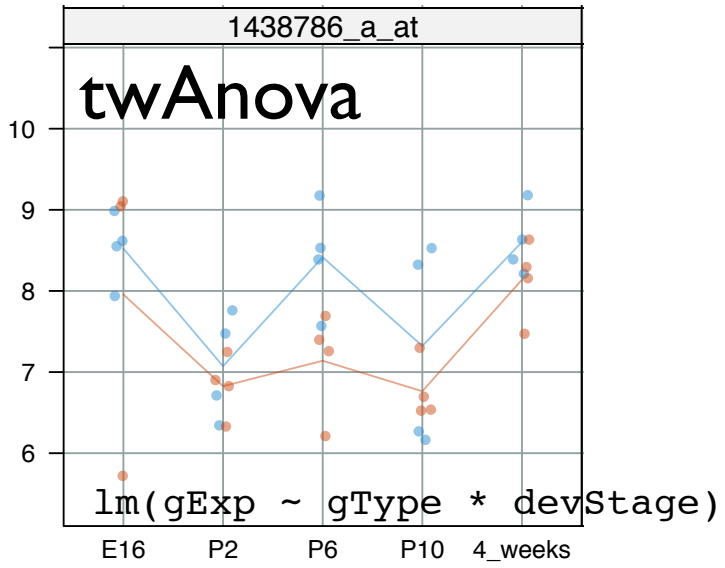
I have fit all the models we've considered to all ~30K probesets.

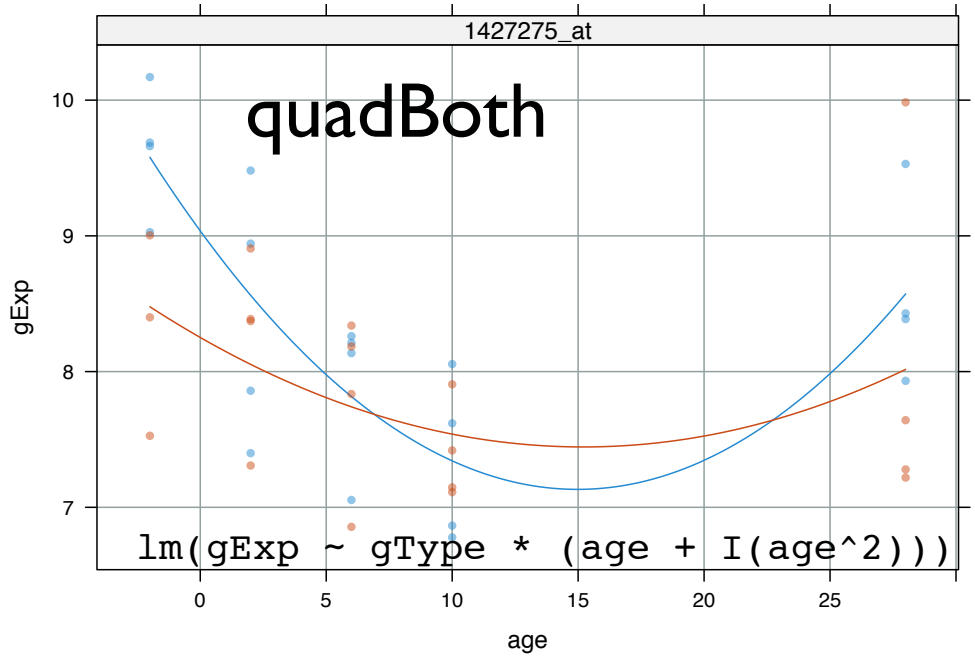
Let's examine some of the results *en masse*.

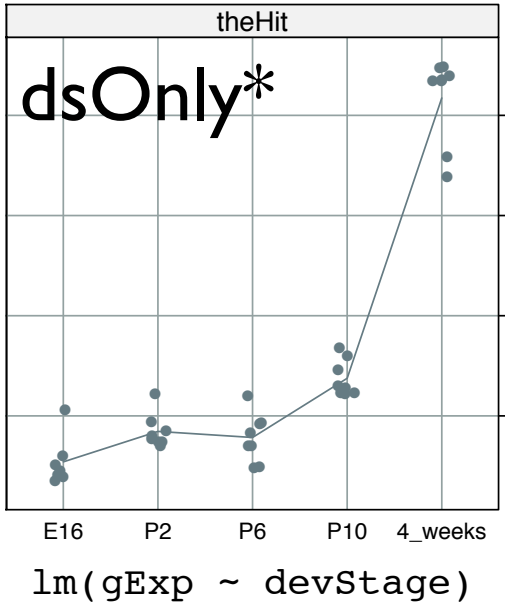
Let this drive home the point that ...

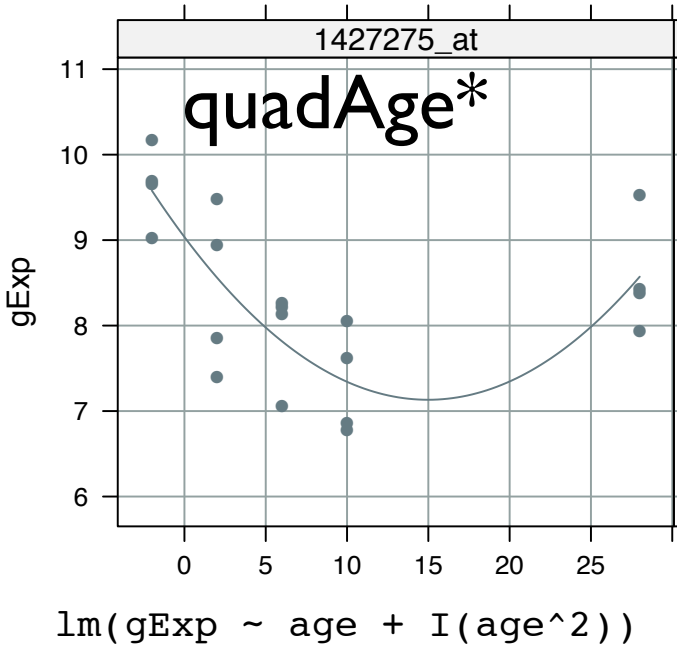
- background variability
- intercepts
- Nrl knockout effects
- devStage effects
- age effects, both linear and quadratic
- and interactions of all the above

differ for each gene.

