BioMM: Biologically-informed Multi-stage Machine learning for identification of epigenetic fingerprints

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Abstract

The identification of reproducible biological patterns from high-dimensional data is a bottleneck for understanding the biology of complex illnesses such as schizophrenia. To address this, we developed a biologically informed, multi-stage machine learning (BioMM) framework. BioMM incorporates biological pathway information to stratify and aggregate high-dimensional biological data. We demonstrate the utility of this method using genome-wide DNA methylation data and show that it substantially outperforms conventional machine learning approaches. Therefore, the BioMM framework may be a fruitful machine learning strategy in high-dimensional data and be the basis for future, integrative analysis approaches.

1 Introduction

The identification of predictive biological signatures from high-dimensional biological data remains a major challenge. This particularly applies for clinically and biologically heterogeneous illnesses, such as schizophrenia [11]. Schizophrenia is defined based on clinical symptom constellations, is highly polygenic and is thought to result from complex gene-environment interactions. As a consequence, effect sizes of individual biological markers is generally low, creating an opportunity for machine learning approaches to identify more predictive biological fingerprints. Polygenic scores integrating common variation across the genome explain a substantial amount of heritable variance, but are not sufficiently predictive for diagnostic applications [15, 13]. Due to the importance of environmental risk [2, 19, 1, 21], analysis of epigenetic modifications, such as DNA methylation [6], has received considerable attention in psychiatric research [14, 18]. The analysis of such epigenetic data entails several challenges, including its sensitivity to confounding factors such as medication, which have thus far limited the utility of this modality for integrative analytics in psychiatry. Due to the high data dimensionality and frequently non-reproducible association signals, it remains to explored whether schizophrenia is characterized by consistent patterns of abnormal DNA methylation. Here, we aimed to develop a novel strategy to derive reproducible biological patterns from DNA methylation data. Our approach builds on the hypothesis that biological stratification can meaningfully reduce the dimensionality of the data. Specifically, inspired by functional genomics studies [16, 9], we built a biologically informed two-stage learning framework. At the first level, machine learning is performed independently on biological predictors (i.e. DNA methylation probes) harboured by gene sets of different ontological categories. This is based on the hypothesis that these gene sets capture at least partially independent, illness associated methylation effects. The predicted scores were then used as input for machine learning at the second level, to identify a predictive methylation pattern across ontological categories. Testing of the algorithm was performed in an independent test dataset, to capture its robustness against cross-dataset heterogeneity. To evaluate the utility of this strategy, we performed a comparative analysis against 5 conventional machine learning approaches.
Table 1: Overview of demographic information

| Meta-information               | Controls | Cases | Sex (m/f)      | Age       |
|-------------------------------|----------|-------|----------------|-----------|
| GEO:GSE80417 (phase 1 cohort) | 322      | 353   | 396/279        | 40.5±15.2 |
| GEO:GSE84727 (phase 2 cohort) | 433      | 414   | 602/245        | 44.6±12.9 |

We demonstrate that the biologically informed, multi-stage machine learning significantly outperforms these conventional approaches.

2 Materials and Methods

2.1 Genome-wide methylation data

Genome-wide profiles of DNA methylation were obtained from two independent cohorts with a total sample number of 1522 (Table 1). Data were downloaded from the GEO database [4]. Detailed descriptions of cohorts and data acquisition can be found elsewhere [7]. We focussed on the overlapping set of autosomal methylation sites to limit the potential influence of sex on machine learning due to the phenomenon of X chromosome inactivation or the existence of an additional X chromosome in female samples. The dataset GSE80417 was used as training set and GSE84727 for testing (independent test set) of machine learning algorithms.

2.2 Adjustment for potential confounders

The data was corrected to account for the influence of potential confounders, which comprised cigarette smoking [3], population structure [10], cellular composition, gender and age. Smoking was quantified from DNA methylation levels as described previously [5, 22]. Population structure was determined from methylation data via Principal Components Analysis. Specifically, the first 10 principal components were considered as covariates. Cellular composition was quantified using the Epigenetic Clock tool (https://dnamage.genetics.ucla.edu/) [8] and included the seven recommended cell types: CD8 naive, CD8p CD28nCD45RAn, PlasmaBlast, CD4T, NK, Mono, Gran. All covariates were used in a linear model to residualize each given methylation probe. This was performed separately for both cohorts and the resulting residuals were used for downstream analysis.

2.3 Gene and ontological assignment

For each gene, we extracted all CpGs within 20Kb upstream and downstream of the transcription start and end sites, respectively. CpG locations were downloaded from the NCBI database for both datasets and gene boundaries from the R library TxDb.Hsapiens.UCSC.hg19.knownGene. Information on gene ontologies was derived from the R library org.Hs.eg.db. In total, 2135 ontological categories (biological processes only), each containing between 10 and 200 genes were used for analysis.

