REGRESSION METHODS

MULTIPLE COMPARISONS

ANOVA: One-Way Model

- What are the groups with differences in means?

MULTIPLE COMPARISONS:

$$\left.\begin{array}{l}\mu_0= \mu_1?\\[2em] \mu_0= \mu_2?\\[2em] \mu_1= \mu_2?\end{array}\right\}\ \text{Pairwise comparisons}$$

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

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
> fit2 = 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(fit2, 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)

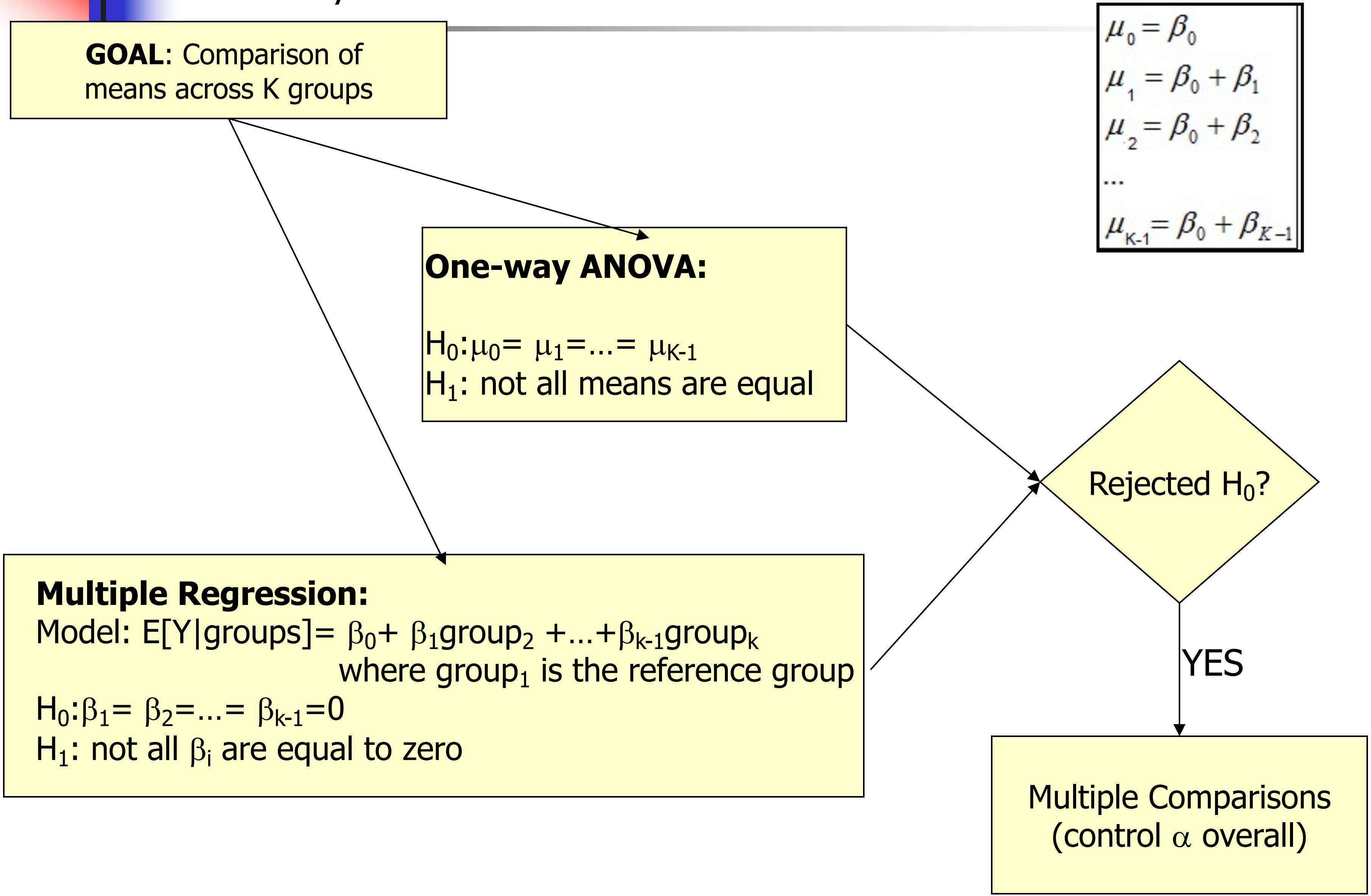
```
> summary(mc, test=adjusted("fdr"))
```

