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Exposing the Brain Proteomic Signatures of Alzheimer's Disease in Diverse Racial Groups: Leveraging Multiple Datasets and Machine Learning

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Abstract

Recent studies have highlighted that the proteome can be used to identify potential biomarker candidates for Alzheimer's disease (AD) in diverse cohorts. Furthermore, the racial and ethnic background of participants is an important factor to consider to ensure the effectiveness of potential biomarkers for representative populations. A promising approach to survey potential biomarker candidates for diagnosing AD in diverse cohorts is the application of machine learning to proteomics datasets. Herein, we leveraged six existing bottom-up proteomics datasets, which included non-Hispanic White, African American/Black, and Hispanic participants, to study protein changes in AD and cognitively unimpaired participants. Machine learning models were applied to these datasets and resulted in the identification of amyloid-β precursor protein (APP) and heat shock protein β-1 (HSPB1) as two proteins that have high ability to distinguish AD; however, each protein's performance varied based upon the racial and ethnic background of the participants. HSPB1 particularly was helpful for generating high areas under the curve (AUCs) for African American/Black participants. Overall, HSPB1 improved the performance of the machine learning models when combined with APP and/or participant age, and it is a potential candidate that should be further explored in AD biomarker discovery efforts.

Graphical Abstract

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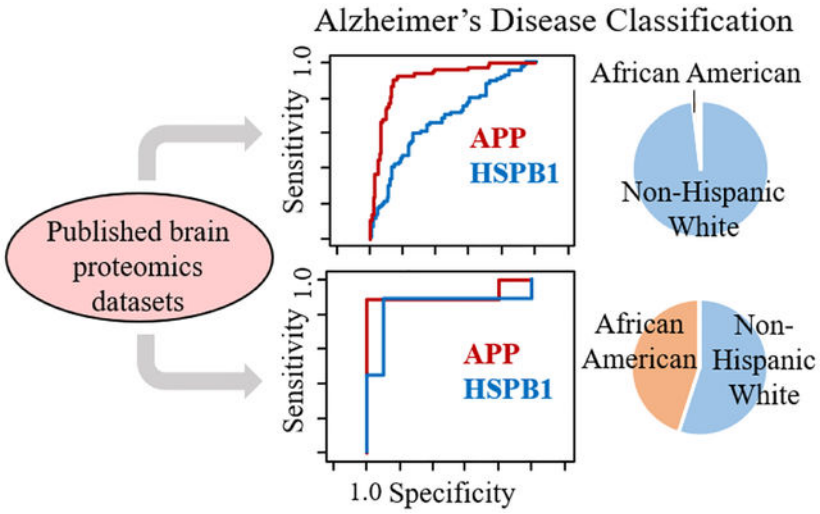

Keywords

Alzheimer's disease; proteomics; machine learning; African American; AUCs; heat shock protein; APP; amyloid-β precursor protein

Introduction

African American/Black and Hispanic adults are more likely to develop Alzheimer's disease (AD) than other racial groups,[1–2] which is a result of complex and interconnected factors related to structural and systemic racism, "lived experiences", social determinants of health, comorbidities, and genetics.[3–10] Reducing these disparities partially requires better understanding of molecular changes in AD. Neuropathological differences in AD hallmarks (amyloid-beta (Aβ) plaques and tau tangles) have not been reported in African American/Black and non-Hispanic White participants.[2, 11–13] Some studies have observed that African American/Black participants are more likely to present with both AD and other dementia pathologies[11, 14–16]; however, this may be dependent on the sampling of participants in the study in terms of community dwelling versus research centers.[17–19] Moreover, potential molecular differences between African American/Black and non-Hispanic White participants have recently been reported, particularly in cerebrospinal fluid (CSF) levels of tau biomarkers for AD.[17, 20–22] CSF levels of total tau and tau phosphorylated at position 181 (p-tau$_{181}$) were lower overall in African American/Black participants than non-Hispanic White participants regardless of cognitive status,[17, 20–22] and furthermore, smaller changes in tau levels occurred in African American/Black participants with cognitive decline.[20] These differences, however, were related to apolipoprotein E (APOE) ε4 status.[17] While such studies have to be replicated, potential differences in biomarker levels based on racial and ethnic background would impact biomarker discovery efforts and biomarker utility.

One promising route forward for establishing AD diagnostic biomarker panels is through incorporating machine learning. In this paradigm, a mathematical model is systematically generated to classify new data based on previous examples of known data.[23–24] Machine

learning can be used in conjunction with protein biomarkers from diseased patients and healthy controls to predict disease status.[24] Machine learning has been previously used for disease classification in AD research.[25–26] For example, various machine learning algorithms including XGBoost,[27] Support Vector Machine (SVM),[28] and the Aristotle Classifier[29] were able to classify brain proteomics data from AD and cognitively normal (CN) groups[30] across two brain regions with high accuracy,[25] and proteins from CSF were also useful in distinguishing the disease.[36] In those studies, however, the impact of racial diversity on the model was not ascertainable because the datasets were relatively small, and the vast majority of the samples were from non-Hispanic White participants.

