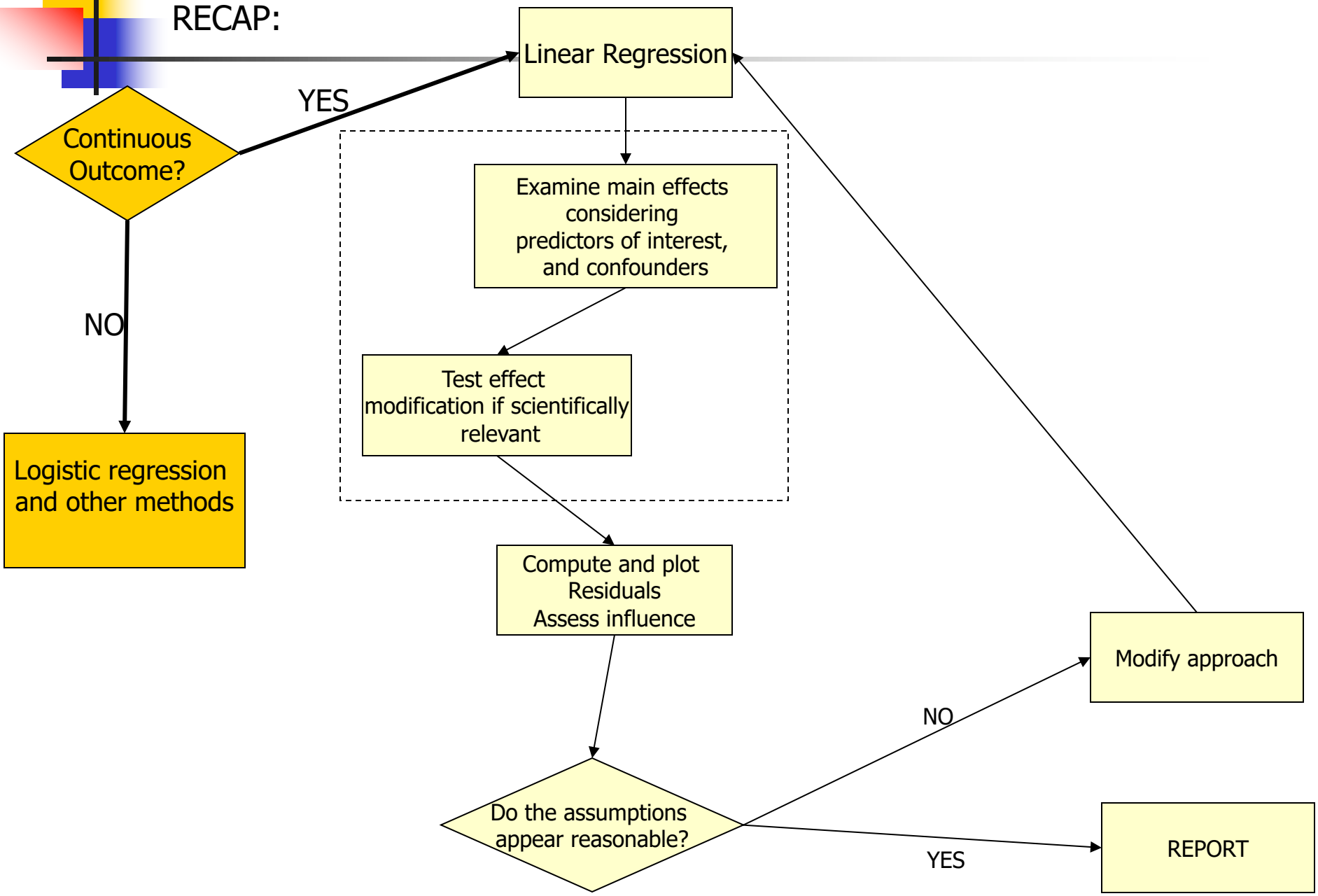
REGRESSION MODELS

ANOVA

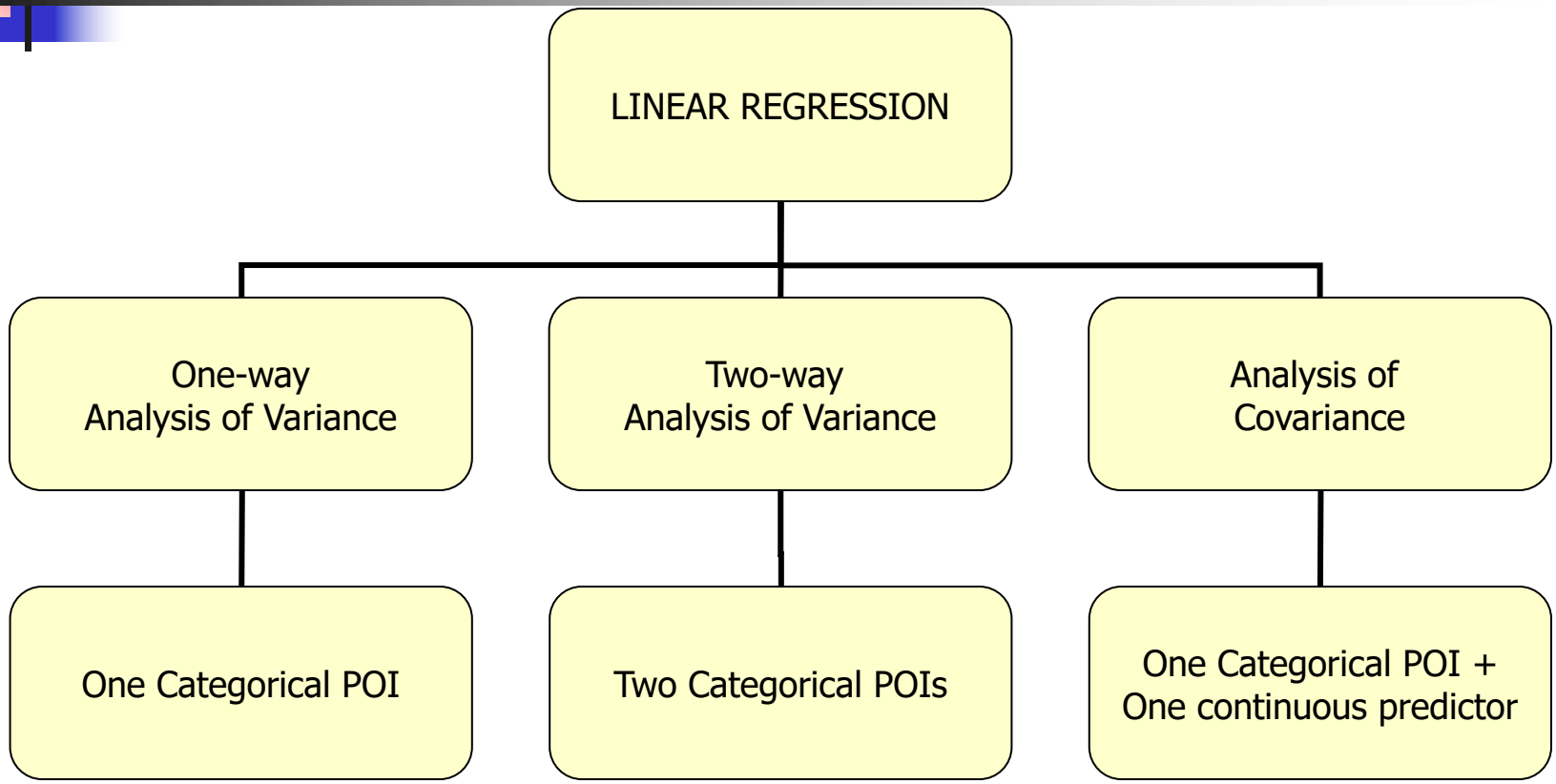
RECAP:


Continuous Outcome?
YES
NO
Linear Regression
Examine main effects considering predictors of interest, and confounders
Test effect modification if scientifically relevant
Logistic regression and other methods
Compute and plot Residuals Assess influence
Do the assumptions appear reasonable?
NO
YES
Modify approach
REPORT

COMING UP NEXT: ANOVA – a special case of linear regression

- What if the independent variables of interest are categorical?
- In this case, comparing the mean of the continuous outcome in the different categories may be of interest
- This is what is called ANalysis Of VAriance
- We will show that it is just a special case of linear regression

ANOVA – a special case of linear regression



Uses dummy variables to represent categorical variables!

Outline

- Motivation: We will consider some examples of ANOVA and show that they are special cases of linear regression
- ANOVA as a regression model
  - Dummy variables
- One-way ANOVA models
  - Contrasts
  - Multiple comparisons
- Two-way ANOVA models
  - Interactions
- ANCOVA models

ANOVA/ANCOVA: Motivation

- Let's investigate if genetic factors are associated with cholesterol levels.
  - Ideally, you would have a <u>confirmatory analysis</u> of scientific hypotheses formulated prior to data collection
  - Alternatively, you could consider an <u>exploratory analysis</u> – hypotheses generation for future studies

ANOVA/ANCOVA: Motivation

- Scientific hypotheses of interest:
  - Assess the effect of rs174548 on cholesterol levels.
  - Assess the effect of rs174548 and sex on cholesterol levels
    - Does the effect of rs174548 on cholesterol differ between males and females?
  - Assess the effect of rs174548 and age on cholesterol levels
    - Does the effect of rs174548 on cholesterol differ depending on subject's age?

ANOVA: One-Way Model
Motivation:

- Scientific question:
  - Assess the effect of rs174548 on cholesterol levels.

Motivation: Example

Here are some descriptive summaries:

```
> tapply(chol, factor(rs174548), mean)
       0        1        2
181.0617 187.8639 186.5000


> tapply(chol, factor(rs174548), sd)
       0        1        2
21.13998 23.74541 17.38333
```

