# CpG methylation data analysis results

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# Introduction

DNA methylation is a process plays a part in gene regulation. Methylation involves the addition or removal of methyl groups to or from the bases and sugars in DNA. methylation occurs on the cytosine of CG locations in the DNA. methylatable CG sequences also called CpG sequences are concentrated in CpG-rich regions called CpG islands, located at the 5'ends of genes. Methylation prevents the formation of certain base pairs, and thereby the accessibility for interactions with other components. methylation adds a hydrophobic character to some of the tRNA regions. that may be important for their interaction with proteins that regulate synthesis. (Klug, William S.;Cummings, Michael R.;Spencer, Charlotte A.;Michael A. Palladino - Concepts of genetics) (Tymoczko, J. L., Berg, J. M. & Stryer, L. (2015, 1 januari). Biochemistry: A Short Course. Macmillan Publishers.)

## Relavance of the project

Because alterations in the genome and activity of the genes are associated with common diseases such as cancer or asthma, it is important to know if smoking can be the cause of this. If this is the case than in further research there can be looked at which CpG sites are affected by smoking, and if there is a relation with the affected CpG sites and the ones associated with diseases like cancer and asthma. (Klug, William S.;Cummings, Michael R.;Spencer, Charlotte A.;Michael A. Palladino - Concepts of genetics)

### 1.1 Goal

Can CpG methylation show a relation with smoking, based on a prediction whether a patient is smoking or non-smoking using differences in CpG site values.

## Data explanation

For this we use the dataset of 683 patients. the dataset has 683 rows and 24 columns containing the patients; age, gender, smoking status and 20 CpG site values. The dataset was already compressed to 20 CpG site values sites per sample. The original dataset was composed for a study titled "Differential DNA methylation in Rheumatoid arthritis" (NCBI series GSE42861) Where the original dataset contained 485577 rows with methylation data per sample (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42861) (https://www.kaggle.com/datasets/thomaskonstantin/cpg-values-of-smoking-and-non-smoking-patients)

# Loading the data

Table 1: An overview from the fist lines of the data (continued below)

| GSM | Smoking.Status | Gender | Age | cg00050873 | cg00212031 |
|---|---|---|---|---|---|
| GSM1051525 | current | f | 67 | 0.6076 | 0.4228 |
| GSM1051526 | current | f | 49 | 0.3451 | 0.5687 |
| GSM1051527 | current | f | 53 | 0.3213 | 0.3609 |
| GSM1051528 | current | f | 62 | 0.2773 | 0.3044 |
| GSM1051529 | never | f | 33 | 0.4136 | 0.1313 |
| GSM1051530 | current | f | 59 | 0.6229 | 0.5017 |

Table 2: Table continues below

| cg00213748 | cg00214611 | cg00455876 | cg01707559 | cg02004872 | cg02011394 |
|---|---|---|---|---|---|
| 0.3725 | 0.6216 | 0.2908 | 0.2671 | 0.1791 | 0.4803 |
| 0.5006 | 0.4986 | 0.3746 | 0.1903 | 0.156 | 0.4181 |
| 0.3527 | 0.3738 | 0.2307 | 0.3147 | 0.1057 | 0.6151 |
| 0.4752 | 0.4863 | 0.2952 | 0.2958 | 0.1113 | 0.301 |
| 0.3675 | 0.7612 | 0.2358 | 0.2505 | 0.1691 | 0.393 |
| 0.2632 | 0.4157 | 0.4752 | 0.2539 | 0.2608 | 0.5098 |

