Exercise 2 SISG Association Mapping

Load the bpdata.csv data file into your R session.

LHON.txt data file into your R session.

Can read the data directly from the website if your computer is connected online

```
bpdata=read.csv("http://faculty.washington.edu/tathornt/sisg/bpdata.csv",header=TRUE)
```

If file is saved on your computer, could also read the data in from the directory that contains the file.

```
bpdata2=read.csv("bpdata.csv",header=TRUE)
```

View the first few lines of the bpdata

```
head(bpdata)
#   X    sex sbp dbp snp1 snp2 snp3 snp4 snp5 snp6 snp7 snp8 snp9 snp10
# 1 1 FEMALE 171  89   CC   TT   TT   TT   CC   GG   AA   TT   TT    CC
# 2 2   MALE 160  99   TT   TT   CC <NA>   CC   AG   AT   CC   CT    CC
# 3 3 FEMALE 142  83   CT   TT   TC   CT   CC   AG   TT   CC   TT    CT
# 4 4   MALE 126  71   CT   TT   CC <NA>   CC   AA   TT   CC   TT    CT
# 5 5 FEMALE 126  82   CT   TT   CC   CC   CC   AA   TT   CC   TT    CT
# 6 6 FEMALE 132  89   CT   TT   CC   CC   CC <NA>   TT   CC   TT    TT
#   snp11 bmi
# 1    TT  25
# 2    CT  35
# 3    TT  34
# 4    CT  32
# 5    CT  34
# 6    CT  25
```

Get information about the types of variables in the LHON data frame

```
str(bpdata)
# 'data.frame': 1000 obs. of  16 variables:
#  $ X    : int  1 2 3 4 5 6 7 8 9 10 ...
#  $ sex  : Factor w/ 2 levels "FEMALE","MALE": 1 2 1 2 1 1 1 1 2 2 ...
#  $ sbp  : int  171 160 142 126 126 132 136 121 120 136 ...
#  $ dbp  : int  89 99 83 71 82 89 58 87 69 88 ...
#  $ snp1 : Factor w/ 3 levels "CC","CT","TT": 1 3 2 2 2 2 3 3 3 3 ...
#  $ snp2 : Factor w/ 3 levels "AA","AT","TT": 3 3 3 3 3 3 3 3 3 3 ...
#  $ snp3 : Factor w/ 3 levels "CC","TC","TT": 3 1 2 1 1 1 1 1 1 1 ...
#  $ snp4 : Factor w/ 3 levels "CC","CT","TT": 3 NA 2 NA 1 1 1 1 2 1 ...
#  $ snp5 : Factor w/ 3 levels "CC","CT","TT": 1 1 1 1 1 1 1 1 1 1 ...
#  $ snp6 : Factor w/ 3 levels "AA","AG","GG": 3 2 2 1 1 NA 2 2 1 1 ...
#  $ snp7 : Factor w/ 3 levels "AA","AT","TT": 1 2 3 3 3 3 2 2 3 3 ...
#  $ snp8 : Factor w/ 3 levels "CC","CT","TT": 3 1 1 1 1 1 1 2 1 1 ...
#  $ snp9 : Factor w/ 3 levels "CC","CT","TT": 3 2 3 3 3 3 2 2 3 3 ...
#  $ snp10: Factor w/ 3 levels "CC","CT","TT": 1 1 2 2 2 3 2 3 2 2 ...
#  $ snp11: Factor w/ 3 levels "CC","CT","TT": 3 2 3 2 2 2 2 3 3 3 ...
#  $ bmi  : int  25 35 34 32 34 25 22 33 21 29 ...
```

1 Perform a linear regression in R of systolic blood pressure (sbp) on SNP3 using lm() for different models.

Additive model

First create a genotype variable with an additive coding based on the counts of the number of T alleles

```r
bpdata$n.minor <- (bpdata$snp3=="TC") +(bpdata$snp3=="CT")+2*(bpdata$snp3=="TT")

head(bpdata$n.minor)
# [1] 2 0 1 0 0 0
```

Now perform a linear regression analysis with the additive genotype coding variable

```r
lm1 = lm(sbp~n.minor, data=bpdata)
summary(lm1)
#
# Call:
# lm(formula = sbp ~ n.minor, data = bpdata)
#
# 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 ***
# n.minor       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
```