Racial bias is a common problem facing machine learning, particularly when racial subgroups are underrepresented,[31] so investigations that address the extent to which machine learning models of AD are effective for all racial groups are necessary. Our laboratories have demonstrated that racial bias in machine learning is relevant to AD proteomics studies. An SVM model trained with proteomics analysis of non-Hispanic White patients' plasma was effective at discriminating AD in multiple datasets, but only for the racial group used to train the model.[26] For African American/Black participants, that specific model was ineffective in distinguishing AD, suggesting that proteomic biomarkers should be established using diverse cohorts. Overall, these studies demonstrate an urgent need for understanding AD-related proteomic changes and ensuring potential biomarkers are evaluated and validated in diverse racial and ethnic participants.

The brain's direct involvement in AD makes it a valuable tissue in which to initially characterize proteomic changes. Analyses in postmortem brain tissue could identify important target proteins that could later be measured in more accessible biological samples such as plasma or CSF. Proteomics has been widely used to study molecular changes in the AD brain, and many proteins have different abundances between AD and CN groups across spatial brain regions.[30, 32–43] However, many existing brain proteomics datasets derived in the United States have included primarily non-Hispanic White participants,[30, 32–36] such that characterization of proteomic changes in AD brain in other racial and ethnic groups has been very limited. Availability of postmortem brain tissue from African American/Black participants is significantly limited due to difficulties around recruitment into AD studies,[44] particularly related to organ donation.[45] Some studies have worked to develop effective strategies for recruiting African American/Black participants into AD research, such as culturally informed storytelling materials, community engagement and AD education, and making CSF and/or organ donation optional instead of required.[46–49]

Recently, we used proteomics to analyze postmortem brain tissue from a cohort that included African American/Black and non-Hispanic White participants. In those studies, despite most proteins changing similarly in both racial groups we identified a subset of proteins with race-specific changes in AD.[37] Others have reported that markers of inflammation and neurodegeneration were increased in AD from the middle temporal gyrus region in African American/Black participants compared to non-Hispanic White participants.[50–51] These studies suggest that there is heterogeneity in protein changes in the brain from AD participants, though these studies have had relatively small sample sizes and require replication.

Herein, we combined multiple, recently-published brain proteomics datasets that are described in the literature[32, 36, 52] with machine learning to accomplish three goals: 1) identify protein features that strongly correlate to AD across multiple datasets, 2) determine the impact of racial demographic on the utility of these features for discriminating AD from CN, and 3) evaluate the degree to which machine learning models successfully distinguish AD from CN samples within specific studies. Our findings show that proteins expressed in the brain can differentiate AD from CN groups across AD brain proteomics datasets, yet the utility of each of the selected proteins for distinguishing AD depends on the cohort diversity. These studies also highlight the need for further studies of heat shock protein β-1 (HSPB1), which we have identified herein as showing particular promise in discriminating AD in African American/Black participants. Finally, the studies point to an urgent need for enhanced diversity in future brain proteomics analyses in AD.

Methods

Cohort details and proteomics dataset selection

Available proteomics datasets of postmortem brain tissue from CN and AD participants were included in this study. Datasets were limited to those analyzed using Tandem Mass Tags (TMT), an isobaric tagging strategy that allows multiplexing of up to 18 samples in a single experiment, for protein quantification.[53–54] This criterion was necessary to ensure that the proteomics sample preparation and analysis process was largely similar for all datasets and resulted in inclusion of six datasets: (1) dorsolateral prefrontal cortex (DLPFC) from the Religious Orders Study and Rush Memory and Aging Project (ROSMAP; N = 192; diagnosis based on Emory strict criteria in 2019)[32]; (2–3) parahippocampal gyrus (PHG; Brodmann area 36) from the Mount Sinai/JJ Peters VA Medical Center Brain Bank (MSBB-Bai, N = 62[36] and MSBB-Full, N = 190[52]); (4–6) hippocampus, inferior parietal lobule (IPL), and globus pallidus (GP) from the University of Pittsburgh Alzheimer Disease Research Center (Pitt ADRC; N = 20).[37] We note that the samples in the MSBB-Bai dataset are also part of the larger MSBB-Full dataset. All cohorts included participants from multiple self-reported racial groups (Table 1). Only findings from AD and CN participants from each dataset were included for machine learning analyses. Participants with asymptomatic AD or mild cognitive impairment were excluded.

