Thema09_Log

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Contents

Introduction

A CpG island or CpG site is a part of the DNA where the GC content is greater than 50%. In this dataset methylation values of certain CpG sites are displayed with also the age, gender and smoking status for 671 people.

Research Question

Is it possible to identify a person's gender, age or status of smoking given their methylation values on CpG islands?

Data exploration

Codebook & Reading the data

```
myData <- read.csv("data/Smoker_Epigenetic_df.csv")
myData <- myData %>% drop_na()

columns = colnames(myData[1:4])
columns <- append(columns, "Columns 5-24")
names <- c("Sample Accessions numbers", "Smoking status", "Gender", "Age", "CG Island")
type <- c("chr", "chr", "chr", "int", "num")
unit <- c(NA, "current/never", "f/m", NA, NA)
descriptions = c("GSM identifier testsubject", "Wether the person is smoking or not", "Gender", "Age",
codebook <- data.frame(columns, names, type, descriptions)
write.csv(codebook, "Codebook.csv", row.names = FALSE)
knitr::kable(codebook)
```

columns	names	type	descriptions
GSM	Sample Accessions numbers	chr	GSM identifier testsubject
Smoking.Status	Smoking status	chr	Wether the person is smoking or not
Gender	Gender	chr	Gender
Age	Age	int	Age
Columns 5-24	CG Island	num	Methylation Rate of CG Island

Summary

```
subsetdata <- head(myData)
dataset <- subsetdata[1:7]
knitr::kable(dataset) %>%
  row_spec(0, bold = TRUE) %>%
  column_spec(1, bold = TRUE)
```

GSM	Smoking.Status	Gender	Age	cg00050873	cg00212031	cg00213748
GSM1051525	current	f	67	0.6075634	0.4228427	0.3724549
GSM1051526	current	f	49	0.3450542	0.5686615	0.5005995
GSM1051527	current	f	53	0.3213497	0.3609091	0.3527315
GSM1051528	current	f	62	0.2772675	0.3044371	0.4752352
GSM1051529	never	f	33	0.4135991	0.1312511	0.3675446
GSM1051530	current	f	59	0.6228599	0.5016849	0.2632270

```
dataset <- subsetdata[c(1, 8:13)]
knitr::kable(dataset) %>%
  row_spec(0, bold = TRUE) %>%
  column_spec(1, bold = TRUE)
```

GSM	cg00214611	cg00455876	cg01707559	cg02004872	cg02011394	cg02050847
GSM1051525	0.6215619	0.2907773	0.2671431	0.1791439	0.4802517	0.3276078
GSM1051526	0.4986067	0.3745909	0.1902743	0.1559775	0.4180809	0.3464627
GSM1051527	0.3738240	0.2306740	0.3147052	0.1057448	0.6151030	0.2375392
GSM1051528	0.4862581	0.2951815	0.2957931	0.1112862	0.3010196	0.3045353
GSM1051529	0.7611667	0.2357703	0.2505265	0.1691084	0.3929746	0.3062257
GSM1051530	0.4157459	0.4751891	0.2539041	0.2607587	0.5097921	0.4052457

```
dataset <- subsetdata[c(1, 14:19)]
knitr::kable(dataset) %>%
  row_spec(0, bold = TRUE) %>%
  column_spec(1, bold = TRUE)
```

GSM	cg02233190	cg02494853	cg02839557	cg02842889	cg03052502	cg03155755
GSM1051525	0.2411204	0.0670696	0.2469934	0.4692396	0.4002466	0.4150313
GSM1051526	0.1754907	0.0469389	0.2367423	0.3074666	0.3770313	0.3973715
GSM1051527	0.2464092	0.0382371	0.2446117	0.3577526	0.3050442	0.5212775
GSM1051528	0.1770279	0.0267163	0.0016414	0.4457390	0.2714746	0.4344920
GSM1051529	0.3017014	0.0370164	0.3343197	0.3950396	0.3265530	0.4300966
GSM1051530	0.3852716	0.0258346	0.3092102	0.3218573	0.5333670	0.5715522

```
dataset <- subsetdata[c(1, 20:24)]
knitr::kable(dataset) %>%
  row_spec(0, bold = TRUE) %>%
  column_spec(1, bold = TRUE)
```

