

Lecture 4: Regression (continued) and multiple regression BIO144 Data Analysis in Biology

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Recap of last week



- ▶ Why use linear regression?
- Fitting the line (least squares).
- ▶ Is the linear model good enough the five assumptions.
- ▶ What if something goes wrong (transformations and handling outliers)?

Overview of this week



Regression continued...

- ▶ How well does the model describe the data: Correlation and R^2
- ▶ Are the parameter estimates compatible with some specific value (t-test)?
- ► What range of parameters values are compatible with the data (confidence intervals)?
- What regression lines are compatible with the data (confidence band)?
- What are plausible values of other data (prediction band)?

Multiple regression:

- ▶ Multiple linear regression $x_1, x_2, ..., x_m$
- Checking assumptions
- $ightharpoonup R^2$ in multiple linear regression
- t-tests, F-tests and p-values

Course material covered today



The lecture material of today is based on the following literature:

- ► Chapters 3.1, 3.2a-q of *Lineare Regression*
- ► Chapters 4.1 4.2f, 4.3a-e of *Lineare Regression*

How good is the regression model?

This is, per se, a difficult question....

One often considered index is the **coefficient of determination (Bestimmtheitsmass)** R^2 . Let us again look at the regression output form the bodyfat example:

```
summary(r.bodyfat)$r.squared
```

```
## [1] 0.5390391
```

Compare this to the squared correlation between the two variables:

```
cor(d.bodyfat$bodyfat,d.bodyfat$bmi)^2
```

```
## [1] 0.5390391
```

 \rightarrow In simple linear regression, R^2 is the squared correlation between the independent and the dependent variable.



- $ightharpoonup R^2$ indicates the proportion of variability of the response variable y that is **explained by the ensemble of all covariates**.
- lts value lies between 0 and 1.

The **larger** R^2

- \Rightarrow the **more** variability of y is captured ("explained") by the covariate
- \Rightarrow the "better" is the model.

(However, it's a bit more complicated, as we will see in the multiple regression later in the lecture today)



 R^2 is also called the *coefficient of determination* or "Bestimmtheitsmass", because it measures the proportion of the reponse's variability that is explained by the ensemble of all explanatory variables:

$$R^2 = SSQ^{(R)}/SSQ^{(Y)} = 1 - SSQ^{(E)}/SSQ^{(Y)}$$

With

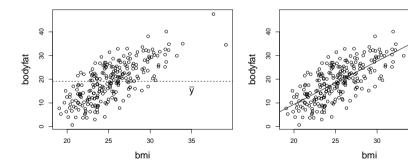
total variability = explained variability + residual variability

$$\sum_{i=1}^{n} (y_i - \overline{y})^2 = \sum_{i=1}^{n} (\hat{y}_i - \overline{y})^2 + \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$$

$$SSQ^{(Y)} = SSQ^{(R)} + SSQ^{(E)}$$



This can be visualized for a model with only one predictor:



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Are the parameter estimates compatible with some specific value (t-test)

Important: $\hat{\beta}_0$ and $\hat{\beta}_1$ are themselves random variables and as such contain uncertainty!

Let us look again at the regression output, this time only for the coefficients. The second column shows the *standard error* of the estimate:

```
summary(r.bodyfat)$coef
```

```
## Estimate Std. Error t value Pr(>|t|)
## (Intercept) -26.984368 2.7689004 -9.745518 3.921511e-19
## bmi 1.818778 0.1083411 16.787522 2.063854e-42
```

→ The logical next question is: what is the distribution of the estimates?

Distribution of the estimators for \hat{eta}_0 and \hat{eta}_1

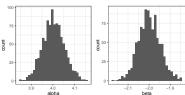
To obtain an idea, we generate data points according to model

$$y_i = 4 - 2x_i + \epsilon_i$$
, $\epsilon_i \sim N(0, 0.5^2)$.

In each round, we estimate the parameters and store them:

```
niter <- 1000
pars <- matrix(NA,nrow=niter,ncol=2)
for (ii in 1:niter){
    x <- rnorm(100)
    y <- 4 - 2*x + rnorm(100,0,sd=0.5)
    pars[ii,] <- lm(y-x)$coef
}</pre>
```

Doing it 1000 times, we obtain the following distributions for $\hat{\beta}_0$ and $\hat{\beta}_1$:



In fact, from theory it is known that

$$\hat{eta}_1 \sim \textit{N}(eta_1, \sigma^{(eta_1)2})$$
 and $\hat{eta}_0 \sim \textit{N}(eta_0, \sigma^{(eta_0)2})$

For formulas of the variances $\sigma^{(\beta_1)2}$ and $\sigma^{(\beta_0)2}$, please consult Stahel 2.2.h.