TMT protein intensity data for all quantified proteins from each dataset were used for these analyses. Quantified proteins from the ROSMAP dataset had < 50% missing TMT intensities. Data for these proteins were normalized to pools (samples containing equal amounts of protein from all samples included in each batch).[32] Both MSBB datasets included TMT quantification at the peptide spectral match (PSM) level, which involved removing PSMs with low intensities prior to normalizing to the median intensity across all PSMs and mean-centering the data. PSMs were averaged per protein to provide protein-level quantification, which was batch-corrected based on the pools.[36, 52] Quantified proteins in the Pitt ADRC dataset were identified across both TMT batches of samples and required that proteins were present for ≥ 80% of channels including the pool channels. Data was normalized to the pool channels.[37] The number of quantified proteins from each dataset

were as follows: ROSMAP = 8,812, MSBB-Bai = 12,148, MSBB-Full = 12,148, Pitt ADRC-hippocampus = 1,414, Pitt ADRC-IPL = 1,487, Pitt ADRC-GP = 1,173.

Univariate analysis

Data analysis was performed in RStudio, using R version 4.0.3. For all univariate analyses, the predictor variable was disease status (control or AD), and the response variable was protein abundance for the protein of interest. All receiver-operating characteristic (ROC) curves and area under the curve (AUC) calculations were calculated directly from the MS data using the package, pROC.[55] Protein identities were matched between datasets using UniProt accession numbers, which were included in the first column or row of each dataset. When the AUC of the entire cohort was specified, all samples in the dataset were used, without regard to racial demographics. When AUCs or fold changes were reported by racial subgroup, the racial identities of the samples along with disease status (CN or AD) were used to stratify samples prior to calculating fold change or AUC. In all cases, fold change was calculated using mean intensity for the group, using the embedded function in R. P-values were calculated using two-tailed t-tests.

Machine learning

Supervised classification was primarily performed with AC.2021, using leave-one-out cross-validation as described previously.[25] This classifier recently has been demonstrated to show enhanced classification performance over Support Vector Machine (SVM) and extreme gradient boosting (XGBoost) on a variety of proteomics datasets classifying AD.[25] Only two hyperparameters are adjustable in the classifier: the number of Repeats, and X, a variable that influences the weighting of each feature. To tune these hyperparameters, the MSBB-Full dataset was used: the full set of patient samples and the three features of interest (APP, HSPB1, and age) were included in the model. First, the Repeats value was set by starting at Repeats = 500 and increasing the value, from 500 to 1000 to 2000, until both the number of misclassified samples and the AUC (to two decimal places) remained constant for three consecutive classifications. To achieve this standard of reproducibility, Repeats set to 2000 was sufficient. Next, the parameter X was tuned, by starting at 1 and increasing through 10. In this case, the optimal value was that which provided the highest AUC (to two decimal places). In cases where two X values provided equivalent AUCs, the better X value was the one that misclassified the fewest samples. This procedure resulted in an X value of 2 being optimal. These hyperparameters (Repeats = 2000; X = 2) were used for all subsequent classifications on all datasets and all feature sets. Hyperparameters were not re-tuned for different datasets, since the tuning of hyperparameters on small datasets could lead to overtraining, and the goal of these studies is to compare results across feature combinations and datasets when a consistent set of machine learning parameters is used.

For each dataset and feature set, a unique model was developed, using AC.2021, X=2, and Repeats=2000, with the resulting Results vector reporting the outcome of a leave-one-out cross-validation. The Results vector shows the strength of the association between each unlabeled sample and its class assignment, and it was used to determine the percent misclassified for each racial group and to calculate the AUC.

In a secondary test, all the datasets and feature sets previously classified using AC.2021 were reclassified using XGBoost, again using leave-one-out cross-validation. In this case, the package, xgboost, was used. The parameters, eta and max_depth, were tuned to optimize the AUC of the MSBB-Full dataset when all three features (age, HSPB1, APP) were included. The optimized parameters were those that produced the highest AUC for the model. The resulting parameters (eta=1; max_depth=3) were used on all subsequent classifications on all feature sets and all datasets. Other parameters included standard choices that were unoptimized, namely: booster = “gbtree”, objective = “binary:logistic”, nrounds =30, eval_metric=“auc”. This set of experiments was completed as a quality control measure to demonstrate that the major outcomes of the original experiments were not influenced by the selection of the classifier. In cases where only one feature was included in the model, for both XGBoost and AC.2021, the feature of interest was listed twice, since at least two features are required for learning with these tools, but the features do not need to be unique.