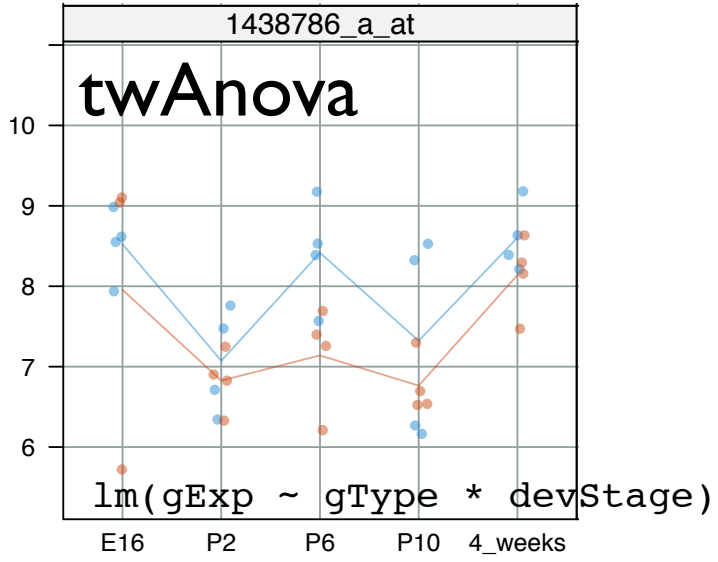


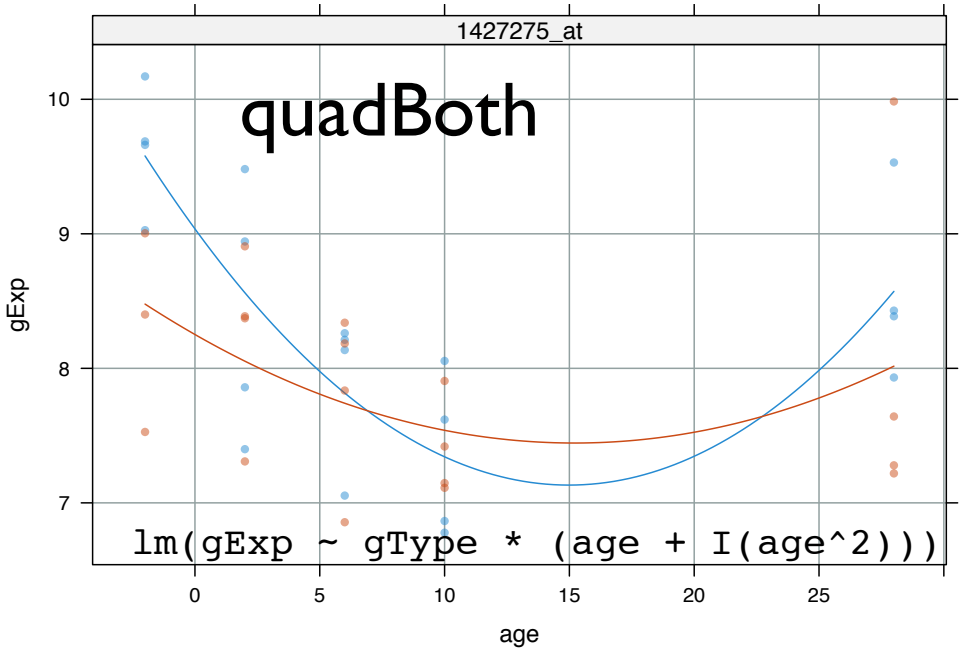


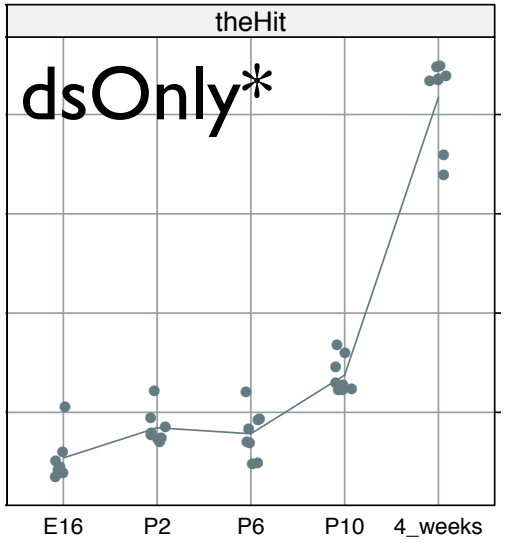




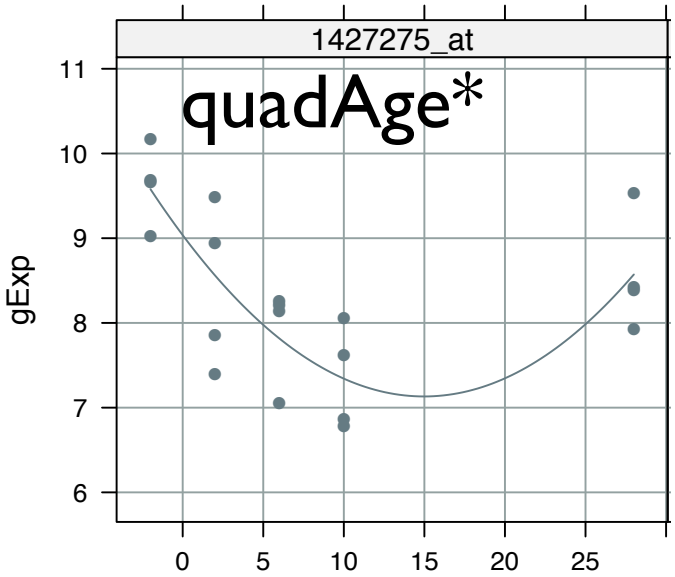
* Figures slightly misleading. Model is fit to all the data, wild type and Nrl knockout, but gType is not used as a covariate.





