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Deep learning analysis of UPLC-MS/MS-based metabolomics data to predict Alzheimer's disease

Kesheng Wang [a,*], Laurie A. Theeke [b], Christopher Liao [c], Nianyang Wang [d], Yongke Lu [e], Danqing Xiao [f], Chun Xu [g,*], The Alzheimer's Disease Neuroimaging Initiative [1]

[a] School of Nursing, Health Sciences Center, West Virginia University, Morgantown, WV 26506, USA
[b] School of Nursing, The George Washington University, Ashburn, VA 20147, USA
[c] Department of Electrical and Computer Engineering, Boston University, MA 02215, USA
[d] Department of Health Policy and Management, School of Public Health, University of Maryland, College Park, MD 20742, USA
[e] Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25755, USA
[f] Department of STEM, School of Arts and Sciences, Regis College, Weston, MA 02493, USA
[g] Department of Health and Biomedical Sciences, College of Health Professions, University of Texas Rio Grande Valley, Brownsville, TX 78520, USA

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ABSTRACT

Objective: Metabolic biomarkers can potentially inform disease progression in Alzheimer's disease (AD). The purpose of this study is to identify and describe a new set of diagnostic biomarkers for developing deep learning (DL) tools to predict AD using Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS/MS)-based metabolomics data.

Methods: A total of 177 individuals, including 78 with AD and 99 with cognitive normal (CN), were selected from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort along with 150 metabolomic biomarkers. We performed feature selection using the Least Absolute Shrinkage and Selection Operator (LASSO). The H2O DL function was used to build multilayer feedforward neural networks to predict AD.

Results: The LASSO selected 21 metabolic biomarkers. To develop DL models, the 21 biomarkers identified by LASSO were imported into the H2O package. The data was split into 70% for training and 30% for validation. The best DL model with two layers and 18 neurons achieved an accuracy of 0.881, F1-score of 0.892, and AUC of 0.873. Several metabolomic biomarkers involved in glucose and lipid metabolism, in particular bile acid metabolites, were associated with *APOE*-ε4 allele and clinical biomarkers (Aβ42, tTau, pTau), cognitive assessments [the Alzheimer's Disease Assessment Scale-cognitive subscale 13 (ADAS13), the Mini-Mental State Examination (MMSE)], and hippocampus volume.

Conclusions: This study identified a new set of diagnostic metabolomic biomarkers for developing DL tools to predict AD. These biomarkers may help with early diagnosis, prognostic risk stratification, and/or early treatment interventions for patients at risk for AD.

1. Introduction

Alzheimer's disease (AD), a progressive, degenerative disorder of the brain, is the most common form of dementia and is characterized by progressive loss of memory and cognitive functions due to continuous neuron damage and the resultant increase in affected areas of the brain [1]. The pathological brain changes of AD include the accumulation of abnormal β-amyloid A(β) plaques (Aβ peptides) and neurofibrillary tangles (tau proteins), as nerve cells degenerate [1–4]. AD and its related dementias currently affect 6.5 million Americans aged 65 and older, and its prevalence is expected to increase to 13.8 million by 2060 which warrants the development of medical breakthroughs to prevent, slow, or cure AD [5]. AD may start years or even decades before clinical symptom onset, therefore it is crucial to identify predictive biomarkers in the

* Corresponding author.
E-mail addresses: kesheng.wang@hsc.wvu.edu (K. Wang), chun.xu@utrgv.edu (C. Xu).

[1] **Note:** Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in the analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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preclinical stage so that medical science can develop strategies to prevent the progression of AD [4,6,7]. In addition, early detection of AD is critically important for drug development and application as well as for the development of diagnostic and therapeutic approaches aiming to prevent loss of function and diminished longevity [8–10]. The metabolic basis of AD is still poorly understood and the relationships between systemic abnormalities in metabolism and AD pathogenesis are unclear but there is potential to identify metabolic biomarkers that are predictive of AD diagnosis and progression.

Machine learning (ML) methods can address high dimensional data, integrate data from different sources, model the etiological and clinical heterogeneity, and discover new biomarkers [8,10,11]. Due to the complex nature of AD, ML methods have the potential to develop new insights into the disease pathophysiology [12,13]. During the last decade, several ML technologies have been used to enhance the diagnosis and prognosis of AD such as Random Forest (RF), Linear Discriminant Analysis (LDA), Decision Trees (DT), and Support Vector Machines (SVM) [8,12–15]. SVM is a commonly used technique to differentiate between AD patients and healthy individuals, and between steady and progressing subtypes of mild cognitive impairment (MCI) [8,13].

Artificial neural networks (ANN) are inspired by the layered structure of the brain's neurons, and their computations could be implemented in biological neurons [16]. ANNs have demonstrated their powerful potential in medical research applications [17] and exceeded the results of other ML algorithms such as RF and SVM since they are capable of modeling complex non-linear relationships via layers of intermediate features [8,18]. ANNs, as a class of ML algorithms, are capable of combining raw inputs into layers of intermediate features, while deep learning (DL) using ANNs has reached unprecedented prediction performance for complex tasks [8,13,15]. Therefore, DL has emerged as a versatile approach for predicting complex biological phenomena and displays tremendous potential in biology and medicine [15,19–21]. DL techniques, such as deep neural networks (DNN) [15], stacked autoencoder (SAE) neural networks [15,22], and convolutional neural networks (CNNs) [15,23,24] have been reported to be more accurate for AD diagnosis in comparison to conventional ML models [12,15]. However, the study of DL-based AD study is still in its early stages, and further studies are needed that incorporate different information sources [8].

