

# Session 01 - Exercises

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Before you begin:

- Make sure that R is installed on your computer
- For this lab, we will use a few R libraries:

Set your working directory to your home directory using in R\*

The data files are in the folder `/data/SISG2022M15/data/`.

## Case-Control Association Testing

### Introduction

We will be using the LHON dataset

([https://raw.githubusercontent.com/joellembatchou/SISG2022\\_Association\\_Mapping/master/data/LHON.txt](https://raw.githubusercontent.com/joellembatchou/SISG2022_Association_Mapping/master/data/LHON.txt)) covered in the lecture notes for this portion of the exercises. The LHON dataset is from a case-control study and includes both phenotype and genotype data for a candidate gene.

Let's first load the LHON data file into the R session.

```
vars    n    mean    sd median trimmed    mad min max range  skew kurtosis
IID*      1 328 164.50 94.83  164.5  164.50 121.57   1 328   327  0.00   -1.21
GEN0*     2 328   2.68  0.56    3.0   2.78   0.00   1  3    2 -1.54    1.38
PHEN0*    3 328   1.73  0.45    2.0   1.78   0.00   1  2    1 -1.02   -0.95

se
IID*    5.24
GEN0*   0.03
PHEN0*  0.02
```

### Exercises

Here are some things to look at:

1. Examine the variables in the dataset:
  - How many observations?

```
[1] 328
```

- How many cases/controls?

CASE CONTROL

89 239

- What is the distribution of the genotypes across cases/controls?