GSM	cg03244189	cg03443143	cg03683899	cg03695421	cg03706273
GSM1051525	0.2214331	0.4758258	0.2077242	0.2091974	0.1299826
GSM1051526	0.2171221	0.5444690	0.1844462	0.1937732	0.0985327
GSM1051527	0.1850495	0.5370600	0.3931231	0.2680030	0.0402481
GSM1051528	0.1654187	0.5079167	0.2812089	0.2178572	0.1015163
GSM1051529	0.1811352	0.4054791	0.3107944	0.2800708	0.0778571
GSM1051530	0.2109749	0.3778239	0.4693609	0.3433317	0.0457791

```r
data_sum <- summary(myData)
data_sum
```

```
##      GSM             Smoking.Status        Gender               Age
##  Length:621         Length:621         Length:621         Min.   :18.00
##  Class :character   Class :character   Class :character   1st Qu.:46.00
##  Mode  :character   Mode  :character   Mode  :character   Median :54.00
##                                                           Mean   :52.59
##                                                           3rd Qu.:61.00
##                                                           Max.   :70.00
##    cg00050873       cg00212031         cg00213748       cg00214611
##  Min.   :0.1186   Min.   :0.006949   Min.   :0.0000   Min.   :0.01247
##  1st Qu.:0.4131   1st Qu.:0.063172   1st Qu.:0.3635   1st Qu.:0.06946
##  Median :0.5052   Median :0.365545   Median :0.4713   Median :0.41575
##  Mean   :0.5600   Mean   :0.309601   Mean   :0.5191   Mean   :0.34106
##  3rd Qu.:0.8144   3rd Qu.:0.459813   3rd Qu.:0.7278   3rd Qu.:0.49745
##  Max.   :0.8989   Max.   :0.709992   Max.   :0.9236   Max.   :0.80606
##    cg00455876       cg01707559         cg02004872         cg02011394
##  Min.   :0.05917   Min.   :0.04333   Min.   :0.002616   Min.   :0.0000
##  1st Qu.:0.29300   1st Qu.:0.11080   1st Qu.:0.042835   1st Qu.:0.4261
##  Median :0.37968   Median :0.23873   Median :0.149332   Median :0.5157
##  Mean   :0.44718   Mean   :0.21435   Mean   :0.155417   Mean   :0.6058
##  3rd Qu.:0.66283   3rd Qu.:0.28061   3rd Qu.:0.242627   3rd Qu.:0.9412
##  Max.   :0.85443   Max.   :0.46999   Max.   :0.473844   Max.   :0.9792
##    cg02050847       cg02233190         cg02494853         cg02839557
##  Min.   :0.05234   Min.   :0.008632   Min.   :0.01162   Min.   :0.00000
##  1st Qu.:0.33963   1st Qu.:0.088375   1st Qu.:0.02865   1st Qu.:0.06384
##  Median :0.42754   Median :0.259817   Median :0.03695   Median :0.35042
##  Mean   :0.54369   Mean   :0.232498   Mean   :0.04077   Mean   :0.30088
##  3rd Qu.:0.95558   3rd Qu.:0.337023   3rd Qu.:0.04677   3rd Qu.:0.45786
##  Max.   :0.98320   Max.   :0.511730   Max.   :0.28947   Max.   :0.82739
##    cg02842889       cg03052502         cg03155755       cg03244189
##  Min.   :0.01346   Min.   :0.0000   Min.   :0.2020   Min.   :0.02972
##  1st Qu.:0.05483   1st Qu.:0.4025   1st Qu.:0.4245   1st Qu.:0.11976
##  Median :0.39757   Median :0.4940   Median :0.4962   Median :0.20397
##  Mean   :0.32362   Mean   :0.5907   Mean   :0.5895   Mean   :0.19552
##  3rd Qu.:0.47385   3rd Qu.:0.9631   3rd Qu.:0.8988   3rd Qu.:0.24921
##  Max.   :0.85625   Max.   :0.9902   Max.   :0.9696   Max.   :0.54074
##    cg03443143       cg03683899         cg03695421       cg03706273
##  Min.   :0.06496   Min.   :0.00788   Min.   :0.0949   Min.   :0.01120
##  1st Qu.:0.40963   1st Qu.:0.06159   1st Qu.:0.2566   1st Qu.:0.03413
##  Median :0.48314   Median :0.34422   Median :0.3208   Median :0.04961
##  Mean   :0.56841   Mean   :0.28442   Mean   :0.3978   Mean   :0.05769
##  3rd Qu.:0.85436   3rd Qu.:0.41866   3rd Qu.:0.5965   3rd Qu.:0.06916
##  Max.   :0.93589   Max.   :0.65925   Max.   :0.8433   Max.   :0.34380
```

Visualization

Distribution plots

Below there are some histograms which visualizes the distribution of smoking status, age and gender.

```
ggplot(myData, aes(x=Smoking.Status)) +
  geom_histogram(stat="count", fill = c("pink", "lightblue"), col = "black") +
  ylim(0, 600) +
  labs(caption = "Figure 1: Number of people who are smoking") +
  theme(plot.caption = element_text(size=16)) +
  theme(plot.caption = element_text(size=16, face="italic")) +
  theme(axis.text = element_text(size = 20)) +
  theme(axis.title = element_text(size = 20, face="bold"))
```

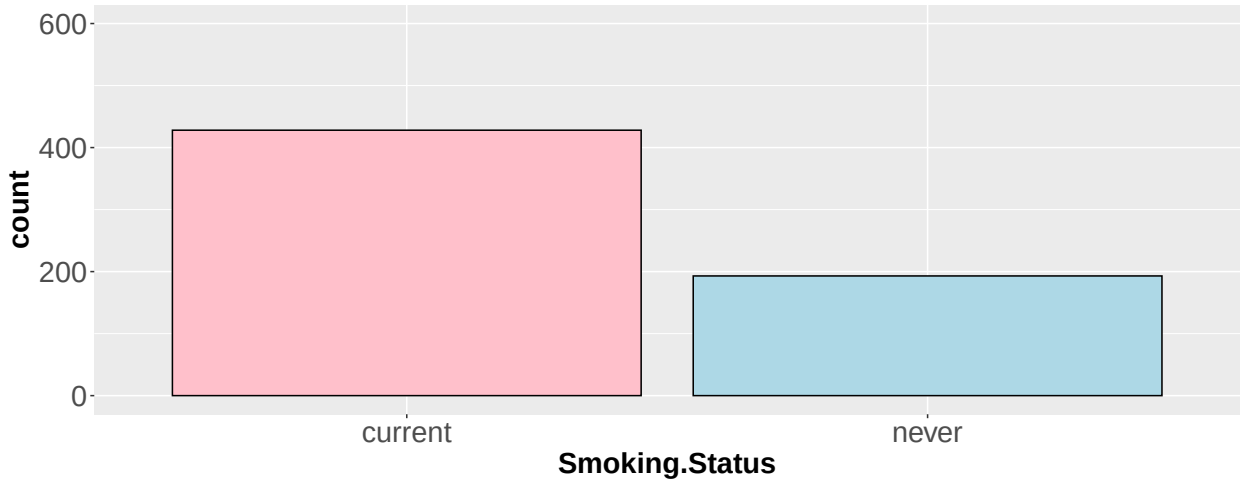


Figure 1: Number of people who are smoking

```
ggplot(myData, aes(x=Gender)) +
  geom_histogram(stat="count", fill = c("pink", "lightblue"), col = "black") +
  ylim(0, 600) +
  labs(caption = "Figure 2: Gender distribution") +
  theme(plot.caption = element_text(size=16)) +
  theme(plot.caption = element_text(size=16, face="italic")) +
  theme(axis.text = element_text(size = 20)) +
  theme(axis.title = element_text(size = 20, face="bold"))
```

