

# Thema09\_Log

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## Introduction

### Smoker epigenetic dataset

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 CpG values?

# EDA

## Codebook

```
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", "Methylation Rate of CG Island")
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

## Data exploration

### Visualization

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

<b>GSM</b>	<b>Smoking.Status</b>	<b>Gender</b>	<b>Age</b>	<b>cg00050873</b>	<b>cg00212031</b>	<b>cg00213748</b>
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)
```

<b>GSM</b>	<b>cg00214611</b>	<b>cg00455876</b>	<b>cg01707559</b>	<b>cg02004872</b>	<b>cg02011394</b>	<b>cg02050847</b>
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)
```

<b>GSM</b>	<b>cg02233190</b>	<b>cg02494853</b>	<b>cg02839557</b>	<b>cg02842889</b>	<b>cg03052502</b>	<b>cg03155755</b>
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)
```

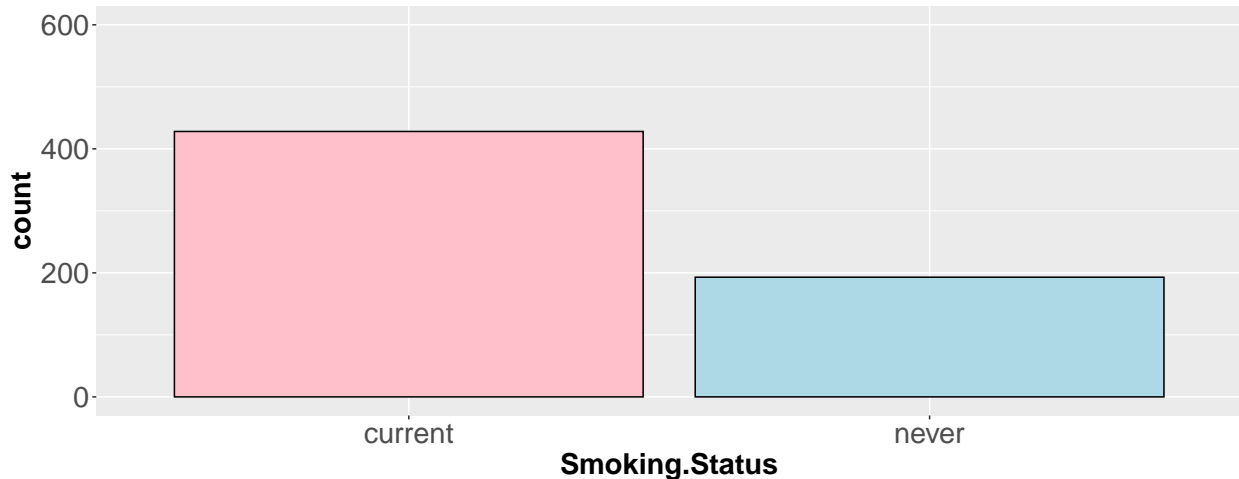
<b>GSM</b>	<b>cg03244189</b>	<b>cg03443143</b>	<b>cg03683899</b>	<b>cg03695421</b>	<b>cg03706273</b>
<b>GSM1051525</b>	0.2214331	0.4758258	0.2077242	0.2091974	0.1299826
<b>GSM1051526</b>	0.2171221	0.5444690	0.1844462	0.1937732	0.0985327
<b>GSM1051527</b>	0.1850495	0.5370600	0.3931231	0.2680030	0.0402481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

```
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
```