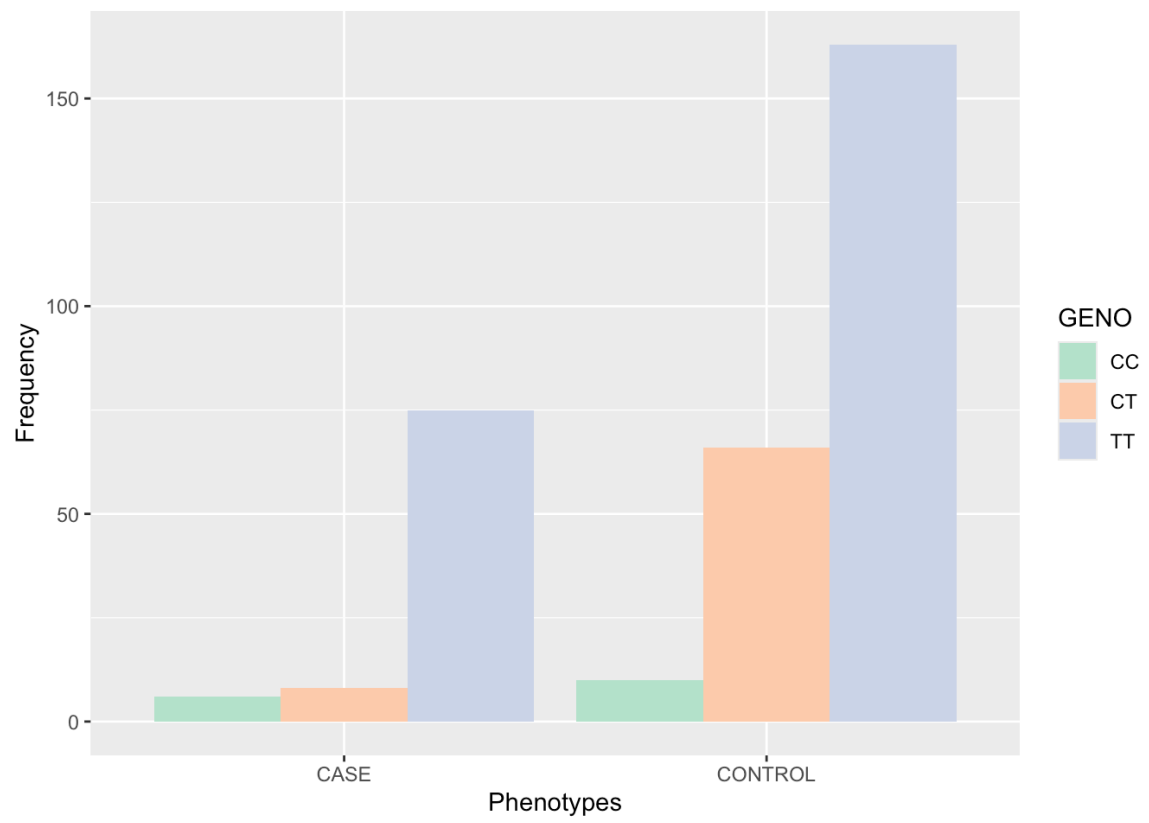
Cell Contents

	N
Chi-square contribution	
N / Row Total	
N / Col Total	
N / Table Total	

Total Observations in Table: 328

PHENO	GENO			Row Total
	CC	CT	TT	
CASE	6	8	75	89
	0.634	7.267	1.682	
	0.067	0.090	0.843	0.271
	0.375	0.108	0.315	
	0.018	0.024	0.229	
CONTROL	10	66	163	239
	0.236	2.706	0.626	
	0.042	0.276	0.682	0.729
	0.625	0.892	0.685	
	0.030	0.201	0.497	
Column Total	16	74	238	328
	0.049	0.226	0.726	

Warning: The dot-dot notation (`..count..`) was deprecated in ggplot2 3.4.0.  
i Please use `after\_stat(count)` instead.  
This warning is displayed once every 8 hours.  
Call `lifecycle::last\_lifecycle\_warnings()` to see where this warning was generated.



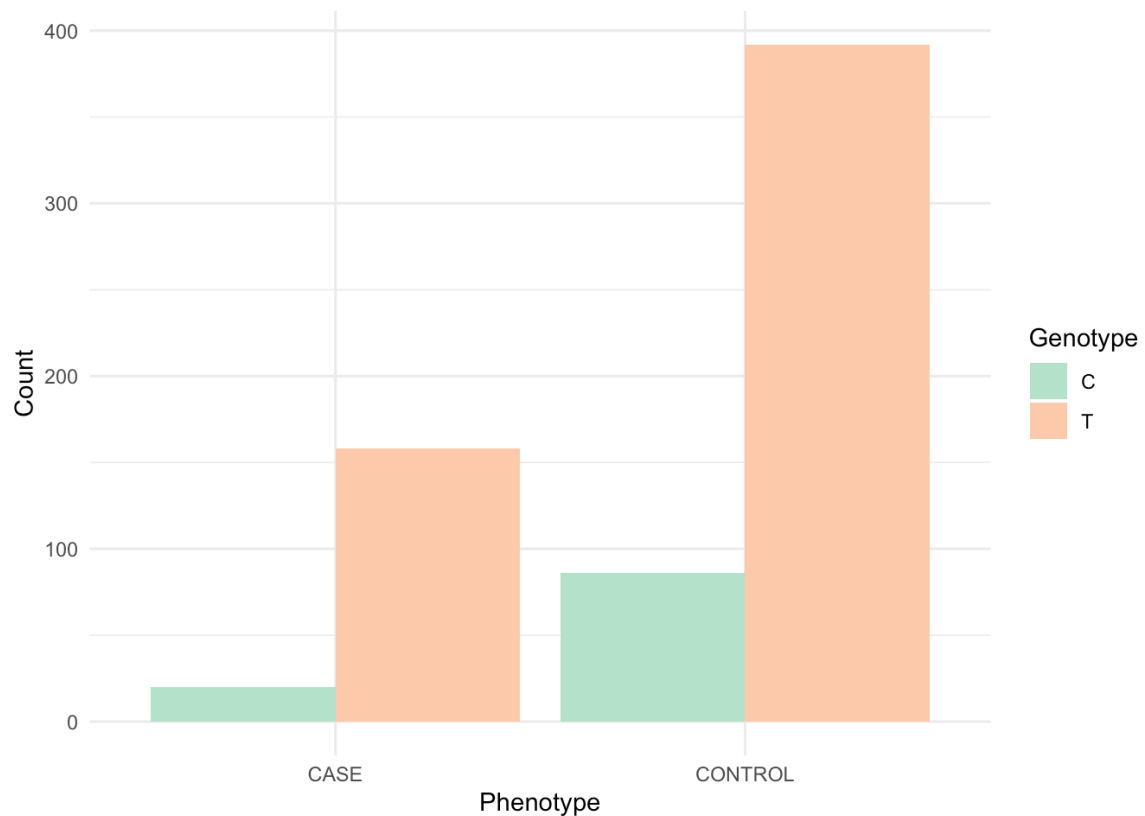
- What about for allele types?

