



COSC681 - AI Project

Classifying smoking history with epigenetic-trained machine
learning

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Abstract

Contents

| | | |
|----------|---|-----------|
| 1 | Introduction | 1 |
| 1.1 | Tobacco Related Health Issues | 1 |
| 1.2 | Self-Reported Smoking Status | 1 |
| 1.3 | Epigenetics | 2 |
| 1.3.1 | DNA Methylation | 2 |
| 1.3.2 | DNAm Platforms | 3 |
| 1.4 | Machine Learning in Epigenetics | 3 |
| 1.4.1 | Applications | 4 |
| 1.5 | Smoking Algorithms | 4 |
| 1.5.1 | Elastic Net Regression | 5 |
| 1.5.2 | DNAmPACKYRS | 5 |
| 1.5.3 | mCigarette | 6 |
| 1.6 | Aim of This Work | 7 |
| 2 | Method | 7 |
| 2.1 | Algorithm | 7 |
| 2.2 | Datasets | 8 |
| 2.3 | Hardware and Software | 9 |
| 2.4 | Pre-Processing | 9 |
| 2.5 | Training | 9 |
| 3 | Results | 9 |
| 3.1 | Model Performance | 9 |
| 3.2 | External Validation Cohort | 15 |
| 3.2.1 | Model Performance | 15 |
| 3.2.2 | Comparison with Prior Results | 15 |
| 4 | Footnotes | 18 |
| 4.1 | Acknowledgements | 18 |
| 4.2 | Ethics Statement | 18 |
| | References | 18 |

List of Figures

| | | |
|---|---|----|
| 1 | Confusion Matrices (Cohort 1) | 10 |
|---|---|----|

| | | |
|----|---|----|
| 2 | Never smoker vs current smoker sub-classifiers performance (Cohort 1) | 10 |
| 3 | Never smoker vs ex smoker sub-classifiers performance (Cohort 1) | 11 |
| 4 | Ex smoker vs current smoker sub-classifiers performance (Cohort 1) | 11 |
| 5 | Class separation of classifier (Cohort 1) | 12 |
| 6 | Boxplots of probability distributions (Cohort 1) | 13 |
| 7 | Confusion Matrices (Cohort 2) | 13 |
| 8 | Never smoker vs current smoker sub-classifiers performance (Cohort 2) | 14 |
| 9 | Never smoker vs ex smoker sub-classifiers performance (Cohort 2) | 14 |
| 10 | Ex smoker vs current smoker sub-classifiers performance (Cohort 2) | 15 |
| 11 | Class separation of classifier (Cohort 2) | 16 |
| 12 | Boxplots of probability distributions (Cohort 2) | 17 |

List of Tables

1 Introduction

1.1 Tobacco Related Health Issues

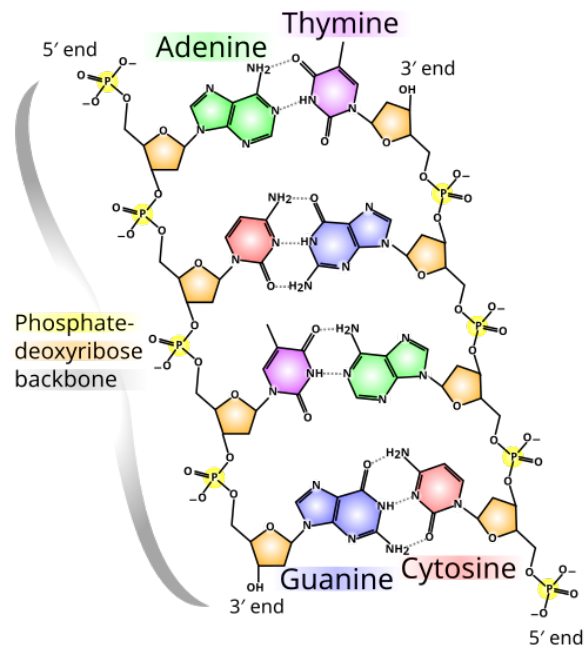
The harms associated with tobacco use are well recognised. Tobacco kills up to half its users who do not quit and more than 8 million people per year, including an estimated 1.3 million non-smokers due to second hand smoke [1]. Smoking causes cancer, heart and lung disease, stroke, type 2 diabetes, and harmful reproductive effects [2]. There is a growing body of evidence suggesting a causal relationship between smoking and mental health issues [3]. Clearly, such negative impacts on patient health due to tobacco use are undesirable, just as they are avoidable. For these reason, tobacco usage is of great concern to health professionals. The World Health Organization asserts that surveillance is key for addressing the tobacco epidemic, as tracking tobacco usage indicates how to shape policy [1].

1.2 Self-Reported Smoking Status

Current surveillance relies on self-reported smoking data. That is, a patient's smoking history is recorded by them personally recalling and reporting. It is a convenient and cost-effective way of collecting smoking statistics. There are two main types of smoking data used to measure tobacco exposure: smoking status and smoking pack-years. Smoking status is label based on the history and habits of tobacco use. Individuals are binned into never-smokers, ex-smokers and current smokers. Smoking pack-years is a calculated score that tries to quantify tobacco use. It is calculated as the number of packs of cigarettes smoked per day multiplied by years of smoking [4]. For example, one pack-year is one pack per day for one year, or half a pack per day for two years. Therefore, smoking pack-years quantifies both the degree of exposure and duration of exposure equally.

Self-reported smoking data has several limitations. Relying on individuals recounting information can introduce bias. Self-reported smoking data is prone to inaccuracy due to stigma, recall bias and a lack of information on second-hand exposure [5, 6]. That is, the social pressure to deny partaking in stigmatised behaviours, forgetting details and information, and not being aware of sources of second-hand exposure can all influence the results of self-reported smoking data. A method of using objective evidence to determine smoking history could overcome these issues. On the other hand, the inaccuracy of self-reported smoking data can differ between population groups. For example, studies suggest that teens are more likely to provide false responses in smoking surveys [5]. Moreover, tobacco consumption differs between social groups, with smoking more prevalent in low-education and low-socio-economic groups [7].

To this end, developing diagnostic tests to collect smoking data that do not share the biases of self-reported methods are of interest for improving the monitoring of health. One such approach is the use of epigenetic biomarkers.