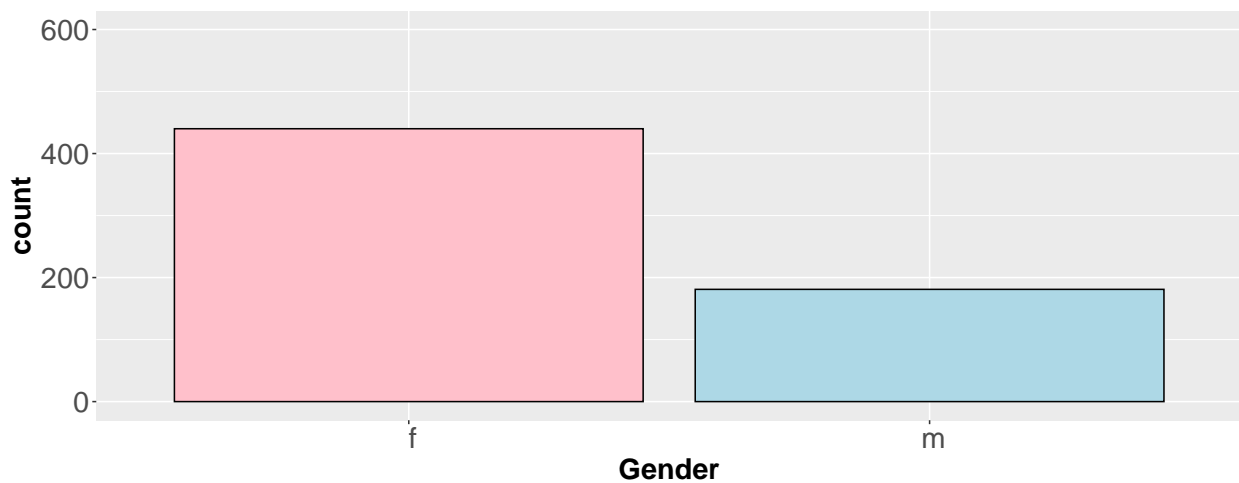
Below there are some histograms which visualizes the distribution of smoking status, age and gender.

```
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"))
```



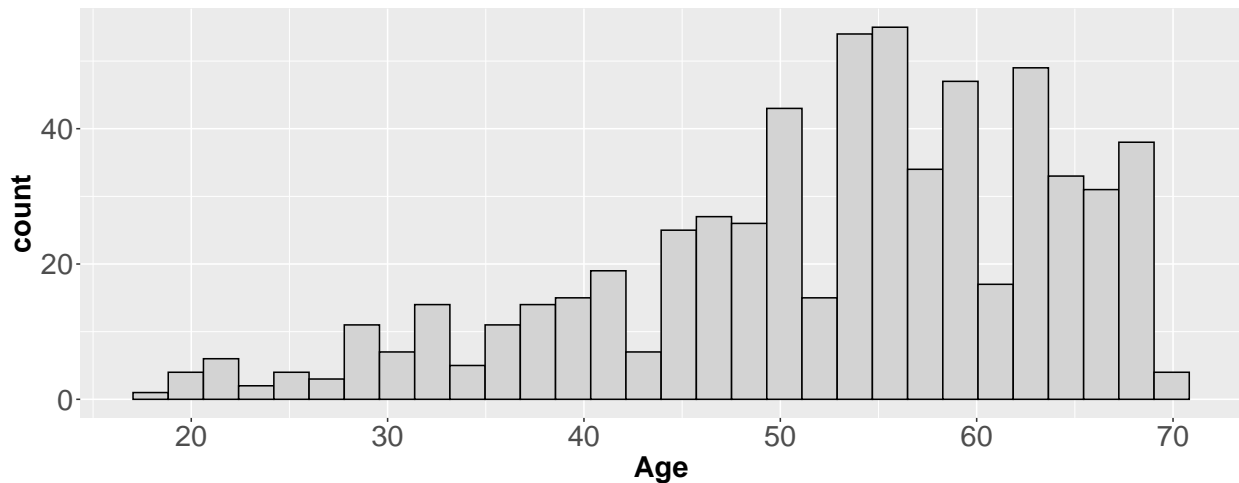
*Figure 1: Number of people who are smoking*

```
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"))
```