# Cell Contents

N
Chi-square contribution
N / Row Total
N / Col Total
N / Table Total

Total Observations in Table: 656

PHENO	C	T	Row Total
CASE	20	158	178
	2.669	0.514	
	0.112	0.888	0.271
	0.189	0.287	
	0.030	0.241	
CONTROL	86	392	478
	0.994	0.192	
	0.180	0.820	0.729
	0.811	0.713	
	0.131	0.598	
Column Total	106	550	656
	0.162	0.838	



- Perform a logistic regression analysis for this data with CC as the reference genotype using the `glm()` function. (Hint: make sure to convert the phenotype to a binary 0/1 variable and specify `family = binomial(link = "logit")` in the `glm` call)

	CASE	CONTROL
0	0	239
1	89	0

Call:  
`glm(formula = BinPheno ~ GENO, family = binomial(link = "logit"), data = LHON.df)`

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-0.5108	0.5164	-0.989	0.3226
GENOCT	-1.5994	0.6378	-2.508	0.0122 *
GENOTT	-0.2654	0.5349	-0.496	0.6197

---

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

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 383.49 on 327 degrees of freedom  
 Residual deviance: 368.48 on 325 degrees of freedom  
 AIC: 374.48

Number of Fisher Scoring iterations: 4

- Obtain odds ratios and confidence intervals for the CT and TT genotypes relative to the CC reference genotype. Interpret.

(Intercept)	GENOCT	GENOTT
0.6000000	0.2020202	0.7668712

	2.5 %	97.5 %
(Intercept)	0.20413356	1.6156811
GENOCT	0.05710635	0.7223515
GENOTT	0.27431485	2.3258908

4. Is there evidence of differences in odds of being a case for the CT and TT genotypes (compared to CC)?

	OR	2.5 %	97.5 %	p_value
(Intercept)	0.6000000	0.20413356	1.6156811	0.32256061
GENOCT	0.2020202	0.05710635	0.7223515	0.01215534
GENOTT	0.7668712	0.27431485	2.3258908	0.61973850

*Extra:* 5. Perform the logistic regression analysis with the additive genotype coding. Obtain odds ratios and confidence intervals. Is there evidence of an association? How does it compare with the 2-parameter model?

Call:  
`glm(formula = BinPheno ~ Dosage, family = binomial(link = "logit"), data = LHON.df)`

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-1.8077	0.4554	-3.970	7.2e-05 ***
Dosage	0.4787	0.2505	1.911	0.0559 .

---

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

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 383.49 on 327 degrees of freedom  
 Residual deviance: 379.47 on 326 degrees of freedom  
 AIC: 383.47

Number of Fisher Scoring iterations: 4

(Intercept)	Dosage
0.1640322	1.6140439

Waiting for profiling to be done...

	2.5 %	97.5 %
(Intercept)	0.06293774	0.3801326
Dosage	1.01029266	2.7133859

Waiting for profiling to be done...

	OR	2.5 %	97.5 %	p_value
(Intercept)	0.1640322	0.06293774	0.3801326	7.201892e-05
Dosage	1.6140439	1.01029266	2.7133859	5.594566e-02

# Association Testing with Quantitative Traits

## Introduction

We will be using the Blood Pressure dataset

([https://raw.githubusercontent.com/joellembatchou/SISG2022\\_Association\\_Mapping/master/data/bpdata.csv](https://raw.githubusercontent.com/joellembatchou/SISG2022_Association_Mapping/master/data/bpdata.csv)) for this portion of the exercises. This dataset contains diastolic and systolic blood pressure measurements for 1000 individuals, and genotype data at 11 SNPs in a candidate gene for blood pressure. Covariates such as gender (sex) and body mass index (bmi) are included as well.