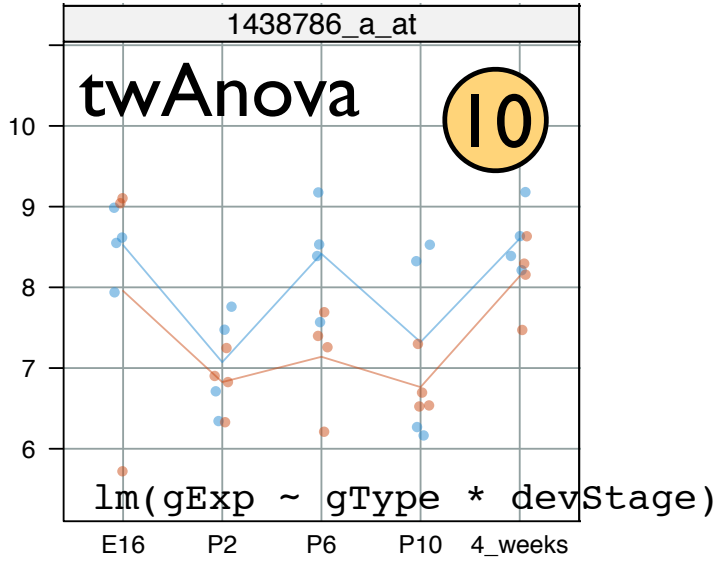


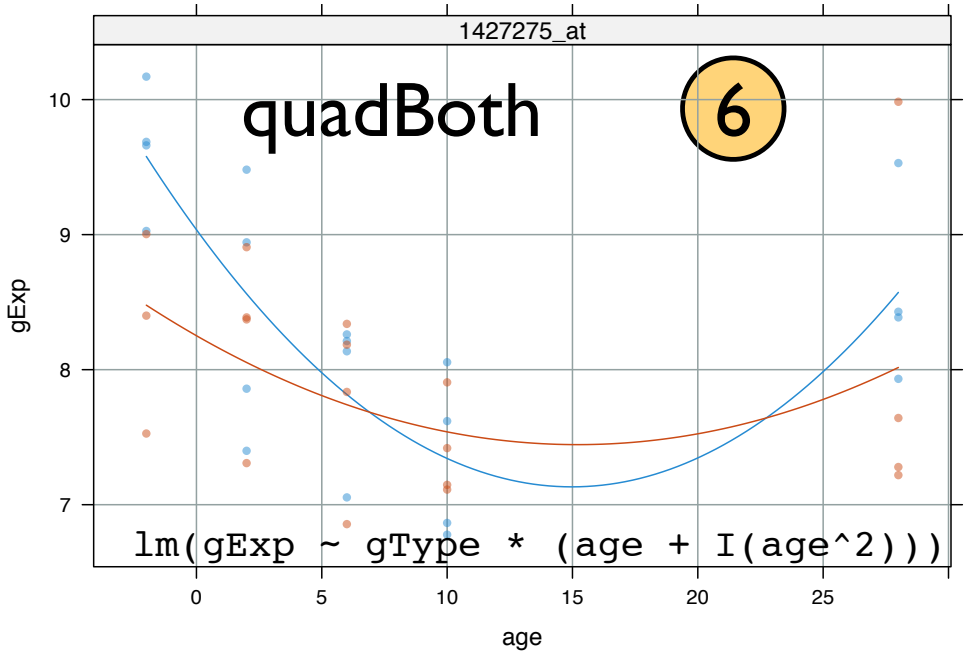
lm(gExp ~ devStage)

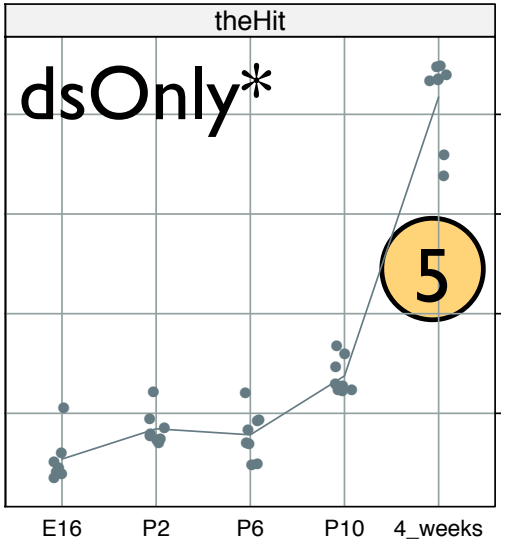


lm(gExp ~ age + I(age^2))

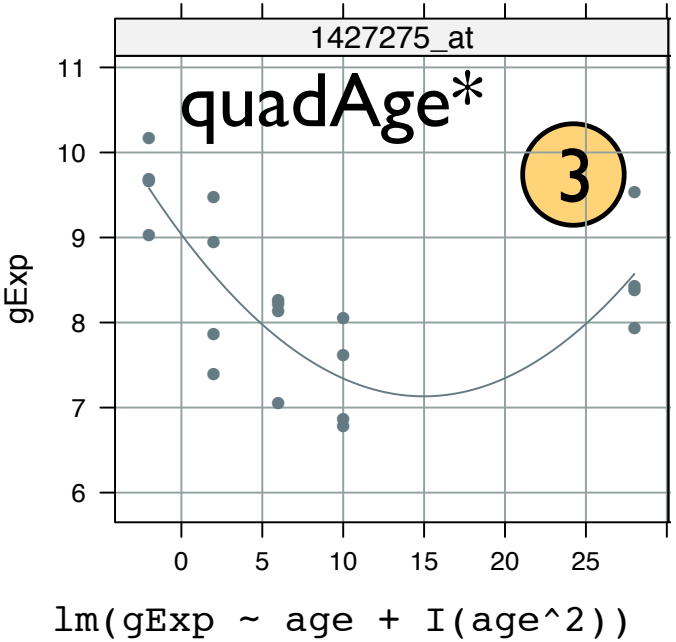
How "big" are these models? How many parameters are we using to specify the mean structure?