Results and Discussion

The overall goal of this study was to understand how inclusion of racial and ethnic diversity in brain proteomics datasets impacts the development of a universal diagnostic biomarker panel for AD. The key challenge to achieve this goal is that a dataset containing large numbers of brain proteomics samples from multiple racial groups does not yet exist. Additionally, use of a single dataset with limited sample size also leads to statistical limitations, is more susceptible to biological noise, and requires further replication. To mitigate these challenges, we employed an experimental design in which multiple existing datasets were leveraged to allow for broader applicability of the findings. We included in this study six publicly available brain proteomics datasets that (1) were analyzed using TMT labeling and (2) contained at least 15% African American/Black participants or at least 50 samples from AD or CN groups (Table 1). The MSBB-Full dataset was chosen as a reference to identify the proteins because it contains N = 190 samples, and the composition of participants was ~12% of African American/Black and ~9% Hispanic.

Each of the 12,148 quantified proteins in the MSBB-Full dataset[52] was assessed for its ability to discriminate AD from CN participants. The performance metric selected was the area under the ROC curve, and only proteins whose AUC was at least 0.8 were considered as highly predictive of the disease state. A total of 13 proteins (Table 2) met this criterion and were further interrogated. We note that the AUC, as a performance metric, ranks proteins somewhat differently than p-values or fold changes. We chose AUC as the primary performance metric because 1) each dataset in this study has different numbers of samples and AUC is independent of study size, and 2) AUC directly measures a protein’s ability to discriminate disease state.

Of the 13 proteins selected, two were present in each of the five remaining datasets: amyloid-β precursor protein (APP) and HSPB1, which are the subject of the remainder of this investigation. To first assess whether these proteins effectively discriminate AD across all the datasets present in the study, ROC curves were evaluated (Figure 1). Both APP and HSPB1, to varying degrees, could be used to differentiate samples from AD and CN

participants. We note that the relative proportion of samples from African American/Black participants in the datasets appears to influence the AUC values obtained for the proteins. The dataset with the lowest proportion of African American/Black participants (ROSMAP) has the largest gap between the ROC curve for APP, which has an AUC of 0.91, and HSPB1, with an AUC of 0.68. By contrast, in the three datasets, Pitt ADRC-hippocampus, -IPL, and -GP, with African American/Black participants comprising at least 40% of the samples, the AUCs of APP and HSPB1 are more similar; in fact, the AUC of HSPB1 (AUC 0.89) in the Pitt ADRC-hippocampus dataset is higher than that of APP (AUC 0.88). APP is noticeably higher than HSPB1 in both MSBB datasets, which have an intermediate number of samples from African American/Black participants. In summary, HSPB1 appears to be a useful indicator of AD, and relative to APP, HSPB1's utility appears to be associated with the number of African American/Black participants in the dataset.

We next considered whether HSPB1 is, in fact, a more useful marker of AD in African American/Black participants than it is in non-Hispanic White participants. If this were to be true, then one would expect to see this difference more readily when the samples in each group are first stratified by racial demographic. We tested this hypothesis by comparing the AUCs for APP and HSPB1 in each dataset within the samples from a given racial group (non-Hispanic White or African American/Black). As predicted based on the data in Figure 1, HSPB1 is a better indicator of AD in African American/Black participants than it is in non-Hispanic White participants (Table 3). In six of six datasets, the AUC for this protein is higher for African American/Black participants. The fold change for HSPB1 is higher in all six datasets for the African American/Black participants. Three of the datasets show fold changes > 20% higher; however, we note that the fold change values are dataset-dependent and a reflection of details of inherent changes in the samples, sample preparation, processing, mass spectrometry acquisition, and data normalization.

By contrast, there is no discernable race-associated difference in performance when evaluating the AUC for APP. In half of the datasets studied, APP has a higher AUC for the samples originating from non-Hispanic White participants (AUC > 0.90) than from those in the African American/Black participants (AUC 0.5–0.85). Yet, in the other half of the datasets, the AUC is higher for the African American/Black participants. APP does appear to have greater changes in expression level in AD for non-Hispanic White participants than in African American/Black participants. In four of six datasets, the fold change for APP is substantially larger (60–80%) in non-Hispanic White participants. Considering the two datasets that did not show a large difference in fold change, one is the "low AD pathology" dataset (Pitt ADRC-GP), where both racial groups show rather slight fold changes. In the other, the MSBB-Bai dataset, only two samples are present in the African American/Black CN group. A fold change value calculated from more samples would likely result in a different outcome. Overall, it is clear that APP has the ability to distinguish AD from CN in both racial groups; however, the level of its discriminating ability varied in each group.

Interestingly, HSPB1 appears to be a better discriminator of AD in African American/Black participants and in pathological regions. HSPB1 shows particular promise as another protein which can potentially help confirm AD diagnosis. It is necessary, however, to evaluate HSPB1 in the context of larger sample sizes from African American/Black adults and also

other racial and ethnic groups. The overall findings for HSPB1 and APP, including which differences are significant ($P < 0.05$), are shown as bar graphs in Figure 2. Here, all five datasets from high-pathology brain regions are included, and a paired t-test was used to determine if race-associated differences are present, based on fold change or AUC. The results reiterate the findings already stated above.