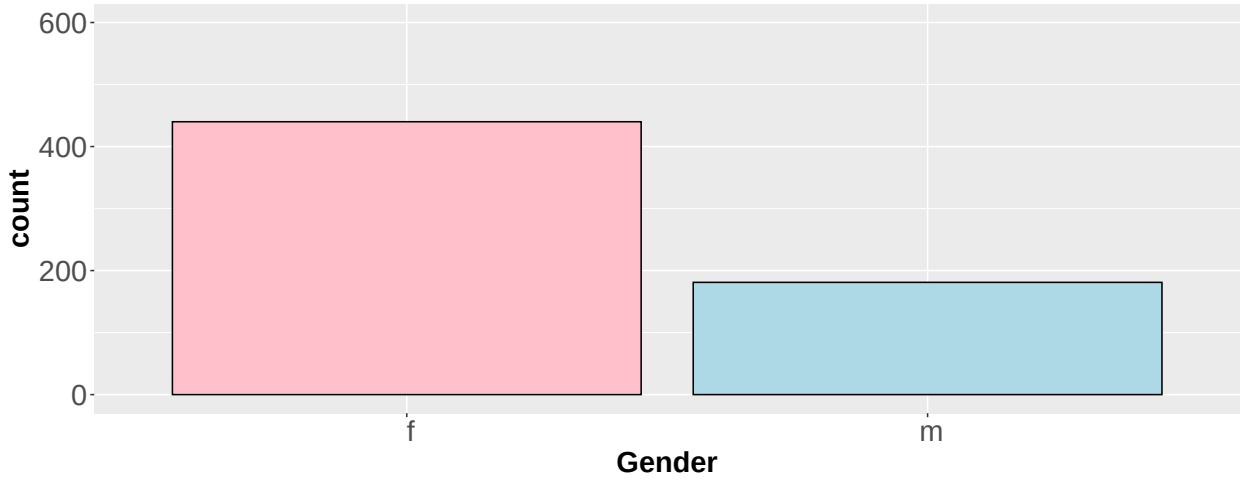


Figure 2: Gender distribution

```
ggplot(myData, aes(x=Age)) +
  geom_histogram(fill = "lightgrey", col = "black") +
  labs(caption = "Figure 3: Age distribution") +
  theme(plot.caption = element_text(size=16, face="italic")) +
  theme(axis.text = element_text(size = 20)) +
  theme(axis.title = element_text(size = 20, face="bold"))
```