lm(gExp ~ devStage)

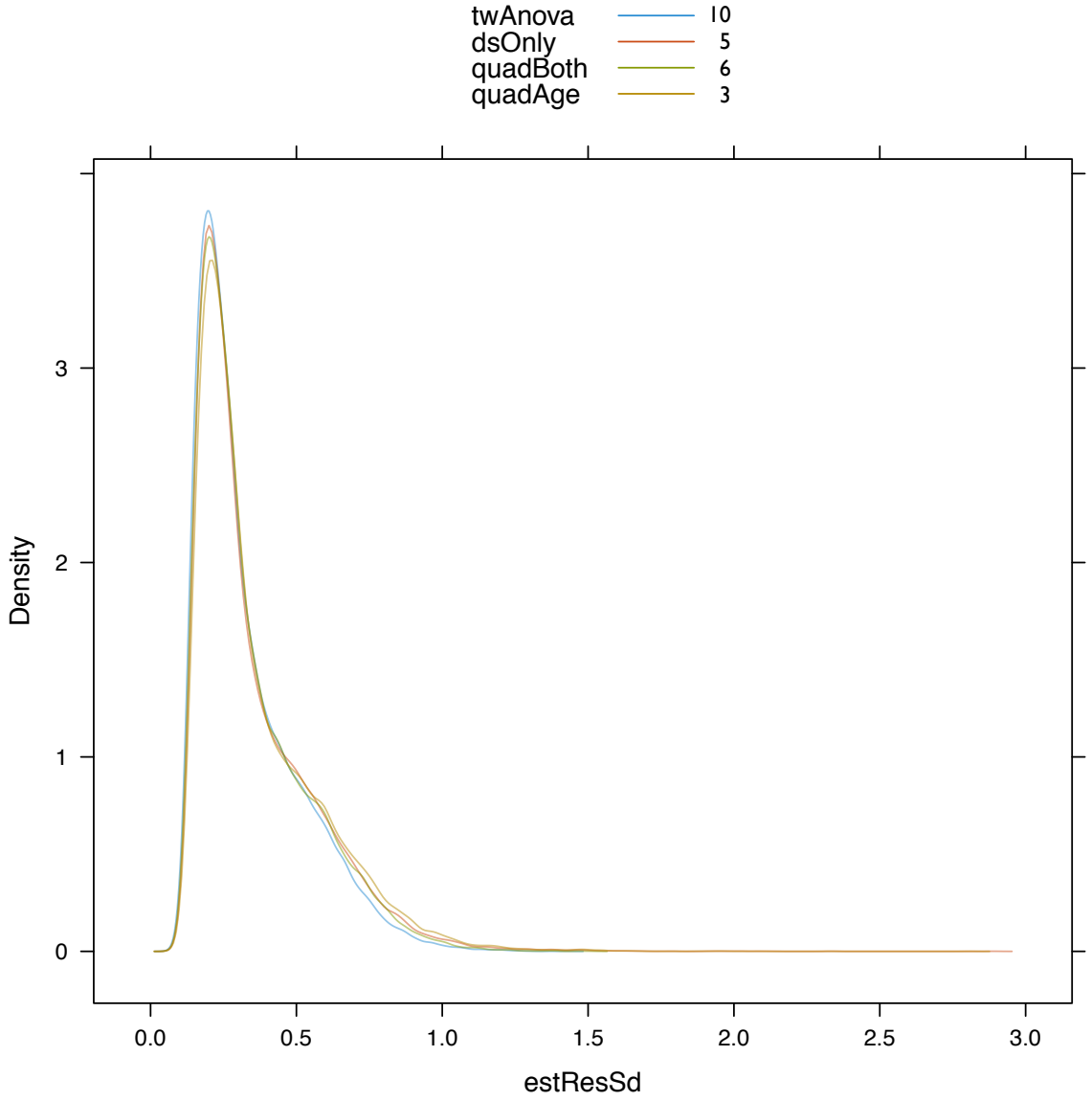


lm(gExp ~ age + I(age^2))

How "big" are these models? How many parameters are we using to specify the mean structure?