Can obtain confidence intervals of the effect estimates using *confint.default()* function

```r
confint.default(lm1)
#                   2.5 %     97.5 %
# (Intercept) 139.0029685 141.832849
# n.minor       0.4750661   4.636205
```

Note that we obtain the same inference on the association with an additive model when the genotype variable is coded based on the number of copies of the C allele

```r
bpdata$n.minorC<- (bpdata$snp3=="TC") + (bpdata$snp3=="CT")+ 2*(bpdata$snp3=="CC")
head(bpdata$n.minorC)
# [1] 0 2 1 2 2 2
lm1a = lm(sbp~n.minorC, data=bpdata)
summary(lm1a)
#
# Call:
# lm(formula = sbp ~ n.minorC, data = bpdata)
#
# 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)  145.529      1.809  80.446   <2e-16 ***
# n.minorC      -2.556      1.062  -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
```

Dominant effect model for T allele.

First create a variable that has a value of 1 for genotypes with at least 1 T allele, and 0 for the genotype that is CC

```
bpdata$domvar <- (bpdata$snp3=="TC") | (bpdata$snp3=="TT")
with(bpdata, table(domvar, snp3))
#        snp3
# domvar   CC  TC  TT
#   FALSE 621   0   0
#   TRUE    0 304  35
```

Now perform the linear regression analysis for the dominant coded genotype variable and obtain confidenc intervals.

```
lm2 <- lm(sbp~domvar, data=bpdata)
summary(lm2)
#
# Call:
# lm(formula = sbp ~ domvar, data = bpdata)
#
# 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 ***
# domvarTRUE     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
confint.default(lm2)
```

```
#                   2.5 %     97.5 %
# (Intercept) 138.9858186 141.870864
# domvarTRUE    0.3624521   5.217443
```

Recessive Model for T allele

```
bpdata$recvar <- (bpdata$snp3=="TT")
with(bpdata, table(recvar, snp3))
#        snp3
# recvar   CC  TC  TT
#   FALSE 621 304   0
#   TRUE    0   0  35
lm3<-lm(sbp~recvar, data=bpdata)
summary(lm3)
#
# Call:
# lm(formula = sbp ~ recvar, data = bpdata)
#
# 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 ***
# recvarTRUE     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
confint.default(lm3)
#                 2.5 %    97.5 %
# (Intercept) 140.06697 142.43465
# recvarTRUE   -1.73658  10.66353
```

2 Degree of freedom model

```
lm4<-lm(sbp~snp3, data=bpdata)
summary(lm4)
#
# Call:
# lm(formula = sbp ~ snp3, data = bpdata)
#
# 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
confint.default(lm4)
#                    2.5 %     97.5 %
# (Intercept) 138.98560975 141.871073
# snp3TC       -0.01405184   5.019211
# snp3TT       -0.96007686  11.531966
```