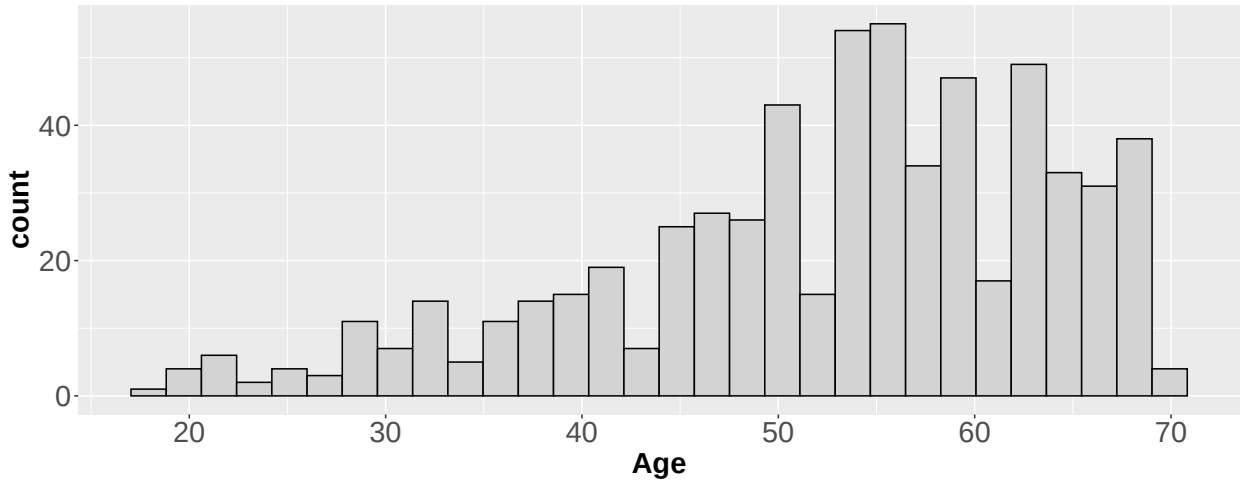


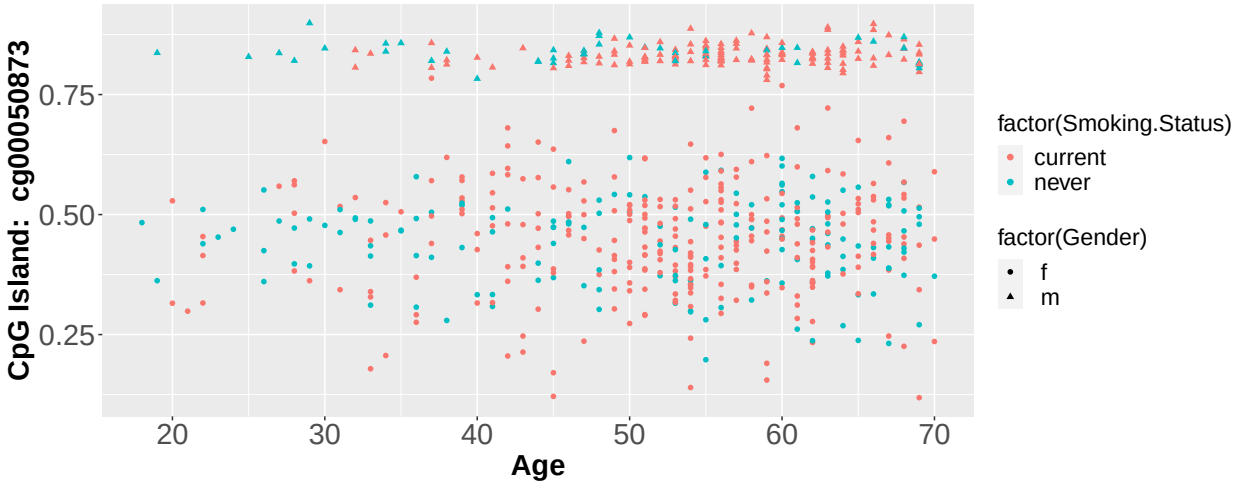
Figure 3: Age distribution

Plotting the data

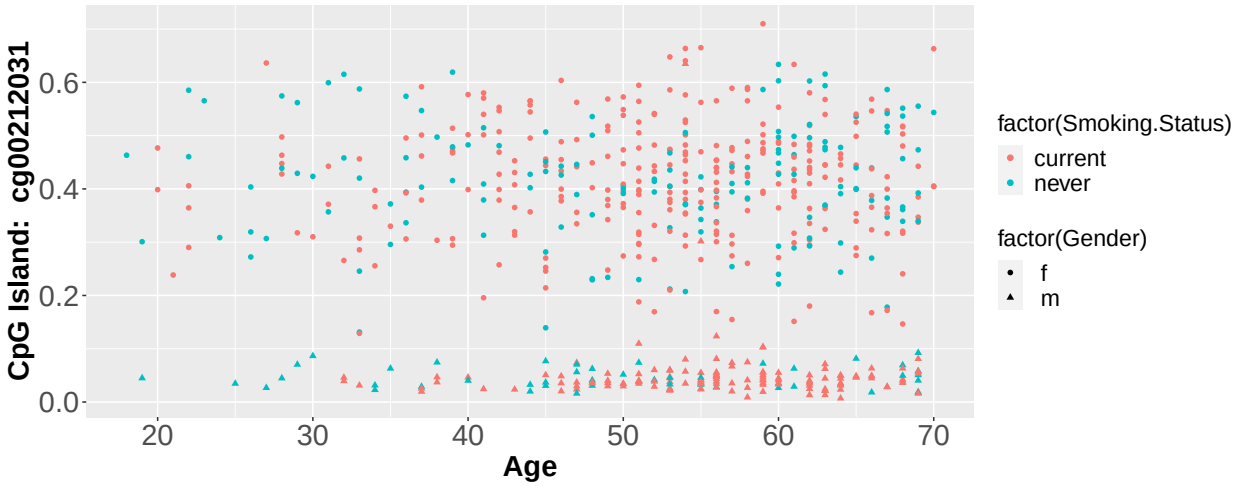
Scatterplots

These are all the CpG sites plotted against age, with smokin status as color groups and gender as shape groups. A few of the CpG islands have been removed, becaused they looked very similar. What stands out here is that you see two groups in almost every graph, one of men and one of women, so apparently the cg methylation rate is different between men and women.

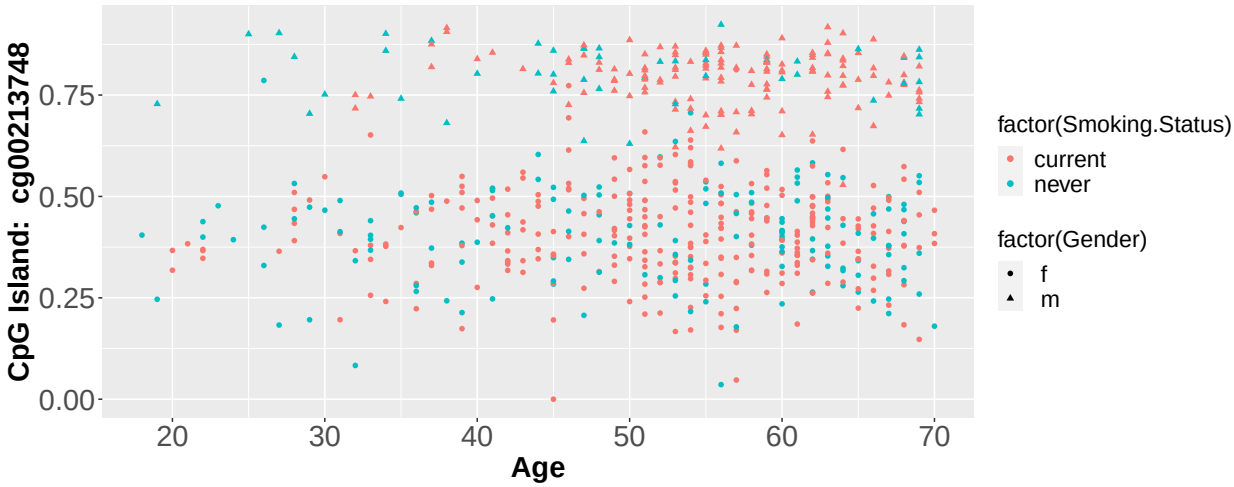
```
plotfunction <- function(cg){
  ggplot(data = myData, mapping = aes_string(x = "Age", y = cg)) +
    geom_point(aes(shape = factor(Gender), color = factor(Smoking.Status))) +
    labs(caption = paste(cg, ": Scatterplot visualizing the methylation rate on this CpG island of diff
    theme(plot.caption = element_text(size=14, face="italic")) +
    theme(axis.text = element_text(size = 20)) +
    theme(axis.title = element_text(size = 20, face="bold")) +
    theme(legend.text = element_text(size = 16)) +
    theme(legend.title = element_text(size=16)) +
    ylab(paste("CpG Island: ", cg))
}
lapply(names(myData[c(5, 6, 7, 9, 10, 15, 20)]), plotfunction)
```



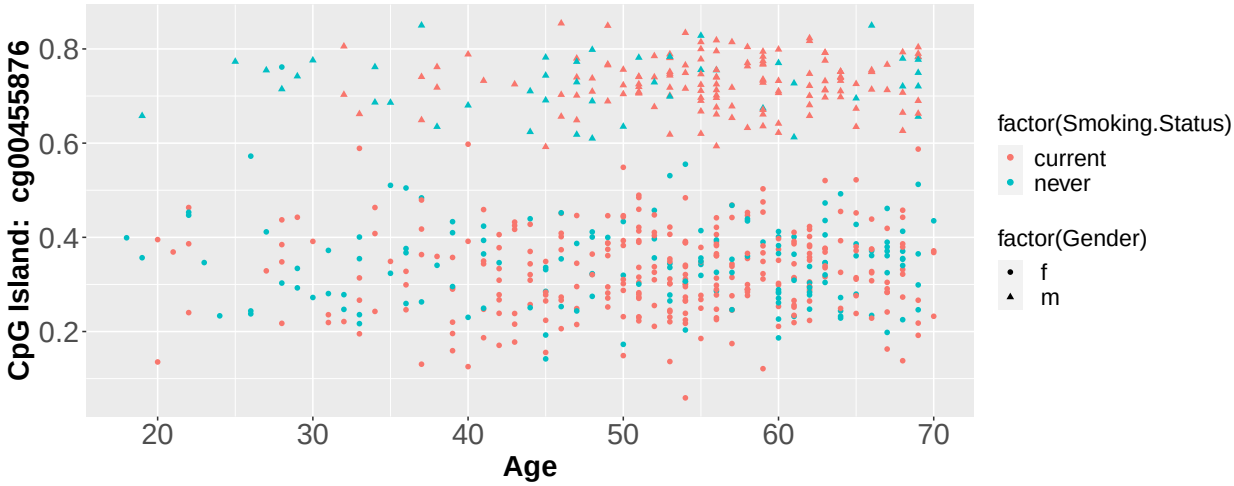
cg00050873 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.