2.4 BioMM algorithm

Input:

1. K: the total number of gene ontological categories (GO).
2. $D_k$: the stage-1 training set $D_k = \{(x_1^k, ..., x_M^k), Y_D)\}$ with M samples for every $k = 1, ..., K$ and a fixed label $Y_D$.
3. $R_k$: the stage-1 independent test set $R_k = \{(x_1^k, ..., x_N^k), Y_R)\}$ with N samples for every $k = 1, ..., K$ and a fixed label $Y_R$.
4. $f^1$: the stage-1 model.
5. $f^2$: the stage-2 model. The random forest algorithm was used in the present study.

Output:

1. $\hat{Y}_{1}^{k_M}$: the stage-1 bootstrapping prediction score with M samples for $k^{th}$ GO.
Table 2: Classification performance (quantified as error rates) of conventional classifiers and BioMM

| Classifiers | BioMM Err<sub>test</sub> | Conventional Err<sub>test</sub> |
|-------------|--------------------------|---------------------------------|
| Random forest | 0.391                    | 0.511                           |
| SVM          | 0.432                    | 0.519                           |
| logit        | 0.482                    | 0.514                           |
| LASSO        | 0.509                    | 0.515                           |
| Elastic      | 0.502                    | 0.514                           |

2. $\hat{Y}_{kN}^1$: the stage-1 independent test prediction score with N samples for $k^{th}$ GO.

3. $D^2$: the stage-2 training set $D^2 = (\hat{Y}_{1M}^1, \ldots, \hat{Y}_{KM}^1, Y_D)$ with M samples and the label $Y_D$.

4. $R^2$: the stage-2 test set $R^2 = (\hat{Y}_{1N}, \ldots, \hat{Y}_{kN}^1, Y_R)$ with N samples and the label $Y_R$.

5. $\hat{Y}_{test}^2$: the stage-2 test estimate.

6. Err<sub>test</sub>: the error rate for the stage-2 test estimate. Err<sub>test</sub> = $L(Y_R, \hat{Y}_{test}^2)$ with the 0-1 loss function $L$.

BioMM 1<sup>st</sup> stage:

1. For each ontological category $k$, repeat the following steps B times (here, B=100):
   a. Draw a bootstrap set from $D_k$ with the same sample size. The out-of-bag samples are used as test set.
   b. Fit a machine learning model $f^1$ to the bootstrapped data.
   c. Predict the model on the test set and $R_k$.

2. For each GO $k$, determine the averaged prediction score $\bar{Y}_{kM}^1 = \frac{1}{B} \sum_{b=1}^{B} \hat{Y}_{kM}^1$ and $\bar{Y}_{kN}^1 = \frac{1}{B} \sum_{b=1}^{B} \hat{Y}_{kN}^1$.

3. Combine $\bar{Y}_{kM}^1$ and $\bar{Y}_{kN}^1$ across all $K$ GOs to generate $D^2$ and $R^2$.

BioMM 2<sup>nd</sup> stage:

1. Fit a machine learning model $f^2$ to $D^2$ with features that are positively correlated with $Y_D$.
2. Predict the model on $R^2$ with the corresponding feature set.
3. Repeat steps 1 and 2 20 times and average prediction scores to obtain $\hat{Y}_{test}^2$.
4. Determine Err<sub>test</sub> to evaluate the model performance.

2.5 Classifier selection

To compare classifiers regarding performance, five different well-known classifiers were used as machine learning models for the first stage of BioMM: random forests, support vector machine (SVM) with the radial basis function kernel, logit regression, the LASSO and elastic net. No variable selection was performed since the categorization based on ontological categories already substantially reduced data dimensionality. At the second stage, the random forest algorithm was applied to capture potential interactions between ontological categories.

2.6 Comparison against conventional machine learning

The same five classifiers were used as conventional machine learning tools to compare performance against the BioMM method. Due to the large data dimensionality, variable selection was performed based on Pearson correlation filtering. The number of selected features (5, 10, 20, 30, 50, 100, 500, 1000 or 3000 CpGs) was determined using bootstrapping in the training data.

3 Results and Discussion

Table 2 shows that BioMM using random forests outperformed conventional and other BioMM approaches, with a classification error rate of 39.1%. The second best classifier was BioMM using
The BioMM method conceptually aligns well with the growing consensus that schizophrenia is not a

wide effect.