Let's first load the file into R.

	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew
V1	1	1000	500.50	288.82	500.5	500.50	370.65	1	1000	999	0.00
sex*	2	1000	1.53	0.50	2.0	1.54	0.00	1	2	1	-0.13
sbp	3	1000	141.42	18.47	140.0	140.72	17.79	87	202	115	0.35
dbp	4	1000	82.61	10.84	82.0	82.60	10.38	47	117	70	-0.03
snp1*	5	988	2.31	0.67	2.0	2.39	1.48	1	3	2	-0.46
snp2*	6	978	2.65	0.54	3.0	2.72	0.00	1	3	2	-1.19
snp3*	7	960	1.39	0.56	1.0	1.32	0.00	1	3	2	1.07
snp4*	8	928	1.75	0.70	2.0	1.69	1.48	1	3	2	0.38
snp5*	9	916	1.20	0.43	1.0	1.11	0.00	1	3	2	1.94
snp6*	10	929	1.52	0.64	1.0	1.42	0.00	1	3	2	0.84
snp7*	11	986	2.44	0.65	3.0	2.53	0.00	1	3	2	-0.73
snp8*	12	984	1.35	0.55	1.0	1.26	0.00	1	3	2	1.29
snp9*	13	966	2.69	0.53	3.0	2.78	0.00	1	3	2	-1.45
snp10*	14	978	1.91	0.71	2.0	1.89	1.48	1	3	2	0.13
snp11*	15	979	2.69	0.51	3.0	2.76	0.00	1	3	2	-1.28
bmi	16	999	30.26	6.23	29.0	29.74	5.93	16	51	35	0.83

	kurtosis	se
V1	-1.20	9.13
sex*	-1.99	0.02
sbp	0.15	0.58
dbp	0.32	0.34
snp1*	-0.80	0.02
snp2*	0.42	0.02
snp3*	0.14	0.02
snp4*	-0.92	0.02
snp5*	2.91	0.01
snp6*	-0.35	0.02
snp7*	-0.51	0.02
snp8*	0.67	0.02
snp9*	1.15	0.02
snp10*	-1.03	0.02
snp11*	0.59	0.02
bmi	0.70	0.20

## Exercises

Here are some things to try:

1. Perform a linear regression of systolic blood pressure ( sbp ) on SNP3 using the `lm()` function. Compare the estimates, confidence intervals and p-values you get using:

- additive (linear) model

```
CC  TC  TT
621 304 35
```

Call:

```
lm(formula = sbp ~ snp3Dosage, data = BP.df)
```

Residuals:

```
      Min       1Q   Median       3Q      Max
-55.974 -12.418  -0.974  10.582  60.582
```

Coefficients:

```
              Estimate Std. Error t value Pr(>|t|)
(Intercept) 140.4179      0.7219 194.506  <2e-16 ***
snp3Dosage    2.5556      1.0615   2.407   0.0163 *
```

---

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

Residual standard error: 18.33 on 958 degrees of freedom

(40 observations deleted due to missingness)

Multiple R-squared: 0.006014, Adjusted R-squared: 0.004976

F-statistic: 5.796 on 1 and 958 DF, p-value: 0.01625

```
(Intercept)  snp3Dosage
140.417909    2.555635
```

```
              2.5 %      97.5 %
(Intercept) 139.0011786 141.834639
snp3Dosage    0.4724342   4.638837
```

```
              Estimate      2.5 %      97.5 %      p_value
(Intercept) 140.417909 139.0011786 141.834639 0.00000000
snp3Dosage    2.555635   0.4724342   4.638837 0.01625073
```

- dominant model

```
      CC  TC  TT
0 621    0    0
1   0 304  35
```



Call:

```
lm(formula = sbp ~ snp3Dom, data = BP.df)
```

Residuals:

Min	1Q	Median	3Q	Max
-56.218	-12.428	-0.823	10.572	60.572

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	140.428	0.736	190.801	<2e-16 ***
snp3Dom	2.790	1.238	2.253	0.0245 *