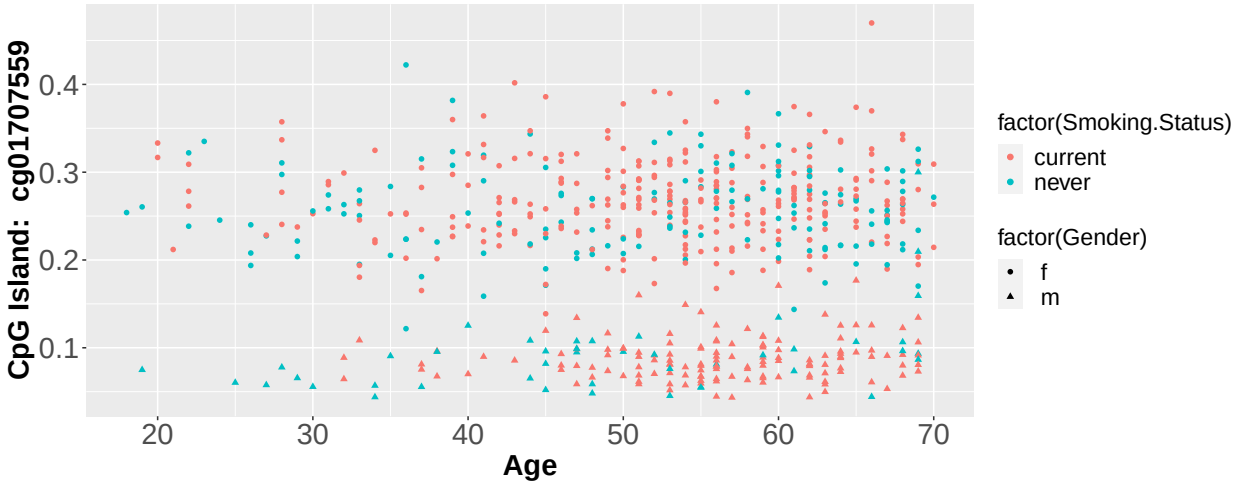
cg00212031 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.



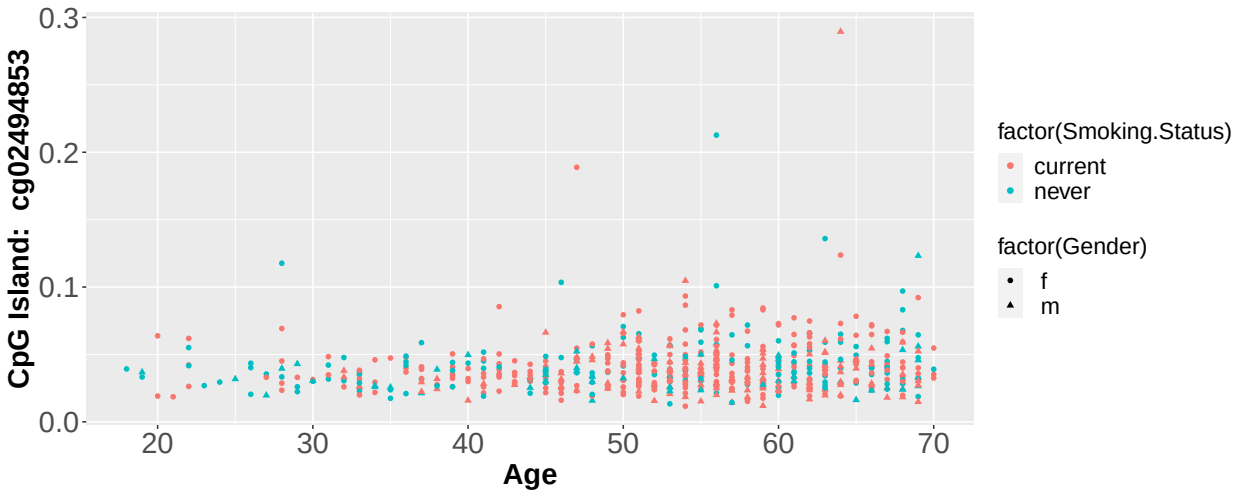
cg00213748 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.



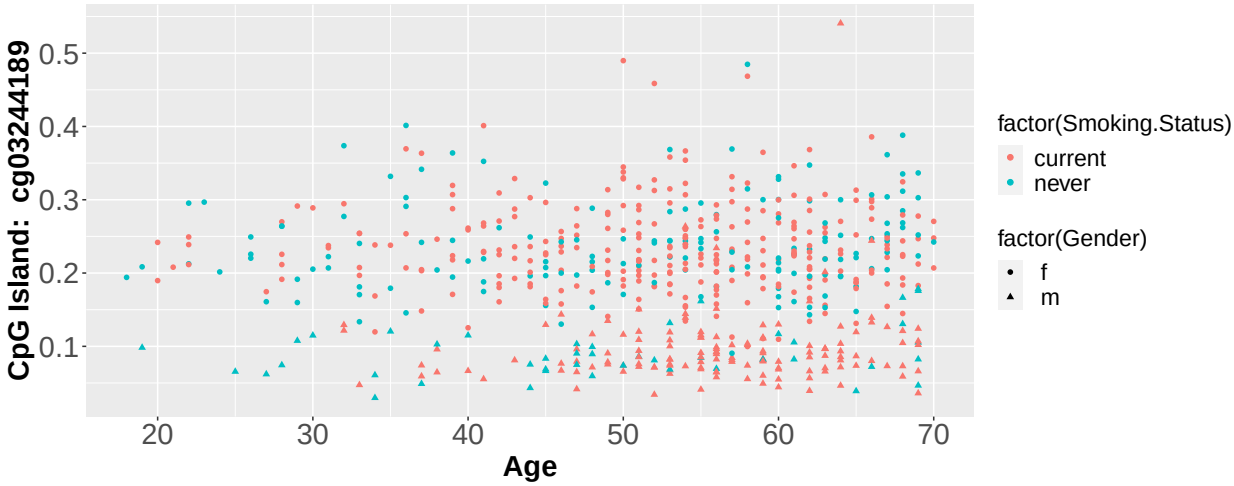
cg00455876 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.



cg01707559 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.



cg02494853 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.