Motivation: Example

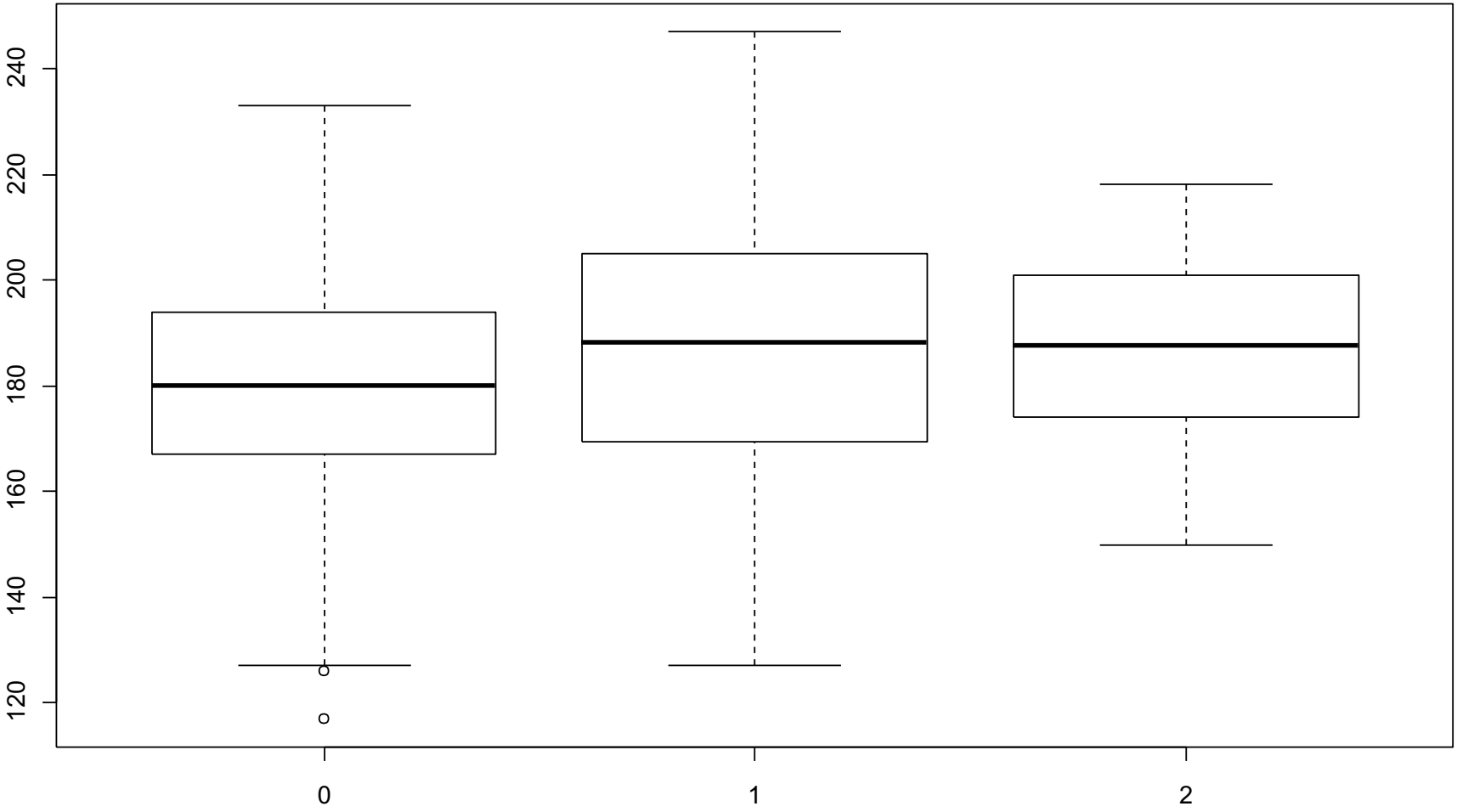
Another way of getting the same results:

```
> by(chol, factor(rs174548), mean)
   factor(rs174548): 0
[1] 181.0617
------------------------------------------------------------
   factor(rs174548): 1
[1] 187.8639
------------------------------------------------------------
   factor(rs174548): 2
[1] 186.5

> by(chol, factor(rs174548), sd)
   factor(rs174548): 0
[1] 21.13998
------------------------------------------------------------
   factor(rs174548): 1
[1] 23.74541
------------------------------------------------------------
   factor(rs174548): 2
[1] 17.38333
```

Motivation: Example

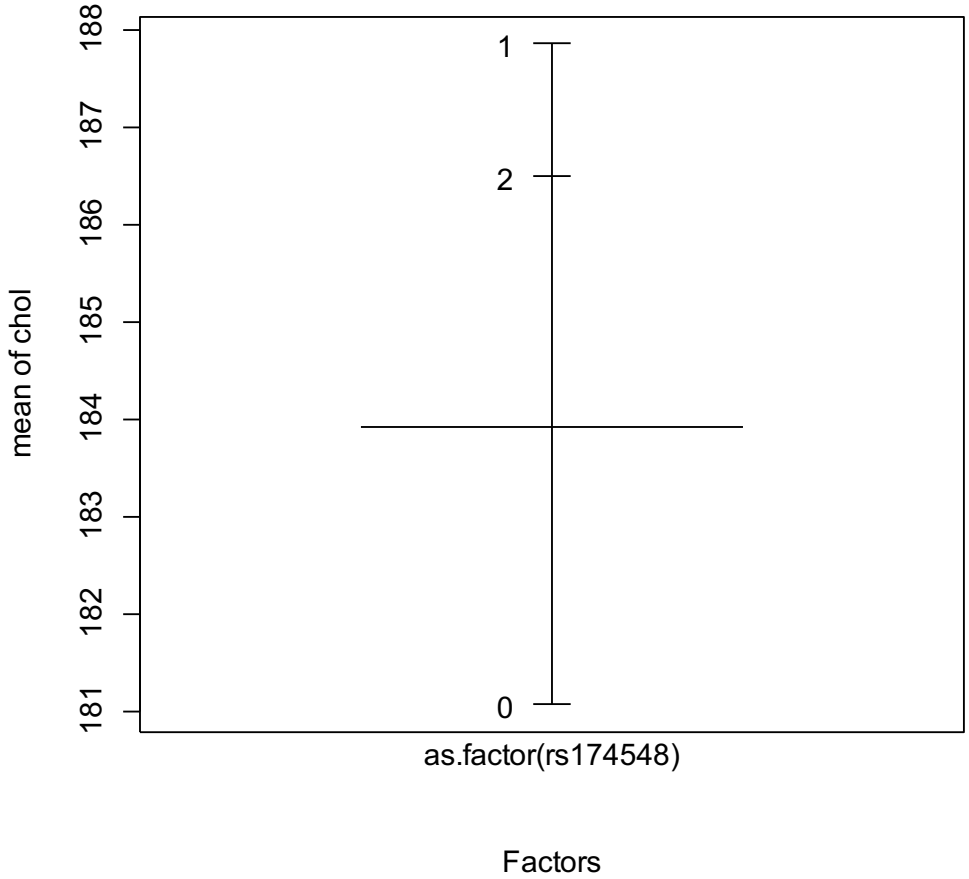
Is rs174548 associated with cholesterol?



R command: `boxplot(chol ~ factor(rs174548))`

Motivation: Example

Another graphical display:



R command:

```
plot.design(chol ~ factor(rs174548))
```

Motivation: Example

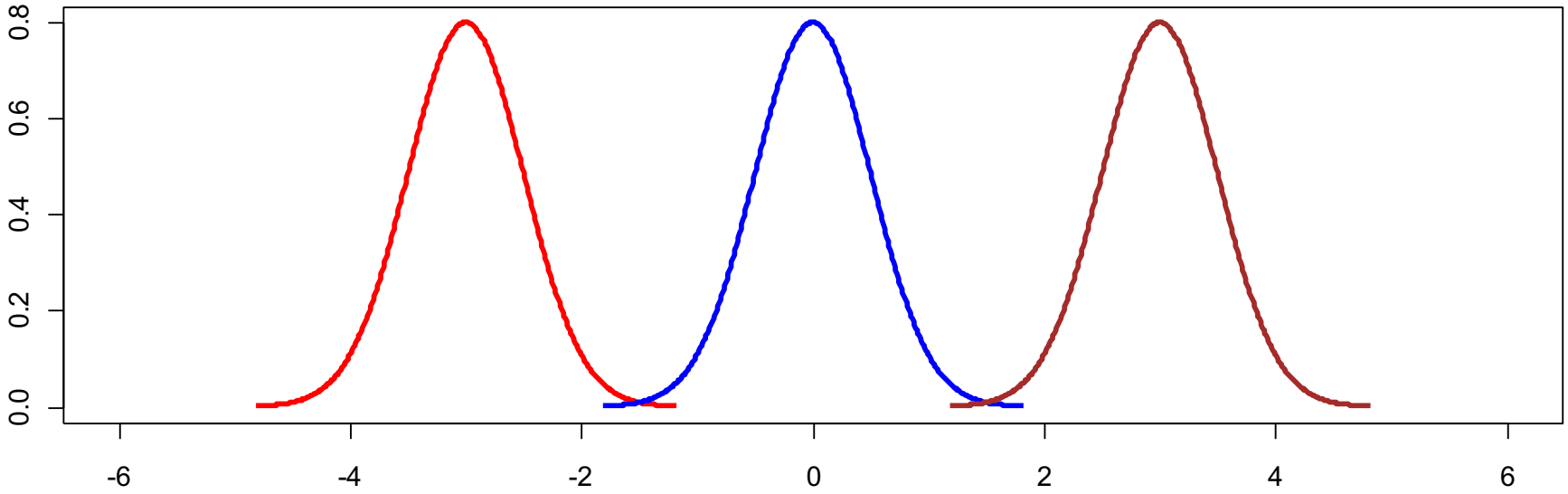
- Feature:
  - How do the mean responses compare across different groups?
    - Categorical/qualitative predictor

REGRESSION MODELS

One-way ANOVA as a regression model

ANalysis Of VAriance Models (ANOVA)

- Compares the means of several populations

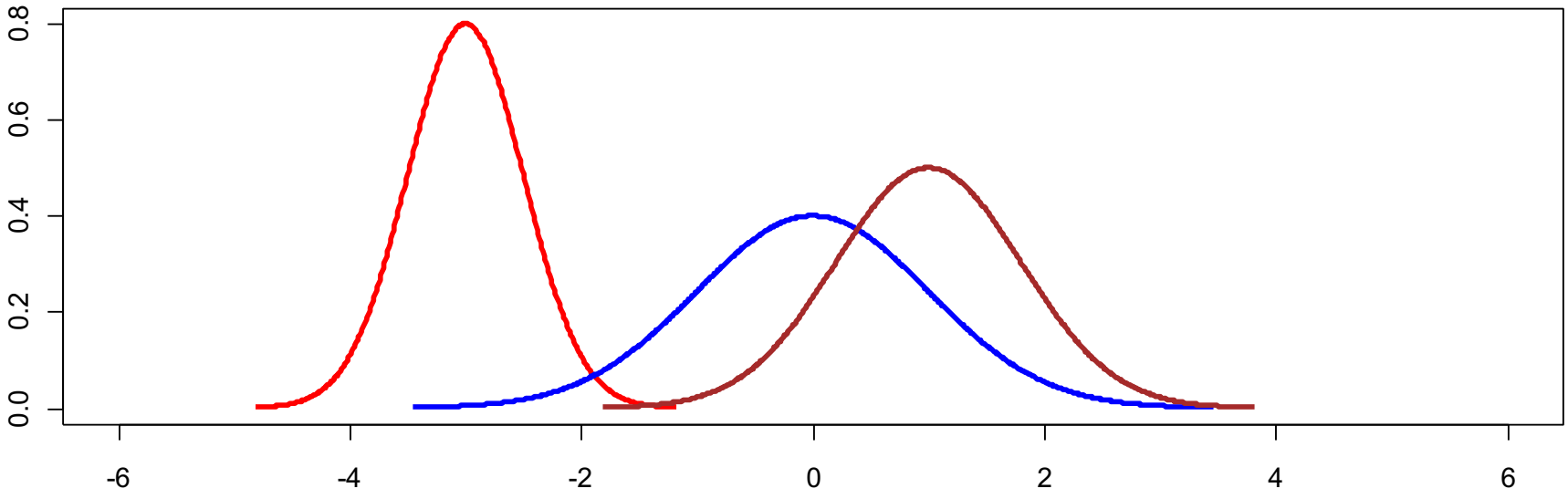


Assumptions for Classical ANOVA Framework:

Independence
Normality
Equal variances

ANalysis Of VAriance Models (ANOVA)

- Compares the means of several populations



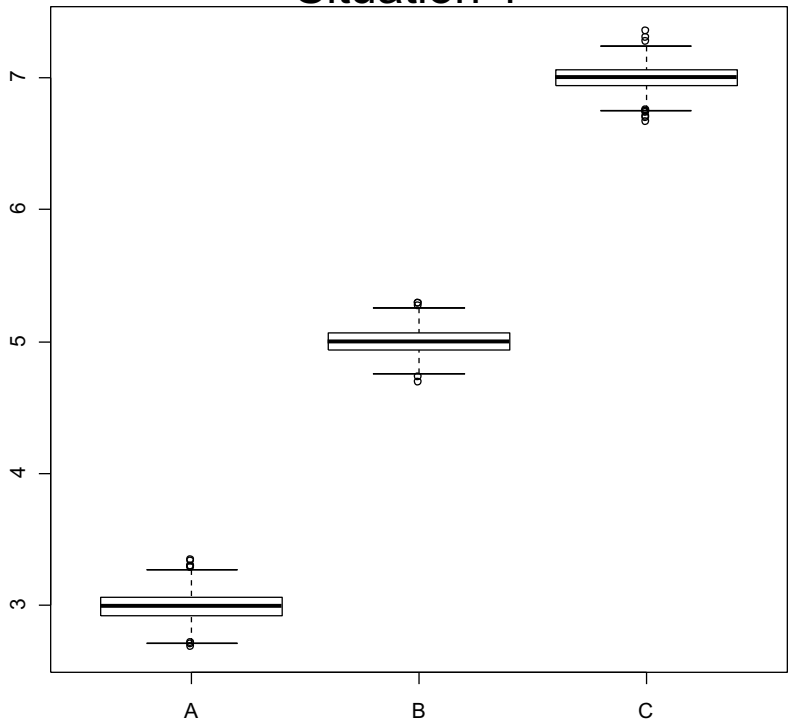
ANalysis Of VAriance Models (ANOVA)

- Compares the means of several populations
  - Counter-intuitive name!

ANalysis Of VAriance Models (ANOVA)

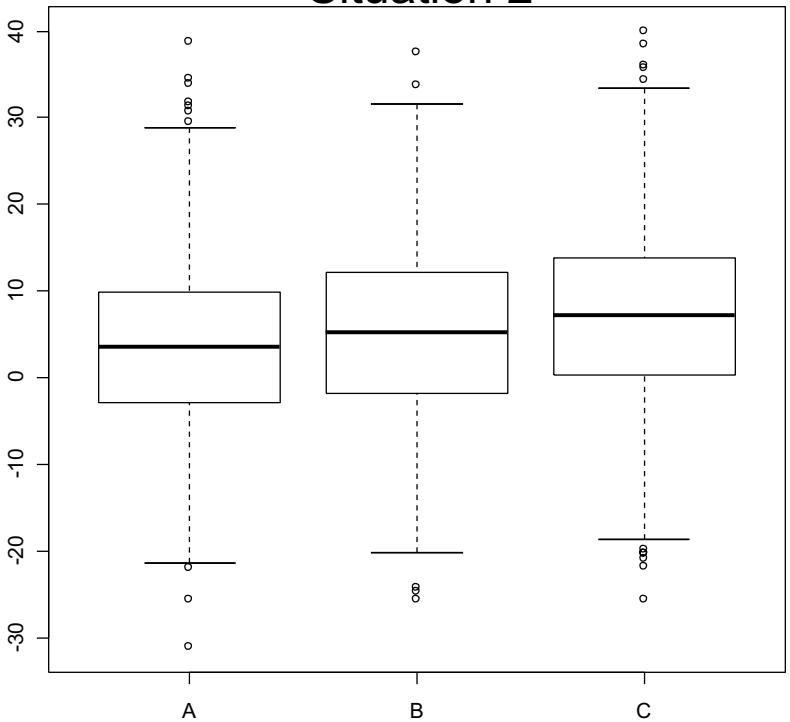
In both data sets, the true population means are: 3 (A), 5 (B), 7(C)

Situation 1



Low variance within groups

Situation 2



High variance within groups

Where do you expect to detect difference between population means?

ANalysis Of VAriance Models (ANOVA)

- Compares the means of several populations
  - Counter-intuitive name!
    - Underlying concept:
      - To assess whether the population means are equal, compares:
        - Variation between the sample means (MSR) to
        - Natural variation of the observations within the samples (MSE).
      - The larger the MSR compared to MSE the more support that there is a difference in the <u>population means</u>!
      - The ratio MSR/MSE is the F-statistic.

- We can make these comparisons with multiple linear regression: the different groups are represented with “dummy” variables

ANOVA as a multiple regression model

- Dummy Variables:
  - Suppose you have a categorical variable C with k categories 0,1, 2, …, k-1. To represent that variable we can construct k-1 dummy variables of the form

$$x_1 = \begin{cases} 1, & \text{if subject is in category } 1 \\ 0, & \text{otherwise} \end{cases}$$

$$x_2 = \begin{cases} 1, & \text{if subject is in category } 2 \\ 0, & \text{otherwise} \end{cases}$$

…

$$x_{k-1} = \begin{cases} 1, & \text{if subject is in category } k\text{-}1 \\ 0, & \text{otherwise} \end{cases}$$

The omitted category (here category 0) is the **reference group**.

ANOVA as a multiple regression model

- Dummy Variables:
  - Back to our motivating example:
    - Predictor: rs174548 (coded 0=C/C, 1=C/G, 2=G/G)
    - Outcome (Y): cholesterol

Let's take C/C as the reference group.

$$x_1 = \begin{cases} 1, & \text{if code } 1\,(\text{C/G}) \\ 0, & \text{otherwise} \end{cases}$$

$$x_2 = \begin{cases} 1, & \text{if code } 2\,(\text{G/G}) \\ 0, & \text{otherwise} \end{cases}$$

ANOVA as a multiple regression model

rs174548	Mean cholesterol	X_1	X_2
C/C	μ_0	0	0
C/G	μ_1	1	0
G/G	μ_2	0	1

ANOVA as a multiple regression model

- Regression with Dummy Variables:
  - Example:

    Model: $E[Y|x_1, x_2] = \beta_0 + \beta_1 x_1 + \beta_2 x_2$

- Interpretation of model parameters?

ANOVA as a multiple regression model

Mean	Regression Model
μ_0	β_0
μ_1	$\beta_0 + \beta_1$
μ_2	$\beta_0 + \beta_2$

ANOVA as a multiple regression model

- Regression with Dummy Variables:
  - Example:

    Model: $E[Y|x_1, x_2] = \beta_0 + \beta_1 x_1 + \beta_2 x_2$

- Interpretation of model parameters?
  - $\mu_0 = \beta_0$: mean cholesterol when rs174548 is C/C
  - $\mu_1 = \beta_0 + \beta_1$: mean cholesterol when rs174548 is C/G
  - $\mu_2 = \beta_0 + \beta_2$: mean cholesterol when rs174548 is G/G

ANOVA as a multiple regression model

- Regression with Dummy Variables:
  - Example:

    Model: $E[Y|x_1, x_2] = \beta_0 + \beta_1 x_1 + \beta_2 x_2$

- Interpretation of model parameters?
  - $\mu_0 = \beta_0$: mean cholesterol when rs174548 is C/C
  - $\mu_1 = \beta_0 + \beta_1$: mean cholesterol when rs174548 is C/G
  - $\mu_2 = \beta_0 + \beta_2$: mean cholesterol when rs174548 is G/G

  - Alternatively
    - β_1: difference in mean cholesterol levels between groups with rs174548 equal to C/G and C/C ($\mu_1 - \mu_0$).

    - β_2: difference in mean cholesterol levels between groups with rs174548 equal to G/G and C/C ($\mu_2 - \mu_0$).

ANOVA: One-Way Model

- Goal:
  - Compare the means of K independent groups (defined by a categorical predictor)
    - Statistical Hypotheses:
      - (Global) Null Hypothesis:

$$H_0: \mu_0 = \mu_1 = \ldots = \mu_{K-1} \text{ or, equivalently,}$$

$$H_0: \beta_1 = \beta_2 = \ldots = \beta_{K-1} = 0$$

      - Alternative Hypothesis:

$$H_1: \text{not all means are equal}$$

  - If the means of the groups are not all equal (i.e. you rejected the above H_0), determine which ones are different (multiple comparisons)

Estimation and Inference

- Global Hypotheses

  H_0: $\mu_1 = \mu_2 = \ldots = \mu_K$ vs. H_1: not all means are equal

  H_0: $\beta_1 = \beta_2 = \ldots = \beta_{K-1} = 0$

- Analysis of variance table

Source	df	SS	MS	F
Regression	K-1	SSR= $\sum_i (\bar{y}_i - \bar{y})^2$	MSR= SSR/(K-1)	MSR/ MSE
Residual	n-K	SSE= $\sum_{i,j} (y_{ij} - \bar{y}_i)^2$	MSE= SSE/n-K	
Total	n-1	SST= $\sum_{i,j} (y_{ij} - \bar{y})^2$		

ANOVA: One-Way Model

- How to fit a one-way model as a regression problem?
  - Need to use "dummy" variables
    - Create on your own (can be tedious!)
    - Most software packages will do this for you
      - R creates dummy variables in the background <u>as long as</u> you state you have a categorical variable (may need to use: factor)

ANOVA: One-Way Model

By hand:
Creating "dummy" variables:

```
> dummy1 = 1*(rs174548==1)
> dummy2 = 1*(rs174548==2)
```

Fitting the ANOVA model:

```
> fit0 = lm(chol ~ dummy1 + dummy2)
> summary(fit0)
Call:
lm(formula = chol ~ dummy1 + dummy2)

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  181.062      1.455 124.411  < 2e-16 ***
dummy1         6.802      2.321   2.930  0.00358 **
dummy2         5.438      4.540   1.198  0.23167
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221,     Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184

> anova(fit0)
Analysis of Variance Table

Response: chol
           Df Sum Sq Mean Sq F value   Pr(>F)
dummy1      1   3624    3624  7.5381 0.006315 **
dummy2      1    690     690  1.4350 0.231665
Residuals 397 190875     481
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ANOVA: One-Way Model

Better:
Let R do it for you!

```
> fit1.1 = lm(chol ~ factor(rs174548))
> summary(fit1.1)
Call:
lm(formula = chol ~ factor(rs174548))

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
                     Estimate  Std. Error t value Pr(>|t|)
(Intercept)           181.062       1.455 124.411  < 2e-16 ***
factor(rs174548)1     6.802         2.321 2.930    0.00358 **
factor(rs174548)2     5.438         4.540 1.198    0.23167
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Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221,     Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184

> anova(fit1.1)
Analysis of Variance Table

Response: chol
                     Df Sum Sq Mean Sq F value  Pr(>F)
factor(rs174548)      2   4314    2157  4.4865 0.01184 *
Residuals           397 190875     481
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ANOVA: One-Way Model

- Your turn!
  - Compare model fit results (fit0 & fit1.1)

    What do you conclude?