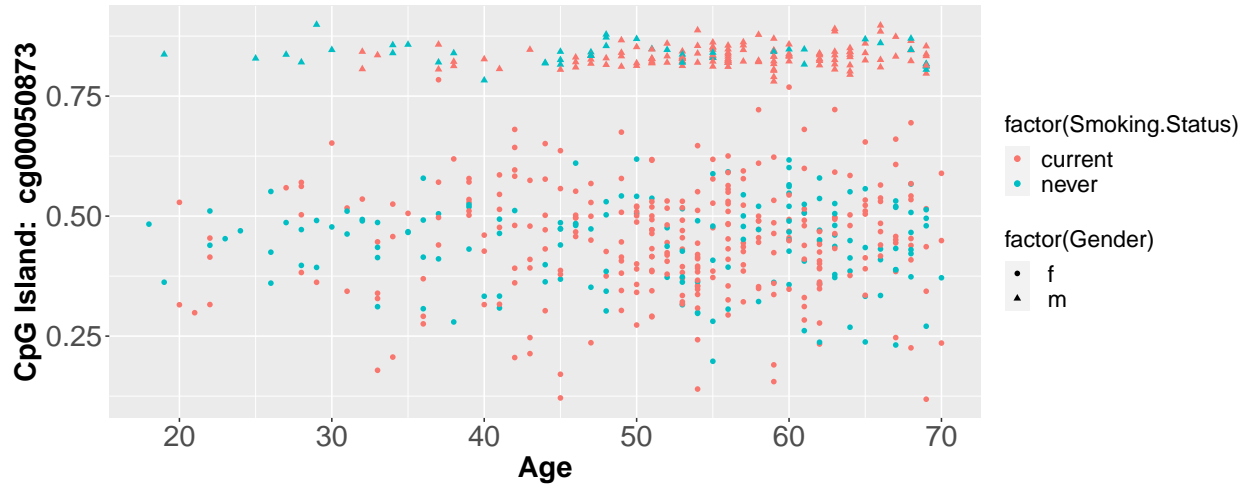


*Figure 3: Age distribution*

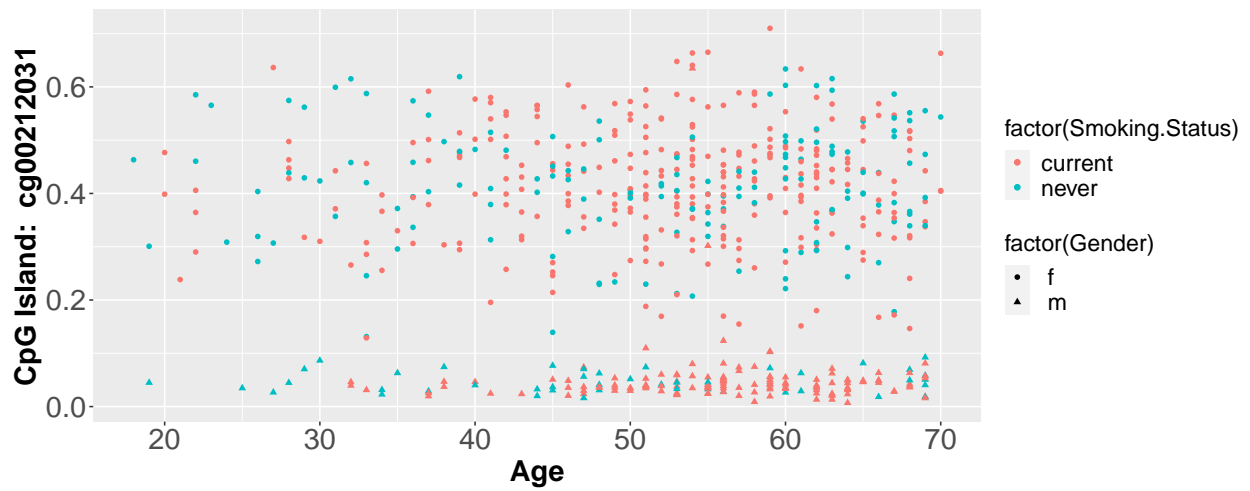
## Plotting the data

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, because 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.