Chemical structure of DNA [8]

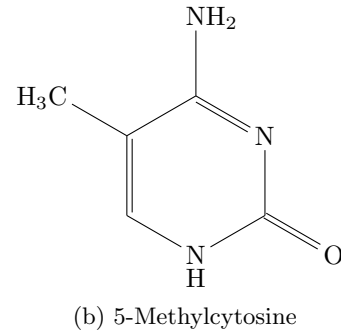
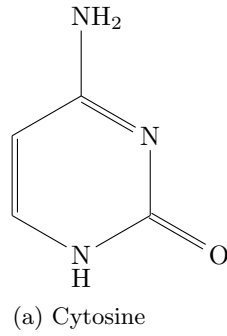
1.3 Epigenetics

Epi- is a Greek prefix meaning upon or on. Therefore, epigenetics is the study of factors on top of or upon genetics. Specifically, it is the study of how environmental factors and behaviours affect, modify and regulate your genetics and their expression. We consider one type of epigenetic modification: DNA methylation.

1.3.1 DNA Methylation

At a high level, DNA is a sequence of letters that provide genetic instructions. Like a human reading a book, strings of these letters are converted into information that tells cells how to function. More precisely, these letters are one of four nucleotide bases: adenine (A), cytosine (C), guanine (G) and thymine (T). To form the sequence, these bases are attached to a deoxyribose sugar and connected by a phosphate molecule, called the sugar-phosphate backbone. Of relevance is the phosphate molecule, specifically for when a cytosine is directly followed by a guanine in the sequence. A phosphate bonding a cytosine and a guanine (called a CpG site) creates a chemical structure which allows methyl groups to attach. This process is an epigenetic modification called DNA methylation (DNAm). As a biomarker, we measure methylation at a CpG site as a float between 0 and 1, measuring the percentage of methylation at that site.

While the genetic sequence of DNA is stable, methylation is not. It is a dynamic state that depends on factors such as behaviours and environmental exposure (**add citation?**). Exposure to such factors



Modification of Cytosine into 5-Methylcytosine caused by DNA methylation

increases methylation of CpG sites, while sufficient lack of exposure causes methylation to decrease over time (**add citation?**). As previously mentioned, DNA methylation affects the expression of genes. Methylation at a CpG site can silence the expression of the gene that site is located in (**add citation?**), where more methylation at a site leads to stronger silencing. Moreover, DNA methylation is not random. There is strong correlation between methylation of specific sites with specific factors (**add citation?**). This means that DNA methylation of CpG sites can be used as a biomarker indicative of the factors that caused it, while also describing changes in cellular function. Therefore, DNA methylation is a biomarker not only useful for reporting on environmental exposures, but also predicting future health outcomes or risks. Examples of this include prediction of cardiovascular diseases [9], neurological diseases [10], type 2 diabetes [11], pace of aging [12], and cancer [13]. Furthermore, DNA methylation is not self-reported, and therefore overcomes the biases associated with self-reported data.

Altogether, this motivates the use of DNA methylation data to develop methods for collecting smoking history of individuals. Work using such data is referred to as an epigenome-wide association study (EWAS).

1.3.2 DNAm Platforms

The human genome contains ~ 28 million CpG sites. This is often a computationally infeasible domain for a dataset, due to massive dataset sizes, processing requirements, and noise contained in the signal. Instead, most EWAS use a biologically relevant and informative subset of CpG sites. There are two commonly chosen platforms used to achieve this: Illumina 450k [14] and Illumina EPIC [15]. 450k was the first platform developed by Illumina, consisting of 485,577 CpG sites chosen for their quality and usefulness. The EPIC platform was developed as a successor, increasing to 865,859 CpG sites. However, only around $> 90\%$ of the sites were retained from 450k.

1.4 Machine Learning in Epigenetics

Broadly, machine learning algorithms are split into one of two tasks: regression or classification. The most significant distinction between these two tasks is the choice of supervised learning labels. Regression

algorithms are trained against continuous, numeric scores, while classification algorithms are trained against discrete class labels. The choice of label in turn determines the output produced by the algorithm. Machine learning has already seen use in many areas of clinical epigenetics. We begin with a review of some developed methods, addressing both classification and regression tasks.

1.4.1 Applications

1. Malta et al. [16] proposed a method for assessing oncogenic dedifferentiation (cells becoming cancerous). This approach seeks to model a "stemness index" which indicates how similar a cell is to stem cell - a trait found in cancerous cells. Of relevance is the developed epigenetic approach using one-class logistic regression. The training features consisted of 219 hypermethylated CpG sites associated with stem cells. Training data only consisted of a single, positive, class: stem cells. The resulting model can then be fed non-stem cells to compare how similar they are to stem cells, i.e. cancerous cells.
2. Adorján et al. [17] proposed a method for using DNA methylation to classify cancer tissues. CpG sites were ranked using a two sample t-test, and then fed into a support vector machine. Models were evaluated using the average of 50 runs of 8-fold cross-validation. The top two CpG sites could classify leukaemia from healthy cells with 84% accuracy, while the top 60 sites achieve 94% accuracy.
3. Dogan et al. [18] proposed a method for integrated genetic and epigenetic classification of coronary heart disease. The training dataset consisted of 1,545 individuals. An approach combining undersampling and ensemble learning [19] was used to address class imbalance, creating 8 training sub-datasets. Point biserial correlation and Pearson correlation were used for feature selection, resulting in 107,799 CpG sites for training. These features were ranked using ROC AUC. Random Forest classifiers were then trained on the 8 training sub-datasets, with majority voting used for ensembling. Hyperparameters were tuned using 10-fold cross-validation. The final model used 4 CpG sites, two genetic variables, age and sex. This achieved an accuracy, sensitivity and specificity of 78%, 0.75 and 0.80, respectively.

1.5 Smoking Algorithms

In the context of smoking, the two most significant machine learning epigenetic scores use Elastic Net regression.