HSPB and APP biomarker panels.

Brain proteomics data are invaluable in the quest to identify useful candidate biomarkers for AD. APP has a well-established role in AD pathogenesis, as APP is cleaved to produce 40- and 42-amino acid Aβ peptides that accumulate in AD's characteristic amyloid plaques.[56–57] While APP has peptides that serve as CSF AD biomarkers, its measurement alone is insufficient to diagnose the disease with high accuracy.[58–59] This study has identified an additional protein, HSPB1, that is highly associated with AD across multiple datasets and shows particular promise for improving the diagnosis of AD in African American/Black participants. HSPB1 has been shown to localize to both amyloid plaques[60] and tau fibrils[61–62] in the brain, and to interact directly with both Aβ[63] and tau[64] *in vitro*, leading to prevention or delay of respective fibril formation. Furthermore, neurons from mice lacking HSPB1 were more sensitive to Aβ toxicity,[60] while *APPswe/PS1dE9* mice overexpressing HSPB1 had improved spatial learning and fewer amyloid plaques in the brain,[65] all of which are consistent with HSPB1 having protective effects against AD in the brain.[66]

To determine the potential benefit of adding HSPB1 into a biomarker panel with APP, machine learning was used to classify four of the datasets (MSBB-Full, ROSMAP, Pitt ADRC-Hippocampus, Pitt ADRC-IPL). Note: these studies were not completed on the Pitt ADRC-GP dataset because this brain region is known to have low AD pathology; also, since the MSBB-Bai dataset is fully represented in MSBB-Full, it was not included in the machine learning studies. The supervised classification relied on different feature combinations, including: APP alone, APP with HSPB1, APP with age, and all three features: APP, HSPB1, age (see Methods). The inclusion of age as an alternative third feature provided an opportunity to assess the relative benefit of HSPB1, as age is the largest risk factor for AD.

In all four datasets, the overall AUC (including all samples/all racial backgrounds) improves when HSPB1 is included in the model, versus a model with APP alone (Table 4). The improvement in AUC is greater in the Pitt ADRC datasets, which have a higher percentage of African American/Black participants. We also assessed the diagnostic accuracy among the racial subgroups. In the MSBB-Full dataset, a total of 23 samples are present in the African American/Black group, and the classification improved from 30% to 22% misclassified when HSPB1 is included as a feature. This difference corresponds to seven vs five samples being misclassified, respectively, so larger studies are still warranted. The inclusion of HSPB1 also substantially improves the classification, from 24% to 6% misclassified, for the Hispanic participants in the MSBB-Full dataset (Table 4).

The utility of HSPB1 for improving classification of samples from non-Hispanic White participants was also studied in MSBB-Full and ROSMAP datasets. The models that include

HSPB1 show consistent improvements in classification: 15 to 13% misclassified in the MSBB- Full dataset and 13 to 11% misclassified in the ROSMAP dataset. These two datasets have 146 and 189 non-Hispanic White participants, respectively. Improvements of classification were difficult to discern in the Pitt ADRC datasets likely because of the small sample sizes.

Finally, we assessed the relative impact of including HSPB1 into the model for predicting AD vs. adding in the participants' age or adding in both the age and HSPB1. Table 4 shows that including age in the model instead of HSPB1 results in a modest classification improvement for non-Hispanic White participants, no improvement for African American/Black participants, and only minimal improvement for Hispanic participants. We note that more samples or more datasets with Hispanic adults represented are needed to support findings about this racial subgroup. Adding in age as a third feature with the set of APP and HSPB1 does not show any improvement in the model over the two protein-only model for the larger datasets (ROSMAP and MSBB-Full). These results, and particularly the results from the MSBB-Full dataset (largest sample size), demonstrate that HSPB1's inclusion is important for distinguishing AD in brain samples from non-White participants, particularly for African American/Black adults.

Finally, to verify that the machine learning outcomes are independent of the classifier used, the entire set of supervised classifications was repeated with XGBoost as the classifier, instead of AC.2021. These results can be found in Supplementary Table 1. Overall, all the key outcomes were replicated using this alternative classifier. One notable difference in comparing the reclassification data to the original AC.2021 results is that the AUCs were lower for 14 of the 16 classifications using XGBoost, and the remaining two classifications had equal AUCs to those generated using AC.2021. This comparison between the classifiers further demonstrates that AC.2021 is a better choice for classifying these data.

Strengths and limitations of the study.