Metabolomics involves the comprehensive analysis of small-molecule metabolites in a given biological matrix and their response to disease, drugs, diet, and lifestyle. It has been shown that by revealing insights into the underlying biochemistry, metabolomics has the potential to successfully differentiate neurodegenerative diseases from healthy controls [25–28]. Metabolomic-based techniques have been used for both early diagnosis of AD and monitoring of appropriate treatment [29]. Traditional ML methods, such as logistic regression, RF, LDA, and SVM, have been used in metabolomics for AD [14,28,30–37]. Recently, DL application in metabolomics in AD has increased [15,22,23,30,34,38,39]. Metabolic biomarkers of AD can potentially inform the disease progression of AD, its mechanisms, and its endophenotypes.

However, metabolic biomarkers are abundant and parts of these metabolic biomarkers are correlated with one another. These correlations can be a result of shared metabolic pathways or regulatory mechanisms, as well as interconnected physiological responses within the body. It is essential to identify the informative and relevant features from a larger collection of features and lead to an improved characterization of the underlying patterns and relations [40,41]. Therefore, the aims of this study are (1) to perform feature selection of metabolomic biomarkers and evaluate the performance in SVM, and (2) to train DL models to predict AD using the H2O package [42].

2. Materials and methods

2.1. Data set

Data used in the preparation of this proposal were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography (PET), CSF biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. The ADNI study began in 2004 as a multicenter that provides services in the United States and Canada. The ADNI is an ongoing, longitudinal, multicenter study designed to develop clinical, imaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. In our study, the merged data was used from several components of ADNI. A letter of exemption was obtained from the Institutional Review Board (IRB) since this study is a secondary analysis of existing data.

2.2. Measures

The study included sociodemographic factors, surveys of cognition, plasma metabolomic biomarkers, cerebral spinal fluid (CSF) clinical biomarkers, and MRI imaging data. Social-demographic factors included gender, age, and educational level. Gender was self-reported as either male or female. Age and years of education were reported as continuous variables. *APOE-ε4* carriers were defined as individuals with at least one ε4 allele (*APOE-ε4*/ε4, ε4/ε3 or ε4/ε2 as *APOE-ε4*–1+), while non-carriers were defined as individuals with no ε4 allele (*APOE-ε4*–0). The Mini-Mental State Examination (MMSE) provides a global measure of mental status, evaluating five cognitive domains: orientation, registration, attention and calculation, recall, and language [43]. The Alzheimer's Disease Assessment Scale-cognitive subscale 13 (ADAS13) was also used and is a 13-items cognitive test where higher scores reflect poorer cognitive performance [44]. The MRI brain structure data was derived from UCSF FreeSurfer datasets. Hippocampus volume (HPV) was generated from UCSF MRI structure data. Clinical CSF biomarkers (Aβ42, tTau, and pTau) were included.

This study selected three metabolomic datasets from ADNI. The first dataset was selected from ADMC U Hawaii ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) Gut Metabolites Serum Longitudinal. There are 104 metabolites in the human serum samples. The second dataset is from AD Metabolomics Consortium Duke Biocrates P180 Kit UPLC, which includes 51 metabolites. Biospecimens of human serum (ADNI samples) were provided by Duke University (the sponsor), and the quantitative measurement of these samples was carried out using both UPLC-MS/MS. The third dataset is from the Targeted UHPLC-MS/MS analysis of High-Value Metabolites in Serum Samples with 22 metabolites available.

AD diagnosis, demographic variables, *APOE*-ε4 genotypes, and clinical CSF biomarkers (Aβ42, tTau, and pTau) were downloaded from the ADNIMERGE data. We merged demographic variables, AD diagnosis, *APOE*-ε4 genotypes, MMSE, ADAS13, clinical CSF biomarkers, HPV, and metabolic biomarkers. Then we excluded the individuals without metabolic biomarkers and individuals with metabolic biomarkers but missing >20% for other variables. The remaining total sample size is 335 with 150 metabolic biomarkers including 78 individuals with AD, 99 with cognitive normal (CN), and 158 with MCI. In the current study, we used AD and CN individuals (Table 1).

2.3. Feature selection of metabolomic data and evaluation using SVM

Before applying the feature selection methods, the Z score was computed for 150 metabolite biomarkers using the mean and standard deviation. We performed feature selection using Least Absolute Shrinkage and Selection Operator (LASSO). The LASSO in the R package

Table 1
Descriptive statistics.

Variable	CN (Mean ± SD or n)	AD (Mean ± SD or n)	t/χ2, p-value
Age	75.5 ± 5.4	74.9 ± 7.9	0.55, 0.559
Gender (n)			0.07, 0.786
Male	50	41	
Female	49	37	
Education	15.7 ± 2.9	15.0 ± 3.0	1.50, 0.137
APOE-ε4 allele (n)			33.91, <0.0001
0	75	25	
+	24	53	
ADAS13	9.6 ± 4.2	28.4 ± 7.7	−20.51, <0.0001
MMSE	29.1 ± 1.1	23.6 ± 1.8	25.2, <0.0001
Hippocampus	7194.5 ± 799.3	5961.1 ± 1064.1	8.81, <0.0001
Aβ42	1156.0 ± 447.2	639.4 ± 302.0	8.75, <0.0001
tTau	236.4 ± 82.3	353.4 ± 136.7	−7.04, <0.0001
pTau	21.9 ± 8.5	35.5 ± 15.7	−7.38, <0.0001

Abbreviations: AD: Alzheimer's disease; CN: Cognitive normal; SD: Standard deviation.
MMSE: Mini-Mental State Examination. ADAS13: Alzheimer's Disease Assessment Scale-cognitive subscale 13. P-value is based on the Chi-square test or independent samples *t*-test.