1.5.1 Elastic Net Regression

Elastic Net [20] is a regularised form of linear regression that includes two additional penalty terms. Given n examples, p features with inputs $x \in \mathbb{R}^{n \times (p+1)}$ and corresponding ground-truth $y \in \mathbb{R}^n$, we find coefficients $\beta \in \mathbb{R}^{p+1}$ that produces an output:

$$\hat{y} = x\beta \in \mathbb{R}^{p+1}$$

and minimises the function:

$$\mathcal{L}(y, \hat{y}, \beta) = \|y - \hat{y}\|_2^2 + \alpha\lambda\|\beta\|_1 + \alpha\frac{1-\lambda}{2}\|\beta\|_2^2$$

where:

$$\|\beta\|_1 = \sum_{i=1}^p |\beta_i|$$

and:

$$\|\beta\|_2 = \sqrt{\sum_{i=1}^p \beta_i^2}$$

We can see this is simply mean-squared error, with the L_1 -norm and L_2 -norm included as penalisation terms on β . The two hyperparameters α and λ control the strength of regularisation and ratio between L_1 -norm and L_2 -norms, respectively. Elastic Net is a combination of two other modifications to linear regression: lasso [21] and ridge [22] regression. The two mathematical regularisation terms have interpretable effects on the convergence of β . Gradient updates for $\|\beta\|_1$ are uniform for all non-zero values of β_i , which typically results in most β_i set to zero, with some having large values. This is referred to as a sparse solution. Gradient updates for $\|\beta\|_2^2$ are large for large β_i , and small for small β_i , which typically results in all β_i having similar values. This is referred to as shrinkage. Together, this results in Elastic Net models promoting the grouping effect, where strongly related features are all included or excluded together. This grouping effect is particularly useful when $p > n$, as it provides better feature selection than lasso [20]. Because of this, Elastic Net sees wide use in epigenetics, with biological age clocks being one area of particular use [23].

1.5.2 DNAmPACKYRS

Lu et al. [24] proposed DNAmPACKYRS as a DNA methylation based score for calculating pack-years. This score was originally developed as surrogate biomarker for use in the DNAm GrimAge and DNAm GrimAge v2 epigenetic clocks [24, 25]. DNAm GrimAge is a regression model for estimating mortality risk. The DNAm GrimAge score is calculated using covariates sex, age, 7 surrogate biomarkers of plasma proteins, and of relevance, the surrogate biomarker for smoking pack-years: DNAmPACKYRS. Elastic

Net regression was used to train both DNAm GrimAge and the surrogate biomarkers of plasma proteins and pack-years. Training data consisted of 1731 individuals from the Framingham Heart Study dataset [26]. Individuals from this dataset had a mean age of 66 years. 54% of individuals were female, leaving 45% as male. The intersection of sites available on Illumina 450k and Illumina EPIC were chosen as the available CpG sites for training. This was to ensure compatibility and future-proofing with new datasets. A total of 450,161 CpG sites were available. 10-fold cross-validation was used for hyperparameter tuning the regularisation strength of the Elastic Net model. This resulted in the DNAmPACKYRS score which used 172 CpG sites.

As surrogate biomarker for lifespan, DNAmPACKYRS performs better than self-reported pack-years. Firstly, DNAmPACKYRS can be used to predict lifespan in never-smokers [24], whereas self-reported pack-years cannot (all self-reported values would be 0). Additionally, DNAmPACKYRS is a more significant predictor of lifespan than self-reported pack-years. Across 4 out of 5 datasets DNAmPACKYRS had a smaller Cox regression p-values when compared to self-reported pack-years [24].

There are some limitations with the DNAmPACKYRS score. Since DNAmPACKYRS is only evaluated as a surrogate biomarker in the GrimAge paper, there is no reporting on its performance of predicting smoking status. However, this can be done independently (see 3.2). When performed, we can see that while DNAmPACKYRS achieves good predictive performance separating never-smokers from current smokers (ROC AUC = 0.991) and ex-smokers from current smokers (ROC AUC = 0.915), the score is not optimised for separating never-smokers from ex-smokers (ROC AUC = 0.798). Additionally, the choice of self-reported pack-years as a regression label introduces a source of inaccuracy, discussed below (see 1.5.3: mCigarette).

Notes for DNAmPACKYRS/mCigarette:

- dataset/cohort/demographic
- algorithms
- result/stat it produces
- limitations

1.5.3 mCigarette

Chybowska et al. [27] proposed mCigarette as a DNA methylation based score for calculating pack-years. This score was developed to overcome the limitations of self-reported smoking data, as well as the modest performance of separating never-smokers from ex-smokers.

Training data consisted of 17,865 individuals from the Generation Scotland dataset [28], which was developed on the Illumina EPIC platform. Individuals from this dataset had a mean age of 47.6 years. 59.1% of the individuals were female. CpG sites were filtered based on statistical association with

tobacco use at a false discovery rate (FDR) < 0.05 , resulting in 18,760 CpG sites per individual. This filtering was ran in a dataset separate to the training dataset, using the Illumina 450k platform. Elastic Net regression was then used to train the mCigarette score. Hyperparameters were tuned using 10-fold cross-validation, which set $\lambda = 0.012577$ and resulting in a model using 1255 CpG sites.

- (?) seeking to address not being able to differentiate never-smokers and ex-smokers
- 17865 individuals
- filtered CpG sites to FDR < 0.05 ($n = 18760$)
- elastic net regression
- 10-fold cross-validation, $\lambda = 0.012577$
- 1255 CpG sites used in model
- in validation cohort, AUCs of Current vs Never: 0.98, Current vs Former: 0.90, Former vs Never: 0.85
- limitation 2: choice of ground truth (sr pack-years) is potentially confusing for the model

1.6 Aim of This Work

- used smoking status label instead of pack-years
- try improve on classification of never vs ex and ex vs current

2 Method

2.1 Algorithm

We begin by transforming Elastic Net from a regression problem into a classification problem. In the binary classification case, this is a straightforward replacement of the mean-square error term in the loss function with a binary-cross entropy error term, alongside transforming the linear prediction into a probability via the sigmoid function. However, in the multiclass classification case the modification also affects the regularisation terms.