To remember:

- $ightharpoonup \hat{\beta}_0$ and $\hat{\beta}_1$ are unbiased estimators of β_0 and β_1 .
- \blacktriangleright the parameters estimates $\hat{\beta}_0$ and $\hat{\beta}_1$ are normally distributed.
- the formulas for the variances depend on the residual variance σ^2 , the sample size n and the variability of X (SSQ^{(X)(*)}).

$$SSQ^{(X)} = \sum_{i=1}^{n} (x_i - \overline{x})^2$$

With all this, we can calculate a standardised measure of the uncertainty in the parameter estimates, known as the standard error, or SE:

Standard error of parameter estimate:
$$se^{(eta_1)} = \sqrt{rac{\hat{\sigma}^2}{SSQ^{(X)}}}$$

Estimated residual variance:
$$\hat{\sigma}^2 = \frac{1}{n-2} \sum_{i=1}^{n} R_i^2$$

Residuals (also sometimes
$$e_i$$
): $R_i = Y_i - (\hat{eta}_0 + \hat{eta}_1 x_i)$

Sum of squares of X:
$$SSQ^{(X)} = \sum_{i=1}^{n} (x_i - \bar{x})^2$$

Let's first go back to the output from the bodyfat example:

```
summary(r.bodyfat)$coef
```

```
## Estimate Std. Error t value Pr(>|t|)
## (Intercept) -26.984368 2.7689004 -9.745518 3.921511e-19
## bmi 1.818778 0.1083411 16.787522 2.063854e-42
```

Besides the estimate and the standard error (which we discussed before), there is a tvalue and a probability Pr(>|t|) that we need to understand.

How do these things help us?

Testing the "effect" of a covariate



Remember: in a statistical test you first need to specify the *null hypothesis*. Here, typically, the null hypothesis is

$$H_0: \quad \beta_1=0.$$

In words: $H_0 =$ "no association"

Here, the alternative hypothesis is given by

$$H_A: \beta_1 \neq 0$$

Remember: To carry out a statistical test, we need a test statistic.

What is a test statistic?

 \rightarrow It is some type of **summary statistic** that follows a known distribution under H_0 . For our purpose, we use the so-called T-**statistic**

$$T = \frac{\hat{\beta}_1 - \beta_{1,H_0}}{se^{(\beta_1)}} . \qquad (1)$$

Again: typically, $\beta_{1,H_0}=0$, so the formula simplifies to $T=\frac{\hat{\beta}_1}{\epsilon_0(\hat{\beta}_1)}$.

Under H_0 , T has a t-distribution with n-2 degrees of freedom (n = number of data points).

(You should try to recall the t-distribution. Check Mat183, keyword: t-test.)



So let's again go back to the bodyfat regression output:

summary(r.bodyfat)\$coef

```
## Estimate Std. Error t value Pr(>|t|)
## (Intercept) -26.984368 2.7689004 -9.745518 3.921511e-19
## bmi 1.818778 0.1083411 16.787522 2.063854e-42
```

Task:

 \rightarrow Please use equation (2) to find out how the first three columns (Estimate, Std. Error and t value) are related! Check by a calculation...

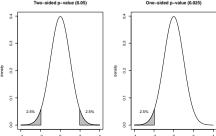
Note: The last column contains the *p*-value of the test of the null hypothesis of $\beta_1 = 0$.

Recap: Formal definition of the p-value

The **formal definition of** p-value is the probability to observe a data summary (e.g., an average) that is at least as extreme as the one observed, given that the Null Hypothesis is correct.

Example (normal distribution): Assume the observed test-statistic leads to a t-value = -1.96

$$\Rightarrow$$
 $Pr(t \ge 1.96) = 0.05$ and $Pr(t \le -1.96) = 0.025$.





The regression output from R indicates that the p-value for BMI is very small (p < 0.0001).

Conclusion: there is **very strong evidence** that the BMI is associated with bodyfat, because p is extremely small (thus it is very unlikely that such a slope $\hat{\beta}_1$ would be seen if there was no association of BMI and body fat).