ANOVA: One-Way Model

```
> fit0 = lm(chol ~ dummy1 + dummy2)
> summary(fit0)
Call:
lm(formula = chol ~ dummy1 + dummy2)

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  181.062      1.455 124.411  < 2e-16 ***
dummy1         6.802      2.321   2.930  0.00358 **
dummy2         5.438      4.540   1.198  0.23167
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Multiple R-squared: 0.0221, Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184
```

```
> fit1.1 = lm(chol ~ factor(rs174548))
> summary(fit1.1)
Call:
lm(formula = chol ~ factor(rs174548))

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           181.062      1.455 124.411  < 2e-16 ***
factor(rs174548)1       6.802      2.321   2.930  0.00358 **
factor(rs174548)2       5.438      4.540   1.198  0.23167
---

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221,  Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184
```

```
> anova(fit0)
Analysis of Variance Table

Response: chol
           Df Sum Sq Mean Sq F value   Pr(>F)
dummy1      1   3624    3624  7.5381 0.006315 **
dummy2      1    690     690  1.4350 0.231665
Residuals 397 190875     481
---
```

```
> anova(fit1.1)
Analysis of Variance Table

Response: chol
                     Df Sum Sq Mean Sq F value  Pr(>F)
factor(rs174548)      2   4314    2157  4.4865 0.01184 *
Residuals           397 190875     481
---
```

ANOVA: One-Way Model

```
> fit0 = lm(chol ~ dummy1 + dummy2)
> summary(fit0)
Call:
lm(formula = chol ~ dummy1 + dummy2)

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  181.062      1.455 124.411  < 2e-16 ***
dummy1         6.802      2.321   2.930  0.00358 **
dummy2         5.438      4.540   1.198  0.23167
---

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221, Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184
```

```
> fit1.1 = lm(chol ~ factor(rs174548))
> summary(fit1.1)
Call:
lm(formula = chol ~ factor(rs174548))

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           181.062      1.455 124.411  < 2e-16 ***
factor(rs174548)1       6.802      2.321   2.930  0.00358 **
factor(rs174548)2       5.438      4.540   1.198  0.23167
---

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221,  Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184
```

```
> anova(fit0)
Analysis of Variance Table

Response: chol
           Df Sum Sq Mean Sq F value   Pr(>F)
dummy1      1   3624    3624  7.5381 0.006315 **
dummy2      1    690     690  1.4350 0.231665
Residuals 397 190875     481
---
```

```
> anova(fit1.1)
Analysis of Variance Table

Response: chol
                    Df Sum Sq Mean Sq F value  Pr(>F)
factor(rs174548)     2   4314    2157  4.4865 0.01184 *
Residuals          397 190875     481
---
```

```
> 1-pf(4.4865,2,397)
[1] 0.01183671
> 1-pf(((3624+690)/2)/481,2,397)
[1] 0.01186096
```

ANOVA: One-Way Model

```
> fit1.1 = lm(chol ~ factor(rs174548))
> summary(fit1.1)
Call:
lm(formula = chol ~ factor(rs174548))

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           181.062      1.455 124.411  < 2e-16
factor(rs174548)1       6.802      2.321   2.930  0.00358
factor(rs174548)2       5.438      4.540   1.198  0.23167
---

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221,  Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184

> anova(fit1.1)
Analysis of Variance Table

Response: chol
                    Df Sum Sq Mean Sq F value  Pr(>F)
factor(rs174548)     2   4314    2157  4.4865 0.01184 *
Residuals          397 190875     481
---
```

- Let's interpret the regression model results!
  - What is the interpretation of the regression model coefficients?

ANOVA: One-Way Model

```
> fit1.1 = lm(chol ~ factor(rs174548))
> summary(fit1.1)
Call:
lm(formula = chol ~ factor(rs174548))

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           181.062      1.455 124.411  < 2e-16
factor(rs174548)1       6.802      2.321   2.930  0.00358
factor(rs174548)2       5.438      4.540   1.198  0.23167
---

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221,  Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184

> anova(fit1.1)
Analysis of Variance Table

Response: chol
                    Df Sum Sq Mean Sq F value  Pr(>F)
factor(rs174548)     2   4314    2157  4.4865 0.01184 *
Residuals          397 190875     481
---
```

- Interpretation:
  - Estimated mean cholesterol for C/C group: 181.062 mg/dl
  - Estimated difference in mean cholesterol levels between C/G and C/C groups: 6.802 mg/dl
  - Estimated difference in mean cholesterol levels between G/G and C/C groups: 5.438 mg/dl

ANOVA: One-Way Model

```
> fit1.1 = lm(chol ~ factor(rs174548))
> summary(fit1.1)
Call:
lm(formula = chol ~ factor(rs174548))

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           181.062      1.455 124.411  < 2e-16
factor(rs174548)1       6.802      2.321   2.930  0.00358
factor(rs174548)2          5.438      4.540   1.198
0.23167
---

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221,  Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184

> anova(fit1.1)
Analysis of Variance Table

Response: chol
                     Df Sum Sq Mean Sq F value  Pr(>F)
factor(rs174548)      2   4314    2157  4.4865 0.01184 *
Residuals           397 190875     481
---
```

- Overall F-test shows a significant p-value. We reject the null hypothesis that the mean cholesterol levels are the same across groups defined by rs174548 (p=0.01184).
  - This does not tell us which groups are different!

    (Need to perform multiple comparisons! More soon…)

ANOVA: One-Way Model

Alternative form: (better if you will perform multiple comparisons)

```
> fit1.2 = lm(chol ~ -1 + factor(rs174548))
> summary(fit1.2)
Call:
lm(formula = chol ~ -1 + factor(rs174548))

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
factor(rs174548)0     181.062      1.455  124.41   <2e-16 ***
factor(rs174548)1     187.864      1.809  103.88   <2e-16 ***
factor(rs174548)2     186.500      4.300   43.37   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.9861,     Adjusted R-squared: 0.986
F-statistic:  9383 on 3 and 397 DF,  p-value: < 2.2e-16

> anova(fit1.2)
Analysis of Variance Table
Response: chol
                     Df   Sum Sq Mean Sq F value    Pr(>F)
factor(rs174548)      3 13534205 4511402  9383.2 < 2.2e-16 ***
Residuals           397   190875     481
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ANOVA: One-Way Model

How about this one?
How is rs174548 being treated now?

Compare model fit results from (fit1.1 & fit2).

```
> fit2 = lm(chol ~ rs174548)
> summary(fit2)

Call:
lm(formula = chol ~ rs174548)

Residuals:
    Min      1Q  Median      3Q     Max 
-64.575 -16.278  -0.575  15.120  60.722 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)  181.575      1.411 128.723  < 2e-16 ***
rs174548       4.703      1.781   2.641  0.00858 ** 
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.95 on 398 degrees of freedom
Multiple R-squared: 0.01723,    Adjusted R-squared: 0.01476 
F-statistic: 6.977 on 1 and 398 DF,  p-value: 0.008583 

> anova(fit2)
Analysis of Variance Table

Response: chol
           Df Sum Sq Mean Sq F value   Pr(>F)   
rs174548    1   3363    3363  6.9766 0.008583 **
Residuals 398 191827     482                    
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ANOVA: One-Way Model

```
> fit2 = lm(chol ~ rs174548)
> summary(fit2)

Call:
lm(formula = chol ~ rs174548)

Residuals:
    Min      1Q  Median      3Q     Max 
-64.575 -16.278  -0.575  15.120  60.722 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)  181.575      1.411 128.723  < 2e-16 ***
rs174548       4.703      1.781   2.641  0.00858 ** 

Residual standard error: 21.95 on 398 degrees of freedom
Multiple R-squared: 0.01723, Adjusted R-squared: 0.01476 
F-statistic: 6.977 on 1 and 398 DF,  p-value: 0.008583 

> anova(fit2)
Analysis of Variance Table

Response: chol
           Df Sum Sq Mean Sq F value   Pr(>F)    
rs174548    1   3363    3363  6.9766 0.008583 ** 
Residuals 398 191827     482                     
```

- Model: $E[Y|x] = \beta_0 + \beta_1 x$

where Y: cholesterol, x: rs174548

- Interpretation of model parameters?
  - β_0: mean cholesterol in the C/C group [estimate: 181.575 mg/dl]
  - β_1: mean cholesterol difference between C/G and C/C – or – between G/G and C/G groups [estimate: 4.703 mg/dl]
- This model presumes differences between "consecutive" groups are the same (in this example, linear dose effect of allele) – more restrictive than the ANOVA model!

Back to the ANOVA model…

ANOVA: One-Way Model

```
> fit1.1 = lm(chol ~ factor(rs174548))
> summary(fit1.1)
Call:
lm(formula = chol ~ factor(rs174548))

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           181.062      1.455 124.411  < 2e-16
factor(rs174548)1       6.802      2.321   2.930  0.00358
factor(rs174548)2       5.438      4.540   1.198  0.23167
---

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221,  Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184

> anova(fit1.1)
Analysis of Variance Table

Response: chol
                     Df Sum Sq Mean Sq F value  Pr(>F)
factor(rs174548)      2   4314    2157  4.4865 0.01184 *
Residuals           397 190875     481
---
```

- We rejected the null hypothesis that the mean cholesterol levels are the same across groups defined by rs174548 (p=0.01184).
  - What are the groups with differences in means?

MULTIPLE COMPARISONS (coming up)

One-Way ANOVA allowing for unequal variances

We can also perform one-way ANOVA allowing for unequal variances:

```
> oneway.test(chol ~ factor(rs174548))

        One-way analysis of means (not assuming equal variances)

data:  chol and factor(rs174548)
F = 4.3258, num df = 2.000, denom df = 73.284, p-value = 0.01676
```

- We reject the null hypothesis that the mean cholesterol levels are the same across groups defined by rs174548 (p=0.01676).
  - What are the groups with differences in means?

MULTIPLE COMPARISONS (coming up)

One-Way ANOVA with robust standard errors

```
> summary(gee(chol ~ factor(rs174548), id=seq(1,length(chol))))
Beginning Cgee S-function, @(#) geeformula.q 4.13 98/01/27
running glm to get initial regression estimate
         (Intercept) factor(rs174548)1    factor(rs174548)2
          181.061674             6.802272             5.438326

 GEE:  GENERALIZED LINEAR MODELS FOR DEPENDENT DATA
 gee S-function, version 4.13 modified 98/01/27 (1998)

Model:
 Link:                      Identity
 Variance to Mean Relation: Gaussian
 Correlation Structure:     Independent

Call:
gee(formula = chol ~ factor(rs174548), id = seq(1, length(chol)))

Summary of Residuals:
         Min           1Q       Median           3Q          Max
-64.06167401 -15.91337769  -0.06167401  14.93832599  59.13605442

Coefficients:
                      Estimate Naive S.E.    Naive z Robust S.E.   Robust z
(Intercept)          181.061674   1.455346 124.411431    1.400016 129.328297
factor(rs174548)1      6.802272   2.321365   2.930290    2.402005   2.831914
factor(rs174548)2      5.438326   4.539833   1.197913    3.624271   1.500530

Estimated Scale Parameter:  480.7932
Number of Iterations:  1
```

Kruskal-Wallis Test

- Non-parametric analogue to the one-way ANOVA
  - Based on ranks

- In our example:

```
> kruskal.test(chol ~ factor(rs174548))

        Kruskal-Wallis rank sum test

data:  chol by factor(rs174548)
Kruskal-Wallis chi-squared = 7.4719, df = 2, p-value = 0.02385
```

  - Conclusion:
    - Evidence that the cholesterol distribution is not the same across all groups.
    - With the global null rejected, you can also perform pairwise comparisons [Wilcoxon rank sum], but adjust for multiplicities!