Given n examples, p features, K classes with inputs $x \in \mathbb{R}^{n \times (p+1)}$ and corresponding ground-truth (as one-hot encoded vectors) $y \in \mathbb{R}^{n \times K}$, we find coefficients $\beta \in \mathbb{R}^{K \times (p+1)}$ that produces logits:

$$z = x\beta^T \in \mathbb{R}^{n \times K}$$

we transform this into probabilities with the softmax function:

$$\text{softmax}(t_1, \dots, t_k) = \begin{bmatrix} \frac{\exp(t_1)}{\sum_{j=1}^K \exp(t_j)} \\ \vdots \\ \frac{\exp(t_k)}{\sum_{j=1}^K \exp(t_j)} \end{bmatrix}$$

The loss function then becomes:

$$\mathcal{L}(y, z, \beta) = -\frac{1}{n} \sum_{i=1}^n y_i \cdot \log(\text{softmax}(z_i)) + \alpha \sum_{k=1}^K (\lambda \|\beta_k\|_1 + (1 - \lambda) \|\beta_k\|_2^2)$$

where the L_1 -norm and L_2 -norm are as before.

Colloquially, this model can be thought of as training K individual logistic regression sub-classifiers, where each sub-classifier is tasked only with predicting one of the K classes. The softmax function ensures that for each example, the K predicted probabilities will all add to 1. Regularisation terms work as before, but are now per-sub-classifier.

Implementation of multi-class Elastic Net logistic regression is provided by scikit-learn [29], via the `linear_model.LogisticRegression` class.

2.2 Datasets

- Cohort 1: discovery
 - Illumina 450k
 - all males
 - mean age 72
 - 235 never smokers, 599 ex smokers, 109 current smokers
 - 90/10 train/test split
 - * 211 never smokers, 539 ex smokers, 98 current smokers in training split
 - * 24 never smokers, 60 ex smokers, 11 current smokers in test split
 - 449,521 CpG sites
- Cohort 2: validation
 - Illumina EPIC
 - 67% of individuals were male (n=658) (female n=326)
 - mean age 68
 - 403 never smokers, 476 ex smokers, 105 current smokers
 - 449,521 CpG sites (intersection with 450k)

2.3 Hardware and Software

- Apple M2
- Memory (and limitations)
- Python version and package versions (sklearn, numpy, scipy)

2.4 Pre-Processing

- Limiting both datasets to the intersection of 450k and EPIC
- Formatting to csv/transposing for ML
- Kruskal-Wallis feature selection

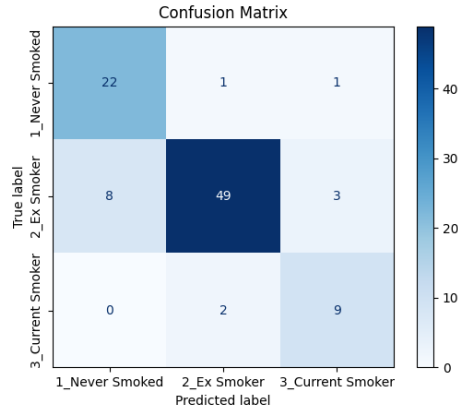
2.5 Training

- Cross Validation
 - 10 folds
 - stratified folds (same proportion of classes in each fold)
 - coarse to fine approach
 - f1-macro scoring (instead of neg log loss)
- choice of optimiser/solver
- choice of loss function
- class weight balancing for gradient updates

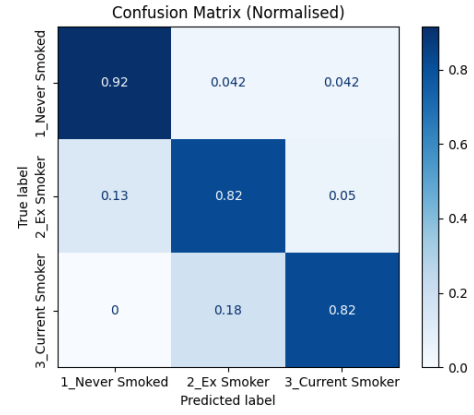
3 Results

3.1 Model Performance

- Confusion Matrices:
 - Good accuracy/tpr across all three classes
-

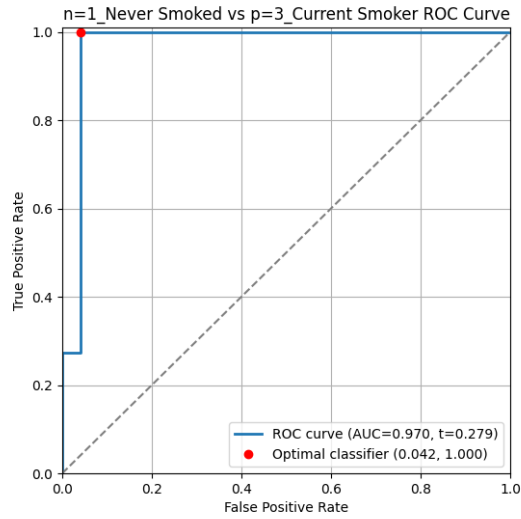


(a) CM

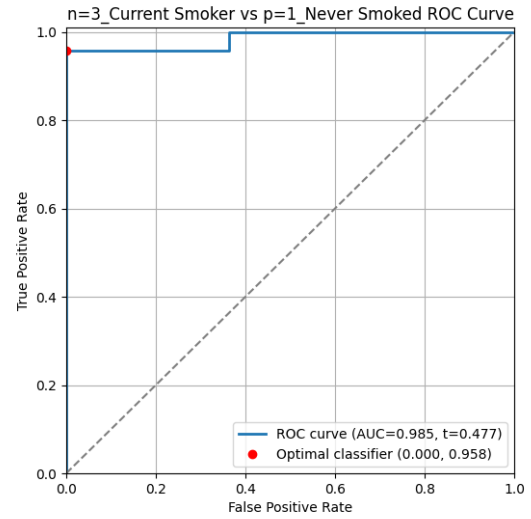


(b) CM Normalised

Figure 1: Confusion Matrices (Cohort 1)