An interesting aspect of the BioMM method is that, in contrast to conventional machine learning,

analysis, which had previously been performed on synthetic features similar to those created by BioMM

merous ontological categories. Besides additional data to remove false-positive associations, other

information, such as with genetic association or expression data.

approach for integration of multiple data modalities that can be mapped via genetic and ontological

help to focus on biologically relevant signals. Finally, we expect the BioMM strategy to be a fruitful

data modalities or biological meta-information, such as tissue specific expression information, may

show illness associations by chance and, therefore, receive high classifier weights at the second

selection procedures may further improve performance [17]. Additionally, especially if larger data

sets are available, other strategies of creating ‘synthetic’ data sets during the first stage may be prefer-

able. Here, we used the oob predictions, but these have been reported to be conservatively biased

[12]. This could impact on the comparability between the synthetic training and test data, which

could, in turn, negatively impact on classifier performance. Another potential concern is that given

the high number of ontological categories used for stratification, some synthetic features will likely

lead to predictions with higher biological reproducibility (1st stage) and accuracy (2nd stage). (IV)

Illness associated methylation differences may be hierarchically organized such that alterations of

individual methylation sites impact first at the pathway level which subsequently leads to a systems-

wide effect.

The BioMM method conceptually aligns well with the growing consensus that schizophrenia is not a

single disease entity, but composed of multiple subtypes with different biological underpinnings. In

contrast to conventional machine learning (as well as the variable selection performed here), which

generally builds on the assumption that all subjects originate from the same population, BioMM

adds a dimension of biological stratification. It specifically exploits the hypothesis that some sub-

jects may have alterations in a given biological process (thereby receiving high prediction scores at

the first stage) and these subgroups of patients are then integrated at the second stage. Therefore,

exploring how first stage predictors distribute across the patient cohorts may give novel insights into

the presence of potential patient subgroups. This may allow a personalized extension functional anal-

ysis, which had previously been performed on synthetic features similar to those created by BioMM

after first stage computations [16, 9]. Notably, these features do not allocate subjects to mutually

exclusive clusters but may elucidate overlapping patient communities depending on the implicated

biological processes. In the present study, we have corrected data for several potential confound-

ing effects that are known to modify DNA methylation levels. However, we cannot exclude that

other factors, such as medication or lifestyle effects, may have introduced bias and led to artificial

illness-associated profiles. Therefore, the present results should be replicated in additional cohorts

that are not affected by these confounders. Results from conventional machine learning, as well as

the BioMM method using some first-stage classifiers, however, demonstrate that these effects did

not lead to obvious signatures that were reproducible across datasets. The resulting signatures may,

therefore, hint at the existence of reproducible methylation signatures in schizophrenia and show

utility for integrative analyses with other data modalities.

From a computational perspective, further improvements to the method may be achieved by variable

selection during the first and second stage. At the second stage, we selected predictors positively

associated with outcome in the training data to prevent confounding, but more advanced variable

selection procedures may further improve performance [17]. Additionally, especially if larger data

sets are available, other strategies of creating ‘synthetic’ data sets during the first stage may be prefer-

able. Here, we used the oob predictions, but these have been reported to be conservatively biased

[12]. This could impact on the comparability between the synthetic training and test data, which

could, in turn, negatively impact on classifier performance. Another potential concern is that given

the high number of ontological categories used for stratification, some synthetic features will likely

show illness associations by chance and, therefore, receive high classifier weights at the second

level. This is particularly a problem if the true biological signal is weak and distributed across nu-

morous ontological categories. Besides additional data to remove false-positive associations, other

data modalities or biological meta-information, such as tissue specific expression information, may

help to focus on biologically relevant signals. Finally, we expect the BioMM strategy to be a fruitful

approach for integration of multiple data modalities that can be mapped via genetic and ontological

information, such as with genetic association or expression data.
4 Conclusion

We have developed here a biologically informed machine learning framework that aims to identify reproducible biological patterns through biological stratification and aggregation of high-dimensional data. This BioMM method outperformed conventional machine learning approaches based on evaluation of prediction in independent test data. This computational framework may allow the exploration of patient subgroup effects and show utility for integrative analyses with other data modalities.

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References

[1] Alan S Brown and Elena J Derkits. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *American Journal of Psychiatry*, 167(3):261–280, 2009.

[2] Elizabeth Cantor-Graae and Jean-Paul Selten. Schizophrenia and migration: a meta-analysis and review. *American Journal of Psychiatry*, 162(1):12–24, 2005.

[3] Jose de Leon and Francisco J Diaz. A meta-analysis of worldwide studies demonstrates an association between schizophrenia and tobacco smoking behaviors. *Schizophrenia research*, 76(2):135–157, 2005.