"glmnet" is used to perform logistic regression when the response is binary. The LASSO method regularizes model parameter λ by shrinking the regression coefficients, reducing some of them to zero. The feature selection phase occurs after the shrinkage, where every non-zero value is selected to be used in the model [45]. The SVM algorithm (linear kernel, radial basis function (RBF) kernel, and polynomial kernel) was applied to develop a model to predict AD [46]. Linear kernel is used when the data is linearly separable, that is, it can be separated using a single line. Linear kernel combines all support vectors linearly to produce the output. The Radial Basis Function (RBF) kernel is usually the first reasonable choice as it can nonlinearly map data into higher dimensional space. The RBF kernel measures the similarity between two data points as a function of the Euclidean distance between them. The polynomial kernel represents the similarity of vectors in a feature space over polynomials of the original variables, allowing learning of nonlinear models, using a polynomial function to transform the input data into a higher dimensional space. The data was split into 70% for training and 30% for validation. To evaluate the performance of feature selection methods, accuracy, recall, specificity, precision, F1-score, and AUC (area under the ROC curve) were used to evaluate the performance of feature selection methods. The F1-score is a harmonic mean that combines both recall and precision.

2.4. Deep learning

Features selected by LASSO were imported into the H2O package (version 3.38.0.1) to develop the DL model [42]. H2O's DL is based on a multi-layer feedforward ANN, also known as DNN or multi-layer perceptron (MLP); this is the most common type of DNN. Fig. 1 illustrates an ANN with two hidden layers, four input variables/neurons, and output. There is no fixed rule for the number of hidden layers and the number of input neurons in the development of DL models. The network can contain a large number of hidden layers consisting of neurons with the tanh, rectifier, or maxout activation functions. The data was split into 70% for training and 30% for validation. First, we performed a grid search to evaluate the accuracy of different models. For hyperparameter tuning, we compared four different activation functions ("Rectifier", "RectifierWithDropout", "Maxout", and "MaxoutWithDropout"), hidden layers from 1 to 3 with different neurons, 10-fold cross-validation, epochs = 100. L1 and L2 penalties were set as l1_opt <− c(0, 0.00001, 0.0001, 0.001, 0.01, 0.1) and l2_opt <− c(0, 0.00001, 0.0001, 0.001, 0.01, 0.1). Search_criteria included strategy = "RandomDiscrete", max_models = 100, max_runtime_secs = 900, stopping_tolerance = 0.001, and stopping_rounds = 15. The models which return higher accuracy were chosen. Then, we focused on several DL models with higher accuracy and compared models with 1–3 hidden layers and different numbers of neurons. To build the DL model, we set activation as "Rectifier", epochs = 100, seed = 1337, reproducible = T, hidden, nfolds = 10, input_dropout_ratio = 0.2, l1 = 1e-6, variable_importances = T. To evaluate different models, the H2O package provides several measures such as accuracy, recall, specificity, precision, F1-score, AUC, Area Under Precision-Recall Curve (AUPRC), and Matthew's correlation coefficient (MCC).

2.5. Statistical analysis

Table 1 presents an overview of the dataset. The categorical variables were presented in their raw values along with the proportions. Continuous variables such as age and education are presented with their mean and standard deviation (SD). The chi-square test was used to examine the associations of categorical variables with AD and CN. An independent samples *t*-test was performed to determine differences in

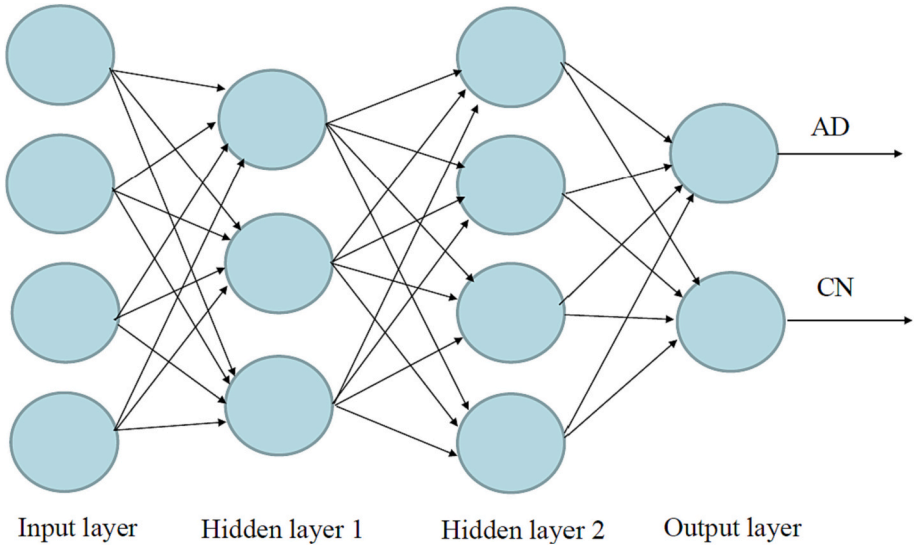


Fig. 1. Visualization of the artificial neural network model with four inputs, two hidden layers, and output.

continuous variables between AD and CN samples and between *APOE*-ε4 genotypes.

To further analyze the relationships among the selected metabolomic biomarkers, three clinical CSF biomarkers (Aβ42, tTau, and pTau), cognitive phenotypes (MMSE and ADAS13), and HPV from MRI, Spearman's rank correlation analyses were performed. All statistical analyses were performed using SAS 9.4.

2.6. Power analysis

Independent samples t-test was used to compare the means of continuous variables between the two groups. Using G.Power [47,48], assuming $\alpha = 0.05$, Cohen's d = 0.50 (moderate effect), sample size for AD and CN being 78 and 99, respectively, the power can reach 90.7%.

3. Results

3.1. Descriptive statistics

The demographic characteristics of the participants are summarized in Table 1. Age, gender, and education did not differ between the CN and AD groups ($p > 0.05$). A significant difference was observed in the higher frequency of the *APOE-ε4* allele presence in the AD group ($p < 0.0001$). The AD group had higher mean values in the ADAS13 but lower mean scores for the MMSE. The AD group had significantly smaller hippocampus volume and less Aβ42, with higher scores for both tTau and pTau proteins (all *p*-values <0.0001).