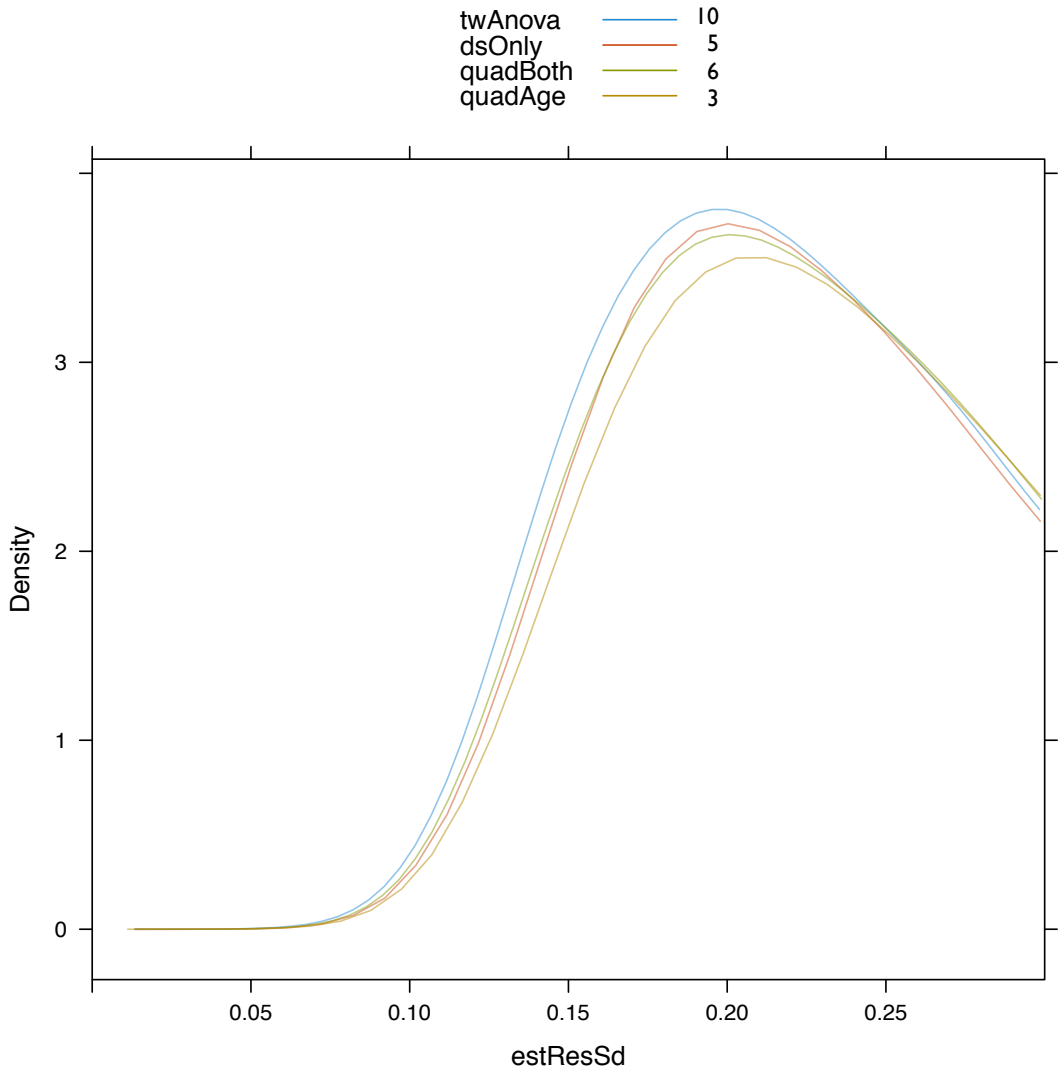
$$y_i = f(x_i;\alpha) + \varepsilon_i, \mathrm{var}(\varepsilon) = \sigma^2$$

Let's look at estimates of the error standard deviation.



$$y_i = f(x_i;\alpha) + \varepsilon_i, \mathrm{var}(\varepsilon) = \sigma^2$$

Let's look at estimates of the error standard deviation.

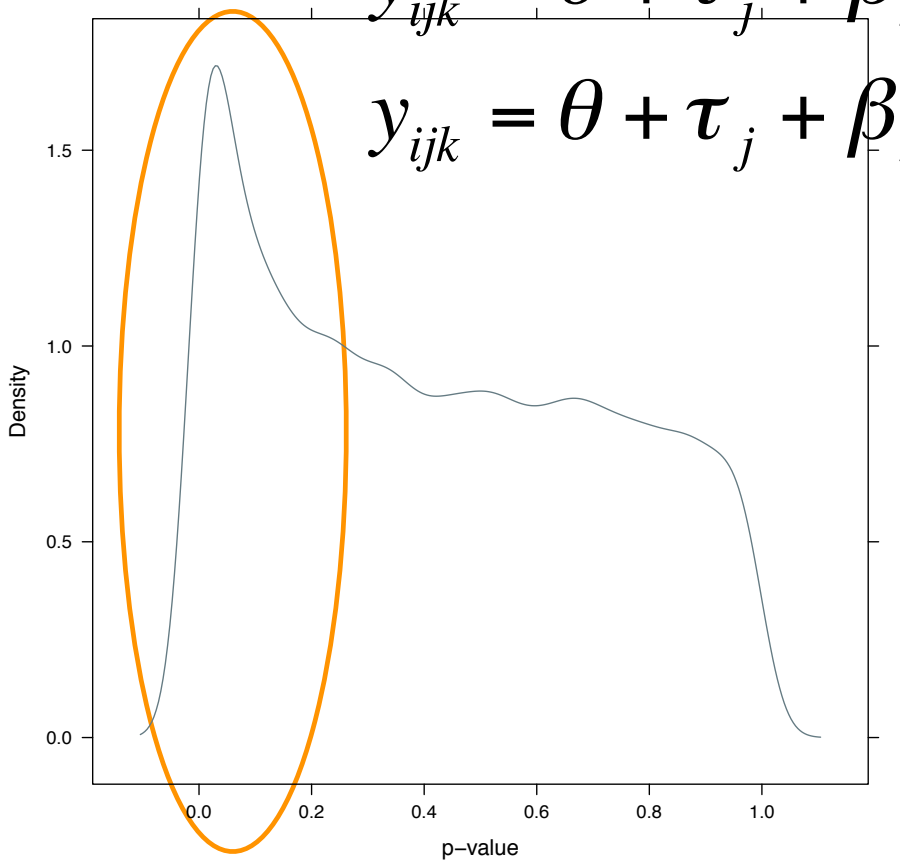


In the two-way ANOVA model, is there evidence for gType * devStage interaction? YES.

```
## this code is fictional but conveys the point
anova(lm(gExp ~ gType * devStage), lm(gExp ~ gType + devStage))
## inspecting the p-values from these F tests
```

$$y_{ijk} = \theta + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk}$$ "big"

$$y_{ijk} = \theta + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk}$$ "small"