This basically answers question 1: "Are the parameters compatible with some specific value?"

A cautionary note on the use of p-values



Maybe you have seen that in statistical testing, often the criterion $p \le 0.05$ is used to test whether H_0 should be rejected. This is often done in a black-or-white manner.

However, we will put a lot of attention to a more reasonable and cautionary interpretation of p-values in this course!

To answer this question, we can determine the confidence intervals of the regression parameters.

Facts we know about $\hat{\beta}_1$

- \triangleright $\hat{\beta}_1$ is estimated with a standard error of $\sigma^{(\beta_1)}$
- ▶ The distribution of $\hat{\beta}_1$ is normal, namely $\hat{\beta}_1 \sim N(\beta_1, \sigma^{(\beta_1)2})$.
- lacktriangle However, since we need to estimate $\sigma^{(\hat{\beta}_1)}$ from the data, we have a t-distribution.

$$[\hat{eta}_1 - c \cdot \hat{\sigma}^{(eta_1)}; \hat{eta}_1 + c \cdot \hat{\sigma}^{(eta_1)}] \; ,$$

where c is the 97.5% quantile of the t-distribution with n-2 degrees of freedom.

Doing this for the bodfat example "by hand" is not hard. We have 241 degrees of freedom:

```
coefs <- summary(r.bodyfat)$coef
beta <- coefs[2,1]
sdbeta <- coefs[2,2]
beta + c(-1,1) * qt(0.975,241) * sdbeta</pre>
```

[1] 1.605362 2.032195

Even easier: directly ask R to give you the Cls.



In summary,

	Coefficient	95%-confidence interval	<i>p</i> -value
Intercept	-26.98	from -32.44 to -21.53	< 0.0001
bmi	1.82	from 1.61 to 2.03	< 0.0001

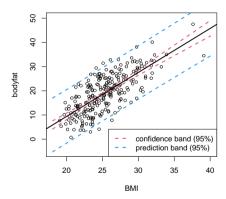
Interpretation: for an increase in the bmi by one index point, roughly 1.82% percentage points more bodyfat are expected, and all true values for β_1 between 1.61 and 2.03 are compatible with the observed data.

Confidence and Prediction Bands



- ▶ Remember: When another sample from the same population was taken, the regression line would look slightly different.
- ▶ There are two questions to be asked:
- 1. Which other regression lines are compatible with the observed data?
 - ⇒ This leads to the **confidence band**.
- 2. Where do future observations with a given x coordinate lie?
 - \Rightarrow This leads to the **prediction band**.

Bodyfat example



Note: The prediction band is much broader than the confidence band.

Calculation of the confidence band



Given a fixed value of x, say x_0 . The question is:

Where does $\hat{y}_0 = \hat{\beta}_0 + \hat{\beta}_1 x_0$ lie with a certain confidence (i.e., 95%)?

This question is not trivial, because both $\hat{\beta}_0$ and $\hat{\beta}_1$ are estimates from the data and contain uncertainty.

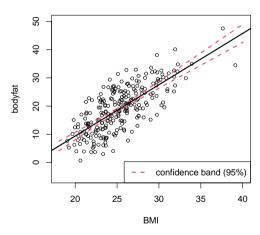
The details of the calculation are given in Stahel 2.4b.

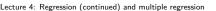
Plotting the confidence interval around all \hat{y}_0 values one obtains the **confidence band** or **confidence band** for **the expected values** of y.

Note: For the confidence band, only the uncertainty in the estimates $\hat{\beta}_0$ and $\hat{\beta}_1$ matters.



Confidence band





Calculations of the prediction band



Given a fixed value of x, say x_0 . The question is:

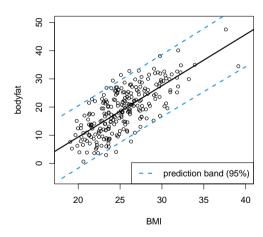
Where does a **future observation** lie with a certain confidence (i.e., 95%)?

To answer this question, we have to consider not only the uncertainty in the predicted value $\hat{y}_0 = \hat{\beta}_0 + \hat{\beta}_1 x_0$, but also the error in the equation $\epsilon_i \sim N(0, \sigma^2)$.