Table 3: Table continues below

| cg02050847 | cg02233190 | cg02494853 | cg02839557 | cg02842889 | cg03052502 |
|---|---|---|---|---|---|
| 0.3276 | 0.2411 | 0.06707 | 0.247 | 0.4692 | 0.4002 |
| 0.3465 | 0.1755 | 0.04694 | 0.2367 | 0.3075 | 0.377 |
| 0.2375 | 0.2464 | 0.03824 | 0.2446 | 0.3578 | 0.305 |
| 0.3045 | 0.177 | 0.02672 | 0.001641 | 0.4457 | 0.2715 |
| 0.3062 | 0.3017 | 0.03702 | 0.3343 | 0.395 | 0.3266 |
| 0.4052 | 0.3853 | 0.02583 | 0.3092 | 0.3219 | 0.5334 |

| cg03155755 | cg03244189 | cg03443143 | cg03683899 | cg03695421 | cg03706273 |
|---|---|---|---|---|---|
| 0.415 | 0.2214 | 0.4758 | 0.2077 | 0.2092 | 0.13 |
| 0.3974 | 0.2171 | 0.5445 | 0.1844 | 0.1938 | 0.09853 |
| 0.5213 | 0.185 | 0.5371 | 0.3931 | 0.268 | 0.04025 |
| 0.4345 | 0.1654 | 0.5079 | 0.2812 | 0.2179 | 0.1015 |
| 0.4301 | 0.1811 | 0.4055 | 0.3108 | 0.2801 | 0.07786 |
| 0.5716 | 0.211 | 0.3778 | 0.4694 | 0.3433 | 0.04578 |

The first four rows are information about the patient and the remaining 20 are genetic information about the CpG sites of the patients. The values show a percentage of methylation in these rows. We deleted the GMS column because is hold unique row identifiers. Fitting a tree with unique row identifiers in a dataset will split every single row in one node, which will give you a high predictive value. > This will cause overfitting.

Table 5: Summary with basic statistics about the data colums (continued below)

| GSM | Smoking.Status | Gender | Age |
|---|---|---|---|
| Length:683 | Length:683 | Length:683 | Min. :18.00 |
| Class :character | Class :character | Class :character | 1st Qu.:47.00 |
| Mode :character | Mode :character | Mode :character | Median :56.00 |
| NA | NA | NA | Mean :53.82 |
| NA | NA | NA | 3rd Qu.:62.00 |
| NA | NA | NA | Max. :80.00 |
| NA | NA | NA | NA |

Table 6: Table continues below

| cg00050873 | cg00212031 | cg00213748 | cg00214611 |
|---|---|---|---|
| Min. :0.1186 | Min. :0.00695 | Min. :0.0000 | Min. :0.01247 |
| 1st Qu.:0.4131 | 1st Qu.:0.06317 | 1st Qu.:0.3635 | 1st Qu.:0.06946 |
| Median :0.5052 | Median :0.36554 | Median :0.4713 | Median :0.41575 |
| Mean :0.5600 | Mean :0.30960 | Mean :0.5191 | Mean :0.34106 |
| 3rd Qu.:0.8144 | 3rd Qu.:0.45981 | 3rd Qu.:0.7278 | 3rd Qu.:0.49745 |
| Max. :0.8989 | Max. :0.70999 | Max. :0.9236 | Max. :0.80606 |
| NA's :62 | NA's :62 | NA's :62 | NA's :62 |

Table 7: Table continues below

| cg00455876 | cg01707559 | cg02004872 | cg02011394 |
|---|---|---|---|
| Min. :0.05917 | Min. :0.04333 | Min. :0.00262 | Min. :0.0000 |
| 1st Qu.:0.29300 | 1st Qu.:0.11080 | 1st Qu.:0.04284 | 1st Qu.:0.4261 |
| Median :0.37968 | Median :0.23873 | Median :0.14933 | Median :0.5157 |
| Mean :0.44718 | Mean :0.21435 | Mean :0.15542 | Mean :0.6058 |
| 3rd Qu.:0.66283 | 3rd Qu.:0.28061 | 3rd Qu.:0.24263 | 3rd Qu.:0.9412 |
| Max. :0.85443 | Max. :0.46999 | Max. :0.47384 | Max. :0.9792 |
| NA's :62 | NA's :62 | NA's :62 | NA's :62 |