3.2. Feature selection

The LASSO software selected 21 biomarkers listed in Table 2 based on the optimal parameter $\ln(\lambda) = -3.32$ (Fig. 2). In addition, among 21 biomarkers selected by LASSO, 8 were significantly associated with AD using the independent *t*-test and 4 cholic acid (CA) species (X3_Hydroxyisovaleric acid, glycohyodeoxycholic acid (GHDCA), hyodeoxycholic acid (HDCA), and isolithocholic acid (ISOLCA)) was associated with *APOE*-ε4 (Table 2 and Table S1). Table S1 lists 150 biomarkers with coefficients using LASSO and *p*-values based on independent *t*-tests comparing means in biomarkers between AD and CN as well as between *APOE*-ε4 genotypes.

3.3. SVM and performance

We evaluated the performance of 21 features selected by LASSO and the whole 150 metabolomic biomarkers using SVM, with the performance statistics (accuracy, recall, specificity, precision, F1-score, and AUC) (Table 3). We used three kernel methods in SVM (linear kernel, RBF kernel, and polynomial kernel models) to develop models for predicting AD. Based on the accuracy, the best model with the highest accuracy was an SVM with a polynomial kernel, trained on 21 biomarkers

Table 2
Features selection using Least Absolute Shrinkage and Selection Operator (LASSO).

Package	Feature selection algorithm	Extracted variables
glmnet in R	Least Absolute Shrinkage and Selection Operator (LASSO)	21 variables: Hippuric acid, L_Malic acid, Adipic acid, L_alpha_Aminobutyric acid, Isocitric acid, X3_Hydroxyisovaleric acid, Docosahexaenoicacid, CA, GHDCA, HDCA, ISOLCA, TCA, UDCA, Asn, Orn, Val, Creatinine, Serotonin, Taurine, Glucose, Lactate

Abbreviations: CA: cholic acid; GHDCA: glycohyodeoxycholic acid; HDCA: hyodeoxycholic acid; ISOLCA: isolithocholic acid; TCA: taurocholic acid; UDCA: ursodeoxycholic acid; Asn: asparagine; Orn: ornithine; Val: valine.

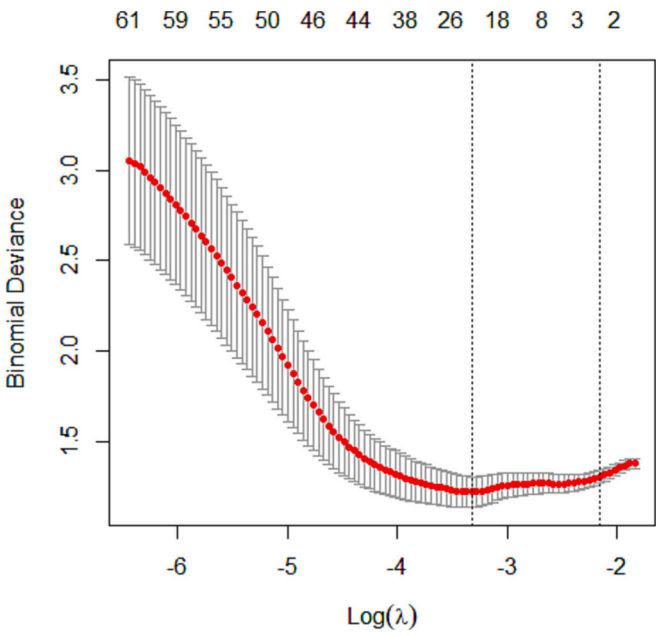


Fig. 2. The LASSO software selected 21 biomarkers based on the optimal parameter $\ln(\lambda) = -3.32$.

selected by LASSO. It achieved an accuracy of 0.755, recall of 0.926, F1-score of 0.793, and AUC of 0.785. Based on the F1-score, the linear kernel SVM model with 21 features based on LASSO is the best with an accuracy of 0.717, recall of 0.926, F1-score of 0.896, and AUC of 0.849.

3.4. Deep learning using the H2O package

Using the 21 metabolomic biomarkers identified by LASSO, we imported them into the H2O package to develop DL models. We performed a grid search with hyperparameter tuning and found that two or three hidden layers achieved higher accuracy. Then, we focused on several DL models with higher accuracy and compared models with 1–3 hidden layers and the number of input neurons from 9 to 21. The performance of different models is listed (Table 4). Based on the accuracy and F1-score, the best model contains two layers and 18 neurons, it achieved the highest accuracy of 0.881, F1-score of 0.892, AUC of 0.873, AUCPR of 0.865, and MCC of 0.784. The variable importance of the best model with two layers and 18 neurons showed the top 8 importance metabolites are hippuric acid, isocitric acid, adipic acid, taurocholic acid (TCA), glucose, GHDCA, valine, and creatinine (Fig. 3), molecules involved in glucose, amino acids, and lipid metabolism. The second-best model with two layers and 20 neurons achieved an accuracy of 0.854, F1-score of 0.847, AUC of 0.851, AUCPR of 0.860, and MCC of 0.771 (Table 4). The AUC curves for models with two layers and 9, 10, 14, 16, 18, and 20 neurons showed that the model with 18 input biomarkers has the highest AUC value, and the model with 20 input biomarkers ranked second (Fig. 4).

3.5. Correlation analysis between metabolomic biomarkers and clinical characteristics

We performed the Spearman correlation analysis between the selected 21 metabolomic biomarkers using LASSO and clinical characteristics. The results are shown in Table S2. Isocitric acid, TCA, Serotonin, and Taurine were significantly correlated with Aβ42. L_alpha_Aminobutyric acid and GHDCA were correlated with tTau and pTau. Hippuric acid, Adipic acid, L_alpha_Aminobutyric acid, Docosahexaenoic acid, GHDCA, HDCA, ISOLCA, Creatinine, Serotonin, Taurine, and Glucose were correlated with ADAS13 and/or MMSE.