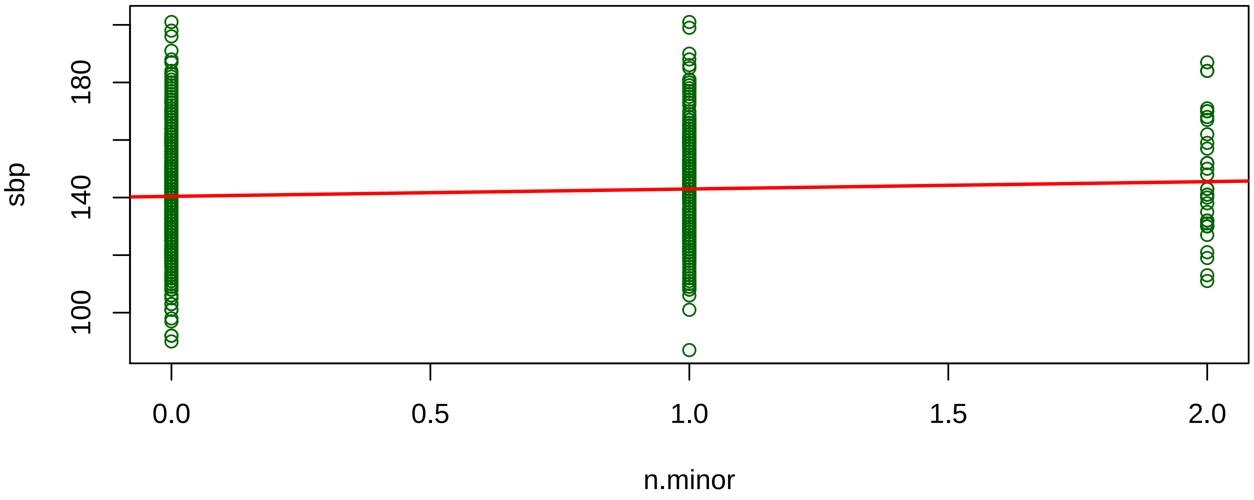
To test the 2 degree of freedom model, can use ANOVA (analysis of variance), which compares the full model to the null model. First need to remove missing data, so comparing models on the same dataset

```
clean.data <- bpdata[complete.cases(bpdata[,c("snp3")]),]
lm4<-lm(sbp~snp3, data=clean.data)
lm.null  <- lm(sbp~1, data=clean.data)
anova(lm.null, lm4)
# Analysis of Variance Table
#
# Model 1: sbp ~ 1
# Model 2: sbp ~ snp3
#   Res.Df    RSS Df Sum of Sq      F  Pr(>F)
# 1    959 323967
# 2    957 322017  2    1950.1 2.8977 0.05563 .
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

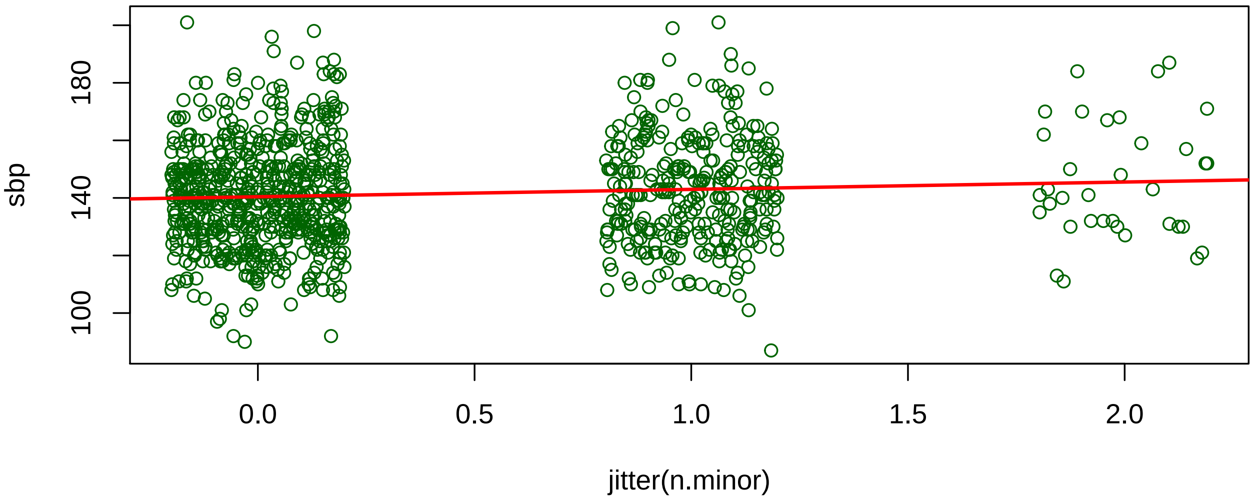
Plot of SBP versus minor allele count for snp3 with linear regression line