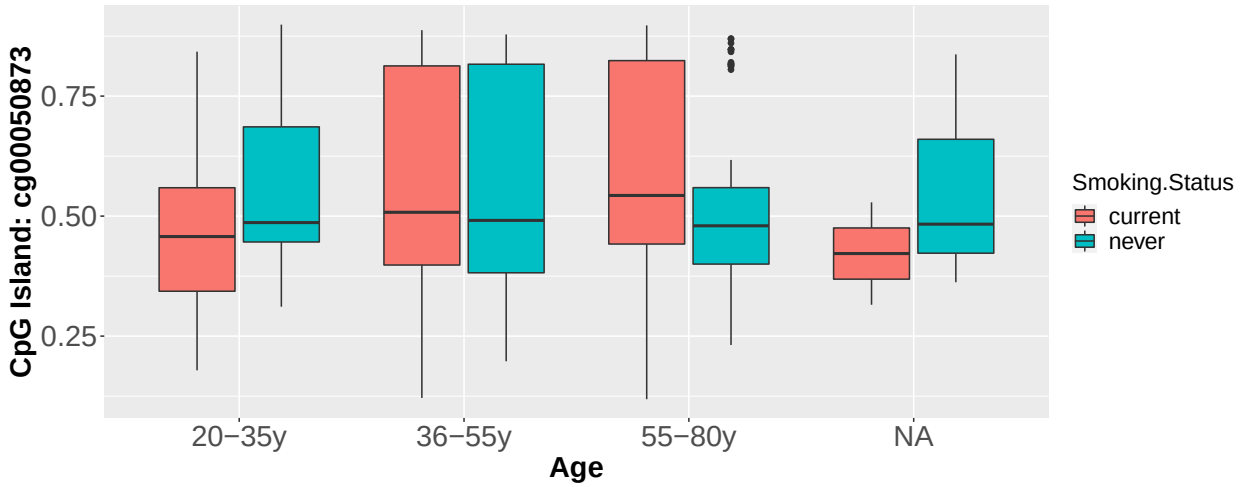


cg03244189 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.

Comparing two variables

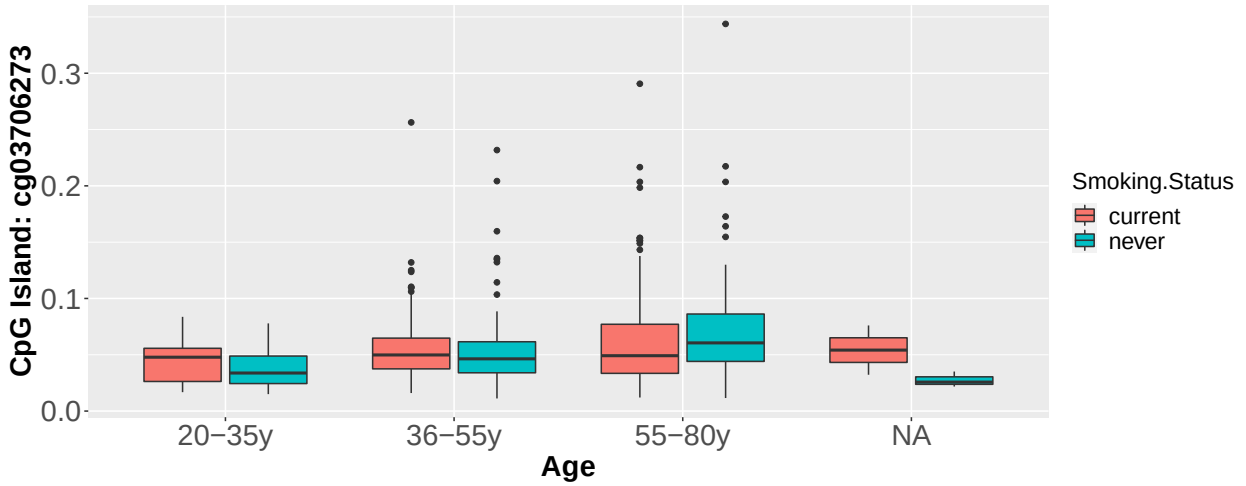
With the age distribution in mind, let's try to plot 2 methylation rates with the age factored as groups.

```
age.groups <- cut(myData$Age, breaks = c(20, 35, 55, 80), labels = c("20-35y", "36-55y", "55-80y"))

myData$ClassAge <- factor(age.groups)

ggplot(data = myData, mapping = aes(x = ClassAge, y = cg00050873, fill = Smoking.Status)) +
    geom_boxplot() +
    labs(caption = paste("cg00050873: Scatterplot visualizing the methylation rate on this CpG island o
    theme(plot.caption = element_text(size=14, face="italic")) +
    theme(axis.text = element_text(size = 20)) +
    theme(axis.title = element_text(size = 20, face="bold")) +
    theme(legend.text = element_text(size = 16)) +
    theme(legend.title = element_text(size=16)) +
    ylab(paste("CpG Island: cg00050873")) +
    xlab("Age")
```



cg00050873: Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.

```
ggplot(data = myData, mapping = aes(x = ClassAge, y = cg03706273, fill = Smoking.Status)) +
    geom_boxplot() +
    labs(caption = paste("cg03706273: Scatterplot visualizing the methylation rate on this CpG island o
    theme(plot.caption = element_text(size=14, face="italic")) +
    theme(axis.text = element_text(size = 20)) +
    theme(axis.title = element_text(size = 20, face="bold")) +
    theme(legend.text = element_text(size = 16)) +
    theme(legend.title = element_text(size=16)) +
    ylab(paste("CpG Island: cg03706273")) +
    xlab("Age")
```



cg03706273: Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.

Data mining

Quality metrics

In this week we define the best algorithm for our data with true positive, false positive, true negative and false negative.