REGRESSION METHODS

MULTIPLE COMPARISONS

ANOVA: One-Way Model

- What are the groups with differences in means?

MULTIPLE COMPARISONS:

$$\left.\begin{array}{l} \mu_0 = \mu_1? \\ \mu_0 = \mu_2? \\ \mu_1 = \mu_2? \end{array}\right\} \text{Pairwise comparisons}$$

$(\mu_1 + \mu_2)/2 = \mu_0? \longrightarrow$ Non-pairwise comparison

Multiple Comparisons: Family-wise error rates

- Illustrating the multiple comparison problem
  - Truth: null hypotheses
  - Tests: pairwise comparisons - each at the 5% level.

What is the probability of rejecting at least one?

#groups = K	2	3	4	5	6	7	8	9	10
#pairwise comparisons C = K(K-1)/2	1	3	6	10	15	21	28	36	45
P(at least one sig) $=1-(1-0.05)^C$	0.05	0.143	0.265	0.401	0.537	0.659	0.762	0.842	0.901

That is, if you have three groups and make pairwise comparisons, each at the 5% level, your family-wise error rate (probability of making at least one false rejection) is over 14%!

Need to address this issue!

Several methods!!!

Multiple Comparisons

- Several methods:
  - None (no adjustment)
  - Bonferroni
  - Holm
  - Hochberg
  - Hommel
  - BH
  - BY
  - FDR
  - …

Available in R

Multiple Comparisons

- **Bonferroni** adjustment: for C tests performed, use level α/C (or multiply p-values by C).
  - Simple
  - Conservative
  - Must decide on number of tests beforehand
  - Widely applicable
  - Can be done without software!

Multiple Comparisons

- FDR (False Discovery Rate)
  - Less conservative procedure for multiple comparisons
  - Among rejected hypotheses, FDR controls the expected proportion of incorrectly rejected null hypotheses (that is, type I errors).

Multiple Comparisons

This option considers all pairwise comparisons

```
> ## call library for multiple comparisons
> library(multcomp)
>
> ## fit model
> fit1 = lm(chol ~ -1 + factor(rs174548))
>
> ## all pairwise comparisons
> ## -- first, define matrix of contrasts
> M = contrMat(table(rs174548), type="Tukey")
> M

         Multiple Comparisons of Means: Tukey Contrasts

       0  1 2
1 - 0 -1  1 0
2 - 0 -1  0 1
2 - 1  0 -1 1
>
> ## -- second, obtain estimates for multiple comparisons
> mc = glht(fit1, linfct =M)
```

Stands for general linear hypothesis testing

Multiple Comparisons

```
> ## -- third, adjust the p-values (or not) for multiple comparisons
> summary(mc, test=adjusted("none"))

         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts


Fit: lm(formula = chol ~ -1 + factor(rs174548))

Linear Hypotheses:
           Estimate Std. Error t value Pr(>|t|)
1 - 0 == 0    6.802      2.321   2.930  0.00358 **
2 - 0 == 0    5.438      4.540   1.198  0.23167
2 - 1 == 0   -1.364      4.665  -0.292  0.77015
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- none method)
```

Multiple Comparisons

```
> summary(mc, test=adjusted("bonferroni"))

         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts


Fit: lm(formula = chol ~ -1 + factor(rs174548))

Linear Hypotheses:
           Estimate Std. Error t value Pr(>|t|)
1 - 0 == 0    6.802      2.321   2.930   0.0107 *
2 - 0 == 0    5.438      4.540   1.198   0.6950
2 - 1 == 0   -1.364      4.665  -0.292   1.0000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- bonferroni method)
```

Multiple Comparisons

```
> summary(mc, test=adjusted("fdr"))

         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts


Fit: lm(formula = chol ~ -1 + factor(rs174548))

Linear Hypotheses:
           Estimate Std. Error t value Pr(>|t|)
1 - 0 == 0    6.802      2.321   2.930   0.0107 *
2 - 0 == 0    5.438      4.540   1.198   0.3475
2 - 1 == 0   -1.364      4.665  -0.292   0.7702
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- fdr method)
```

Multiple Comparisons

- What about using other adjustment methods?
  - For example, we used:

```
> summary(mc, test=adjusted("bonferroni"))
```

(all pairwise comparisons, with Bonferroni adjustment)

```
> summary(mc, test=adjusted("fdr"))
```

(all pairwise comparisons, with FDR adjustment)

  - Other options are:
    - `summary(mc, test=adjusted("holm"))`
    - `summary(mc, test=adjusted("hochberg"))`
    - `summary(mc, test=adjusted("hommel"))`
    - `summary(mc, test=adjusted("BH"))`
    - `summary(mc, test=adjusted("BY"))`

Results, in this particular example, are basically the same, but they don't need to be! Different criteria could lead to different results!

Summary:

GOAL: Comparison of means across K groups

One-way ANOVA:

$H_0: \mu_0 = \mu_1 = \ldots = \mu_{K-1}$

H_1: not all means are equal

Multiple Regression:

Model: $E[Y|\text{groups}] = \beta_0 + \beta_1 \text{group}_2 + \ldots + \beta_{k-1} \text{group}_k$

where group_1 is the reference group

$H_0: \beta_1 = \beta_2 = \ldots = \beta_{k-1} = 0$

H_1: not all β_i are equal to zero

Rejected H_0?

YES

Multiple Comparisons (control α overall)

e.g. Bonferroni: α/#comparisons

Relationships:

$$\mu_0 = \beta_0$$
$$\mu_1 = \beta_0 + \beta_1$$
$$\mu_2 = \beta_0 + \beta_2$$
$$\ldots$$
$$\mu_{K-1} = \beta_0 + \beta_{K-1}$$

REGRESSION METHODS

Two-way ANOVA models

ANOVA: Two-Way Model
Motivation:

- Scientific question:
  - Assess the effect of rs174548 and sex on cholesterol levels.

ANOVA: Two-Way Model

- Factors: A and B
- Goals:
  - Test for main effect of A
  - Test for main effect of B
  - Test for interaction effect of A and B

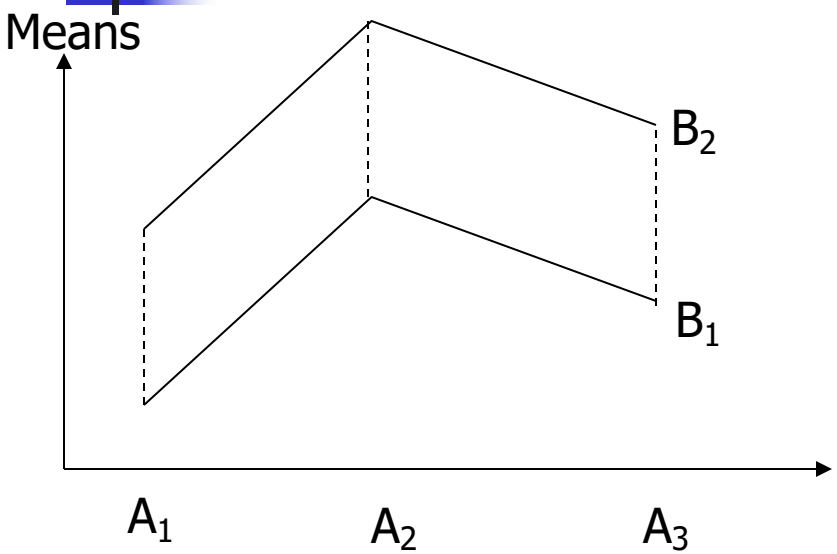
ANOVA: Two-Way Model

- To simplify discussion, assume that factor A has three levels, while factor B has two levels

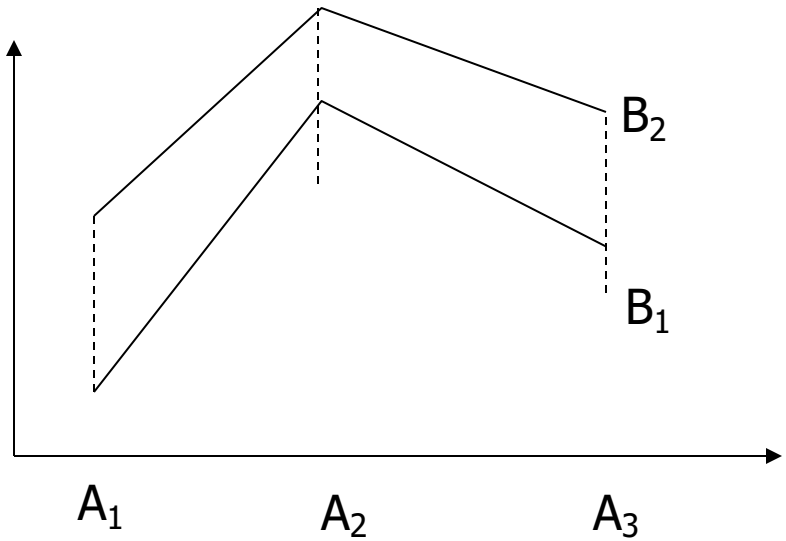
Factor A

Factor B		A_1	A_2	A_3
	B_1	μ_{11}	μ_{21}	μ_{31}
	B_2	μ_{12}	μ_{22}	μ_{32}

ANOVA: Two-Way Model



Parallel lines = No interaction



Lines are not parallel = Interaction

ANOVA: Two-Way Model

- Recall:
  - Categorical variables can be represented with “dummy” variables
  - Interactions are represented with “cross-products”

ANOVA: Two-Way Model

- Model 1:

  $E[Y|A_2, A_3, B_2] = \beta_0 + \beta_1 A_2 + \beta_2 A_3 + \beta_3 B_2.$

  - What are the means in each combination-group?

	A_1	A_2	A_3
B_1	$\mu_{11}=\beta_0$	$\mu_{21}=\beta_0+\beta_1$	$\mu_{31}=\beta_0+\beta_2$
B_2	$\mu_{12}=\beta_0+\beta_3$	$\mu_{22}=\beta_0+\beta_1+\beta_3$	$\mu_{32}=\beta_0+\beta_2+\beta_3$

ANOVA: Two-Way Model

- Model 1:

  $E[Y|A_2, A_3, B_2] = \beta_0 + \beta_1 A_2 + \beta_2 A_3 + \beta_3 B_2.$

	A_1	A_2	A_3
B_1	$\mu_{11}=\beta_0$	$\mu_{21}=\beta_0+\beta_1$	$\mu_{31}=\beta_0+\beta_2$
B_2	$\mu_{12}=\beta_0+\beta_3$	$\mu_{22}=\beta_0+\beta_1+\beta_3$	$\mu_{32}=\beta_0+\beta_2+\beta_3$

Model with no interaction:

- Difference in means between groups defined by factor B does not depend on the level of factor A.
- Difference in means between groups defined by factor A does not depend on the level of factor B.