```
plot(sbp~n.minor, data=bpdata,col="darkgreen")
abline(lm1, col="red", lwd=2)
```



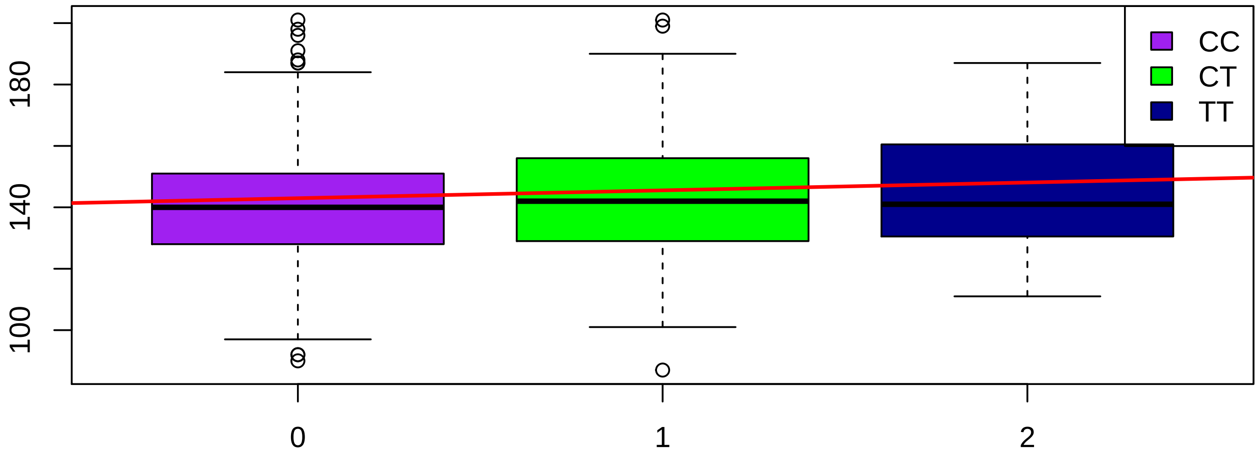
Using *jitter()* adds noise to the points so that we can better see how many observations there are within each genotype group

```
plot(sbp~jitter(n.minor), data=bpdata,col="darkgreen")
abline(lm1, col="red", lwd=2)
```



Below is a boxplot with a regression line

```
boxplot(sbp~n.minor,data=bpdata, col=c("purple","green","darkblue"))
legend("topright",c("CC","CT","TT"),fill=c("purple","green","darkblue"))
abline(lm1, lwd=2,col="red")
```



3. Now redo the linear regression analysis of sbp from question 2 for the additive model, but this time adjust for sex and bmi. Do the results change?

```
lm1adj = lm(sbp~n.minor+sex+bmi, data=bpdata)
summary(lm1adj)
#
# Call:
# lm(formula = sbp ~ n.minor + sex + bmi, data = bpdata)
#
# Residuals:
#    Min     1Q Median     3Q    Max
# -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 ***
# n.minor       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
confint.default(lm1adj)
#                   2.5 %       97.5 %
# (Intercept) 139.9685971 151.73899665
# n.minor       0.5691929   4.70213658
# sexMALE      -7.0815507  -2.47004973
# bmi          -0.2842037   0.08745441
```

4. Perform a linear regression for additive model using all SNPs

```
lmall<- lm(sbp~snp1+snp2+snp3+snp4+snp5+snp6+snp7+snp8+snp9+snp10+snp11,data=bpdata)
summary(lmall)
#
# Call:
# lm(formula = sbp ~ snp1 + snp2 + snp3 + snp4 + snp5 + snp6 +
#     snp7 + snp8 + snp9 + snp10 + snp11, data = bpdata)
#
# 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
```

Below is the model above with sex and bmi included as predictors

```
lmalladj<- lm(sbp~snp1+snp2+snp3+snp4+snp5+snp6+snp7+snp8+snp9+snp10+snp11+sex+bmi,data=bpdata)
summary(lmalladj)
#
# Call:
# lm(formula = sbp ~ snp1 + snp2 + snp3 + snp4 + snp5 + snp6 +
#     snp7 + snp8 + snp9 + snp10 + snp11 + sex + bmi, data = bpdata)
```