The key strength of this study is leveraging datasets from multiple laboratories to better understand how AD is manifested at the protein level and which proteins are important to consider as biomarker candidates for the disease. Each of the datasets used in this study had a similar TMT quantitation strategy, which helps to minimize potential sample processing- and mass spectrometry-related differences. The detection of both APP and HSPB1 in all six datasets is an asset to this study. The general agreement of magnitude of protein change in AD across laboratories and determined by the TMT quantitation strategy supports the reliability of the proteomics results. Finally, the application of machine learning, following the univariate analysis, is a strength, as it identifies the most robust potential protein candidates that can determine AD. The analysis of each protein individually and as a panel demonstrated that the two proteins combined gave more diagnostic utility than using either individually.

The major limitation of this study is the lack of larger and/or more diverse proteomics datasets to conduct machine learning analyses. Even though two of the datasets in this study had ~200 samples, the vast majority of those samples were from non-Hispanic White participants. Assessing the value of biomarkers in sub-populations of participants introduces

the challenge of potentially working with low sample sizes. Yet, as this work shows, this type of analysis is critical for generating biomarker panels that are effective for the full population, since some proteins have differential diagnostic utility in sub-populations. While this study focused on racial subgroups, similar subpopulations should be investigated across other parameters, including socioeconomic status, comorbidities, education, lifestyle factors, and other social determinants of health. This study, in addition to others that attempt to undertake the challenging problem of assessing racial sub-populations, would benefit from larger datasets containing a greater representation of African American/Black, Hispanic, and other racial and ethnic participants.

This study focused on two proteins, APP and HSPB1, that were detected in six publicly available datasets but selected from a set of 13 proteins in the MSBB-Full dataset, which served as a reference in this study. Whether there are other shared proteins that could be useful biomarkers would require further analysis and selection from different reference datasets and those with larger numbers and diverse participants.

Conclusions

Leveraging six different brain proteomics datasets, we identified potential AD biomarkers that could serve a racially diverse American population. This combination of datasets and attention to racial subgroup analysis allowed us to identify HSPB1 as a protein that correlates strongly to AD in multiple studies and does so with higher accuracy in samples from African American/Black than non-Hispanic White participants. Furthermore, combining APP and HSPB1 does a better job of discriminating AD than APP alone for all racial groups. HSPB1 should be considered as a potential biomarker candidate for other tissues such as CSF and plasma. Future efforts to identify other potential biomarker candidates using the machine learning strategies presented herein and to replicate the combined value of HSPB1 and APP as diagnostic biomarkers should be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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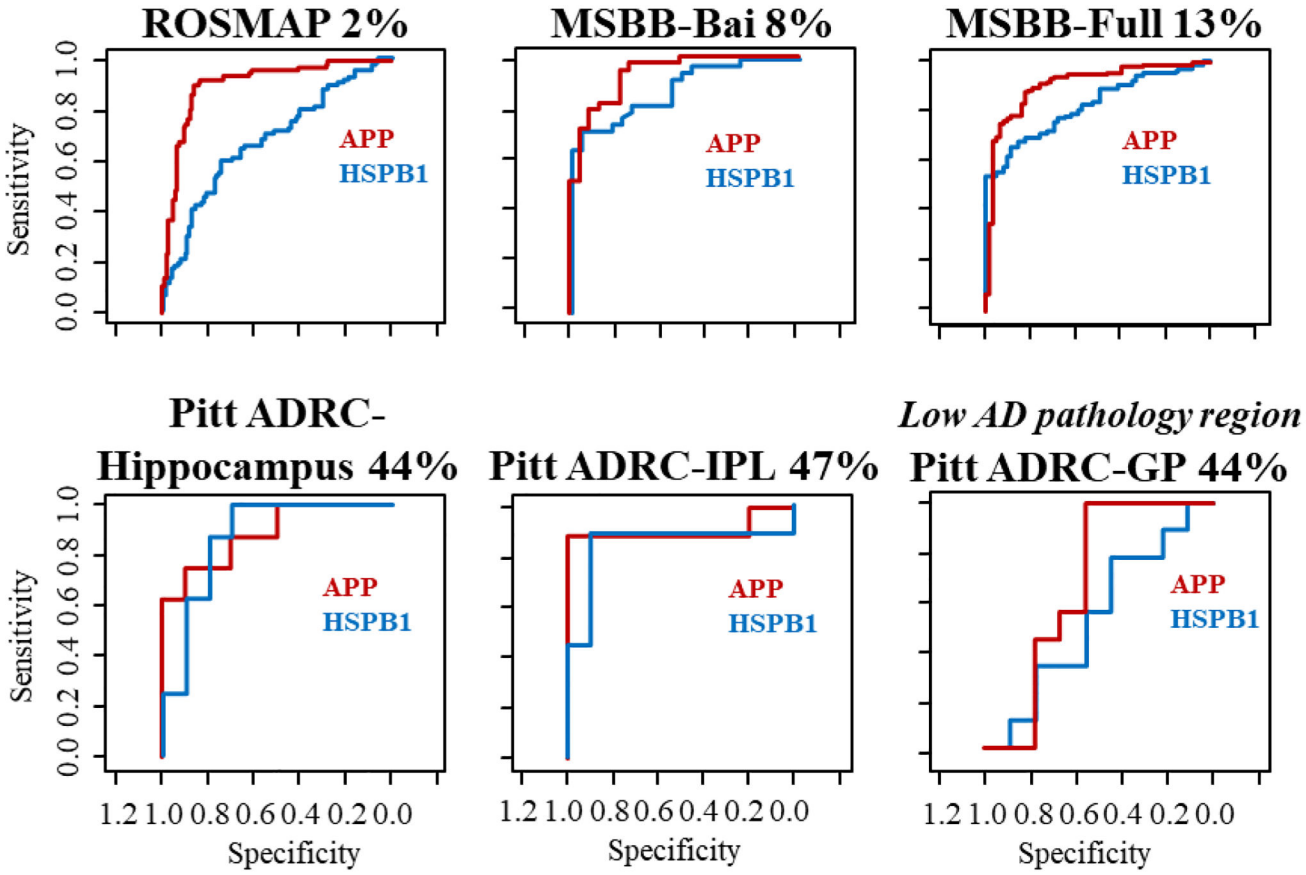