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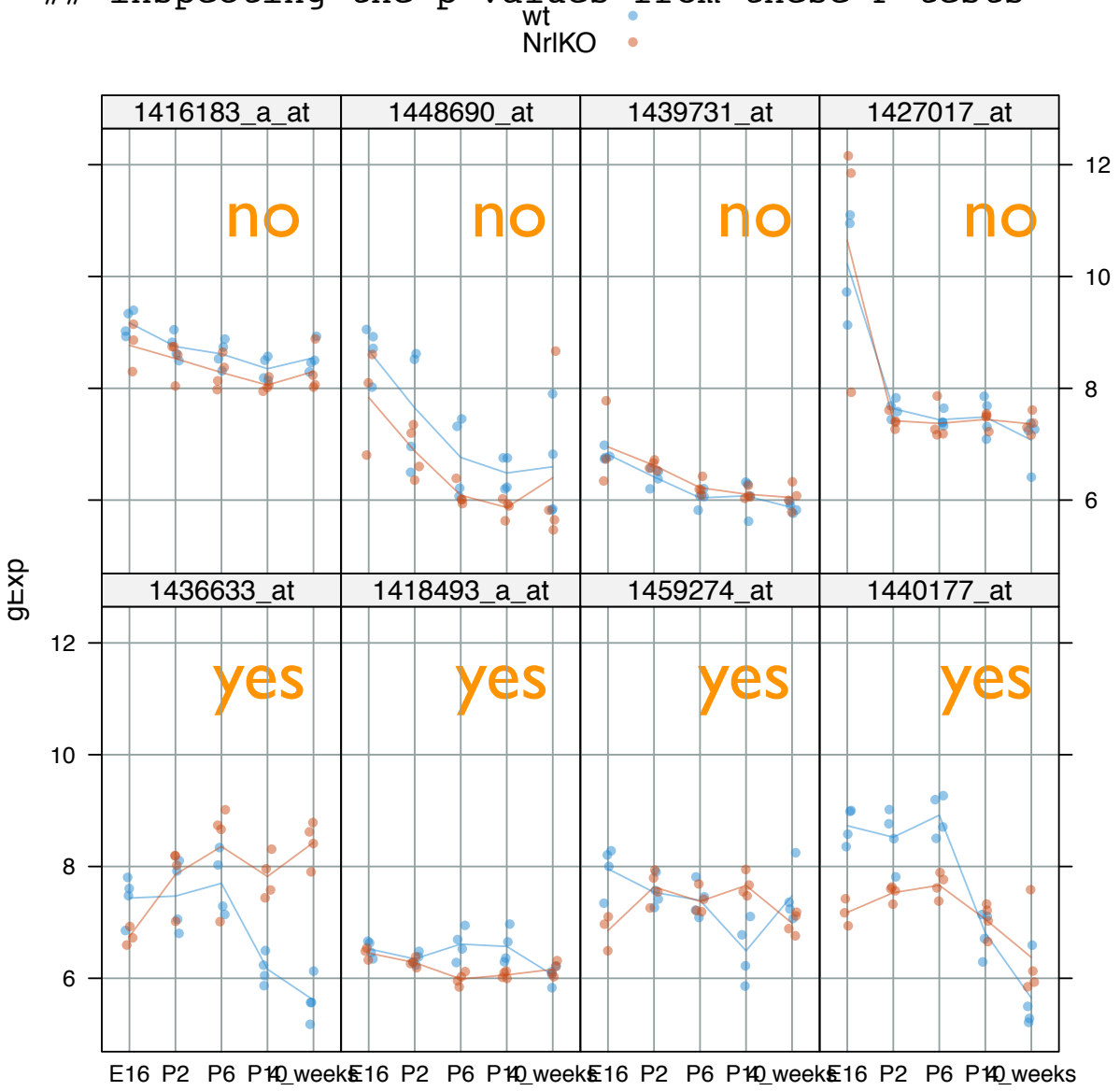

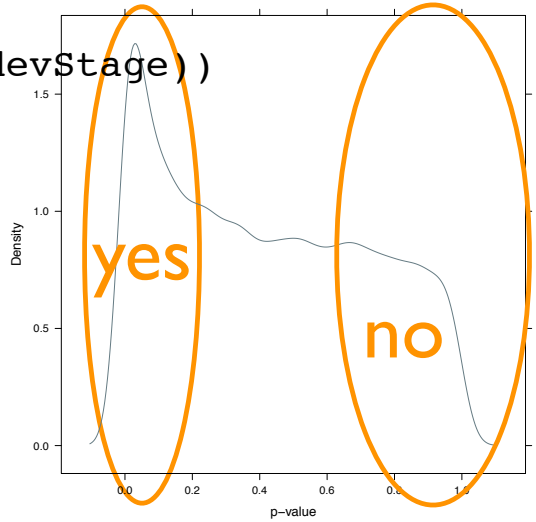

interaction?

In the two-way ANOVA model, is there evidence that genotype matters? YES.