---

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

Residual standard error: 18.34 on 958 degrees of freedom

(40 observations deleted due to missingness)

Multiple R-squared: 0.005269, Adjusted R-squared: 0.00423

F-statistic: 5.074 on 1 and 958 DF, p-value: 0.02451

(Intercept)	snp3Dom
140.428341	2.789948

	2.5 %	97.5 %
(Intercept)	138.9839938	141.872689
snp3Dom	0.3593814	5.220514

	Estimate	2.5 %	97.5 %	p_value
(Intercept)	140.428341	138.9839938	141.872689	0.00000000
snp3Dom	2.789948	0.3593814	5.220514	0.02450948

- recessive model

	CC	TC	TT
0	621	304	0
1	0	0	35

Call:

```
lm(formula = sbp ~ snp3Rec, data = BP.df)
```

Residuals:

Min	1Q	Median	3Q	Max
-54.251	-12.501	-1.251	10.749	59.749

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	141.251	0.604	233.854	<2e-16 ***
snp3Rec	4.463	3.163	1.411	0.159

---

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

Residual standard error: 18.37 on 958 degrees of freedom

(40 observations deleted due to missingness)

Multiple R-squared: 0.002074, Adjusted R-squared: 0.001032

F-statistic: 1.991 on 1 and 958 DF, p-value: 0.1586

(Intercept)	snp3Rec
141.250811	4.463475

	2.5 %	97.5 %
(Intercept)	140.065471	142.43615
snp3Rec	-1.744423	10.67137

	Estimate	2.5 %	97.5 %	p_value
(Intercept)	141.250811	140.065471	142.43615	0.0000000
snp3Rec	4.463475	-1.744423	10.67137	0.1585706

- 2 parameter model

CC	TC	TT
621	304	35

```
Call:
lm(formula = sbp ~ snp3, data = BP.df)

Residuals:
    Min       1Q   Median       3Q      Max
-55.931 -12.428  -0.931  10.572  60.572

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 140.4283    0.7361 190.773  <2e-16 ***
snp3TC       2.5026     1.2840   1.949   0.0516 .
snp3TT       5.2859     3.1868   1.659   0.0975 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 18.34 on 957 degrees of freedom
(40 observations deleted due to missingness)
Multiple R-squared:  0.006019, Adjusted R-squared:  0.003942
F-statistic: 2.898 on 2 and 957 DF, p-value: 0.05563
```