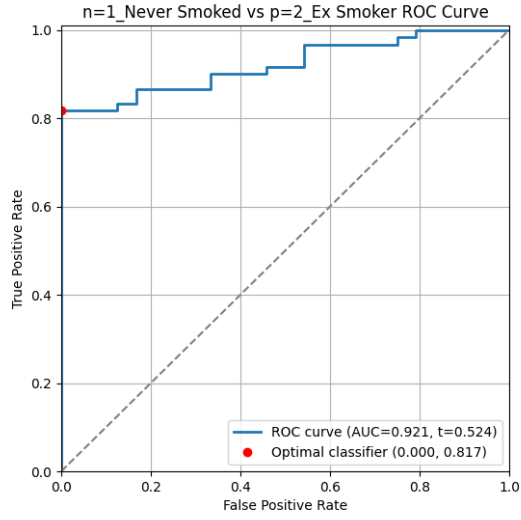


(a) Separation of never vs current smokers by current smoker sub-classifier

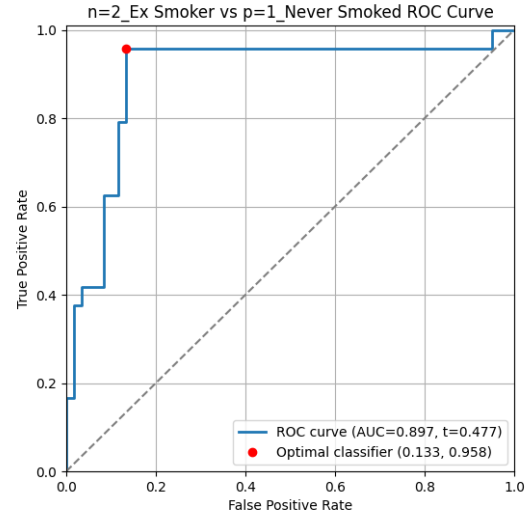


(b) Separation of never vs current smokers by never smoker sub-classifier

Figure 2: Never smoker vs current smoker sub-classifiers performance (Cohort 1)

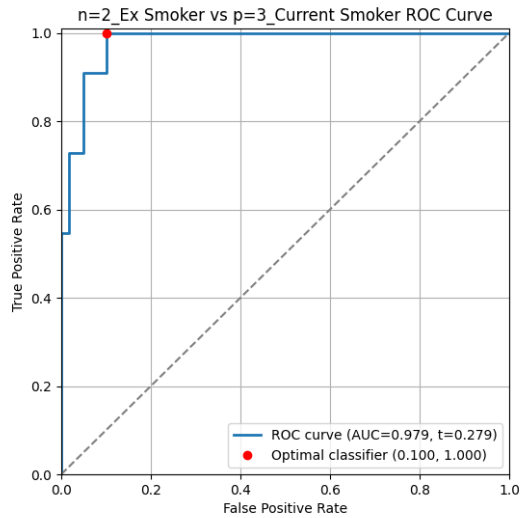


(a) Separation of never vs ex smokers by ex smoker sub-classifier

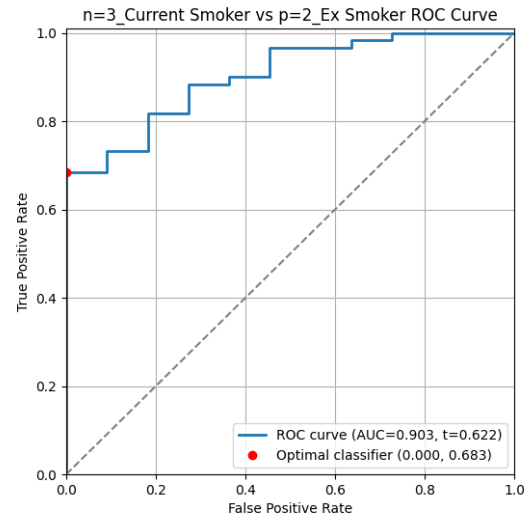


(b) Separation of never vs ex smokers by never smoker sub-classifier

Figure 3: Never smoker vs ex smoker sub-classifiers performance (Cohort 1)



(a) Separation of ex vs current smokers by current smoker sub-classifier



(b) Separation of ex vs current smokers by ex smoker sub-classifier

Figure 4: Ex smoker vs current smoker sub-classifiers performance (Cohort 1)