Table 3
Performance comparison of metabolomic biomarkers using support vector machines (SVM).

Features	Model	Accuracy	Recall	Specificity	Precision	F1-score	ROC
All 150 features	Linear kernel	0.642	0.733	0.522	0.667	0.698	0.622
	RBF kernel	0.642	0.833	0.391	0.641	0.724	0.557
	Polynomial kernel	0.679	0.800	0.522	0.686	0.739	0.603
21 features based on LASSO	Linear kernel	0.717	0.926	0.500	0.867	0.896	0.849
	RBF kernel	0.717	0.815	0.615	0.688	0.746	0.837
	Polynomial kernel	0.755	0.926	0.577	0.694	0.793	0.785

Abbreviations: SVM: support vector machines; LASSO: Least Absolute Shrinkage and Selection Operator; RBF: Radial basis function; F1-score: a harmonic mean that combines both recall and precision; AUC: Area under the ROC (receiver operating characteristics) curve.

Table 4
Performance comparison of metabolomic biomarkers using deep learning.

Number of neurons in the hidden layer	Hidden Layer	Accuracy	Recall	Specificity	Precision	F1-score	AUC	AUPRC	MCC
9	c(9)	0.800	0.843	0.766	0.774	0.785	0.743	0.707	0.611
	c(9,9)	0.851	0.808	0.807	0.862	0.810	0.789	0.777	0.725
	c(9,9,9)	0.734	0.901	0.564	0.667	0.748	0.695	0.681	0.539
10	c(10)	0.752	0.950	0.528	0.700	0.780	0.728	0.697	0.591
	c(10,10)	0.854	0.925	0.745	0.810	0.852	0.842	0.795	0.703
	c(10,10,10)	0.762	0.930	0.563	0.688	0.782	0.746	0.770	0.583
11	c(11)	0.773	0.858	0.628	0.723	0.775	0.720	0.732	0.616
	c(11,11)	0.767	0.858	0.636	0.697	0.761	0.727	0.735	0.568
	c(11,11,11)	0.816	0.904	0.695	0.774	0.820	0.776	0.771	0.641
12	c(12)	0.796	0.898	0.662	0.758	0.797	0.802	0.780	0.648
	c(12,12)	0.811	0.848	0.733	0.798	0.808	0.757	0.758	0.666
	c(12,12,13)	0.824	0.921	0.679	0.791	0.817	0.786	0.773	0.663
13	c(13)	0.841	0.942	0.726	0.772	0.841	0.819	0.772	0.693
	c(13,13)	0.848	0.866	0.797	0.795	0.827	0.829	0.773	0.678
	c(13,13,13)	0.791	0.942	0.622	0.737	0.819	0.798	0.812	0.651
14	c(14)	0.798	0.889	0.702	0.802	0.812	0.787	0.761	0.645
	c(14,14)	0.846	0.896	0.788	0.813	0.843	0.842	0.842	0.692
	c(14,14,14)	0.835	0.866	0.748	0.845	0.836	0.790	0.797	0.668
15	c(15)	0.783	0.882	0.667	0.737	0.791	0.753	0.743	0.613
	c(15,15)	0.828	0.902	0.742	0.805	0.830	0.783	0.765	0.684
	c(15,15,15)	0.772	0.844	0.673	0.744	0.770	0.751	0.742	0.593
16	c(16)	0.800	0.913	0.656	0.744	0.811	0.790	0.814	0.644
	c(16,16)	0.855	0.932	0.749	0.809	0.856	0.826	0.817	0.715
	c(16,16,16)	0.822	0.940	0.676	0.758	0.830	0.790	0.762	0.698
17	c(17)	0.798	0.896	0.653	0.764	0.810	0.769	0.783	0.645
	c(17,17)	0.728	0.904	0.515	0.671	0.764	0.721	0.743	0.537
	c(17,17,17)	0.847	0.830	0.804	0.843	0.828	0.922	0.836	0.674
18	c(18)	0.809	0.883	0.714	0.781	0.812	0.769	0.761	0.617
	c(18,18)	0.881	0.973	0.793	0.841	0.892	0.873	0.865	0.784
	c(18,18,18)	0.767	0.926	0.628	0.744	0.796	0.728	0.727	0.650
19	c(19)	0.781	0.948	0.610	0.722	0.811	0.791	0.793	0.595
	c(19,19)	0.797	0.875	0.664	0.802	0.811	0.752	0.787	0.656
	c(19,19,19)	0.837	0.942	0.708	0.805	0.852	0.806	0.823	0.747
20	c(20)	0.802	0.883	0.681	0.779	0.808	0.775	0.771	0.649
	c(20,20)	0.854	0.879	0.790	0.858	0.847	0.851	0.860	0.771
	c(20,20,20)	0.819	0.876	0.713	0.788	0.818	0.719	0.778	0.676
21	c(21)	0.846	0.925	0.719	0.827	0.856	0.852	0.864	0.702
	c(21,21)	0.774	0.920	0.626	0.720	0.787	0.758	0.761	0.579
	c(21,21,21)	0.824	0.908	0.691	0.773	0.823	0.824	0.833	0.647

Abbreviations: F1-score: a harmonic mean that combines both recall and precision; AUC: Area under the ROC (receiver operating characteristics) curve; AUPRC: Area Under the Precision-Recall Curve; MCC: Matthews correlation coefficient.

L_Malic acid, GHDCA, Valine, Serotonin, and Glucose were correlated with hippocampus volume. GHDCA, a bile acid derivative, was found to be correlated with tTau and pTau, cognitive test, and hippocampus volume.