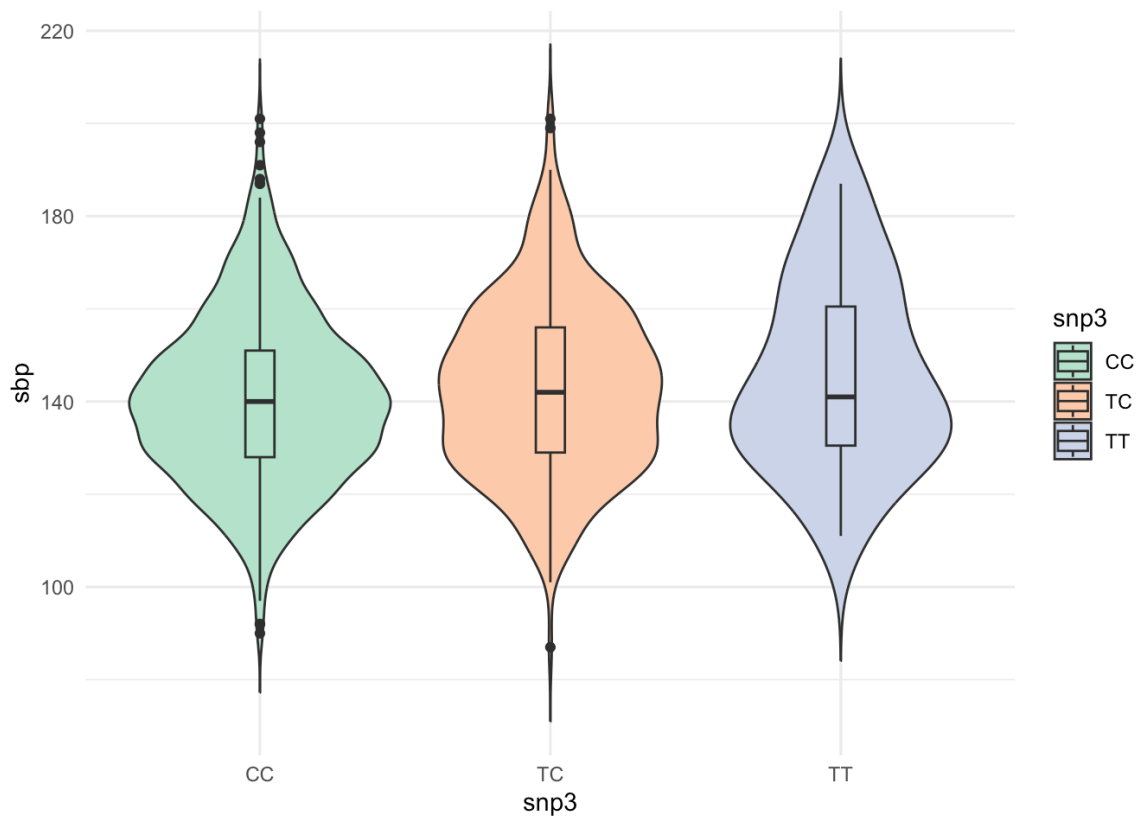
```
(Intercept)      snp3TC      snp3TT
140.428341      2.502580      5.285944
```

```
                2.5 %      97.5 %
(Intercept) 138.98378278 141.872900
snp3TC      -0.01723871   5.022398
snp3TT      -0.96798634  11.539875
```

```
            Estimate      2.5 %      97.5 %      p_value
(Intercept) 140.428341 138.98378278 141.872900 0.00000000
snp3TC       2.502580  -0.01723871   5.022398 0.05158487
snp3TT       5.285944  -0.96798634  11.539875 0.09750441
```

(Hint: for each case, first add a new column to the data frame, containing the ‘predictor’ variable you need. Then do the regression using `lm()` )

2. Provide a plot illustrating the relationship between sbp and the three genotypes at SNP3.



For question 3 and 4 below, R also has a ‘formula’ syntax, frequently used when specifying regression models with many predictors. To regress an outcome *y* on several covariates, the syntax is:

```
outcome ~ covariate1 + covariate2 + covariate3
```

3. Now redo the linear regression analysis of *sbp* from question 1 for the additive model, but this time adjust for *sex* and *bmi*. Do the results change?

Call:

```
lm(formula = sbp ~ snp3Dosage + sex + bmi, data = BP.df)
```

Residuals:

	Min	1Q	Median	3Q	Max
Residuals	-58.83	-12.81	-0.82	11.58	57.80

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	145.85380	3.00271	48.574	< 2e-16 ***
snp3Dosage	2.63566	1.05434	2.500	0.0126 *
sexMALE	-4.77580	1.17642	-4.060	5.32e-05 ***
bmi	-0.09837	0.09481	-1.038	0.2997

---

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

Residual standard error: 18.19 on 955 degrees of freedom

(41 observations deleted due to missingness)

Multiple R-squared: 0.02402, Adjusted R-squared: 0.02096

F-statistic: 7.836 on 3 and 955 DF, p-value: 3.608e-05

(Intercept)	snp3Dosage	sexMALE	bmi
145.85379689	2.63566473	-4.77580021	-0.09837466

	2.5 %	97.5 %
(Intercept)	139.9611289	151.74646484
snp3Dosage	0.5665706	4.70475889
sexMALE	-7.0844766	-2.46712378
bmi	-0.2844395	0.08769022

#### Results with Covariates

	Estimate	2.5 %	97.5 %	p_value
(Intercept)	145.85379689	139.9611289	151.74646484	2.761186e-260
snp3Dosage	2.63566473	0.5665706	4.70475889	1.259244e-02
sexMALE	-4.77580021	-7.0844766	-2.46712378	5.318310e-05
bmi	-0.09837466	-0.2844395	0.08769022	2.997326e-01