(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

Relationships:

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

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

REGRESSION METHODS

Two-way ANOVA models

ANOVA: Two-Way Model Motivation:

- Scientific question:
    - Assess the effect of rs174548 and diabetes 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

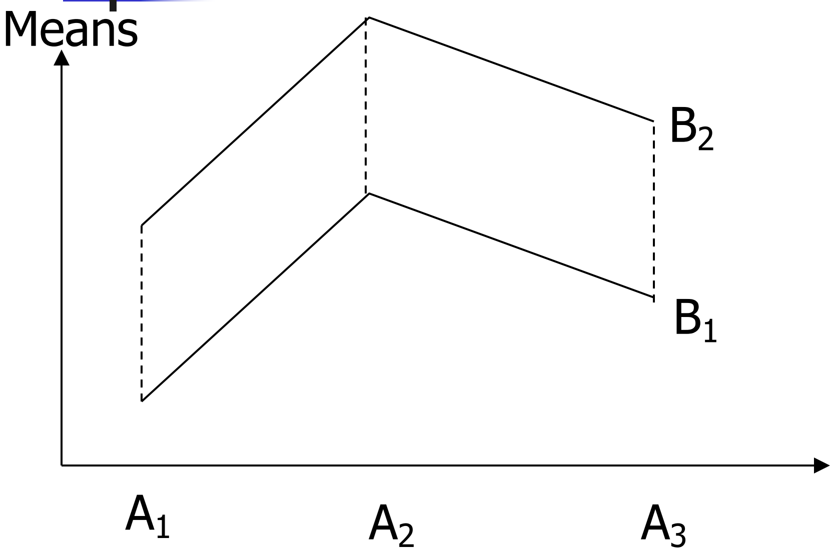
ANOVA: Two-Way Model

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

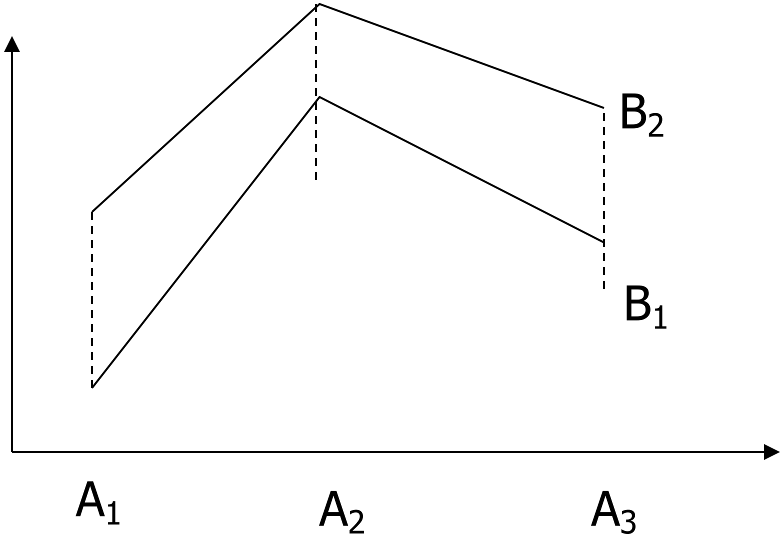
Factor A

		A_1	A_2	A_3
Factor B	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:

  $E[Y|A_2, A_3, B_2] = \beta_0 + \beta_1 A_2 + \beta_2 A_3 + \beta_3 B_2 + \beta_4 A_2 B_2 + \beta_5 A_3 B_3$

  - 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(DM) + factor(rs174548))
> summary(fit1)
Call:
lm(formula = chol ~ factor(DM) + 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(DM)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(DM))
> anova(fit0,fit1)
Analysis of Variance Table

Model 1: chol ~ factor(DM)
Model 2: chol ~ factor(DM) + 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(DM) + factor(rs174548))
> summary(fit1)
Call:
lm(formula = chol ~ factor(DM) + factor(rs174548))

Residuals:
    Min      1Q  Median      3Q     Max 
-66.653 -14.463  -0.601  15.445  57.635 

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)    
(Intercept)        175.365      1.786  98.208  < 2e-16 ***
factor(DM)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 

> anova(fit0,fit1)
Analysis of Variance Table

Model 1: chol ~ factor(DM)
Model 2: chol ~ factor(DDM) + 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 people without diabetes in C/C group:

    175.365 mg/dl
  - Estimated difference in mean cholesterol levels between people with and without diabetes adjusted by genotype:

    11.053 mg/dl
  - Estimated difference in mean cholesterol levels between C/G and C/C groups adjusted by diabetes status:

    7.236 mg/dl
  - Estimated difference in mean cholesterol levels between G/G and C/C groups adjusted by diabetes status:

    5.184 mg/dl

  - There is evidence that cholesterol is associated with diabetes (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 diabetes status, 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 diabetes status
      - (this is because the model does not have an interaction between diabetes and genotype!)

ANOVA: Two-Way Model (with interaction)

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

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

Residuals:
    Min      1Q  Median      3Q     Max 
-70.529 -13.604  -0.974  14.171  54.882 

Coefficients:
                              Estimate Std. Error t value Pr(>|t|)    
(Intercept)                   178.1182     2.0089  88.666  < 2e-16 ***
factor(DM)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(DM)1:factor(rs174548)1  12.7398     4.4650   2.853  0.00456 ** 
factor(DM)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: 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: Model comparison