[4] Ron Edgar, Michael Domrachev, and Alex E Lash. Gene expression omnibus: Ncbi gene expression and hybridization array data repository. *Nucleic acids research*, 30(1):207–210, 2002.

[5] Hannah R Elliott, Therese Tillin, Wendy L McArdle, Karen Ho, Aparna Duggirala, Tim M Frayling, George Davey Smith, Alun D Hughes, Nish Chaturvedi, and Caroline L Relton. Differences in smoking associated dna methylation patterns in south asians and europeans. *Clinical epigenetics*, 6(1):4, 2014.

[6] Dennis R Grayson and Alessandro Guidotti. The dynamics of dna methylation in schizophrenia and related psychiatric disorders. *Neuropsychopharmacology*, 38(1):138–166, 2013.

[7] Eilis Hannon, Emma Dempster, Joana Viana, Joe Burrage, Adam R Smith, Ruby Macdonald, David St Clair, Colette Mustard, Gerome Breen, Sebastian Herman, et al. An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential dna methylation. *Genome biology*, 17(1):176, 2016.

[8] Steve Horvath. Dna methylation age of human tissues and cell types. *Genome biology*, 14(10):3156, 2013.

[9] C Liu, CA Bousman, C Pantelis, E Skafidas, D Zhang, W Yue, and IP Everall. Pathway-wide association study identifies five shared pathways associated with schizophrenia in three ancestral distinct populations. *Translational psychiatry*, 7(2):e1037, 2017.

[10] Jingyu Liu, Kent Hutchison, Nora Perrone-Bizzozero, Marilee Morgan, Jing Sui, and Vince Calhoun. Identification of genetic and epigenetic marks involved in population structure. *PloS one*, 5(10):e13209, 2010.

[11] John McGrath, Sukanta Saha, David Chant, and Joy Welham. Schizophrenia: a concise overview of incidence, prevalence, and mortality. *Epidemiologic reviews*, 30(1):67–76, 2008.

[12] Matthew W Mitchell. Bias of the random forest out-of-bag (oob) error for certain input parameters. 2011.

[13] The Network, Pathway Analysis Subgroup of the Psychiatric Genomics Consortium, et al. Psychiatric genome-wide association study analyses implicate neuronal, immune and histone pathways. *Nature neuroscience*, 18(2):199, 2015.
[14] Masaki Nishioka, Miki Bundo, Kiyoto Kasai, and Kazuya Iwamoto. DNA methylation in schizophrenia: progress and challenges of epigenetic studies. *Genome medicine*, 4(12):96, 2012.

[15] Schizophrenia Working Group of the Psychiatric Genomics Consortium et al. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, 511(7510):421–427, 2014.

[16] Qinxin Pan, Ting Hu, James D Malley, Angeline S Andrew, Margaret R Karagas, and Jason H Moore. A system-level pathway-phenotype association analysis using synthetic feature random forest. *Genetic epidemiology*, 38(3):209–219, 2014.

[17] Claudia Perlich and Grzegorz Świrszcz. On cross-validation and stacking: Building seemingly predictive models on random data. *ACM SIGKDD Explorations Newsletter*, 12(2):11–15, 2011.

[18] Tania L Roth, Farah D Lubin, Monsheel Sodhi, and Joel E Kleinman. Epigenetic mechanisms in schizophrenia. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1790(9):869–877, 2009.

[19] Cheryl D Swofford, John W Kasckow, Geri Scheller-Gilkey, and Lawrence B Inderbitzin. Substance use: a powerful predictor of relapse in schizophrenia. *Schizophrenia research*, 20(1):145–151, 1996.

[20] Makoto Takahashi, Hiroshi Hayashi, Yuichiro Watanabe, Kazushi Sawamura, Naoki Fukui, Junzo Watanabe, Tsuyoshi Kitajima, Yoshio Yamanouchi, Nakao IWata, Katsuyoshi Mizukami, et al. Diagnostic classification of schizophrenia by neural network analysis of blood-based gene expression signatures. *Schizophrenia research*, 119(1):210–218, 2010.

[21] Gregory B Teague, Robert E Drake, and Stephen J Bartels. Stress and schizophrenia: A review of research models and findings. *Stress and Health*, 5(3):153–165, 1989.

[22] Sonja Zeilinger, Brigitte Kühnel, Norman Klopp, Hansjörg Baurecht, Anja Kleinschmidt, Christian Gieger, Stephan Weidinger, Eva Lattka, Jerzy Adamski, Annette Peters, et al. Tobacco smoking leads to extensive genome-wide changes in DNA methylation. *PloS one*, 8(5):e63812, 2013.