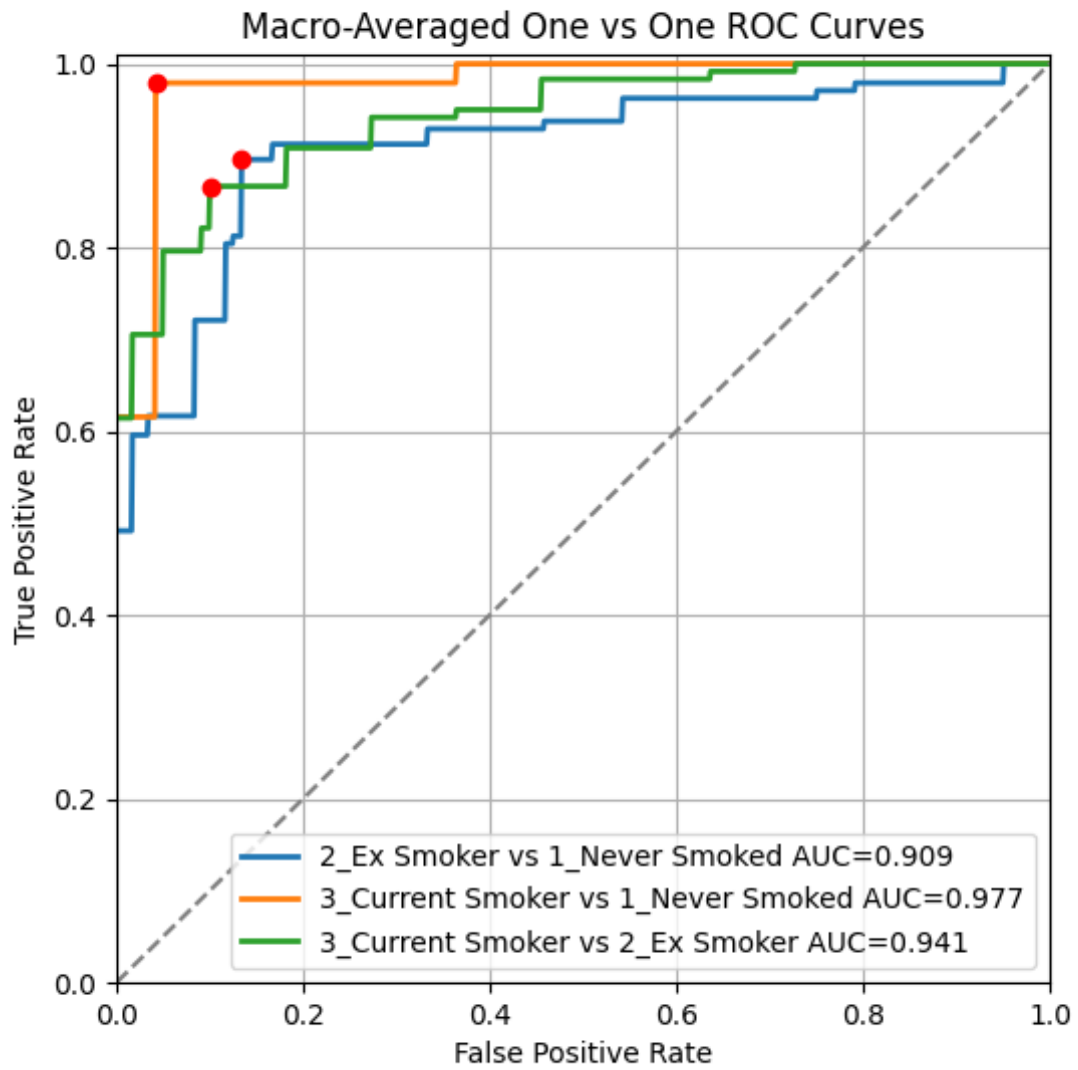
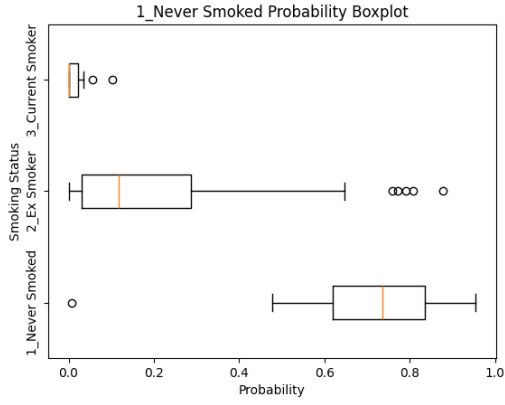
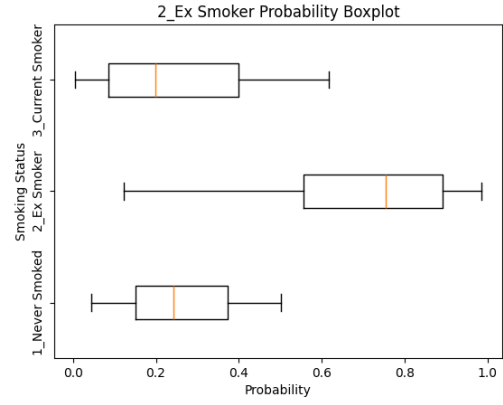


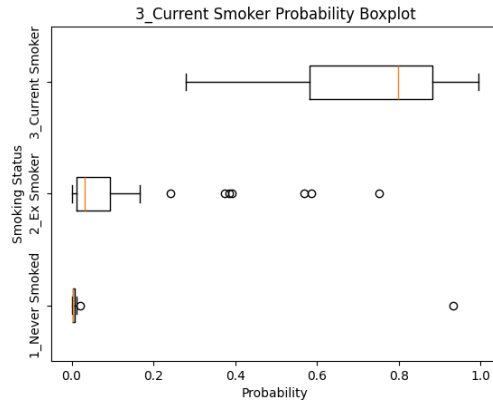
Figure 5: Class separation of classifier (Cohort 1)



(a) Never smoker sub-classifier probabilities across all three classes

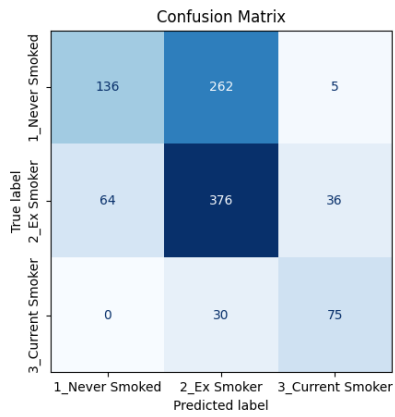


(b) Ex smoker sub-classifier probabilities across all three classes

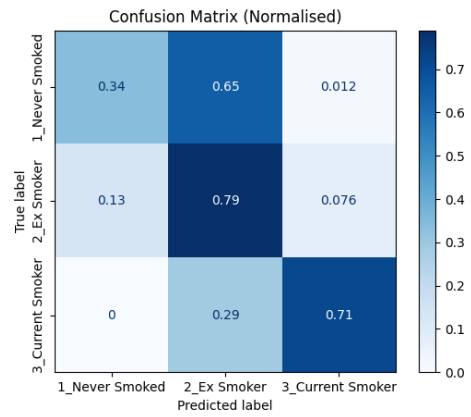


(c) Current smoker sub-classifier probabilities across all three classes

Figure 6: Boxplots of probability distributions (Cohort 1)