ANOVA: Two-Way Model

- Model 2:

  $E[Y|A_2, A_3, B_2] = \beta_0 + \beta_1 A_2 + \beta_2 A_3 + \beta_3 B_2 + \beta_4 A_2 B_2 + \beta_5 A_3 B_2$

  - What are the means in each combination-group?

	A_1	A_2	A_3
B_1	$\mu_{11}=\beta_0$	$\mu_{21}=\beta_0+\beta_1$	$\mu_{31}=\beta_0+\beta_2$
B_2	$\mu_{12}=\beta_0+\beta_3$	$\mu_{22}=\beta_0+\beta_1+\beta_3+\beta_4$	$\mu_{32}=\beta_0+\beta_2+\beta_3+\beta_5$

ANOVA: Two-Way Model

- Three (possible) tests
  - Interaction of A and B (may want to start here)
    - Rejection would imply that differences between means of A depends on the level of B (and vice-versa) so stop
  - Main effect of A
    - Test only if no interaction
  - Main effect of B
    - Test only if no interaction

[Note: If you have one observation per cell, you cannot test interaction!]

ANOVA: Two-Way Model

- Model without interaction

  $E[Y|A_2, A_3, B_2] = \beta_0 + \beta_1 A_2 + \beta_2 A_3 + \beta_3 B_2$.

  How do we test for main effect of factor A?

  H_0: $\beta_1 = \beta_2 = 0$ vs. H_1: β_1 or β_2 not zero

  How do we test for main effect of factor B?

  H_0: $\beta_3 = 0$ vs. H_1: β_3 not zero

ANOVA: Two-Way Model

- Model with interaction:

  $E[Y|A_2, A_3, B_2] = \beta_0 + \beta_1 A_2 + \beta_2 A_3 + \beta_3 B_2 + \beta_4 A_2 B_2 + \beta_5 A_3 B_2$

  How do we test for interactions?

$$\begin{cases} H_0: \beta_4 = \beta_5 = 0 \quad \text{vs.} \\ H_1: \beta_4 \text{ or } \beta_5 \text{ not zero} \end{cases}$$

IMPORTANT:

If you reject the null, do not test main effects!!!

ANOVA: Two-Way Model (without interaction)

```
> fit1 = lm(chol ~ factor(sex) + factor(rs174548))
> summary(fit1)
Call:
lm(formula = chol ~ factor(sex) + factor(rs174548))

Residuals:
     Min       1Q   Median       3Q      Max
-66.6534 -14.4633  -0.6008  15.4450  57.6350

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           175.365      1.786  98.208  < 2e-16 ***
factor(sex)1           11.053      2.126   5.199 3.22e-07 ***
factor(rs174548)1       7.236      2.250   3.215  0.00141 **
factor(rs174548)2       5.184      4.398   1.179  0.23928
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.24 on 396 degrees of freedom
Multiple R-squared: 0.08458,    Adjusted R-squared: 0.07764
F-statistic:  12.2 on 3 and 396 DF,  p-value: 1.196e-07

> fit0 = lm(chol ~ factor(sex))
> anova(fit0,fit1)
Analysis of Variance Table

Model 1: chol ~ factor(sex)
Model 2: chol ~ factor(sex) + factor(rs174548)
  Res.Df    RSS Df Sum of Sq     F   Pr(>F)
1    398 183480
2    396 178681  2    4799.1 5.318 0.005259 **
```

ANOVA: Two-Way Model (without interaction)

```
> fit1 = lm(chol ~ factor(sex) + factor(rs174548))
> summary(fit1)
Call:
lm(formula = chol ~ factor(sex) + factor(rs174548))

Residuals:
     Min       1Q   Median       3Q      Max
-66.6534 -14.4633  -0.6008  15.4450  57.6350

Coefficients:
                      Estimate Std. Error t value Pr(>|t|)
(Intercept)            175.365      1.786  98.208  < 2e-16 ***
factor(sex)1            11.053      2.126   5.199 3.22e-07 ***
factor(rs174548)1        7.236      2.250   3.215  0.00141 **
factor(rs174548)2        5.184      4.398   1.179  0.23928

Residual standard error: 21.24 on 396 degrees of freedom
Multiple R-squared: 0.08458,    Adjusted R-squared: 0.07764
F-statistic:  12.2 on 3 and 396 DF,  p-value: 1.196e-07

> anova(fit0,fit1)
Analysis of Variance Table

Model 1: chol ~ factor(sex)
Model 2: chol ~ factor(sex) + factor(rs174548)
  Res.Df    RSS Df Sum of Sq     F   Pr(>F)
1    398 183480
2    396 178681  2    4799.1 5.318 0.005259 **
```

- Interpretation of results:
  - Estimated mean cholesterol for male C/C group: 175.37 mg/dl
  - Estimated difference in mean cholesterol levels between females and males adjusted by genotype: 11.053 mg/dl
  - Estimated difference in mean cholesterol levels between C/G and C/C groups adjusted by sex: 7.236 mg/dl
  - Estimated difference in mean cholesterol levels between G/G and C/C groups adjusted by sex: 5.184 mg/dl

- There is evidence that cholesterol is associated with sex ($p < 0.001$).
- There is evidence that cholesterol is associated with genotype ($p=0.005$)

ANOVA: Two-Way Model (without interaction)

- In words:
  - Adjusting for sex, the difference in mean cholesterol comparing C/G to C/C is 7.236 and comparing G/G to C/C is 5.184.
    - This difference does not depend on sex
      - (this is because the model does not have an interaction between sex and genotype!)

ANOVA: Two-Way Model (with interaction)

```
> fit2 = lm(chol ~ factor(sex) * factor(rs174548))
> summary(fit2)

Call:
lm(formula = chol ~ factor(sex) * factor(rs174548))

Residuals:
     Min       1Q   Median       3Q      Max
-70.5286 -13.6037  -0.9736  14.1709  54.8818

Coefficients:
                                    Estimate Std. Error t value Pr(>|t|)
(Intercept)                         178.1182     2.0089  88.666  < 2e-16 ***
factor(sex)1                          5.7109     2.7982   2.041  0.04192 *
factor(rs174548)1                     0.9597     3.1306   0.307  0.75933
factor(rs174548)2                    -0.2015     6.4053  -0.031  0.97492
factor(sex)1:factor(rs174548)1       12.7398     4.4650   2.853  0.00456 **
factor(sex)1:factor(rs174548)2       10.2296     8.7482   1.169  0.24297
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.07 on 394 degrees of freedom
Multiple R-squared: 0.1039,     Adjusted R-squared: 0.09257
F-statistic:  9.14 on 5 and 394 DF,  p-value: 3.062e-08
```

ANOVA: Model comparison

```
> anova(fit1,fit2)
Analysis of Variance Table

Model 1: chol ~ factor(sex) + factor(rs174548)
Model 2: chol ~ factor(sex) * factor(rs174548)
  Res.Df    RSS  Df Sum of Sq      F  Pr(>F)
1    396 178681
2    394 174902   2      3779 4.2564 0.01483 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

ANOVA: Two-Way Model (with interaction)

```
> fit2 = lm(chol ~ factor(sex) * factor(rs174548))
> summary(fit2)

Call:
lm(formula = chol ~ factor(sex) * factor(rs174548))

Residuals:
     Min       1Q   Median       3Q      Max
-70.5286 -13.6037  -0.9736  14.1709  54.8818

Coefficients:
                                    Estimate Std. Error t value Pr(>|t|)
(Intercept)                         178.1182     2.0089  88.666  < 2e-16 ***
factor(sex)1                          5.7109     2.7982   2.041  0.04192 *
factor(rs174548)1                     0.9597     3.1306   0.307  0.75933
factor(rs174548)2                    -0.2015     6.4053  -0.031  0.97492
factor(sex)1:factor(rs174548)1       12.7398     4.4650   2.853  0.00456 **
factor(sex)1:factor(rs174548)2       10.2296     8.7482   1.169  0.24297
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.07 on 394 degrees of freedom
Multiple R-squared: 0.1039,     Adjusted R-squared: 0.09257
F-statistic:  9.14 on 5 and 394 DF,  p-value: 3.062e-08
```

```
> anova(fit1,fit2)
Analysis of Variance Table

Model 1: chol ~ factor(sex) + factor(rs174548)
Model 2: chol ~ factor(sex) *    factor(rs174548)
  Res.Df    RSS  Df Sum of Sq      F  Pr(>F)
1    396 178681
2    394 174902   2      3779 4.2564 0.01483 *
---

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Interpretation of results:

- Estimated mean cholesterol for male C/C group:

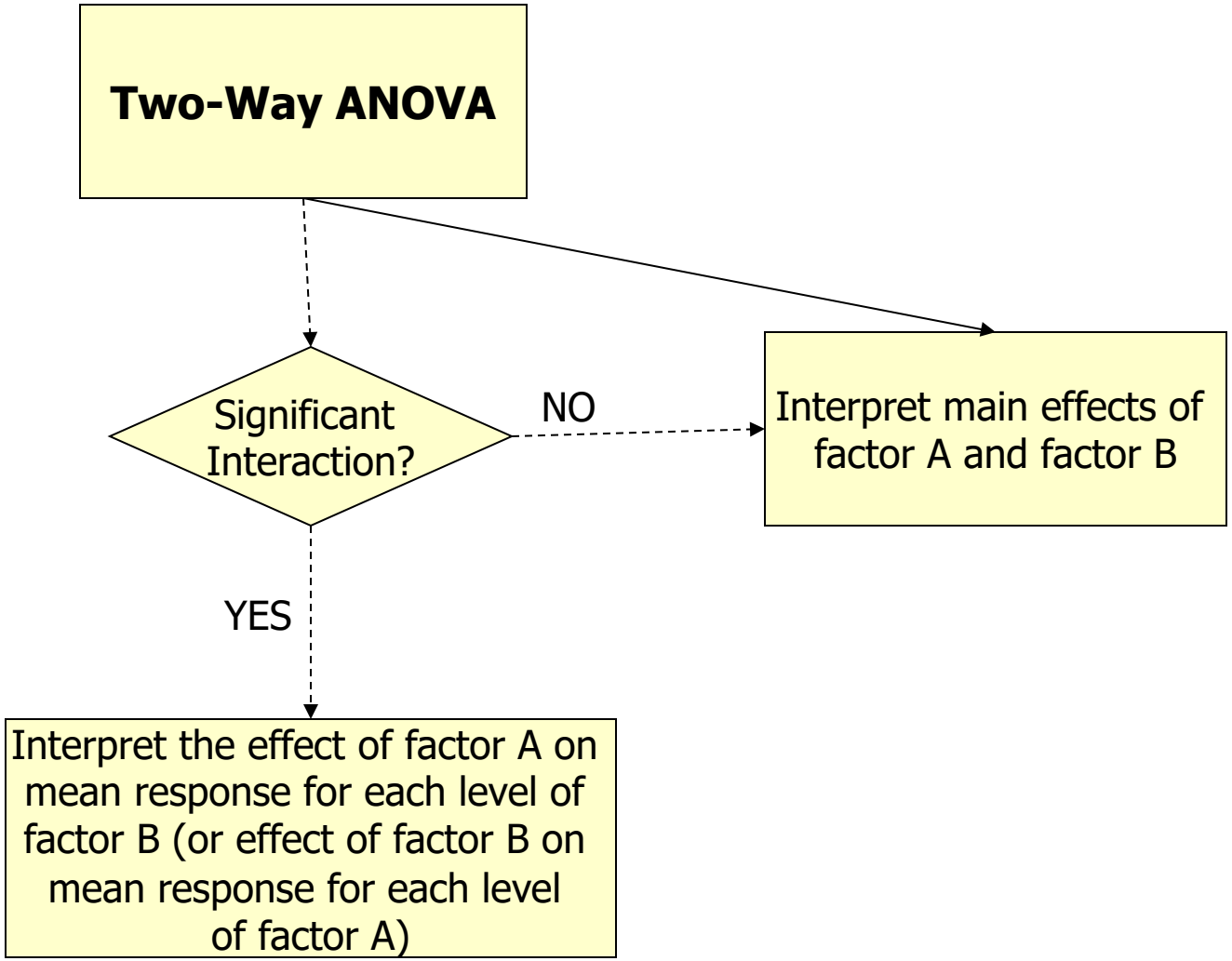
  178.12 mg/dl
- Estimated mean cholesterol for female C/C group? (178.12 + 5.7109) mg/dl
- Estimated mean cholesterol for male C/G group:

  (178.12 +0.9597) mg/dl
- Estimated mean cholesterol for female C/G group:

  (178.12 + 5.7109 + 0.9597 + 12.7398) mg/dl
- …

- There is evidence for an interaction between sex and genotype (p= 0.015)

SUMMARY:


Two-Way ANOVA
Significant Interaction?
NO
Interpret main effects of factor A and factor B
YES
Interpret the effect of factor A on mean response for each level of factor B (or effect of factor B on mean response for each level of factor A)

ANalysis of COVAriance Models (ANCOVA) Motivation:

- Scientific question:
  - Assess the effect of rs174548 on cholesterol levels adjusting for age

ANalysis of COVAriance Models (ANCOVA)

- ANOVA with one or more continuous variables
  - Equivalent to regression with “dummy” variables and continuous variables
  - Primary comparison of interest is across k groups defined by a categorical variable, but the k groups may differ on some other potential predictor or confounder variables [also called covariates].

ANalysis of COVAriance Models (ANCOVA)

- To facilitate discussion assume
  - Y: continuous response (e.g. cholesterol)
  - X: continuous variable (e.g. age)
  - Z: dummy variable (e.g. indicator of C/G or G/G versus C/C)

- Model: $Y = \beta_0 + \beta_1 X + \beta_2 Z + \beta_3 \boxed{XZ} + \varepsilon$

Interaction term

Note that:

$$Z = 0 \Rightarrow E[Y \mid X, Z = 0] = \beta_0 + \beta_1 X$$

$$Z = 1 \Rightarrow E[Y \mid X, Z = 1] = (\beta_0 + \beta_2) + (\beta_1 + \beta_3) X$$

This model allows for different intercepts/slopes for each group.

ANCOVA

- Testing coincident lines: $H_0 : \beta_2 = 0, \beta_3 = 0$
  - Compares overall model with reduced model

$$Y = \beta_0 + \beta_1 X + \varepsilon$$

- Testing parallelism: $H_0 : \beta_3 = 0$
  - Compares overall model with reduced model

$$Y = \beta_0 + \beta_1 X + \beta_2 Z + \varepsilon$$

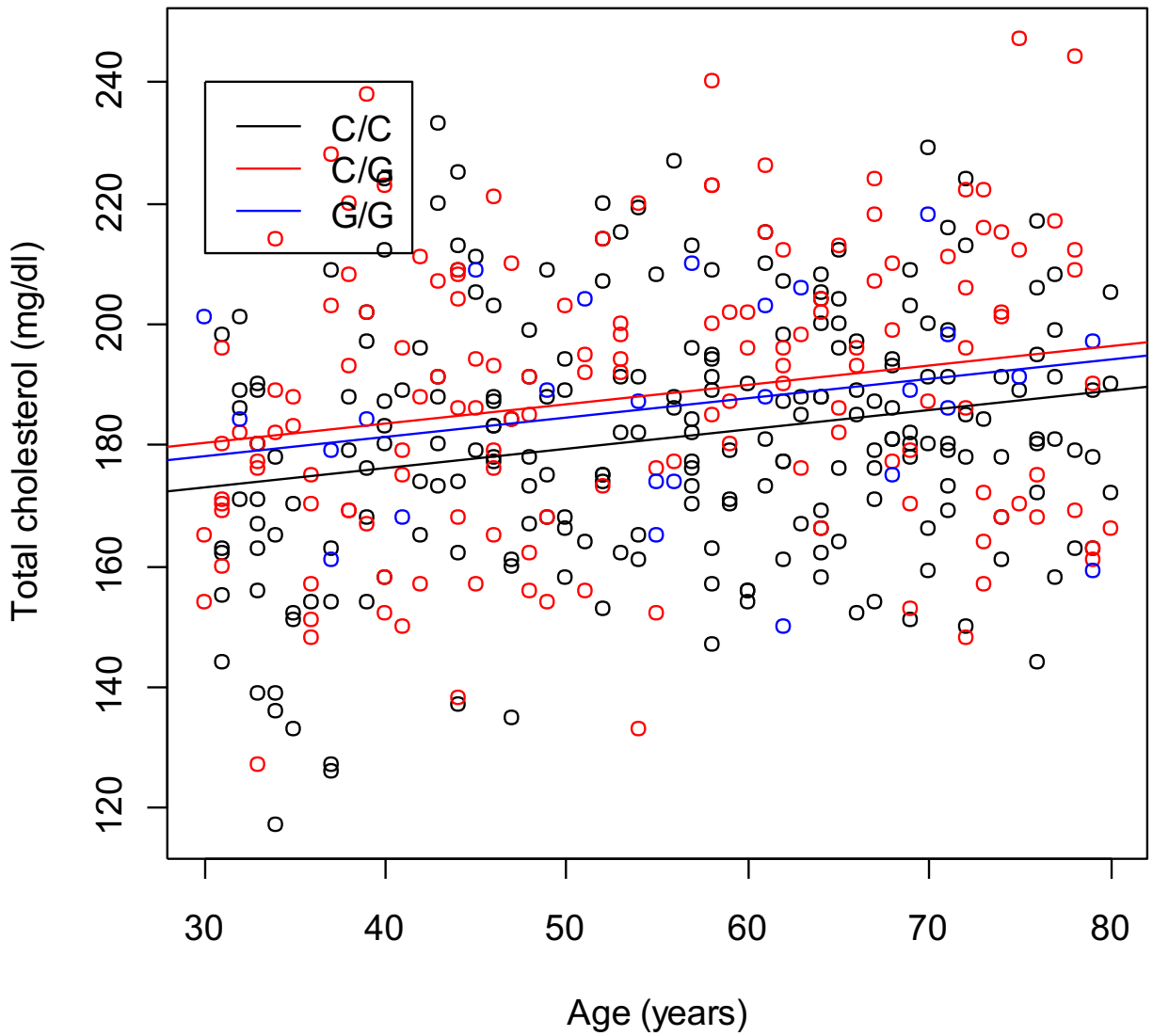
ANCOVA

```
> fit0 = lm(chol ~ factor(rs174548))
> summary(fit0)
Call:
lm(formula = chol ~ factor(rs174548))

Residuals:
      Min        1Q    Median        3Q       Max
-64.06167 -15.91338  -0.06167  14.93833  59.13605

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)           181.062      1.455 124.411  < 2e-16 ***
factor(rs174548)1       6.802      2.321   2.930  0.00358 **
factor(rs174548)2       5.438      4.540   1.198  0.23167
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.93 on 397 degrees of freedom
Multiple R-squared: 0.0221,     Adjusted R-squared: 0.01718
F-statistic: 4.487 on 2 and 397 DF,  p-value: 0.01184

> anova(fit0)
Analysis of Variance Table
Response: chol
                     Df Sum Sq Mean Sq F value  Pr(>F)
factor(rs174548)      2   4314    2157  4.4865 0.01184 *
Residuals           397 190875     481
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ANCOVA

```
> fit1 = lm(chol ~ factor(rs174548) + age)
> summary(fit1)
Call:
lm(formula = chol ~ factor(rs174548) + age)

Residuals:
     Min       1Q   Median       3Q      Max 
-57.2089 -14.4293   0.4443  14.2652  55.8985 

Coefficients:
                      Estimate Std. Error t value Pr(>|t|)    
(Intercept)          163.28125    4.36422  37.414  < 2e-16 ***
factor(rs174548)1      7.30137    2.27457   3.210  0.00144 ** 
factor(rs174548)2      5.08431    4.44331   1.144  0.25321    
age                    0.32140    0.07457   4.310 2.06e-05 ***

Residual standard error: 21.46 on 396 degrees of freedom
Multiple R-squared: 0.06592,    Adjusted R-squared: 0.05884 
F-statistic: 9.316 on 3 and 396 DF,  p-value: 5.778e-06

> anova(fit0,fit1)
Analysis of Variance Table

Model 1: chol ~ factor(rs174548)
Model 2: chol ~ factor(rs174548) + age
  Res.Df    RSS Df Sum of Sq      F    Pr(>F)    
1    397 190875                                  
2    396 182322  1    8552.9 18.577 2.062e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ANCOVA


C/C
C/G
G/G
Total cholesterol (mg/dl)
120
140
160
180
200
220
240
30
40
50
60
70
80
Age (years)

ANCOVA

```
> fit2 = lm(chol ~ factor(rs174548) * age)
> summary(fit2)
Call:
lm(formula = chol ~ factor(rs174548) * age)

Residuals:
     Min       1Q   Median       3Q      Max 
-57.5425 -14.3002   0.7131  14.2138  55.7089 

Coefficients:
                          Estimate Std. Error t value Pr(>|t|)    
(Intercept)              164.14677    5.79545  28.323  < 2e-16 ***
factor(rs174548)1          3.42799    8.79946   0.390  0.69707    
factor(rs174548)2         16.53004   18.28067   0.904  0.36642    
age                        0.30576    0.10154   3.011  0.00277 ** 
factor(rs174548)1:age      0.07159    0.15617   0.458  0.64692    
factor(rs174548)2:age     -0.20255    0.31488  -0.643  0.52043    

Residual standard error: 21.49 on 394 degrees of freedom
Multiple R-squared: 0.06777,    Adjusted R-squared: 0.05594 
F-statistic: 5.729 on 5 and 394 DF,  p-value: 4.065e-05
```