#### Results without Covariates

	Estimate	2.5 %	97.5 %	p_value
(Intercept)	140.417909	139.0011786	141.834639	0.00000000
snp3Dosage	2.555635	0.4724342	4.638837	0.01625073

- What proportion of the heritability of `sbp` is explained by all 11 SNPs combined? (contrast categorical coding vs additive coding for the genotypes)

#### Categorical Coding

Call:

```
lm(formula = sbp ~ snp1 + snp2 + snp3 + snp4 + snp5 + snp6 +  
    snp7 + snp8 + snp9 + snp10 + snp11, data = BP.df)
```

Residuals:

Min	1Q	Median	3Q	Max
-50.722	-11.967	-0.703	11.021	61.704

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	133.1726	12.4033	10.737	<2e-16 ***
snp1CT	-1.7048	4.5991	-0.371	0.711
snp1TT	1.9319	8.2839	0.233	0.816
snp2AT	0.7347	5.5923	0.131	0.896
snp2TT	-0.5118	6.9317	-0.074	0.941
snp3TC	4.7672	5.0211	0.949	0.343
snp3TT	6.6913	9.7904	0.683	0.495
snp4CT	-0.4778	3.5501	-0.135	0.893
snp4TT	2.3431	6.4874	0.361	0.718
snp5CT	1.1896	3.0462	0.391	0.696
snp5TT	-2.2787	7.5490	-0.302	0.763
snp6AG	-3.0266	2.0697	-1.462	0.144
snp6GG	2.1230	4.6650	0.455	0.649
snp7AT	-3.0873	3.9148	-0.789	0.431
snp7TT	-2.6319	4.3146	-0.610	0.542
snp8CT	-1.5509	3.6318	-0.427	0.669
snp8TT	-2.5507	7.3228	-0.348	0.728
snp9CT	6.0693	7.6170	0.797	0.426
snp9TT	4.7385	7.4517	0.636	0.525
snp10CT	1.4330	1.6466	0.870	0.384
snp10TT	1.9810	2.0699	0.957	0.339
snp11CT	4.8005	6.5175	0.737	0.462
snp11TT	4.0226	9.2775	0.434	0.665

---

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

Residual standard error: 18.2 on 707 degrees of freedom

(270 observations deleted due to missingness)

Multiple R-squared: 0.02633, Adjusted R-squared: -0.003965

F-statistic: 0.8691 on 22 and 707 DF, p-value: 0.6372

	Estimate	2.5 %	97.5 %	p_value
(Intercept)	133.1725671	108.820890	157.524245	5.131083e-25
snp1CT	-1.7048318	-10.734443	7.324779	7.109834e-01
snp1TT	1.9318980	-14.332013	18.195809	8.156641e-01
snp2AT	0.7346616	-10.244868	11.714191	8.955201e-01
snp2TT	-0.5118275	-14.121058	13.097403	9.411599e-01
snp3TC	4.7671827	-5.090918	14.625283	3.427288e-01
snp3TT	6.6912706	-12.530460	25.913001	4.945449e-01
snp4CT	-0.4777530	-7.447752	6.492246	8.929866e-01
snp4TT	2.3430912	-10.393724	15.079906	7.180747e-01
snp5CT	1.1896284	-4.791101	7.170358	6.962657e-01
snp5TT	-2.2787219	-17.099774	12.542330	7.628481e-01
snp6AG	-3.0265667	-7.090080	1.036946	1.440994e-01
snp6GG	2.1230367	-7.035788	11.281861	6.491746e-01
snp7AT	-3.0872887	-10.773220	4.598642	4.305930e-01
snp7TT	-2.6319274	-11.102829	5.838974	5.420516e-01
snp8CT	-1.5509162	-8.681351	5.579519	6.694831e-01
snp8TT	-2.5507399	-16.927782	11.826302	7.276973e-01
snp9CT	6.0693019	-8.885403	21.024006	4.258307e-01
snp9TT	4.7385208	-9.891650	19.368692	5.250505e-01
snp10CT	1.4329754	-1.799872	4.665823	3.844573e-01
snp10TT	1.9809712	-2.082851	6.044793	3.388680e-01
snp11CT	4.8005159	-7.995478	17.596510	4.616364e-01
snp11TT	4.0225845	-14.192153	22.237323	6.647219e-01

Let's check the model if we had used additive coding for all SNPs.