4. Discussion

This study reports the development of a DL model for the prediction of AD using non-invasive UPLC-MS/MS-based metabolomics data. A total of 21 of 150 metabolomic biomarkers were selected by the LASSO algorithm, which showed the best model with the highest accuracy in an SVM with a polynomial kernel. Furthermore, the DL model (with two layers and 18 neurons) trained using the H2O package and the 21 metabolomic biomarkers identified by LASSO achieved a higher accuracy, F1-score, and AUC than the SVM model. Multiple metabolomic biomarkers were associated with *APOE*-ε4 genotypes and existing clinical characterics of AD (Aβ42, tTau, pTau, ADAS13, MMSE, and hippocampus volume). This is clinically relevant since these metabolomic biomarkers can be obtained in minimally invasive ways and have the potential to be used in a diagnostic profile for AD prior to the recognition of cognitive impairment. The findings regarding hippocampus volume are interesting and require further exploration. Current studies have identified that aging and memory loss, particularly verbal memory function loss, are related to right greater than left hippocampal shrinkage [49] but in people with AD, reports of hippocampus volume have been reported as general loss of tissue [50].

A wide variety of ML and DL methods have been applied to classify and subtype patients, predict progression, identify biomarkers, and

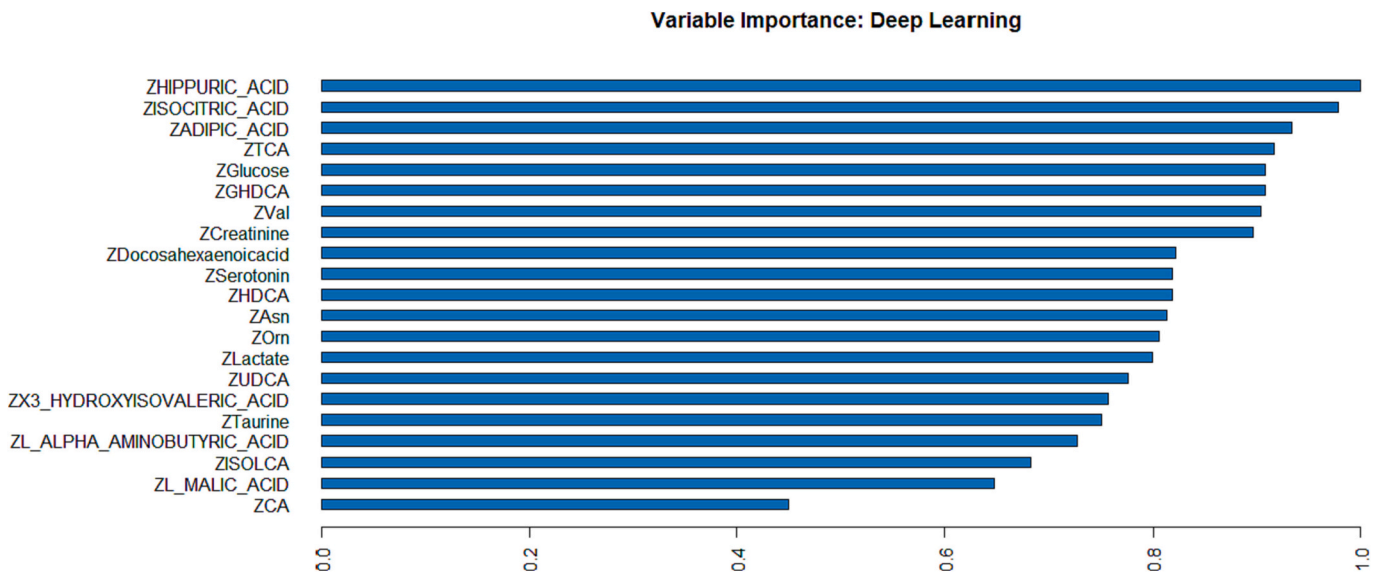


Fig. 3. Variable importance of deep learning model with two hidden layers and 18 input biomarkers in hidden layers.

explore drug repurposing [10–12,51]. One benefit of the DL approach over typical ML methods is that the reliability of DL techniques grows with the phases of learning. DL methods tend to outperform conventional ML techniques when more information is available [52]. DL, with its simple architecture, has the potential for appropriate applications in clinical practice.

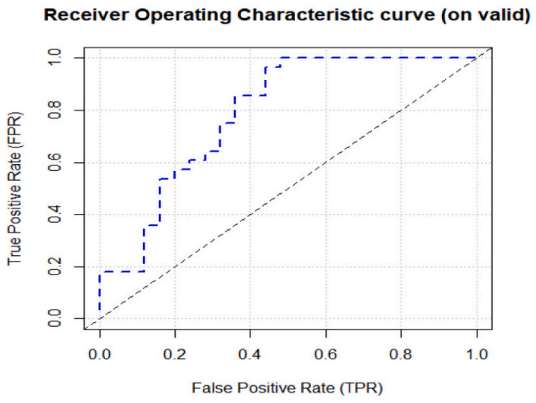
Though it has been suggested over the last 10 years that metabolomics has the potential to play a key role in the early diagnosis of AD [53], there is a paucity of publications on metabolomics and DL in general and a metabolomic profile has not been developed for the early detection of AD. This is partly due to the fact that part of metabolomic variables are highly correlated, owing to their extensive cross-linking in biochemical processes. Consequently, feature selection is burdensome, and predictive modeling is challenging [54]. Considering the high-dimensional context, as well as the high degree of metabolite-metabolite interaction, found in untargeted metabolomics data [55], logistic regression, together with feature selection algorithms such as LASSO, RF, and SVM, are now being increasingly applied to the analysis of metabolomics data [56,57]. For example, one study used the LASSO algorithm followed by SVM and RF, to select eight metabolic features to differentiate stable MCI subjects from MCI subjects who later develop AD, with an overall average accuracy of 73.5% [14]. Another study used correlation-based feature selection and LASSO methods to develop biomarker panels from urine metabolomics samples and then train an SVM to distinguish healthy controls from patients with AD [28]. In the present study, we found that LASSO feature selection resulted in the best overall prediction performance when used for SVM training. LASSO can remove highly correlated features. If a group of predictors are highly correlated, LASSO picks only one of the predictors and shrinks the others to zero [45]. Therefore, the 21 biomarkers selected by LASSO do not show a strong correlation among them. Table S2 reveals the Spearman correlations among these 21 biomarkers and clinical variables.