This is the reason why the **prediction band is always wider than the confidence band.**



Prediction band



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That is regression done (at least for our current purposes)

- ▶ Why use (linear) regression?
- ► Fitting the line (= parameter estimation)
- ▶ Is linear regression good enough model to use?
- What to do when things go wrong?
- ► Transformation of variables/the response.
- Handling of outliers.
- ▶ Goodness of the model: Correlation and R^2
- Tests and confidence intervals
- Confidence and prediction bands

(Homework and Practical class: Presentation of findings)

Multiple linear regression



Multiple continuous explanatory variables.

- ▶ Question 1: Are the explanatory variables (i.e. more than one) associated with the response?
- Question 2: Which variables are associated with the response?
- Question 3: What proportion of variability is explained?

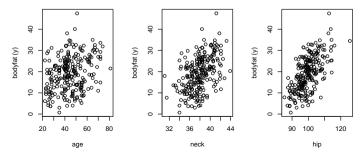
Bodyfat example

We have so far modeled bodyfat in dependence of bmi, that is:

$$(bodyfat)_i = \beta_0 + \beta_1 \cdot bmi_i + \epsilon_i.$$

However, other explanatory variables might also be relevant for an accurate prediction of bodyfat.

Examples: Age, neck fat (Nackenfalte), hip circumference, abdomen circumference etc.





Multiple linear regression is when we have more than one explanatory variable. We can then ask three questions:

- 1. Is the ensemble of all explanatory variables associated with the response?
- 2. If yes, which explanatory variables are associated with the response?
- 3. What proportion of response variability $(SSQ^{(Y)})$ is explained by the model?

Multiple linear regression model



The idea is simple: Just extend the linear model by additional predictors.

▶ Given several influence explanatory variables $x_i^{(1)}, \ldots, x_i^{(m)}$, the straightforward extension of the simple linear model is

$$y_i = \beta_0 + \beta_1 x_i^{(1)} + \beta_2 x_i^{(2)} + \ldots + \beta_m x_i^{(m)} + \epsilon_i$$
 with $\epsilon_i \sim N(0, \sigma^2)$.

▶ The parameters of this model are $\beta = (\beta_0, \beta_1, \dots, \beta_m)$ and σ^2 .

The components of β are again estimated using the **least squares** method. Basically, the idea is (again) to minimize

$$\sum_{i=1}^{n} e_i^2$$

with

$$e_i = y_i - (\beta_0 + \beta_1 x_i^{(1)} + \beta_2 x_i^{(2)} + \ldots + \beta_m x_i^{(m)})$$

It is a bit more complicated than for simple linear regression, see Section 3.4 of the Stahel script.

Some **linear algebra** is needed to understand these sections; we look at this in Lecture 7.





Let us regress the proportion (%) of bodyfat (from last week) on the predictors **bmi** and **age** simultaneously. The model is thus given as

$$(bodyfat)_i = \beta_0 + \beta_1 \cdot bmi_i + \beta_2 \cdot age_i + \epsilon_i$$
,
with $\epsilon_i \sim N(0, \sigma^2)$.



Multiple linear regression with R

Let's now fit the model with R, and quickly glance at the output:

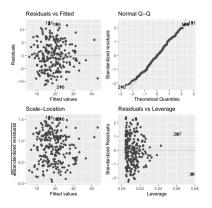
```
r.bodyfatM <- lm(bodyfat ~ bmi + age, d.bodyfat)
summary(r.bodyfatM)</pre>
```

```
##
## Call:
## lm(formula = bodyfat ~ bmi + age, data = d.bodyfat)
## Residuals:
       Min
                 10 Median
                                           Max
## -12.0415 -3.8725 -0.1237 3.9193 12.6599
## Coefficients:
               Estimate Std. Error t value Pr(>|t|)
## (Intercept) -31.25451
                           2.78973 -11.203 < 2e-16 ***
                1.75257 0.10449 16.773 < 2e-16 ***
## bmi
## age
                0.13268
                           0.02732
                                    4.857 2.15e-06 ***
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
## Residual standard error: 5.329 on 240 degrees of freedom
## Multiple R-squared: 0.5803, Adjusted R-squared: 0.5768
## F-statistic: 165.9 on 2 and 240 DF, p-value: < 2.2e-16
```



Model checking

Before we look at the results, we must check if the modelling assumptions are fulfilled (check our 'chute before we jump):



This seems ok, so continue with answering questions 1-3.