# SNP 1

	0	1	2
CC	119	0	0
CT	0	444	0
TT	0	0	425

# SNP 2

	0	1	2
AA	30	0	0
AT	0	285	0
TT	0	0	663

# SNP 3

	0	1	2
CC	621	0	0
TC	0	304	0
TT	0	0	35

# SNP 4

	0	1	2
CC	368	0	0
CT	0	421	0
TT	0	0	139

# SNP 5

	0	1	2
CC	742	0	0
CT	0	162	0
TT	0	0	12

# SNP 6

	0	1	2
AA	521	0	0
AG	0	335	0
GG	0	0	73

# SNP 7

	0	1	2
AA	85	0	0
AT	0	381	0
TT	0	0	520

# SNP 8

	0	1	2
CC	678	0	0
CT	0	271	0
TT	0	0	35

# SNP 9

	0	1	2
CC	30	0	0
CT	0	239	0
TT	0	0	697

# SNP 10



	0	1	2
CC	296	0	0
CT	0	475	0
TT	0	0	207

SNP 11

	0	1	2
CC	20	0	0
CT	0	264	0
TT	0	0	695

Call:

```
lm(formula = sbp ~ snp1Dosage + snp2Dosage + snp3Dosage + snp4Dosage +
    snp5Dosage + snp6Dosage + snp7Dosage + snp8Dosage + snp9Dosage +
    snp10Dosage + snp11Dosage, data = BP.df.dos)
```

Residuals:

Min	1Q	Median	3Q	Max
-53.638	-12.849	-0.522	11.032	61.683

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	137.36286	9.09141	15.109	<2e-16 ***
snp1Dosage	1.88456	4.03838	0.467	0.641
snp2Dosage	-1.95639	2.96674	-0.659	0.510
snp3Dosage	4.60730	4.65652	0.989	0.323
snp4Dosage	0.05946	3.11138	0.019	0.985
snp5Dosage	-0.26494	2.58719	-0.102	0.918
snp6Dosage	-1.17284	1.80185	-0.651	0.515
snp7Dosage	-0.28939	1.78362	-0.162	0.871
snp8Dosage	0.70702	2.78030	0.254	0.799
snp9Dosage	2.17197	2.54774	0.853	0.394
snp10Dosage	0.60685	1.01229	0.599	0.549
snp11Dosage	-0.39009	4.15347	-0.094	0.925

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


Residual standard error: 18.17 on 718 degrees of freedom

(270 observations deleted due to missingness)

Multiple R-squared: 0.01418, Adjusted R-squared: -0.0009268

F-statistic: 0.9386 on 11 and 718 DF, p-value: 0.5022

	Estimate	2.5 %	97.5 %	p_value
(Intercept)	137.36286195	119.513941	155.211783	5.463978e-45
snp1Dosage	1.88455556	-6.043884	9.812996	6.408836e-01
snp2Dosage	-1.95638699	-7.780906	3.868132	5.098245e-01
snp3Dosage	4.60729872	-4.534719	13.749316	3.227861e-01
snp4Dosage	0.05945758	-6.049037	6.167953	9.847589e-01
snp5Dosage	-0.26493620	-5.344303	4.814431	9.184654e-01
snp6Dosage	-1.17284174	-4.710367	2.364684	5.153132e-01
snp7Dosage	-0.28939265	-3.791133	3.212348	8.711547e-01
snp8Dosage	0.70701756	-4.751477	6.165512	7.993403e-01
snp9Dosage	2.17196761	-2.829934	7.173869	3.942159e-01
snp10Dosage	0.60685115	-1.380549	2.594251	5.490393e-01
snp11Dosage	-0.39009298	-8.544499	7.764313	9.251992e-01

 Session information