Table 8: Table continues below

| cg02050847 | cg02233190 | cg02494853 | cg02839557 |
|---|---|---|---|
| Min. :0.05234 | Min. :0.00863 | Min. :0.01162 | Min. :0.00000 |
| 1st Qu.:0.33963 | 1st Qu.:0.08838 | 1st Qu.:0.02865 | 1st Qu.:0.06384 |
| Median :0.42754 | Median :0.25982 | Median :0.03695 | Median :0.35042 |
| Mean :0.54369 | Mean :0.23250 | Mean :0.04077 | Mean :0.30088 |
| 3rd Qu.:0.95558 | 3rd Qu.:0.33702 | 3rd Qu.:0.04677 | 3rd Qu.:0.45786 |
| Max. :0.98320 | Max. :0.51173 | Max. :0.28947 | Max. :0.82739 |
| NA's :62 | NA's :62 | NA's :62 | NA's :62 |

Table 9: Table continues below

| cg02842889 | cg03052502 | cg03155755 | cg03244189 | cg03443143 | cg03683899 | cg03695421 | cg03706273 |
|---|---|---|---|---|---|---|---|
| Min. :0.01346 | Min. :0.0000 | Min. :0.2020 | Min. :0.02972 | Min. :0.06496 | Min. :0.00788 | Min. :0.0949 | Min. :0.01120 |
| 1st Qu.:0.05483 | 1st Qu.:0.4025 | 1st Qu.:0.4245 | 1st Qu.:0.11976 | 1st Qu.:0.40963 | 1st Qu.:0.06159 | 1st Qu.:0.2566 | 1st Qu.:0.03413 |
| Median :0.39757 | Median :0.4940 | Median :0.4962 | Median :0.20397 | Median :0.48314 | Median :0.34422 | Median :0.3208 | Median :0.04961 |
| Mean :0.32362 | Mean :0.5907 | Mean :0.5895 | Mean :0.19552 | Mean :0.56841 | Mean :0.28442 | Mean :0.3978 | Mean :0.05769 |
| 3rd Qu.:0.47385 | 3rd Qu.:0.9631 | 3rd Qu.:0.8988 | 3rd Qu.:0.24921 | 3rd Qu.:0.85436 | 3rd Qu.:0.41866 | 3rd Qu.:0.5965 | 3rd Qu.:0.06916 |
| Max. :0.85625 | Max. :0.9902 | Max. :0.9696 | Max. :0.54074 | Max. :0.93589 | Max. :0.65925 | Max. :0.8433 | Max. :0.34380 |
| NA's :62 | NA's :62 | NA's :62 | NA's :62 | NA's :62 | NA's :62 | NA's :62 | NA's :62 |

When we look at the summary of the patient data we see in the column of the methylation data that there are 62 missing values (NA's). These are the same rows where the gender column has a capital F for female and M for male. these rows will be deleted because it gives us no information for the CpG sites on the genetic attributes.

# Data exploratory analysis

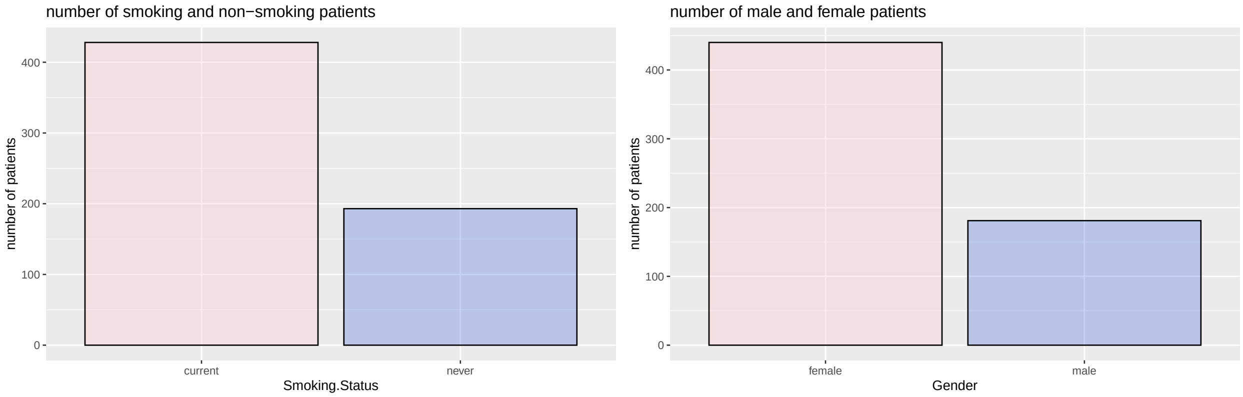


Figure 1: Comparison distribution smoking status and gender

In the figure above we see a distribution overview of the gender and smoking status of the patients. We see a high number of patients are female and the majority is smoking. We will not remove data to get an even distribution of the data, but we do need to keep this in mind when using data mining.