Figure 1. APP and HSPB1 receiver-operating characteristic (ROC) curves in brain datasets as a function of the percentage of African American/Black participants.

The AUCs for APP and HSPB1, respectively, in each dataset are: ROSMAP (0.91, 0.68); MSBB-Bai (0.93, 0.88); MSBB-Full (0.91, 0.82); Pitt ADRC-hippocampus (0.88, 0.89); Pitt ADRC-IPL (0.91, 0.84); Pitt ADRC-GP (0.67, 0.53). Note: the GP is a brain region with low AD pathology.

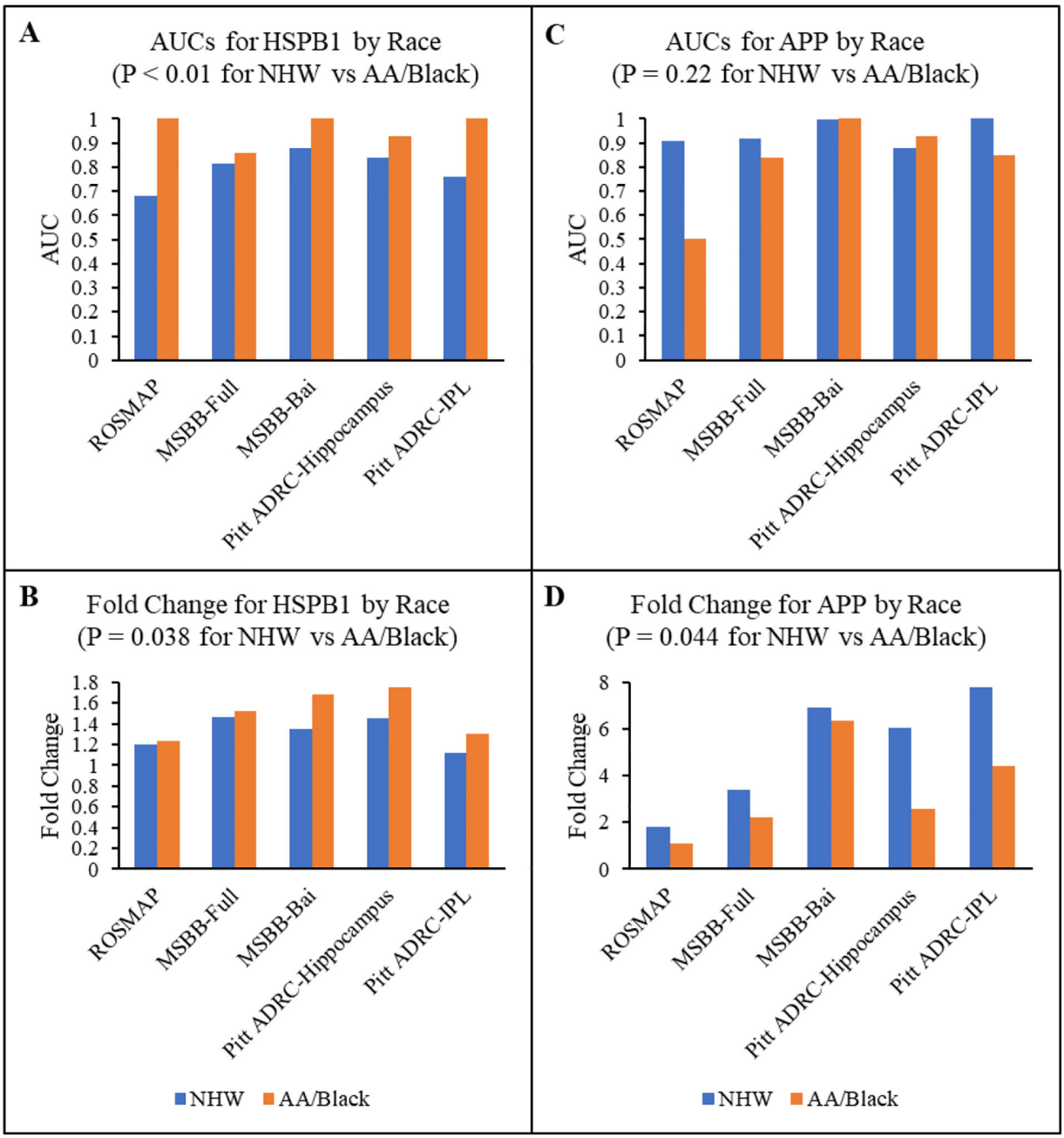

Figure 2. Assessment of HSPB1 and APP for discriminating AD in five datasets obtained from brain regions with high AD pathology.

(A) AUCs for HSPB1; **(B)** fold change for HSPB1; **(C)** AUCs for APP; **(D)** fold change for APP. P-values were calculated using paired t-tests. Abbreviations: AUC, are under the curve; HSPB1, heat shock protein β-1; NHW, non-Hispanic White; AA, African American; ROSMAP, Religious Orders Study and Rush Memory and Aging Project; MSBB, Mount Sinai Brain Bank; Pitt ADRC, University of Pittsburgh Alzheimer Disease Research Center; IPL, inferior parietal lobule; APP, amyloid-β precursor protein.

Table 1.

Demographics of patients in proteomics datasets.

	ROSMAP		MSBB-Bai		MSBB-Full[a]		Pitt ADRC-Hippocampus		Pitt ADRC-IPL		Pitt ADRC-GP	
	CN	**AD**	**CN**	**AD**	**CN**	**AD**	**CN**	**AD**	**CN**	**AD**	**CN**	**AD**
N	84	108	23	39	63	127	8	10	9	10	9	9
Age at death[b]	85 ± 5	88 ± 3	81 ± 10	84 ± 10	81 ± 8	80 ± 7	65 ± 12	82 ± 8	67 ± 13	82 ± 8	67 ± 13	81 ± 8
Sex (M/F)	34/50	26/82	12/11	12/27	29/34 2 ND	41/84 2 ND	5/3	5/5	6/3	5/5	6/3	5/4