ANCOVA

```
> fit0 = lm(chol ~ age)
> summary(fit0)

Call:
lm(formula = chol ~ age)

Residuals:
    Min      1Q  Median      3Q     Max 
-60.453 -14.643  -0.022  14.659  58.995 

Coefficients:
             Estimate Std. Error t value Pr(>|t|)    
(Intercept) 166.90168    4.26488  39.134  < 2e-16 ***
age           0.31033    0.07524   4.125 4.52e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.69 on 398 degrees of freedom
Multiple R-squared:  0.04099,	Adjusted R-squared:  0.03858 
F-statistic: 17.01 on 1 and 398 DF,  p-value: 4.522e-05

> anova(fit0,fit2)
Analysis of Variance Table

Model 1: chol ~ age
Model 2: chol ~ factor(rs174548) * age
  Res.Df    RSS Df Sum of Sq      F  Pr(>F)
1    398 187187                           
2    394 181961  4    5226.6 2.8293 0.02455 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

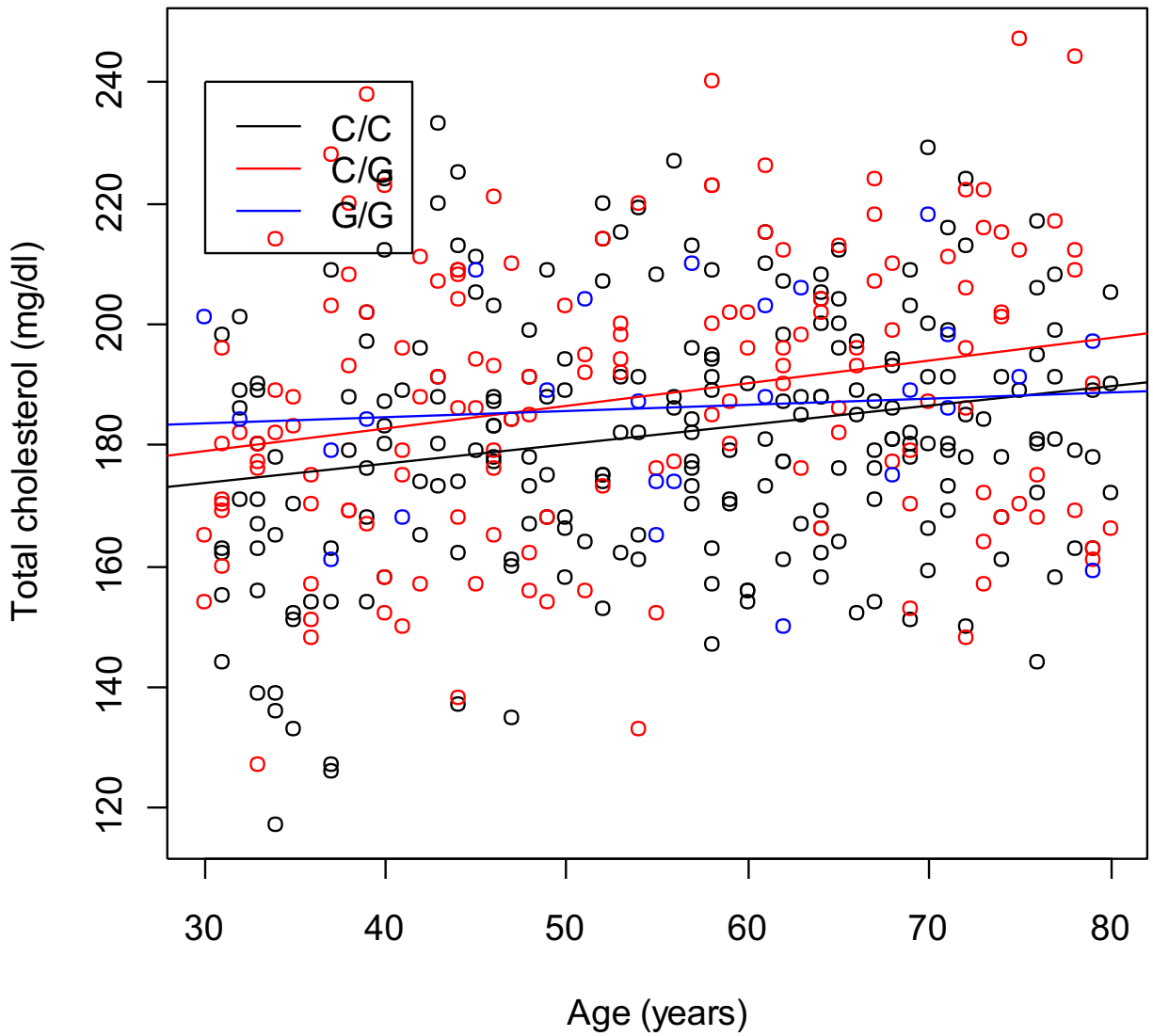
Test of coincident lines

ANCOVA

Test of parallel lines

```
> anova(fit1,fit2)
Analysis of Variance Table

Model 1: chol ~ factor(rs174548) + age
Model 2: chol ~ factor(rs174548) * age
  Res.Df    RSS Df Sum of Sq     F Pr(>F)
1    396 182322
2    394 181961  2    361.11 0.391 0.6767
```

ANCOVA


C/C
C/G
G/G
Total cholesterol (mg/dl)
120
140
160
180
200
220
240
30
40
50
60
70
80
Age (years)

ANCOVA

- In summary:
  - If the slopes are not equal, then age is an effect modifier

$$E[Y \mid x, z] = \beta_0 + \beta_1 x + \beta_2 (CG) + \beta_3 (GG) + \beta_4 (x * CG) + \beta_5 (x * GG)$$

  - If the slopes are the same,

$$E[Y \mid x, z] = \beta_0 + \beta_1 x + \beta_2 (CG) + \beta_3 (GG)$$

ANCOVA

- If the slopes are the same,

$$E[Y \mid x, z] = \beta_0 + \beta_1 x + \beta_2 (CG) + \beta_3 (GG)$$

  - then one can obtain adjusted means for the three genotypes using the mean age over all groups
    - For example, the adjusted means for the three groups would be

$$\overline{Y}_1(\text{adj}) = \hat{\beta}_0 + \bar{x}\hat{\beta}_1$$

$$\overline{Y}_2(\text{adj}) = (\hat{\beta}_0 + \hat{\beta}_2) + \bar{x}\hat{\beta}_1$$

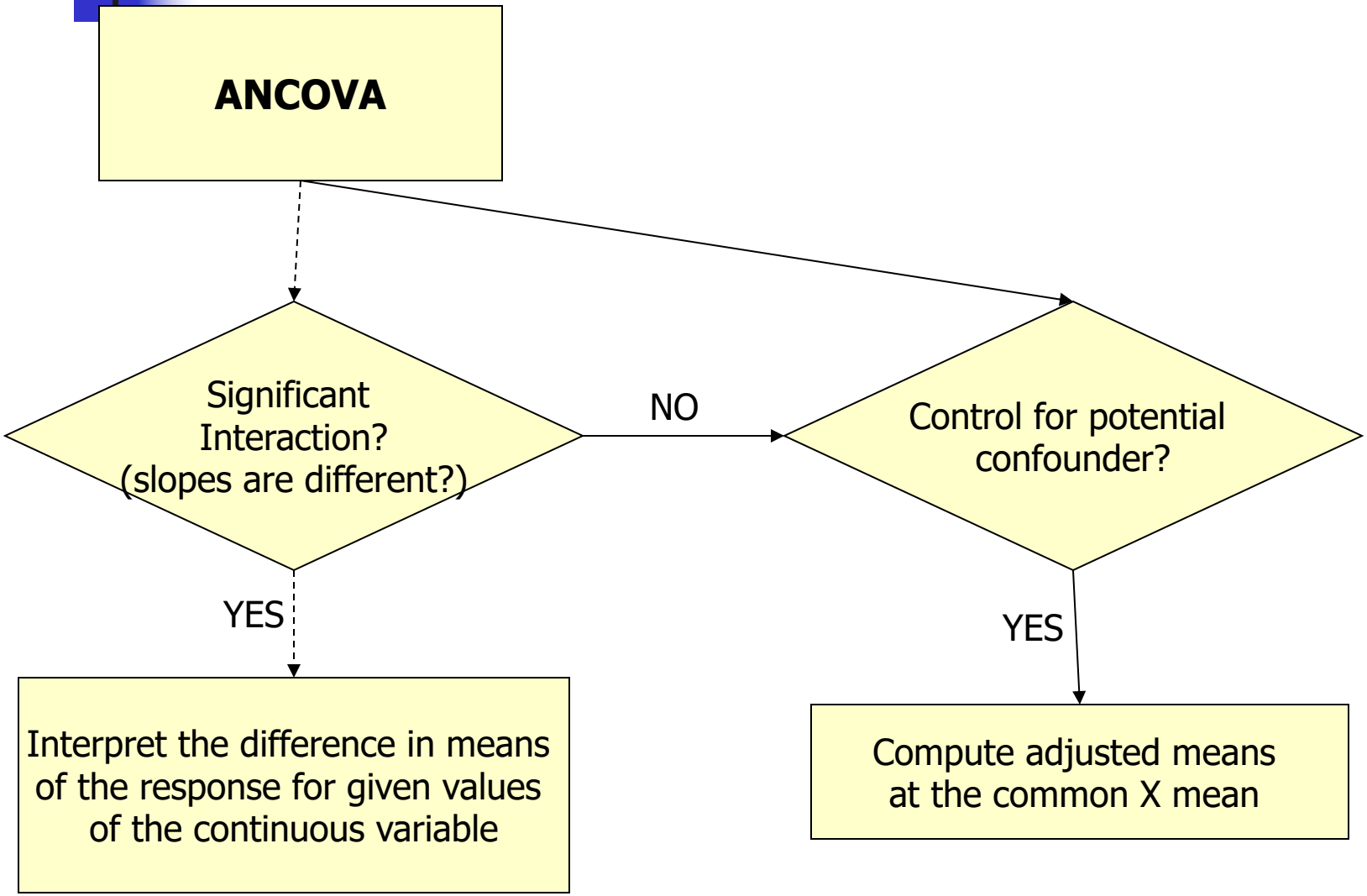
$$\overline{Y}_3(\text{adj}) = (\hat{\beta}_0 + \hat{\beta}_3) + \bar{x}\hat{\beta}_1$$

ANCOVA

```
> ## mean cholesterol for different genotypes adjusted by age
> predict(fit1, new=data.frame(age=mean(age),rs174548=0))
       1
180.9013
> predict(fit1, new=data.frame(age=mean(age),rs174548=1))
       1
188.2026
> predict(fit1, new=data.frame(age=mean(age),rs174548=2))
       1
185.9856
```

```
> ## mean cholesterol for different genotypes adjusted by age
> mean(predict(fit1, new=data.frame(age=age,rs174548=0)))
180.9013
> mean(predict(fit1, new=data.frame(age=age,rs174548=1)))
188.2026
> mean(predict(fit1, new=data.frame(age=age,rs174548=2)))
185.9856
```

SUMMARY:


ANCOVA
Significant Interaction? (slopes are different?)
NO
Control for potential confounder?
YES
YES
Interpret the difference in means of the response for given values of the continuous variable
Compute adjusted means at the common X mean

Summary

We have considered:

- ANOVA and ANCOVA
  - Interpretation
  - Estimation
  - Interaction

- Multiple comparisons

Exercise

- Work on Exercise 9-12
  - Try each exercise on your own
  - Make note of any questions or difficulties you have
  - At **3:30ET??** we will meet as a group to go over the solutions and discuss your questions