Question 1: Are the explanatory variables associated with the response?

To answer question 1, we need to perform a so-called F-test. The results of the test are displayed in the final line of the regression summary. Here, it says:

So apparently (and we already suspected that) the model has some explanatory power.

*The F-statistic and -test is briefly recaptured in 3.1.f) of the Stahel script, but see also Mat183 chapter 6.2.5. It uses the fact that

$$\frac{SSQ^{(R)}/m}{SSQ^{(E)}/(n-p)} \sim F_{m,n-p}$$

follows an F-distribution with m and (n-p) degrees of freedom, where m are the number of variables, n the number of data points, p the number of β -parameters (typically m+1). $SSQ^{(E)} = \sum_{i=1}^n R_i^2$ is the squared sum of the residuals, and $SSQ^{(R)} = SSQ^{(Y)} - SSQ^{(E)}$ with $SSQ^{(y)} = \sum_{i=1}^n (y_i - \overline{y})^2$.

n is the number of data points

m is the number of explanatory variables in the regression model

p is the number of beta parameters estimated (e.g. intercept, plus a slope for each explanatory variable, hence p=m+1)

And the degrees of freedom for error are n-p



Question 2: Which variables are associated with the response?

summary(r.bodyfatM)\$coef

```
## (Intercept) -31.2545057 2.78973238 -11.203406 1.039096e-23
## bmi 1.7525705 0.10448723 16.773060 2.600646e-42
## age 0.1326767 0.02731582 4.857137 2.149482e-06
```

To answer this question, again look at the *t*-tests, for which the *p*-values are given in the final column. Each *p*-value refers to the test for the null hypothesis $\beta_0^{(j)} = 0$ for explanatory variable $x^{(j)}$.

As in simple linear regression, the T-statistic for the j-th explanatory variable is calculated as

$$T_{j} = \frac{\hat{\beta}_{j}}{se^{(\beta_{j})}} , \qquad (2)$$

with $se^{(\beta_j)}$ given in the second column of the regression output.

The distribution of this statistic is $T_j \sim t_{n-p}$.

Therefore: A "small" p-value indicates that the variable is relevant in the model University of Zurich ""

Here, we have

- \triangleright p < 0.001 for bmi
- p < 0.001 for age

Thus both, bmi and age seem to be associated with bodyfat.

Again, a 95% CI for $\hat{\beta}_i$ can be calculated with R:

confint(r.bodyfatM)

```
## (Intercept) -36.7499929 -25.7590185
## bmi 1.5467413 1.9583996
## age 0.0788673 0.1864861
```

(The CI is again $[\hat{\beta} - c \cdot \sigma^{(\beta)}; \hat{\beta} + c \cdot \sigma^{(\beta)}]$, where c is the 97.5% quantile of the t-distribution with n-p degrees of freedom; compare to slides 38-40 of last week).

!However!:

The p-value and T-statistics should only be used as a **rough guide** for the "significance" of the coefficients.

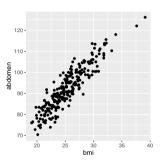
For illustration, let us extend the model a bit more, including also neck, hip and abdomen:

	Coefficient	95%-confidence interval	<i>p</i> -value
Intercept	-7.75	from -22.13 to 6.63	0.29
bmi	0.43	from -0.03 to 0.88	0.066
age	0.015	from -0.04 to 0.07	0.60
neck	-0.80	from -1.18 to -0.43	< 0.0001
hip	-0.32	from -0.53 to -0.11	0.003
abdomen	0.84	from 0.67 to 1.00	< 0.0001

It is now much less clear how strongly age (p=0.60) and bmi (p=0.07) are associated with bodyfat.

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Basically, the problem is that the variables in the model are correlated and therefore explain similar aspects of bodyfat. **Example:** Abdomen (Bauchumfang) seems to be a relevant predictor and it is obvious that abdomen and BMI are correlated:



This problem of collinearity is at the heart of many confusions of regression analysis, and we will talk about such issues later in the course (lectures 8 and 9).

Please see also IC: practical 4 (milk example) for an analysis and more thoughts.

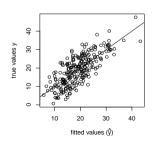


Question 3: Which proportion of variability is explained?