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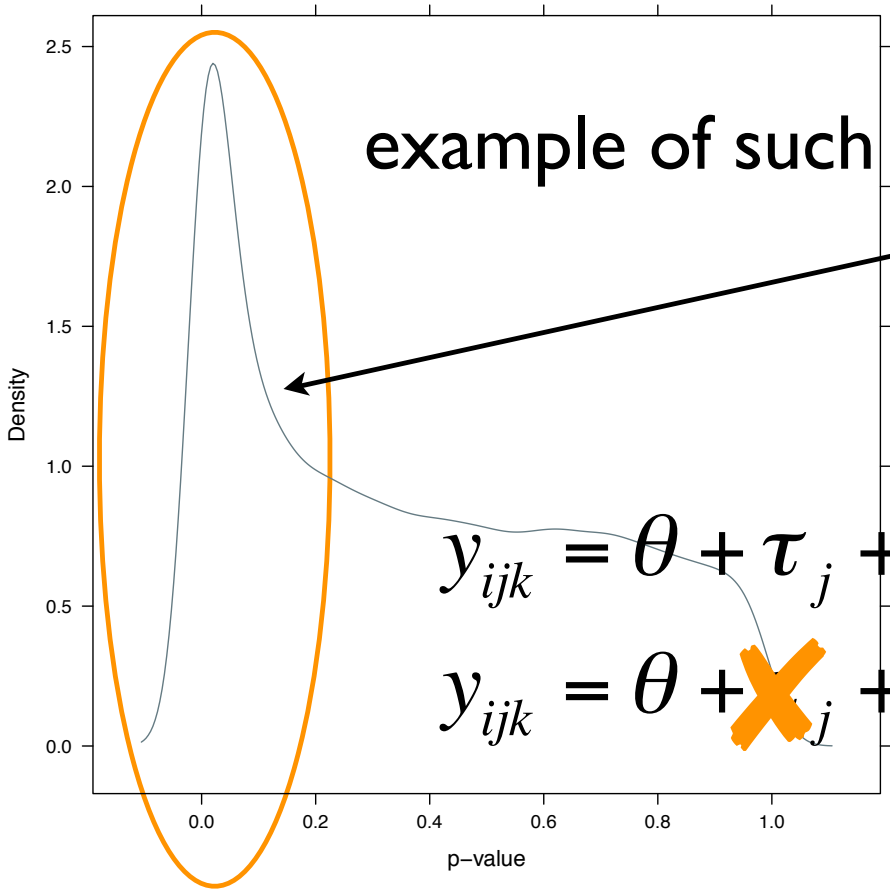
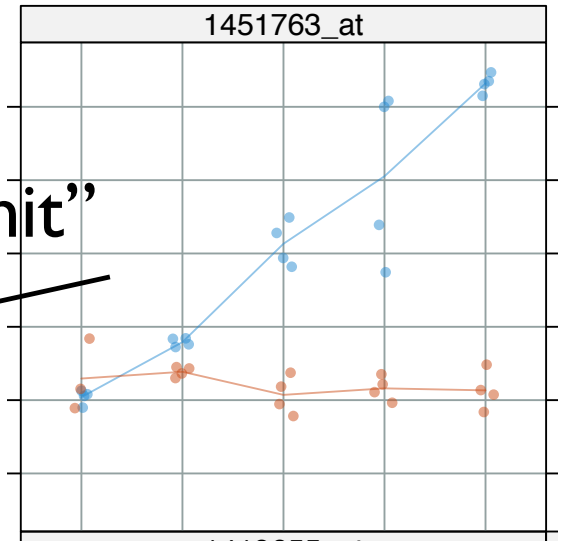


example of such a "hit"



$$y_{ijk} = \theta + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk}$$ "big"