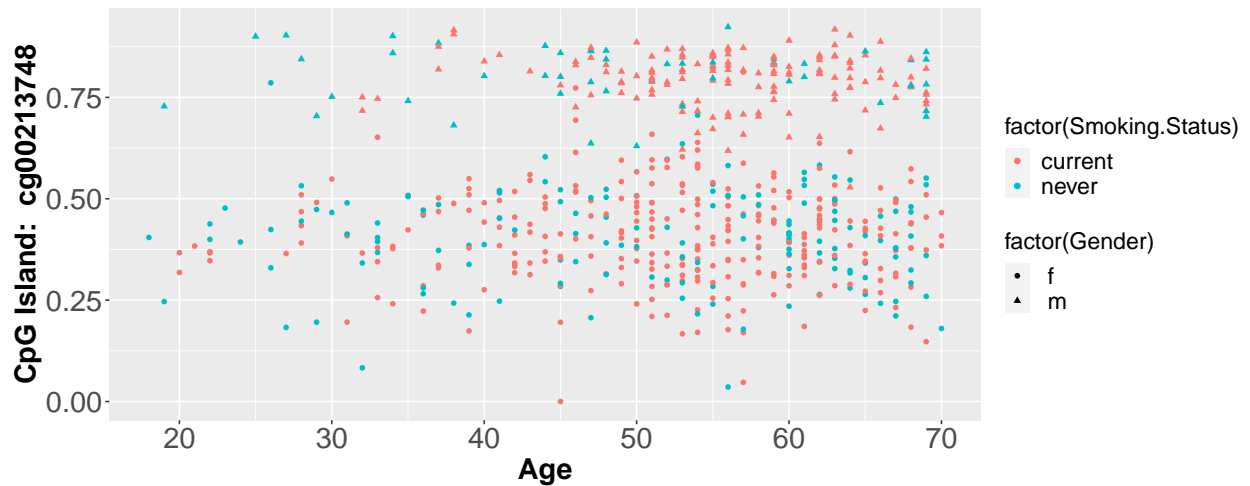
```
plotfunction <- function(cg){
  ggplot(data = myData, mapping = aes_string(x = "Age", y = cg)) +
    geom_point(aes(shape = factor(Gender), color = factor(Smoking.Status))) +
    labs(caption = paste(cg, ": Scatterplot visualizing the methylation rate on this CpG island of diff
    theme(plot.caption = 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.*

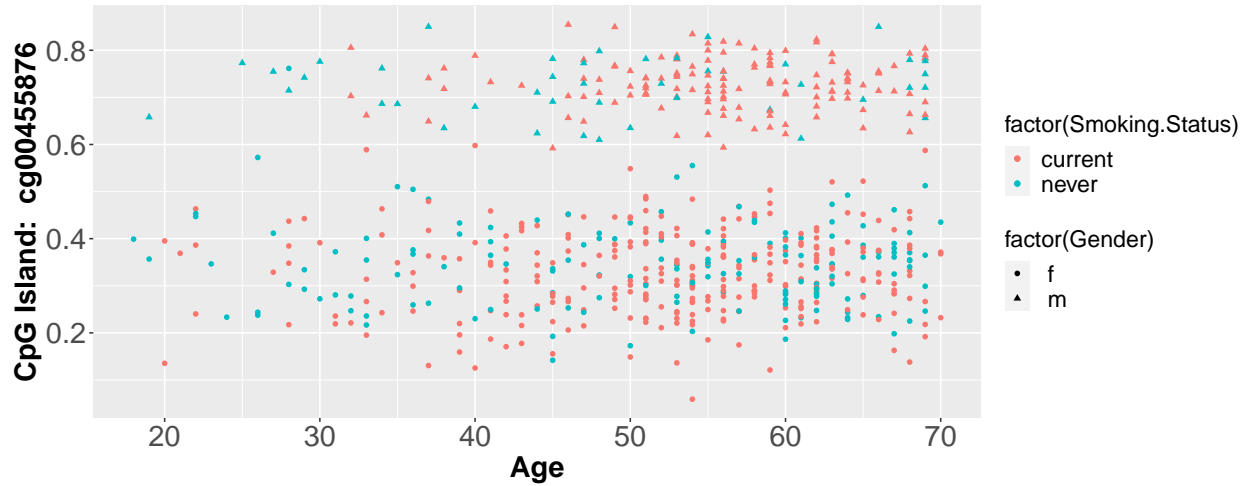


*cg00212031 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.*

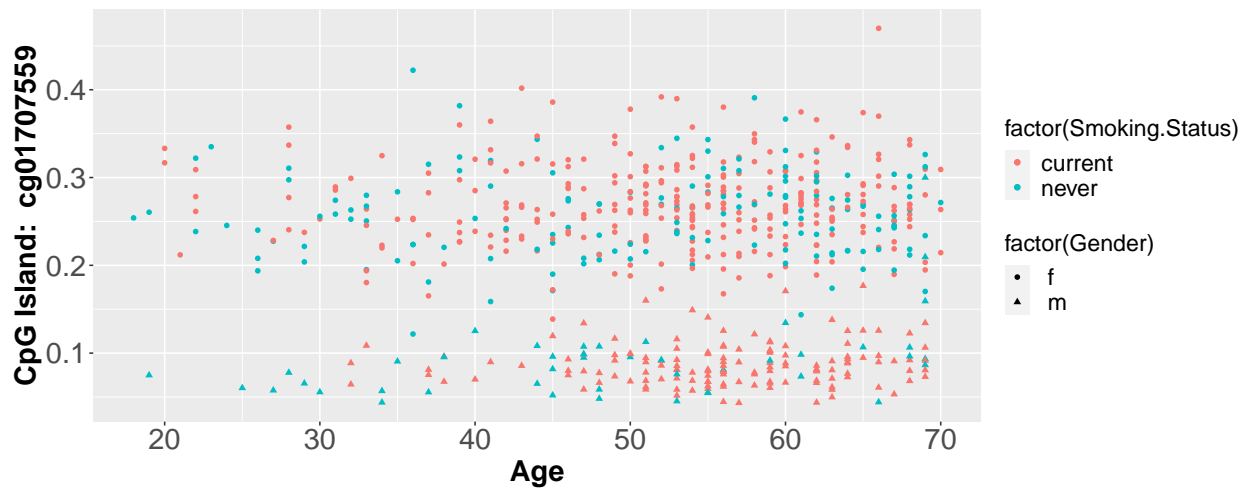