Previous studies have shown that DL techniques were more accurate than traditional ML algorithms such as RF and SVM [12,15,18]. More specifically, metabolomic data is very high-dimensional and hard for non-DL methods to handle. Consequently, DL could aid in revealing hidden relationships and help the clinician in the decision-making process of patient selection in an individualized way [58]. DL has been used in metabolomics in AD [15,22,30,34,38,39]. In addition, the H2O package in R is an open-source ML platform that supports the most widely used ML models and advanced models, such as multilayer feed-forward ANNs with multiple hidden layers [40,41]. Several studies have used the H2O package for building DL models. For example, one study built an H2O DL model to predict 12-month esophageal variceal bleeding based on endoscopic images and clinical variables [59]. Another study exploited the ability of DL algorithms using the H2O package to combine multi-omics data in prostate cancer [60]. Interestingly, one study found the DL framework in H2O has the highest AUC in classifying estrogen receptor status (positive or negative) in breast cancer patients, compared to the other ML algorithms using metabolomics data [61]. Until now, no single study has used H2O DL for AD. In this present study, we compared DL models with different hidden layers and various numbers of input neurons for predicting AD using the H2O DL function. The best model had two layers and 18 input neurons/metabolomic biomarkers and the second-best model with two layers and 20 input neurons/biomarkers.

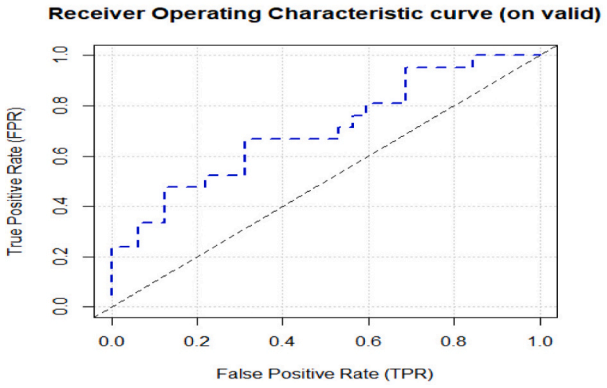
Although the metabolic basis of AD is poorly understood, metabolic biomarkers have been shown to be sensitive to AD and have an enormous impact on developing methods, which will improve life quality and slow the progression of the disease [25–30]. The ADNI collected metabolomics datasets to investigate the relationship between metabolites and disease susceptibility and progression [62,63]. Previous studies have shown that AD etiology may start over 10 years before clinical symptom onset [4,6,7], while metabolomics has the potential to successfully differentiate neurodegenerative diseases from healthy controls [25–28]. Furthermore, metabolomic-based techniques have been used for both early diagnosis of AD and monitoring of appropriate treatment [29]. The findings of our present study have revealed a significant metabolic biomarker that holds immense potential in effectively classifying individuals with AD and CN individuals. Moreover, newly identified biomarkers show promising capabilities in predicting the onset of AD during its early stages.

The findings from this current study are relevant to findings from several papers that provided intensive comments on the application of DL in metabolomics in AD [10,15,30,38,39]. One study reported that combining brain structure data with metabolite levels of the frontal and parietal brain regions (a total of 14 metabolic data) can improve the model classification efficiency of AD using SAE neural network [22]. Another study compared metabolites in blood to CSF biomarkers for specificity for AD and reported that the plasma metabolites matched the AUC for CSF biomarkers of amyloid, pTau, andtTau [34]. The present study selected 21 metabolic biomarkers using LASSO and identified the neural network model with 2 hidden layers and 18 input neurons as the best model. The top six biomarkers are Hippuric acid Isocitric acid,

(a)



(b)



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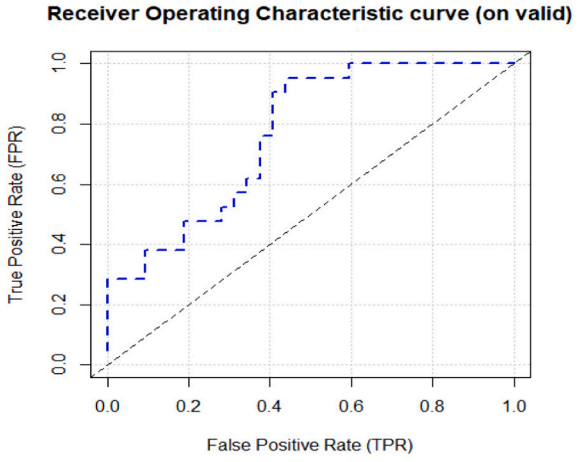

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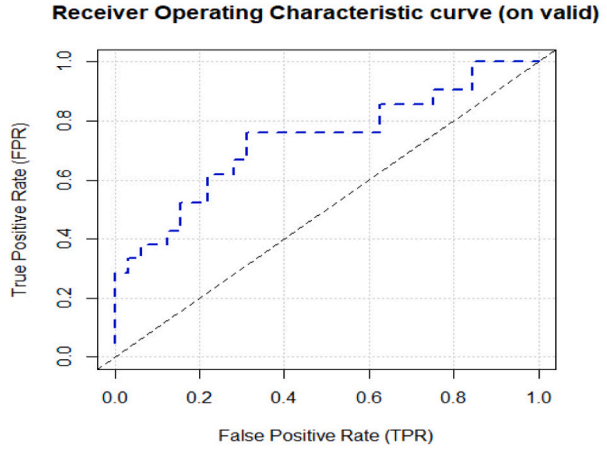
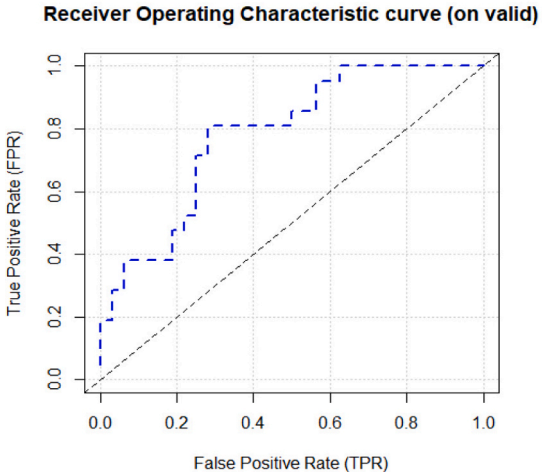