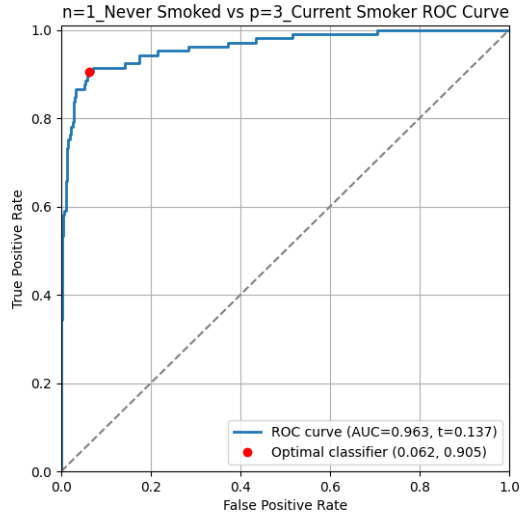


(a) CM

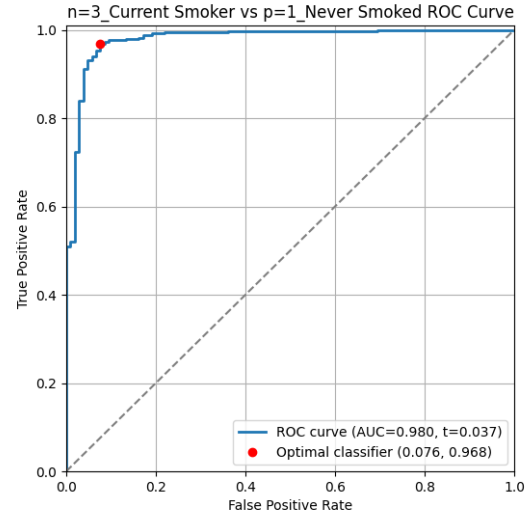


(b) CM Normalised

Figure 7: Confusion Matrices (Cohort 2)

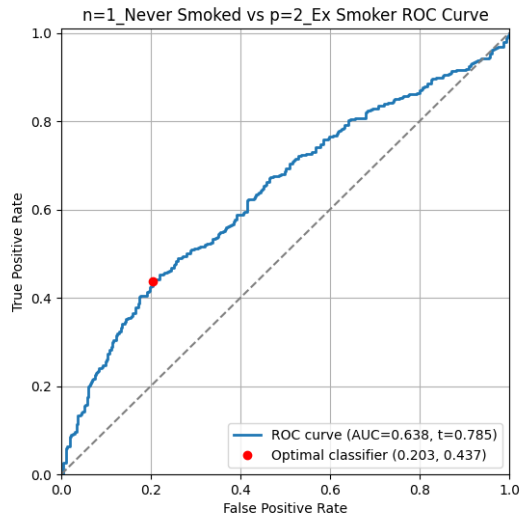


(a) Separation of never vs current smokers by current smoker sub-classifier

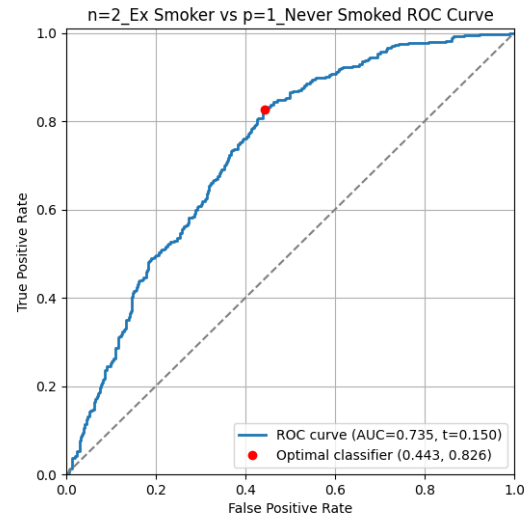


(b) Separation of never vs current smokers by never smoker sub-classifier

Figure 8: Never smoker vs current smoker sub-classifiers performance (Cohort 2)

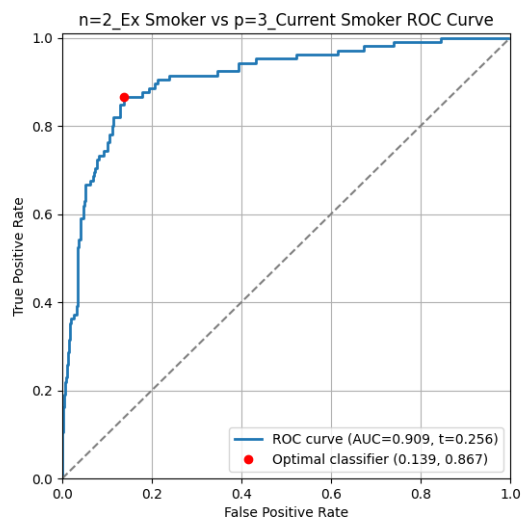


(a) Separation of never vs ex smokers by ex smoker sub-classifier

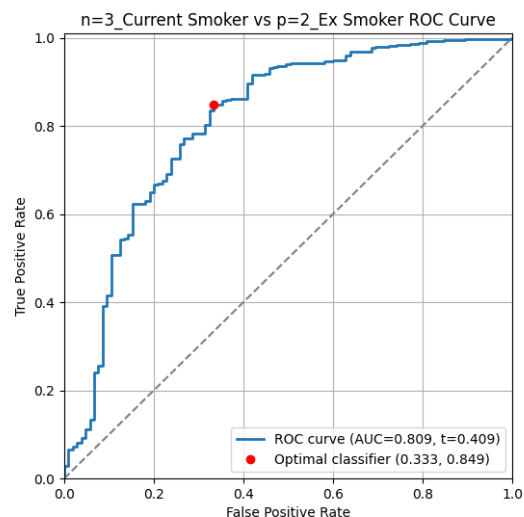


(b) Separation of never vs ex smokers by never smoker sub-classifier

Figure 9: Never smoker vs ex smoker sub-classifiers performance (Cohort 2)



(a) Separation of ex vs current smokers by current smoker sub-classifier



(b) Separation of ex vs current smokers by ex smoker sub-classifier

Figure 10: Ex smoker vs current smoker sub-classifiers performance (Cohort 2)

3.2 External Validation Cohort

3.2.1 Model Performance

3.2.2 Comparison with Prior Results

Choices made in development

- class weight balancing
- feature pruning for cohorts, ensuring compatability
- direction of discovery/validation cohorts, sex being a cofounder
- k-fold cross validation, fold size, using stratified folds
- coarse-to-fine cross-validation strategy
- choice of scoring metric (f1-macro)