*cg00213748 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.*

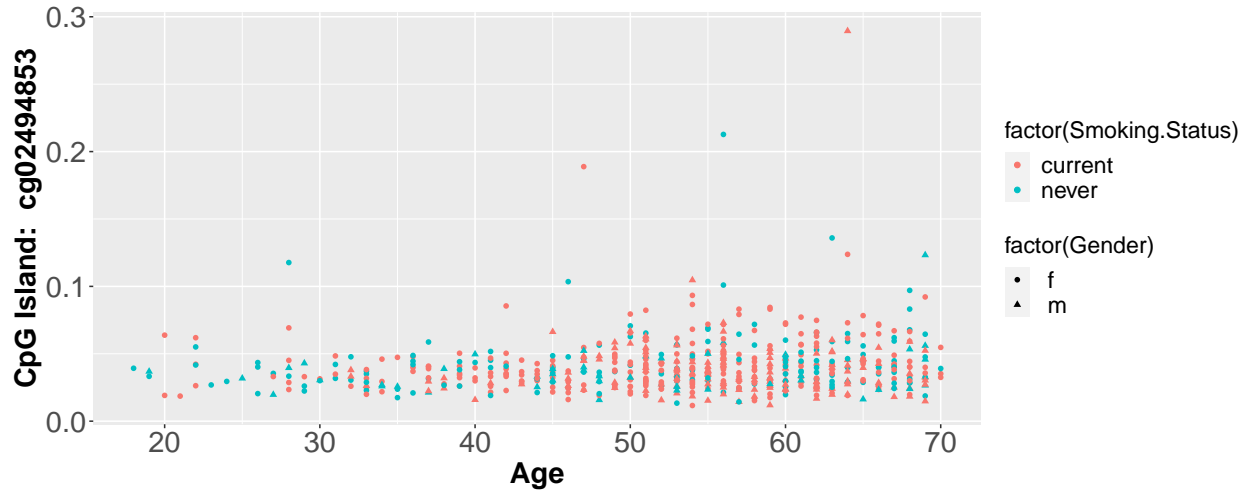




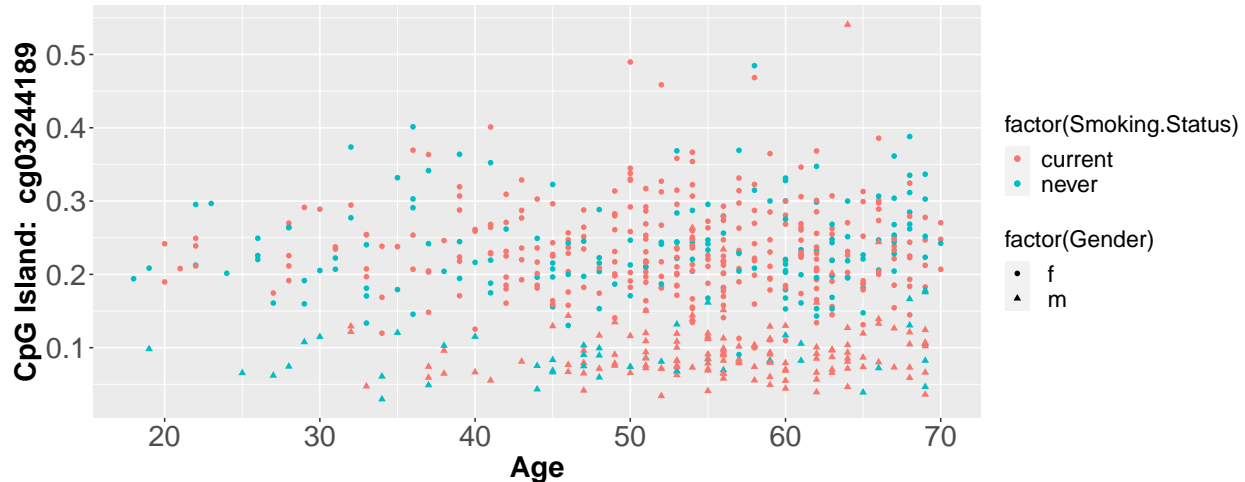
*cg00455876 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.*



*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.*

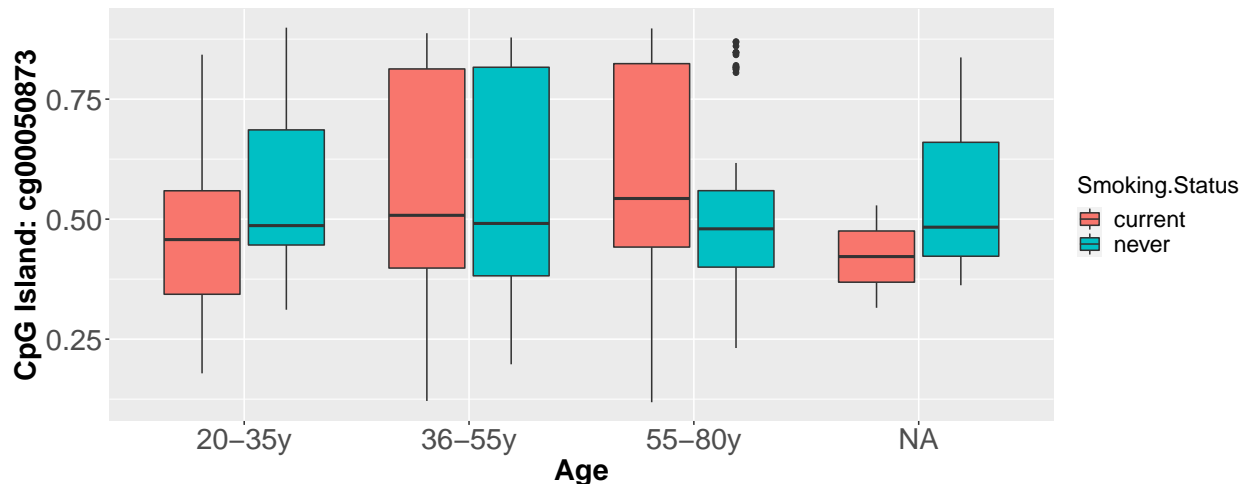


cg03244189 : Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.

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 of different ages and genders.")) +
  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")
```