To answer this question, we can look at the multiple \mathbb{R}^2 (see Stahel 3.1.h). It is a generalized version of \mathbb{R}^2 for simple linear regression:

 R^2 for multiple linear regression is defined as the squared correlation between (y_1, \ldots, y_n) and $(\hat{y}_1, \ldots, \hat{y}_n)$, where the \hat{y} are the fitted values

$$\hat{y}_i = \hat{\beta}_0 + \hat{\beta}_1 x^{(1)} + \ldots + \hat{\beta}_m x^{(m)}$$



Let us look at the R^2 s from the three bodyfat models





model r.bodyfat: $y \sim bmi$

model r.bodyfatM: $y \sim bmi + age$

model r.bodyfatM2: $y \sim bmi + age + neck + hip + abdomen$:

summary(r.bodyfat)\$r.squared

[1] 0.5390391

[1] 0.5802956

summary(r.bodyfatM)\$r.squared

summary(r.bodyfatM2)\$r.squared

[1] 0.718497

The models explain 54%, 58% and 72% of the total variability of ν . It thus seems that larger models are "better". However, R^2 does always increase when new variables are included, but this does not mean that the model is more reasonable.

Adjusted R^2



When the sample size n is small with respect to the number of variables m included in the model, an adjusted R^2 gives a better ("fairer") estimation of the actual variability that is explained by the explanatory variables:

$$R_a^2 = 1 - (1 - R^2) \frac{n-1}{n-m-1}$$

Why R_a^2 ?

It penalizes for adding more variables if they do not really improve the model!

Note: R_a may decrease when a new variable is added.

Interpretation of the coefficients



Apart from model checking and thinking about questions 1-3, it is probably even **more important to understand what you** *see.* Look at the output and ask yourself:

What does the regression output actually mean?

	Coefficient	95%-confidence interval	<i>p</i> -value
Intercept	-31.25	from -36.75 to -25.76	< 0.0001
bmi	1.75	from 1.55 to 1.96	< 0.0001
age	0.13	from 0.08 to 0.19	< 0.0001

Table 1: Parameter estimates of model 2.

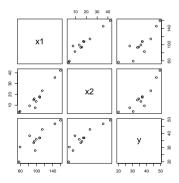
Task in teams: Interpret the coefficients, 95% CIs and p-values.

Example: Catheter Data

Catheter length (y) for heart surgeries depending on two characteristic variables $x^{(1)}$ and $x^{(2)}$ of the patients.

Aim: estimate y from $x^{(1)}$ and $x^{(2)}$ (n = 12).

Again look at the data first $(x^{(1)})$ and $x^{(2)}$ are highly correlated!):



Regression results with both variables: $R^2=0.81$, $R^2=0.76$, F-test p=0.0006. Zurich Turich Turich

	Coefficient	95%-confidence interval	<i>p</i> -value
Intercept	21.09	from 1.25 to 40.93	0.04
×1	0.077	from -0.25 to 0.40	0.61
×2	0.43	from -0.41 to 1.26	0.28

With x_1 only: $R^2 = 0.78$, $R_a^2 = 0.75$, F-test p = 0.0002

		Coefficient	95%-confidence interval	<i>p</i> -value
Interc	ept	12.13	from 2.66 to 21.59	0.017
	$\times 1$	0.24	from 0.15 to 0.33	0.0002

With x_2 only: $R^2 = 0.80, R_a^2 = 0.78$, F-test p = 0.0001

	Coefficient	95%-confidence interval	<i>p</i> -value
Intercept	25.63	from 21.16 to 30.09	< 0.0001
x2	0.62	from 0.40 to 0.83	< 0.0001

Questions to consider:

- 1. Is x_1 an important explanatory variable?
- 2. Is x_2 an important explanatory variable?
- 3. Are both explanatory variables needed in the model?
- 4. Interpretation of the results?

Recap



- \triangleright How well does the model describe the data: Correlation and R^2
- ▶ Are the parameter estimates compatible with some specific value (t-test)?
- ► What range of parameters values are compatible with the data (confidence intervals)?
- What regression lines are compatible with the data (confidence band)?
- What are plausible values of other data (prediction band)?

Multiple regression:

- ▶ Multiple linear regression $x_1, x_2, ..., x_m$
- Checking assumptions
- $ightharpoonup R^2$ in multiple linear regression
- t-tests, F-tests and p-values

Next steps



- ► Homework.
- ► Practical.
- ▶ Then week 5: Binary/categorical explanatory variables, and interactions