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

Model 1: chol ~ factor(DM) + factor(rs174548)
Model 2: chol ~ factor(DM) * 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
```

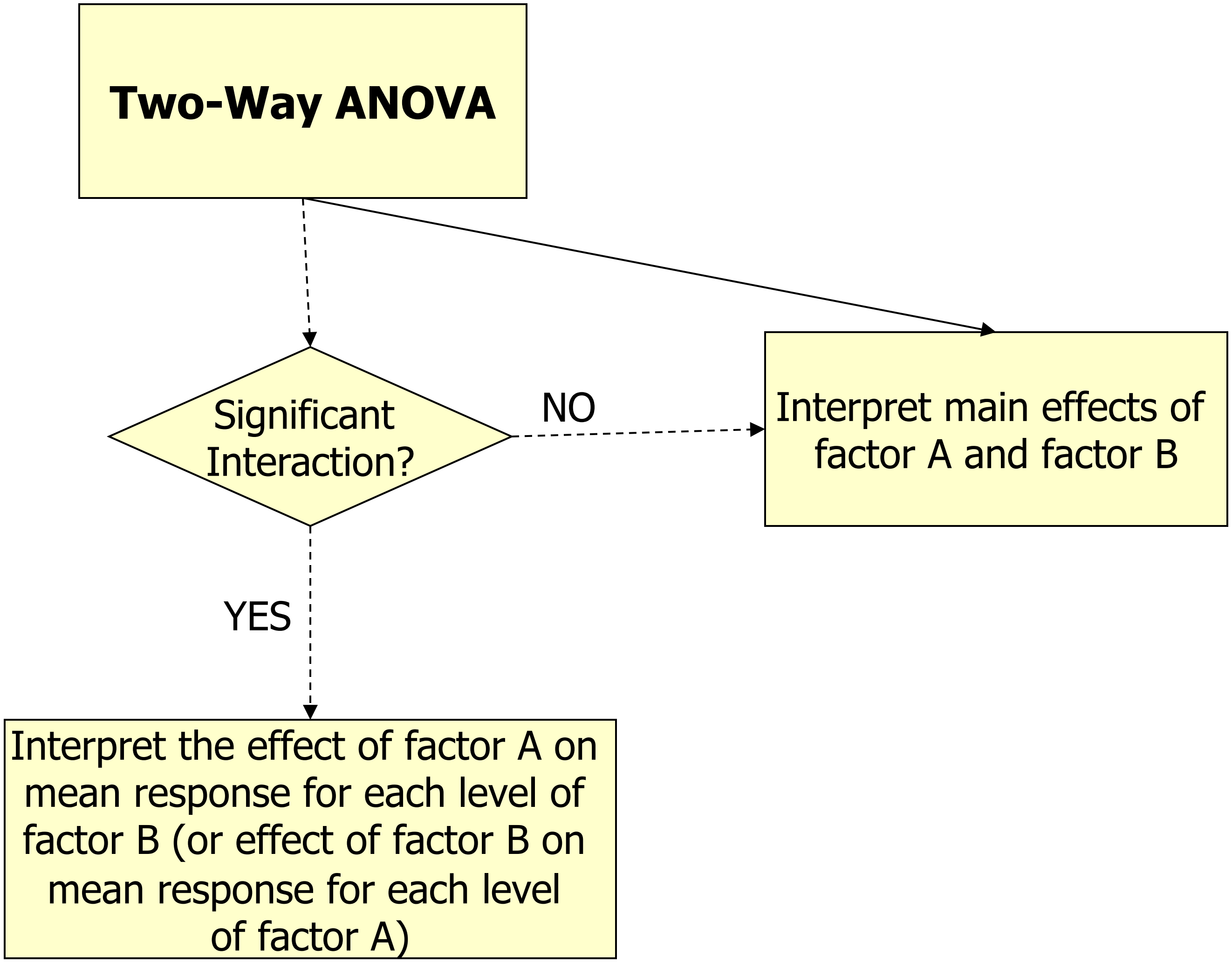
ANOVA: Two-Way Model (with interaction)

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

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

Residuals:
    Min      1Q  Median      3Q     Max 
-70.529 -13.604  -0.974  14.171  54.882 

Coefficients:
                              Estimate Std. Error t value Pr(>|t|)    
(Intercept)                   178.1182     2.0089  88.666  < 2e-16 ***
factor(DM)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(DM)1:factor(rs174548)1  12.7398     4.4650   2.853  0.00456 ** 