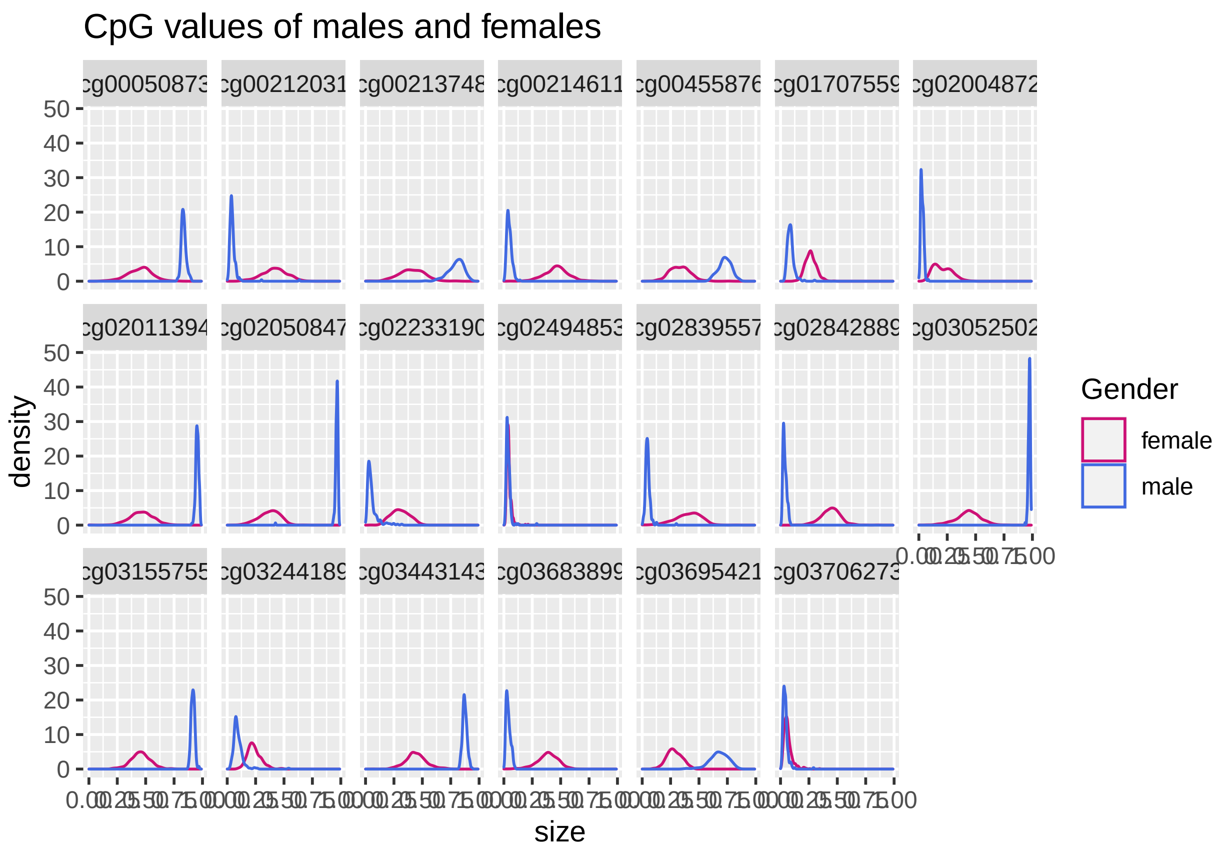


Figure 2: CpG values in percentages comparison of males and females

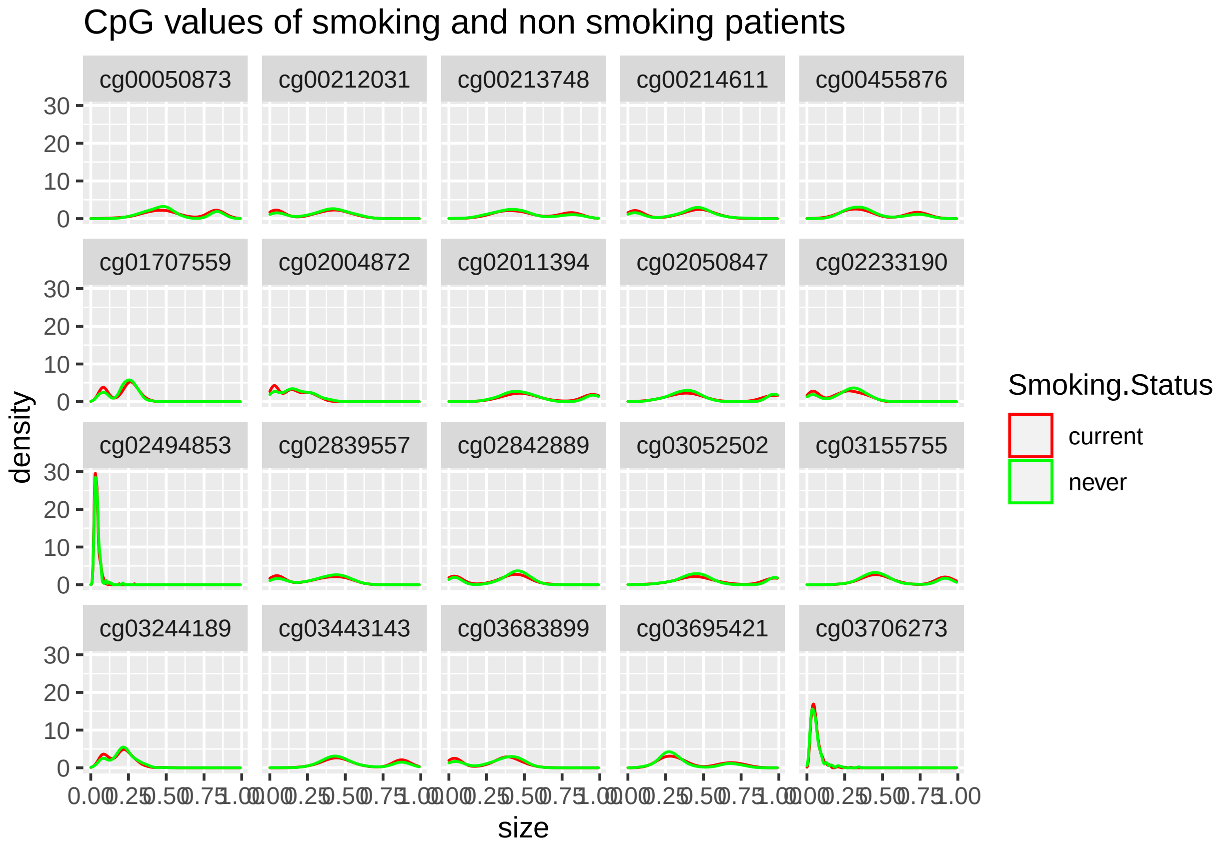
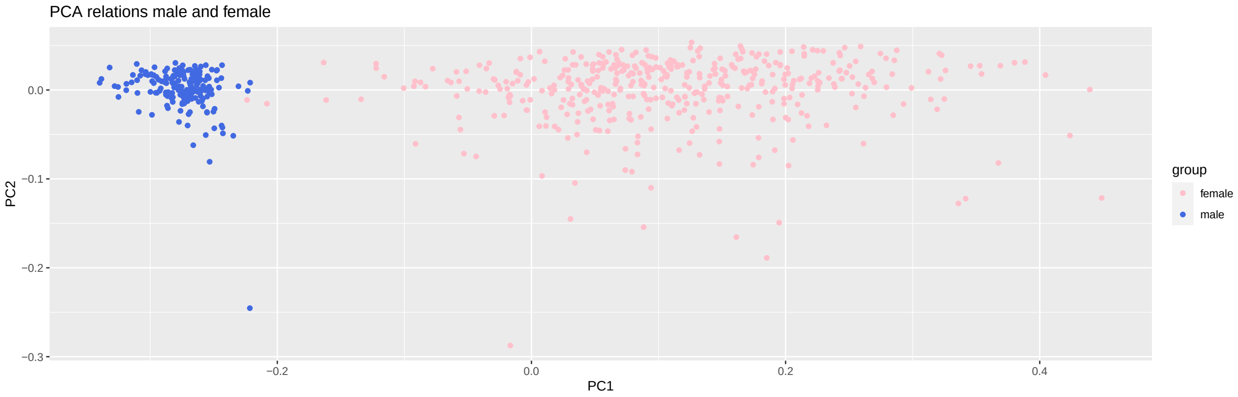
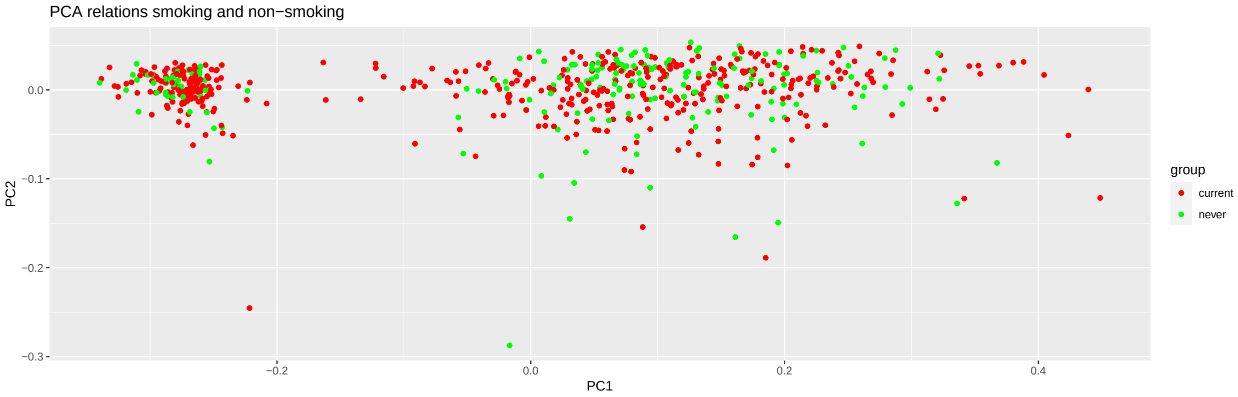


Figure 3: CpG values in percentages comparison of smoking and non smoking patients

As you can see in figure 2, the male and female CpG values are very different from each other. the green and red curve are on top of each other which means that there is not much difference in the CpG values for smoking and non smoking patients. This gives us already a lot of information for the research goal. The bimodal double curve in this graph stands for male and female, we observed that in figure 2 where we compared the males and female.





A principal component analysis gives an extra confirmation of the underlying groups that we saw in the CpG value differences. The PCA aims to show pattern in multivariate data. In figure 4 and 5 we see two groups that show a relation in the data. There is a clear difference in males and females. The smokers and non smokers are distributed in both these groups.

## Discussion

Male and female methylation is shown to be different, we cant go deeper in detail in explaining these differences because we do not know the associated genes to the CpG locations in our dataset

## Conclusion

The goal was to understand the given dataset and to clean the data. it is yet to discover if CpG methylation can show a relation with smoking, based on a prediction whether a patient is smoking or non-smoking using differences in CpG site values. The data shows good prospect for the use of machine learning because of the the patterns and correlation that is found in the data. We already saw that there is a difference in CpG values for gender. Smoking status had not yet shown a clear relation with the CpG values. This will be researched further using machine learning.