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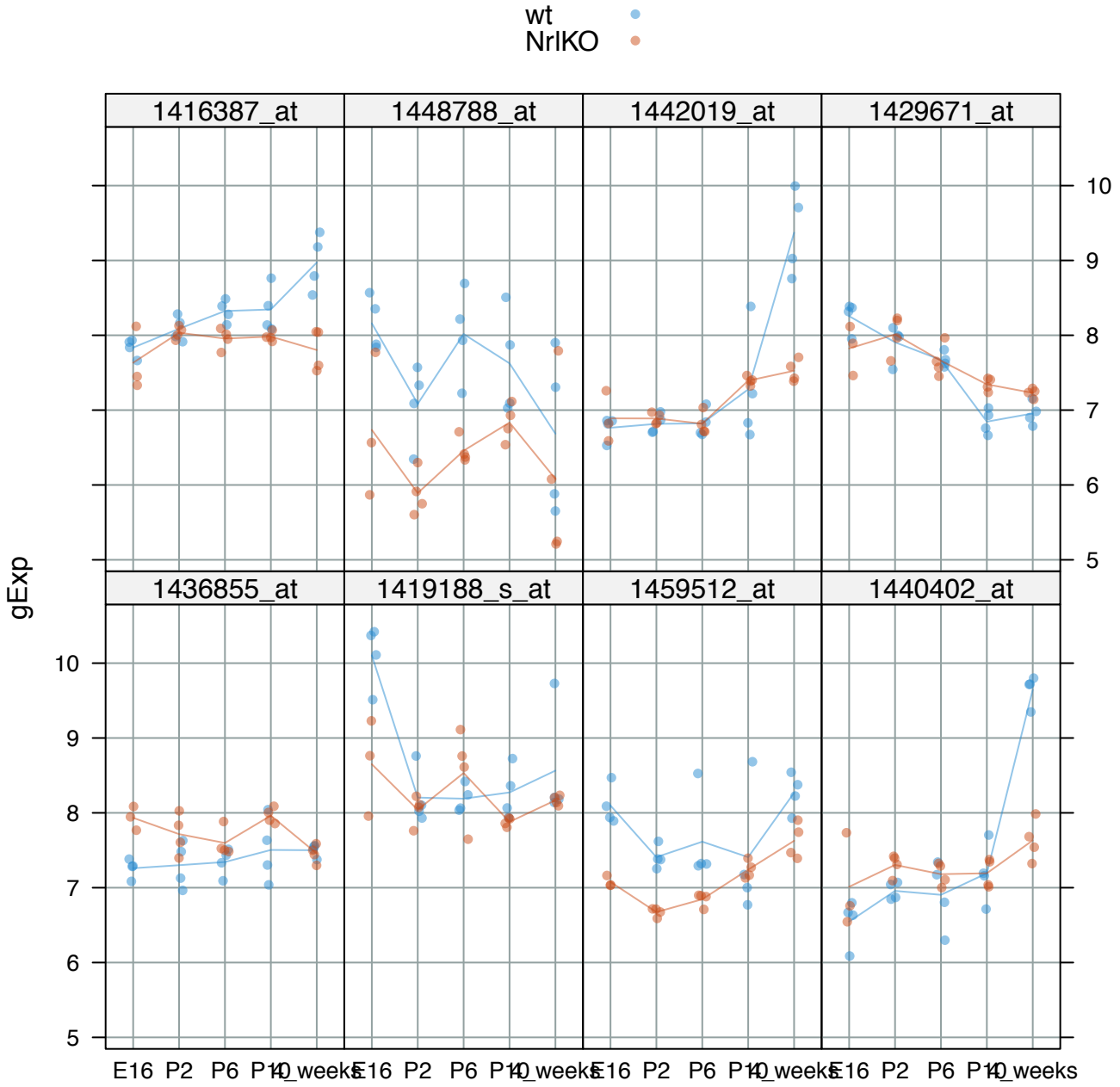
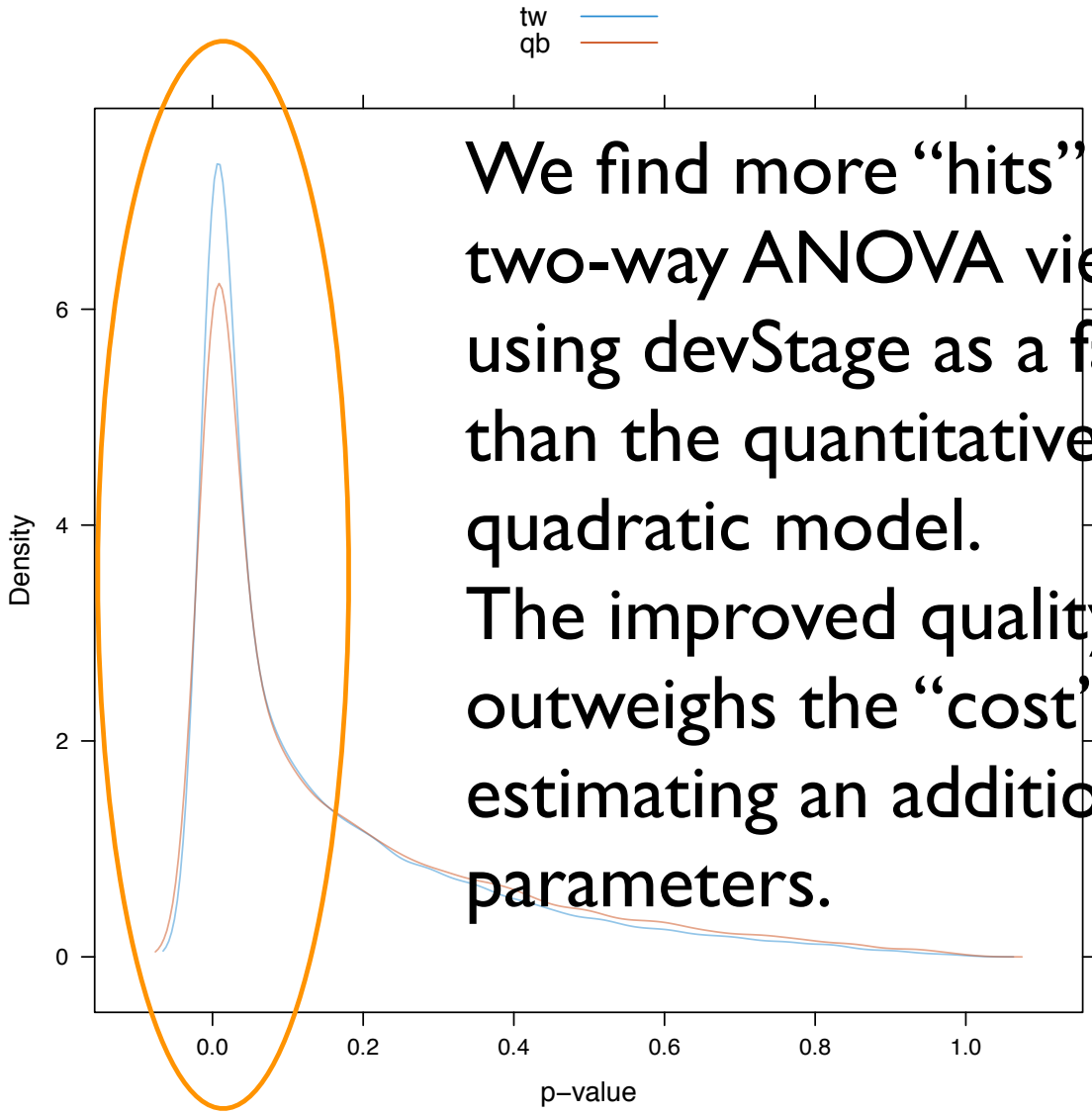
more "gType" hits within the ANOVA models



Looking at evidence of any differential expression at all (overall F test) in the two-way ANOVA model vs. the quadratic.



We find more "hits" with the two-way ANOVA viewpoint, i.e. using devStage as a factor rather than the quantitative age and a quadratic model.

The improved quality of fit outweighs the "cost" of estimating an additional 4 parameters.

where to next? ... Wednesday

in many studies, the # replicates is small relative to # params being estimated

can lead to crazy small estimates of error variance
which leads to crazy large test statistics
which leads to crazy small p-values
which leads to "hits" where the observed phenomenon is rather subtle

which leads to people saying the platform and/or analysis method and/or analyst is bad

moderating the variance estimates can be very helpful --> limma!

where to next? ... following Wednesday

multiple testing, large scale inference

analysis of high-throughput data results in thousands of "gene-wise" hypothesis tests

often, "gene-wise" analysis is relatively simple

BUT a recurring and thorny issue is how to handle thousands of p-values, each for a separate hypothesis test

how to guard against crazy # false positives?

which error rate is more relevant ... rate at which null genes are 'discovered' or rate at which 'discoveries' are null?