```
#
# Residuals:
#     Min      1Q  Median      3Q     Max
# -53.174 -12.355  -0.629  11.250  59.791
#
# Coefficients:
#              Estimate Std. Error t value Pr(>|t|)
# (Intercept) 137.91819   12.98670  10.620   <2e-16 ***
# snp1CT       -1.12301    4.60167  -0.244   0.8073
# snp1TT        2.63061    8.28860   0.317   0.7511
# snp2AT        0.22237    5.58086   0.040   0.9682
# snp2TT       -1.03410    6.91365  -0.150   0.8811
# snp3TC        4.96410    5.01294   0.990   0.3224
# snp3TT        7.37868    9.78154   0.754   0.4509
# snp4CT       -0.35549    3.54132  -0.100   0.9201
# snp4TT        2.22937    6.46966   0.345   0.7305
# snp5CT        1.05751    3.04173   0.348   0.7282
# snp5TT       -2.05836    7.53421  -0.273   0.7848
# snp6AG       -3.03433    2.06696  -1.468   0.1425
# snp6GG        1.98216    4.66029   0.425   0.6707
# snp7AT       -3.48175    3.91557  -0.889   0.3742
# snp7TT       -3.03391    4.31639  -0.703   0.4824
# snp8CT       -1.71031    3.62926  -0.471   0.6376
# snp8TT       -2.22421    7.31597  -0.304   0.7612
# snp9CT        7.33391    7.61822   0.963   0.3360
# snp9TT        5.95797    7.45023   0.800   0.4242
# snp10CT       1.20427    1.64451   0.732   0.4642
# snp10TT       1.77744    2.06794   0.860   0.3903
# snp11CT       3.98814    6.50793   0.613   0.5402
# snp11TT       3.10665    9.27131   0.335   0.7377
# sexMALE      -3.42337    1.35814  -2.521   0.0119 *
# bmi          -0.09882    0.11020  -0.897   0.3702
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#
# Residual standard error: 18.15 on 704 degrees of freedom
#   (271 observations deleted due to missingness)
# Multiple R-squared:  0.03622, Adjusted R-squared:  0.003361
# F-statistic: 1.102 on 24 and 704 DF,  p-value: 0.3345
```

Below is another way to do the regression analysis in R without having to write all of the predictors:

```
lm5<- lm(sbp~.,data=bpdata[,c(2:3,5:16)])
summary(lm5)
#
# Call:
# lm(formula = sbp ~ ., data = bpdata[, c(2:3, 5:16)])
#
# Residuals:
#     Min      1Q  Median      3Q     Max
# -53.174 -12.355  -0.629  11.250  59.791
#
# Coefficients:
#              Estimate Std. Error t value Pr(>|t|)
# (Intercept) 137.91819   12.98670  10.620   <2e-16 ***
# sexMALE      -3.42337    1.35814  -2.521   0.0119 *
# snp1CT       -1.12301    4.60167  -0.244   0.8073
# snp1TT        2.63061    8.28860   0.317   0.7511
# snp2AT        0.22237    5.58086   0.040   0.9682
# snp2TT       -1.03410    6.91365  -0.150   0.8811
# snp3TC        4.96410    5.01294   0.990   0.3224
# snp3TT        7.37868    9.78154   0.754   0.4509
# snp4CT       -0.35549    3.54132  -0.100   0.9201
# snp4TT        2.22937    6.46966   0.345   0.7305
# snp5CT        1.05751    3.04173   0.348   0.7282
# snp5TT       -2.05836    7.53421  -0.273   0.7848
# snp6AG       -3.03433    2.06696  -1.468   0.1425
# snp6GG        1.98216    4.66029   0.425   0.6707
# snp7AT       -3.48175    3.91557  -0.889   0.3742
# snp7TT       -3.03391    4.31639  -0.703   0.4824
# snp8CT       -1.71031    3.62926  -0.471   0.6376
# snp8TT       -2.22421    7.31597  -0.304   0.7612
# snp9CT        7.33391    7.61822   0.963   0.3360
# snp9TT        5.95797    7.45023   0.800   0.4242
# snp10CT       1.20427    1.64451   0.732   0.4642
# snp10TT       1.77744    2.06794   0.860   0.3903
# snp11CT       3.98814    6.50793   0.613   0.5402
# snp11TT       3.10665    9.27131   0.335   0.7377
# bmi          -0.09882    0.11020  -0.897   0.3702
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#
# Residual standard error: 18.15 on 704 degrees of freedom
#   (271 observations deleted due to missingness)
# Multiple R-squared:  0.03622, Adjusted R-squared:  0.003361
# F-statistic: 1.102 on 24 and 704 DF,  p-value: 0.3345
```