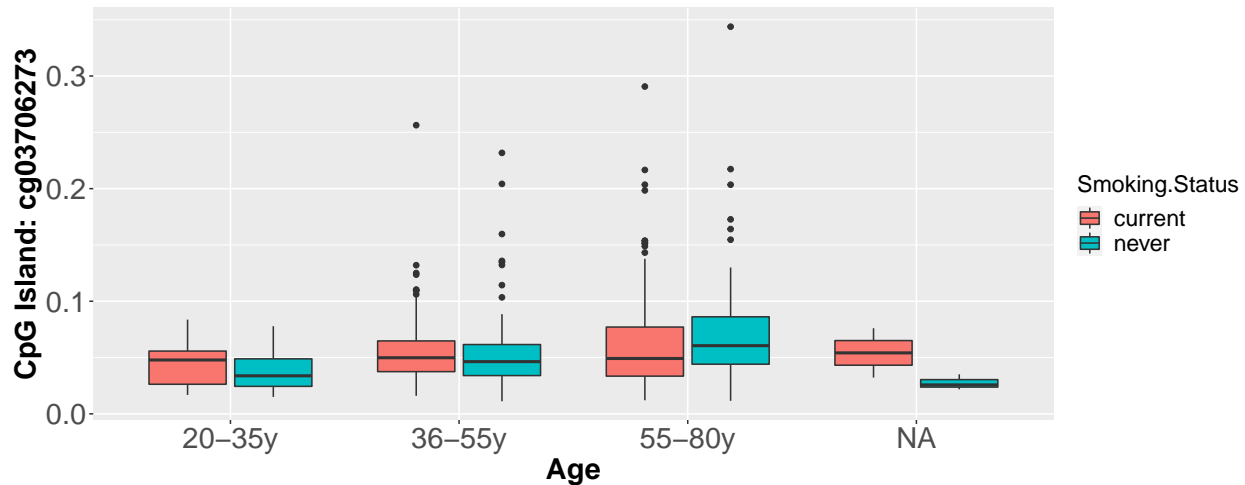
cg00050873: Scatterplot visualizing the methylation rate on this CpG island of different ages and genders.

```
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 of different ages and genders."))
```

```

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.*

## Results

These PCA plots show quite the same thing as previous graphs. But there is a slight difference in smoking status where the current smoking group is slightly to the left. This can also be, because there is a huge different in the number of current smokers and non-smokers. In the age graph, all males are left and all females are right with no exceptions. The graph is a bit scaled to make it more readable.