Comparisons to other models in validation cohort

- obviously ROC curves, etc.
- how many features selected during training
-

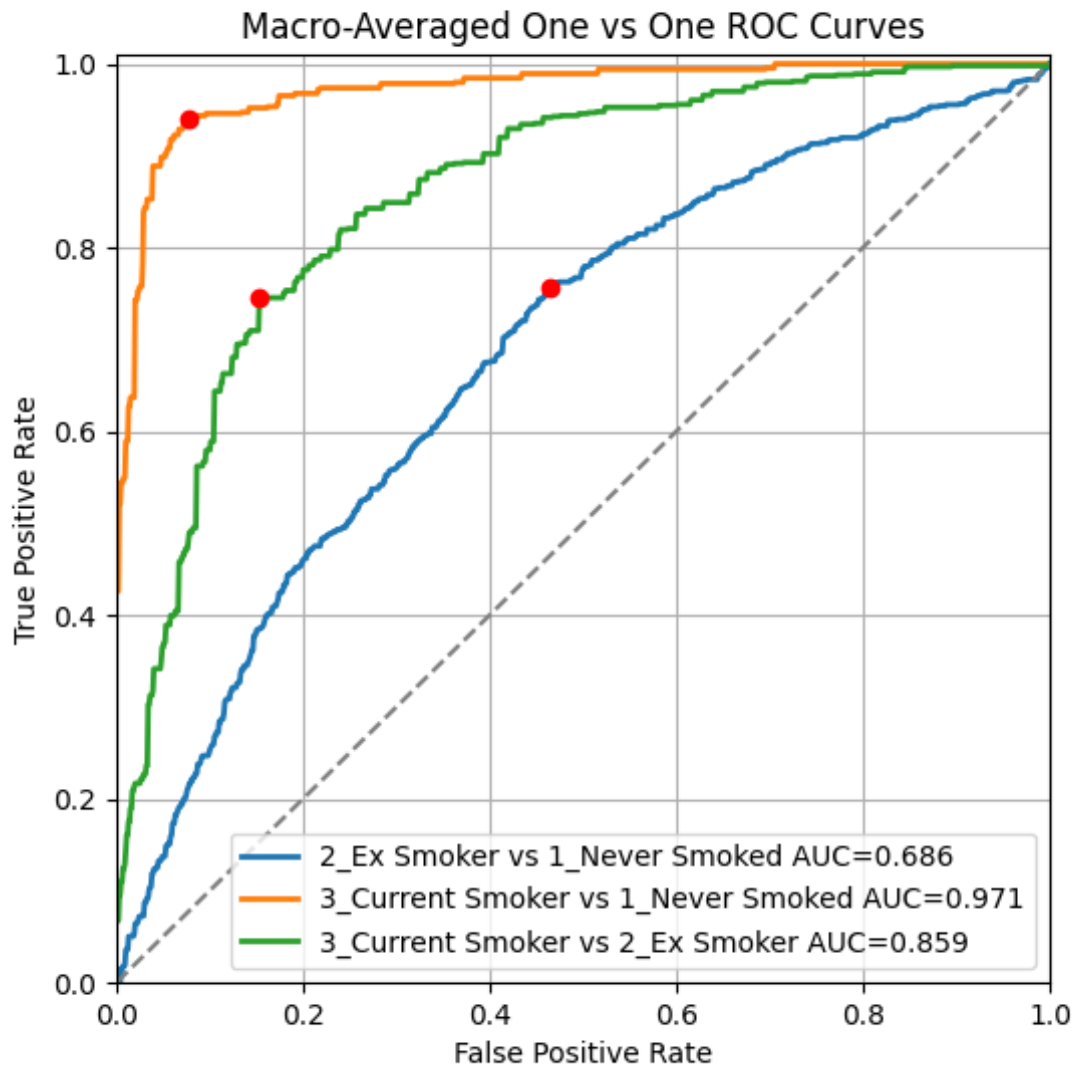
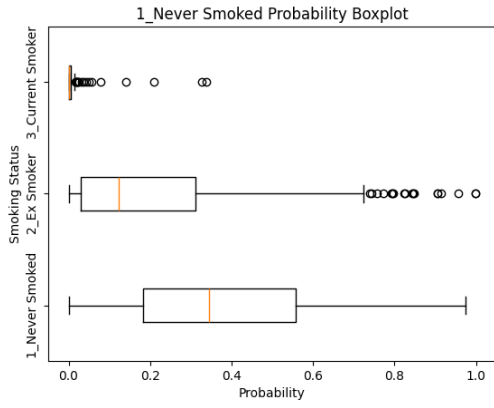
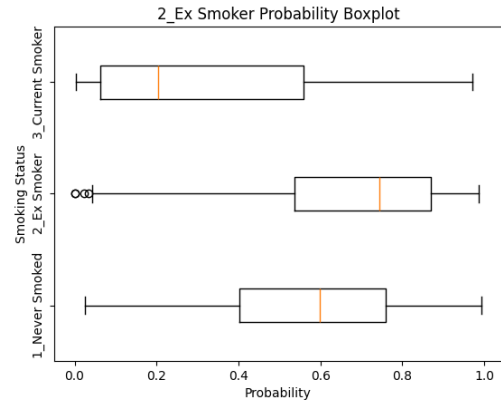


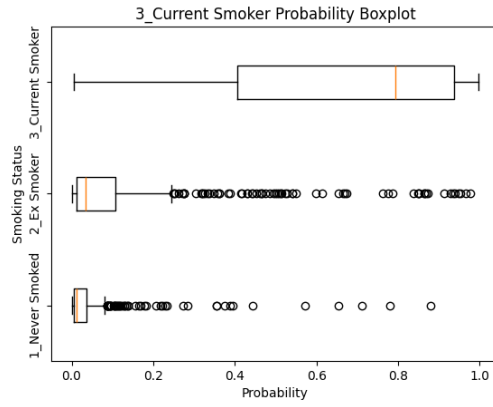
Figure 11: Class separation of classifier (Cohort 2)



(a) Never smoker sub-classifier probabilities across all three classes



(b) Ex smoker sub-classifier probabilities across all three classes



(c) Current smoker sub-classifier probabilities across all three classes

Figure 12: Boxplots of probability distributions (Cohort 2)

Limitations of my model vs other models

- trained only on males
- much smaller dataset size (n)
- comparison of distributions of sex, age, etc in training cohorts (potential confounders)

4 Footnotes

4.1 Acknowledgements

4.2 Ethics Statement

All participants gave written informed consent, and the study was approved by the national ethics committee. Data was anonymised and only age, biological sex and self-reported smoking values were extracted for comparison with matching whole blood DNA methylation values.

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