factor(DM)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(DM) + factor(rs174548)
Model 2: chol ~ factor(DM) *  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 people without diabetes in C/C group:
    178.12 mg/dl
  - Estimated mean cholesterol for people with diabetes in C/C group:
    (178.12 + 5.7109) mg/dl
  - Estimated mean cholesterol for people without diabetes in C/G group:
    (178.12 +0.9597) mg/dl
  - Estimated mean cholesterol for people with diabetes in C/G group:
    (178.12 + 5.7109 + 0.9597 + 12.7398) mg/dl
  - …
  - There is evidence for an interaction between diabetes 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

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

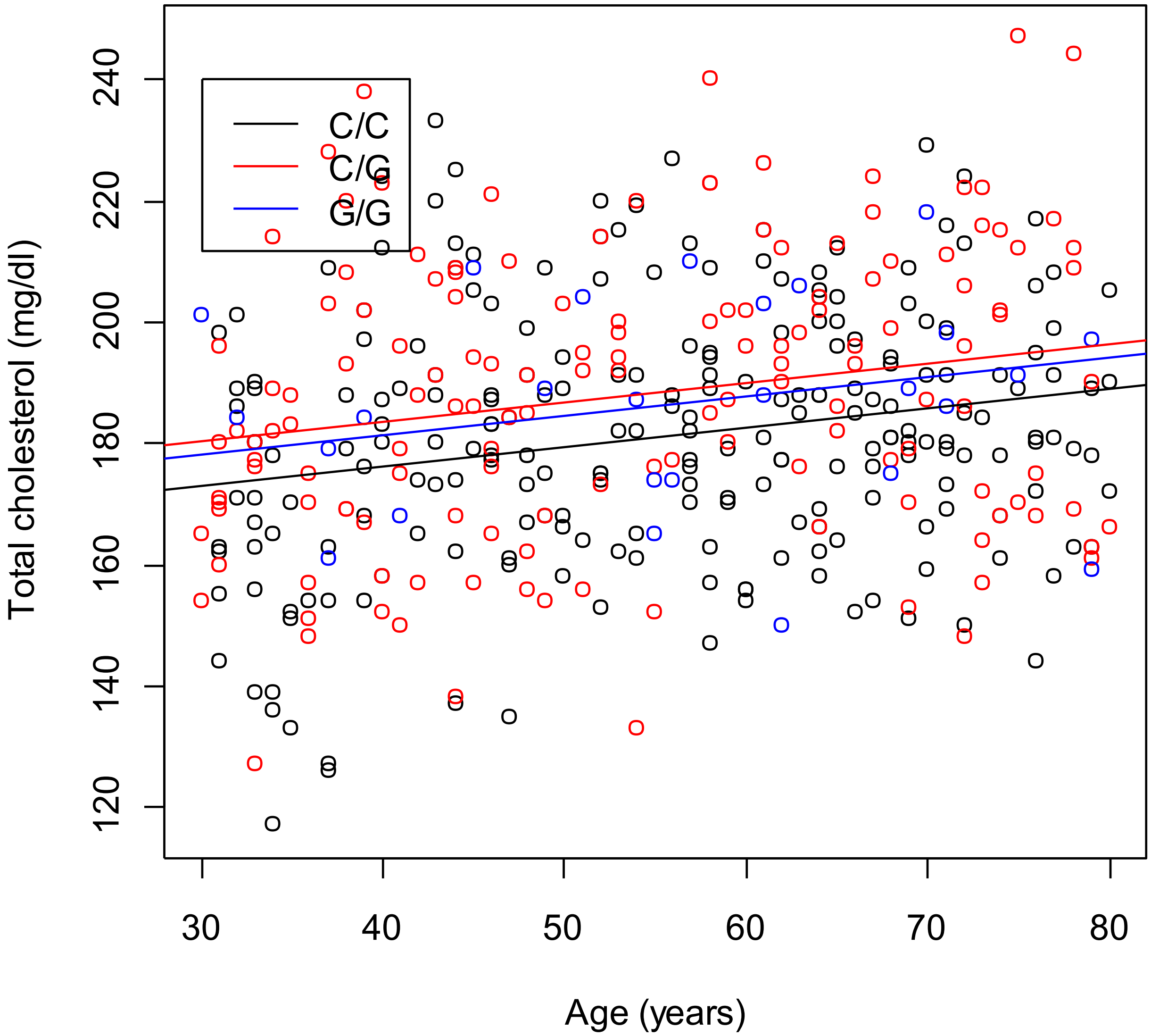
ANCOVA