```

## Importance of components:
##               PC1      PC2      PC3      PC4      PC5      PC6      PC7
## Standard deviation  0.7601 0.14718 0.10089 0.09257 0.09146 0.08919 0.08813
## Proportion of Variance 0.8360 0.03135 0.01473 0.01240 0.01210 0.01151 0.01124
## Cumulative Proportion 0.8360 0.86739 0.88212 0.89452 0.90662 0.91813 0.92937
##               PC8      PC9      PC10     PC11     PC12     PC13     PC14
## Standard deviation  0.08618 0.08128 0.07387 0.07271 0.07201 0.06779 0.06360
## Proportion of Variance 0.01075 0.00956 0.00790 0.00765 0.00750 0.00665 0.00585
## Cumulative Proportion 0.94012 0.94968 0.95758 0.96523 0.97273 0.97938 0.98524
##               PC15     PC16     PC17     PC18     PC19     PC20
## Standard deviation  0.05677 0.04934 0.04035 0.03801 0.03522 0.01522
## Proportion of Variance 0.00466 0.00352 0.00236 0.00209 0.00179 0.00034
## Cumulative Proportion 0.98990 0.99342 0.99578 0.99787 0.99966 1.00000

```



## Conclusion and Discussion

The data shows that there are significant differences in methylation on most CpG islands between males and females. Underlying relations between smoking or age are not significant enough. However the smoking data shows a slight difference between current and never smokers in the PCA plot. There are no weird outliers or other weird points in the data. The data is not suitable enough to partition in classes, because age isn't really influencing the methylation rate. Even when the data is factored, there is no significant pattern in the plot. So there is probably no relation between age and methylation rate. So to refer to the research question: Using the methylation rate of certain CpG islands, you can say that it is independant from age and somewhat dependant on smoking status and extremely dependant on gender.