Algorithms

The algorithms we are going to compare are: ZeroR, OneR, J48, NaiveBayes, IBK, Simple Logistics, SMO and Random Forest. Below you can see a table with the most important results of all algorithms.

```
ML_dataLoc_Smoke <- "data/Smoker_Epigenetic_dfExperimentSmoke.csv"
ML_dataLoc_Gender <- "data/Smoker_Epigenetic_dfExperimentGender.csv"
files <- list(ML_dataLoc_Smoke, ML_dataLoc_Gender)

count <- 0
for (ML_dataLoc in files)
{
  count <- count + 1
  if (count == 1)
  {
    file <- "Smoke"
  }
  else
  {
    file <- "Gender"
  }

  ML_data <- read.csv(ML_dataLoc)
  ML_ZeroR <- ML_data[c(1:100),]
  ML_OneR <- ML_data[c(101:200),]
  ML_J48 <- ML_data[c(201:300),]
  ML_IBK <- ML_data[c(301:400),]
  ML_NaiveBayes <- ML_data[c(401:500),]
  ML_SimpleLog <- ML_data[c(501:600),]
  ML_SMO <- ML_data[c(601:700),]
  ML_RandomForest <- ML_data[c(701:800),]
  algos <- list(ML_ZeroR, ML_OneR, ML_J48, ML_IBK, ML_NaiveBayes, ML_SimpleLog, ML_SMO, ML_RandomForest

  avgs_pc <- list()
  avgs_pi <- list()
  avgs_tp <- list()
  avgs_fp <- list()
  avgs_tn <- list()
  avgs_fn <- list()
  avgs_pr <- list()
  avgs_rc <- list()
  avgs_roc <- list()

  for (algo in algos) {
    percent_correct <- algo[3]
    avg <- lapply(percent_correct, mean)
    avgs_pc[[length(avgs_pc) + 1]] <- avg
  }
  for (algo in algos) {
    percent_incorrect <- algo[4]
    avg <- lapply(percent_incorrect, mean)
    avgs_pi[[length(avgs_pi) + 1]] <- avg
  }
  for (algo in algos) {
```

```
  TP <- algo[5]
  avg <- lapply(TP, sum)
  avgs_tp[[length(avgs_tp) + 1]] <- avg
}
for (algo in algos) {
  FP <- algo[6]
  avg <- lapply(FP, sum)
  avgs_fp[[length(avgs_fp) + 1]] <- avg
}
for (algo in algos) {
  TN <- algo[7]
  avg <- lapply(TN, sum)
  avgs_tn[[length(avgs_tn) + 1]] <- avg
}
for (algo in algos) {
  FN <- algo[8]
  avg <- lapply(FN, sum)
  avgs_fn[[length(avgs_fn) + 1]] <- avg
}
for (algo in algos) {
  precision <- algo[9]
  avg <- lapply(precision, mean)
  avgs_pr[[length(avgs_pr) + 1]] <- avg
}
for (algo in algos) {
  recall <- algo[10]
  avg <- lapply(recall, mean)
  avgs_rc[[length(avgs_rc) + 1]] <- avg
}
for (algo in algos) {
  roc_area <- algo[11]
  avg <- lapply(roc_area, mean)
  avgs_roc[[length(avgs_roc) + 1]] <- avg
}
avgs_df <- data.frame(row.names = c("ZeroR", "OneR", "J48", "IBK", "NaiveBayes", "SimpleLogistics", "S

vec_pc <- unlist(avgs_pc)
vec_pi <- unlist(avgs_pi)
vec_tp <- unlist(avgs_tp)
vec_fp <- unlist(avgs_fp)
vec_tn <- unlist(avgs_tn)
vec_fn <- unlist(avgs_fn)
vec_pr <- unlist(avgs_pr)
vec_rc <- unlist(avgs_rc)
vec_roc <- unlist(avgs_roc)

avgs_df$percent_correct_avgs <- vec_pc
avgs_df$percent_incorrect_avgs <- vec_pi
avgs_df$TP_sum <- vec_tp
avgs_df$FP_sum <- vec_fp
avgs_df$TN_sum <- vec_tn
avgs_df$FN_sum <- vec_fn
avgs_df$precision_avgs <- vec_pr
```

```
  avgs_df$recall_avgs <- vec_rc
  avgs_df$ROC_Area_avgs <- vec_roc

  knitr::kable(avgs_df)
  filepath <- paste("data/Smoker_Epigenetic_df_Summary_", file, ".csv", sep = "")

  write.csv(avgs_df, file = filepath)
}
```

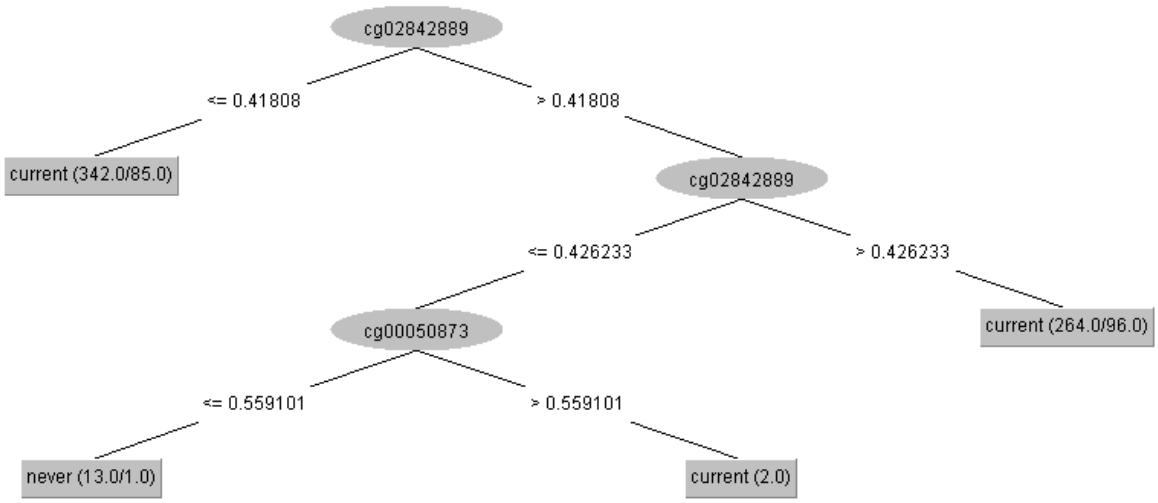


Figure 1: The generated tree of the J48 algorithm with the smoking status as class. Apparently these CpG islands say the most about whether a person smokes or not.

Conclusion & Discussion

So far we don't have a concrete answer to the research question, because the algorithms aren't extremely overwhelming. So for now it isn't really possible to determine whether someone is smoking or not, because the data is too much spreaded out and not really divided into groups.

It's quite obvious that the percentage of correctly classified instances of every algorithm except for NaiveBayes are very close to eachother, the only significant lower scores according to weka are NaiveBayes and OneR, the best (not really significant) algorithm are surprisingly ZeroR, J48, SimpleLogistics and SMO. However it's quite remarkable that the ROC-area and the precision are the highest on NaiveBayes. So even though the results are disappointing, this is the best algorithm according to these quality metrics.

The Data with the gender as class is quite more interesting, these algorithms have a way higher area under the curve and correctly classified instance percentage.

For the Java wrapper, it is probably the best to use the data with the gender class, because this is way more reliable than the smoking status. Even though it sounds a bit odd to predict gender with the methylation rate of certain CpG islands.

One possible purpose for the use of the gender class may be for forensic research. If some suspect need to be found, it's a way to find out if the suspect is male or female.