(e)



(f)

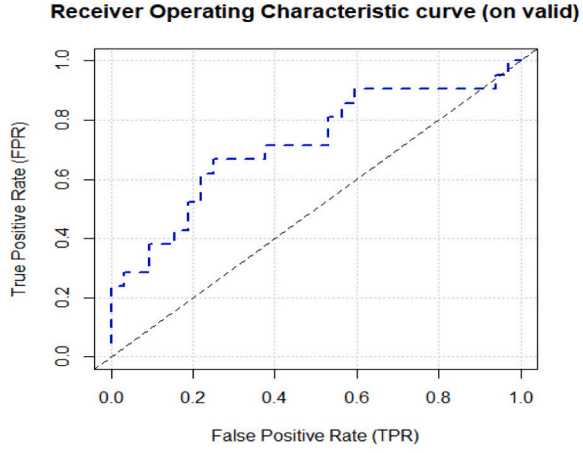


Fig. 4. AUC curves in the validation data using H2O. (a) model with two layers and nine input biomarkers; (b) model with two layers and ten input biomarkers; (c) model with two layers and 14 input biomarkers; (d) model with two layers and 16 input biomarkers; (e) model with two layers and 18 input biomarkers; (f) model with two layers and 20 input biomarkers.

Adipic acid, TCA, Glucose, and GHDCA (Fig. 2). Hippuric acid was correlated with ADAS13. Isocitric acid and TCA were correlated with Aβ42. Adipic acid was correlated with MMSE. Glucose was correlated with MMSE and Hippocampus. GHDCA was correlated with most of the clinical biomarkers, tTau, pTau, ADAS13, MMSE, and Hippocampus in the present study. Among glucose, amino acid, and lipid metabolism, lipid homeostasis has been mostly perturbed in multifactorial pathophysiology in AD [10,31,33,64,65]. Indeed, a lipid regulator drug has shown improvement in AD pathology using a mouse model of AD [51]. Our present study shows that GHDCA, implicated in the neutral synthesis pathway of bile acid metabolism, is consistently correlated with changes in biomarkers, brain structure hippocampus, and cognitive performance. Our finding that the alteration of both glucose metabolism and bile acid metabolism is implicated in AD is not surprising, as these two metabolisms and their disorders are closely linked [66]. Glucose is the major energy source in the brain. Cholesterol metabolism is associated with the development of AD, with many studies focused on APOE because APOE transports cholesterol. Interestingly, bile acid is synthesized from cholesterol. With the proficiency in analytical chemistry to profile the metabolites, a multiomics approach such as lipidomics along with proteomics combined with intelligent DL method will show us multiple biomarkers combinations as signatures, defining different stages of progression of AD in support of precision medicine.

This study makes several contributions. First, we performed feature selection using LASSO and identified 21 biomarkers for SVM and DL to predict AD. Second, simulations confirmed that 18 hidden neurons with 2 hidden layers resulted in the best DNN model. Additionally, we performed cluster analysis to examine the complex relationships among these metabolomic biomarkers and found that they were correlated with meaningful clinical variables such as clinical CSF biomarkers (Aβ42, tTau, and pTau), ADAS13, MMSE, and hippocampus volume.

Several limitations need to be acknowledged. First, the sample size ($n = 177$) is relatively small. We merged several components from ADNI, whereas fewer individuals had CSF biomarkers or metabolomic biomarker values. However, the power is still high (90.7%). Second, the current study is cross-sectional, using the baseline data. Future studies will investigate the variation in metabolic biomarkers on the longitudinal progression of AD. Third, the present study focused on the feature selection of metabolomic biomarkers, we did not compare the DL algorithm and SVM methods with other ML tools. In addition, this study focused on non-invasive metabolic data. The conversion of non-image data to image-like data could boost prediction metrics [67]. Finally, future studies can incorporate heterogeneity into the analysis and develop methods for addressing issues in multi-source data integration so that findings can be translatable to apply to future clinical practice [12].

5. Conclusions

In the present study, we performed feature selection using LASSO and found the 21 metabolomic biomarkers selected by the LASSO algorithm showing the highest accuracy in the SVM to predict AD. Using DL modeling of the 21 metabolomic biomarkers selected by the LASSO, we constructed DNN models to predict AD and found that the best DL model with two layers and 18 neurons achieved the highest accuracy. Some of the metabolites are correlated with clinical CSF biomarkers (Aβ42, tTau, and pTau), cognitive measures (MMSE and ADAS13), and Hippocampus volume. The metabolites are part of the glucose, amino acid, and lipid metabolisms. These findings can be used to inform early diagnosis, prognostic risk stratification, and/or early treatment or preventive interventions for individuals at risk for AD. In addition, the model provides new insights into the use of metabolomics and imaging in the care of people with AD.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2023.120812.

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Institutional review board statement

There was an Institutional Review Board exemption for the current study due to secondary data analysis. Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu).

Informed consent statement

Informed consent was obtained from all subjects involved in the original study. Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu).

Declaration of Competing Interest

The authors declare no conflicts of interest.

Data availability

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu).

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