```
> 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
Age (years)
30
40
50
60
70
80

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
```

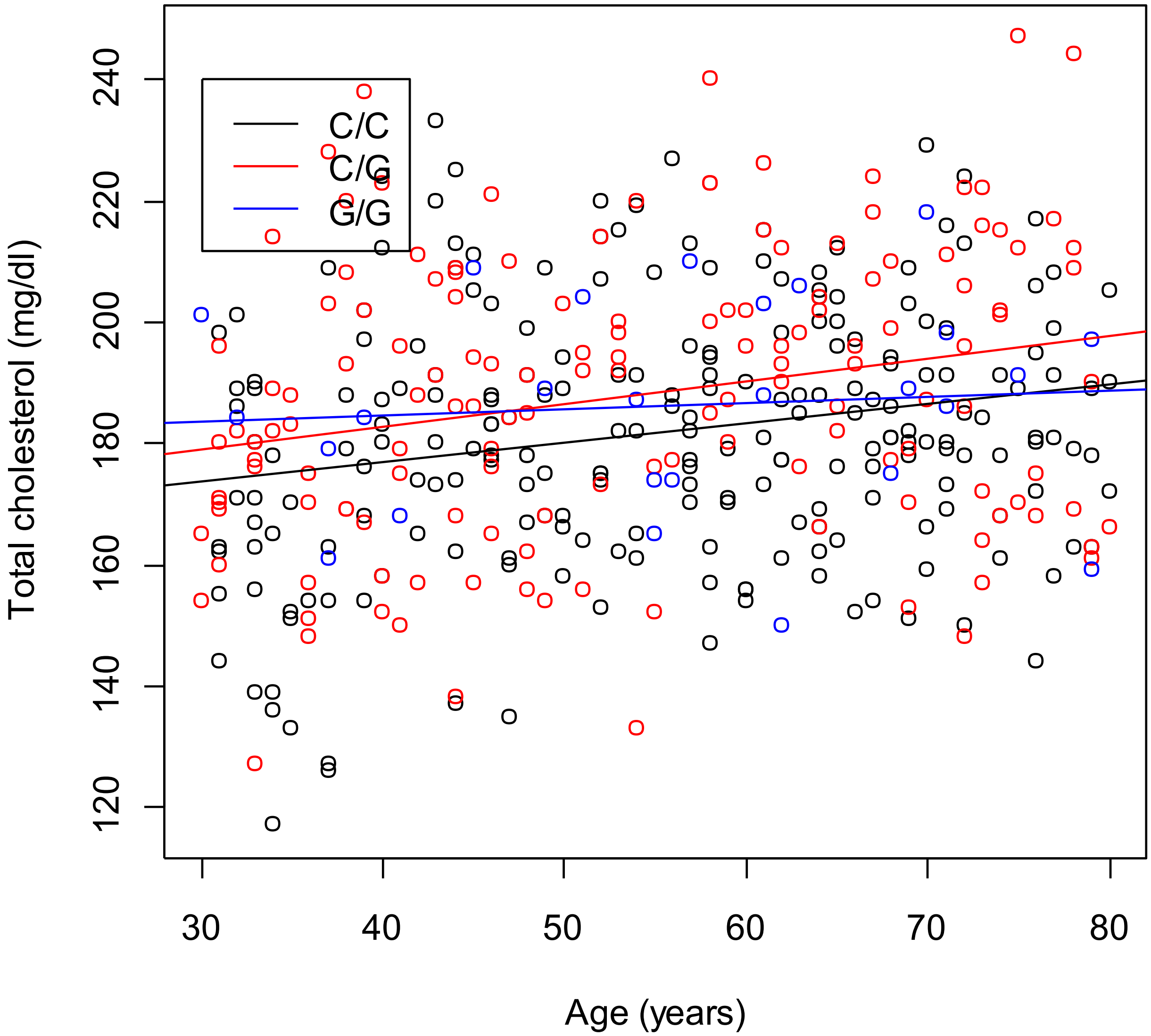
Test of coincident lines

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

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

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

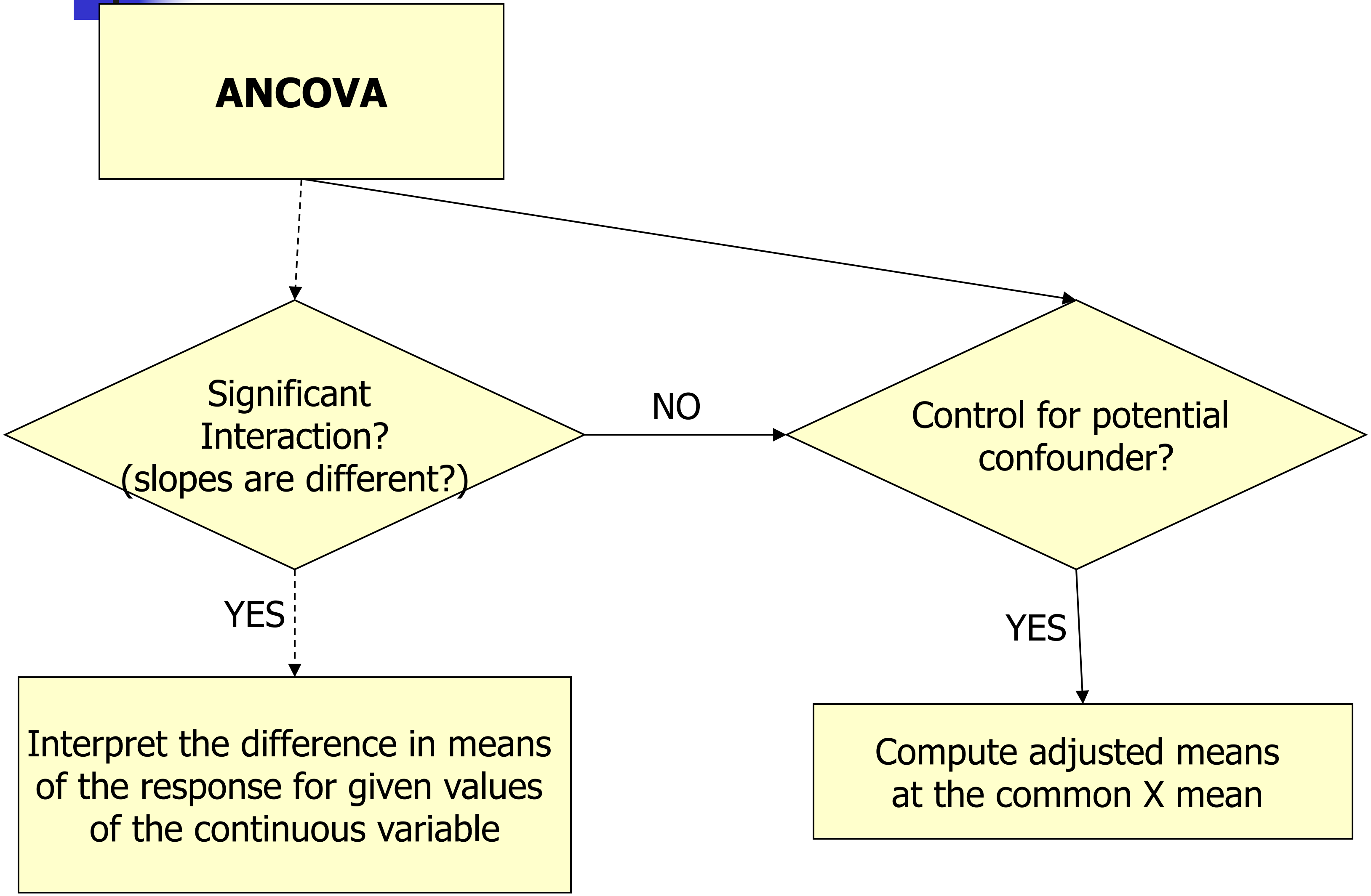
$$\overline{\mathrm{Y}}_3(\mathrm{adj}) = (\hat{\beta}_0 + \hat{\beta}_3) + \bar{x}\hat{\beta}_1$$

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

Interpret the difference in means of the response for given values of the continuous variable

YES

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 **1:15PT** we will meet as a group to go over the solutions and discuss your questions