After some research I found out what the reason for this remarkable difference is. These differences have everything to do with the diversity of the transcriptomic and proteomic profiles in the two sexes. [1]

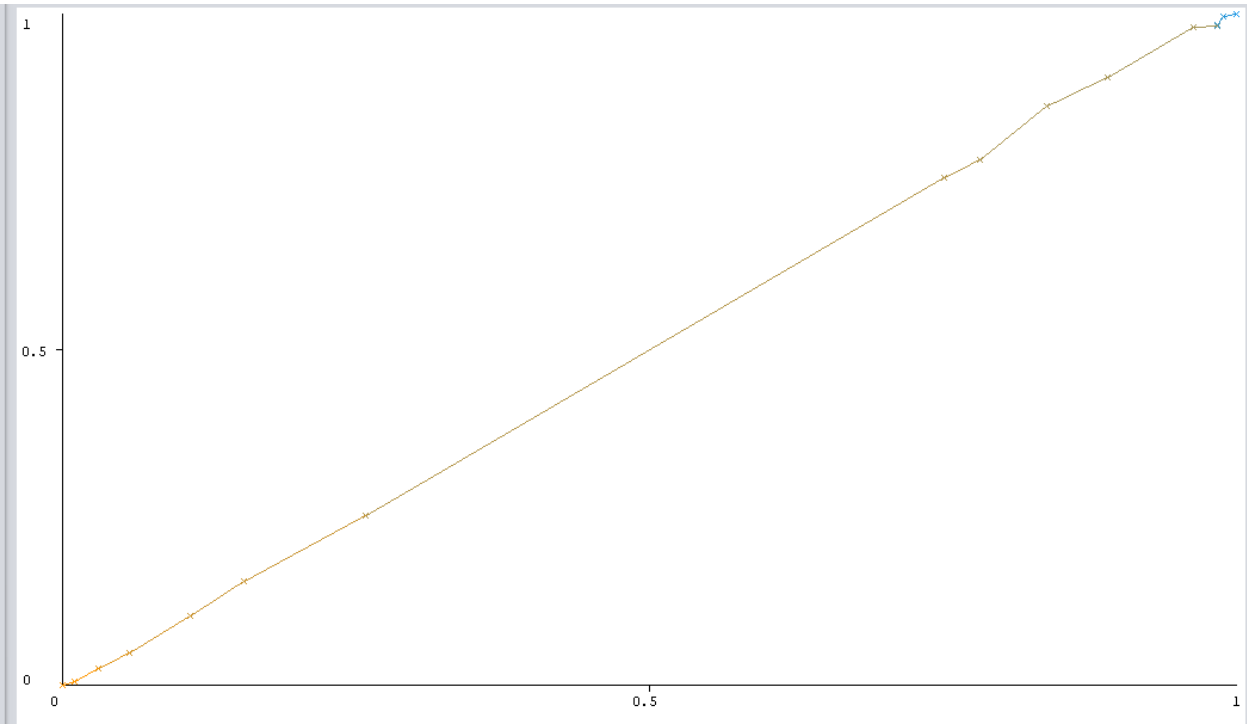


Figure 2: The ROC curve of the J48 algorithm using the current class. Not much is going on here, this algorithms gives almost no information.

Sources

[1] Yusipov, I., Bacalini, M. G., Kalyakulina, A., Krivonosov, M., Pirazzini, C., Gensous, N., Ravaioli, F., Milazzo, M., Giuliani, C., Vedunova, M., Fiorito, G., Gagliardi, A., Polidoro, S., Garagnani, P., Ivanchenko, M., & Franceschi, C. (2020). Age-related DNA methylation changes are sex-specific: a comprehensive assessment. Aging, 12(23), 24057–24080. https://doi.org/10.18632/aging.202251

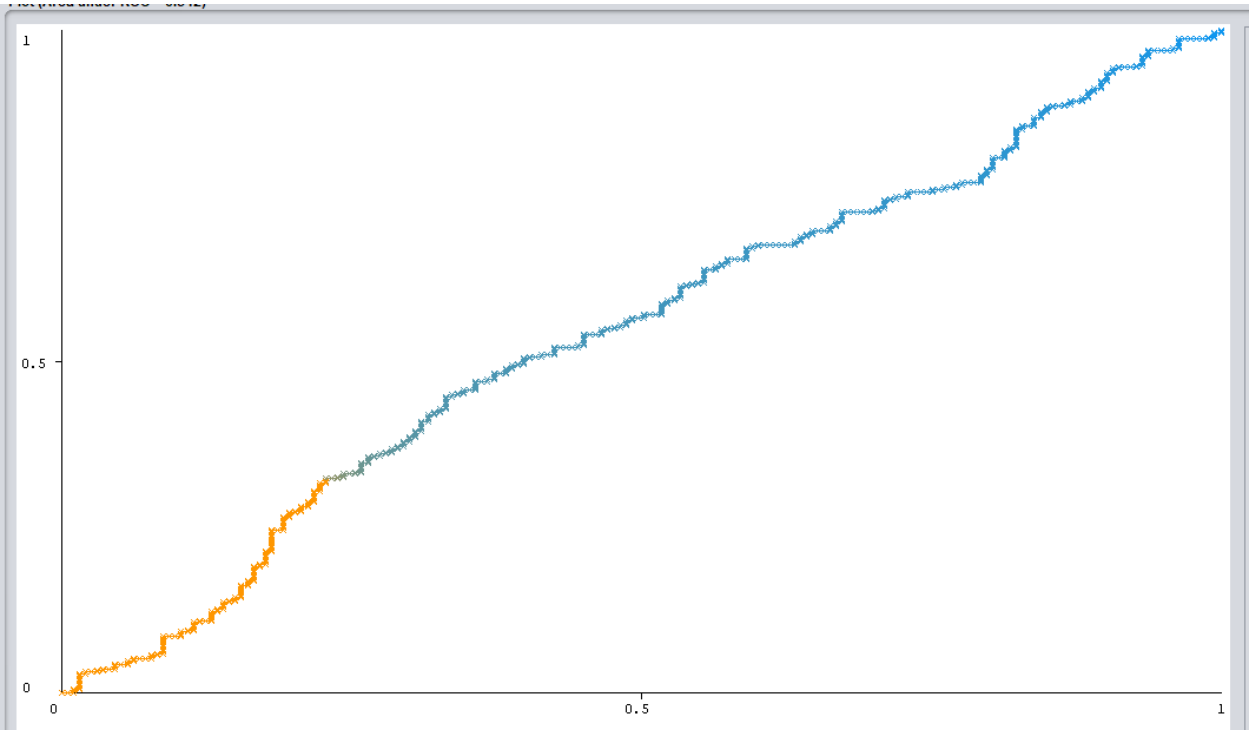


Figure 3: The ROC curve of the NaiveBayes algorithm using the current class. It's still quite straight, but not as straight as the J48 ROC curve