Race[c]	83 W 1 B	106 W 2 B	17 W 2 B 4 H	34 W 3 B 2 H	44 W 7 B 10 H 2 ND	102 W 16 B 7 H 2 ND	5 W 3 B	5 W 5 B	5 W 4 B	5 W 5 B	5 W 4 B	5 W 4 B

[a] ND indicates no data was available for N participants in the designated variable.

[b] Reported as mean ± standard deviation.

[c] Race was self-reported.

W = non-Hispanic White; B = African American/Black; H = Hispanic. Abbreviations: CN, cognitively normal; AD, Alzheimer's disease; ROSMAP, Religious Orders Study and Rush Memory and Aging Project; MSBB, Mount Sinai Brain Bank; Pitt ADRC, University of Pittsburgh Alzheimer Disease Research Center; IPL, inferior parietal lobule; GP, globus pallidus.

Table 2.

List of proteins discriminating AD from CN participants in MSBB-Full dataset.[a]

UniProt accession number	Protein name	AUCs	p-value	Fold change[b]
P21741	Midkine	0.93	1.78E-17	3.53
P05067-108	Amyloid-β precursor protein	0.91	6.39E-16	3.35
Q9H4F8	SPARC-related modular calcium-binding protein 1	0.90	3.37E-16	1.66
O95631	Netrin-1	0.85	3.23E-11	1.54
Q96CG8	Collagen triple helix repeat-containing protein 1	0.84	2.74E-13	1.79
Q8N474	Secreted frizzled-related protein 1	0.84	6.03E-13	1.55
P04792	Heat shock protein β-1	0.82	9.32E-12	1.46
P05362	Intercellular adhesion molecule 1	0.82	4.88E-10	1.70
E7EMC7	Sequestosome-1	0.81	5.07E-10	1.30
F8WE04	N/A	0.81	1.87E-11	1.44
Q96AQ6	Pre-B-cell leukemia transcription factor-interacting protein 1	0.81	5.94E-10	1.33
P17948-2	Vascular endothelial growth factor receptor 1 isoform 2	0.81	4.72E-11	1.21
Q9NPD7	Neuritin	0.80	3.66E-12	0.74

[a]Proteins discriminating AD and CN participants with AUCs > 0.80.

[b]Mean fold change value of AD/CN participants.

Table 3.

Performance metrics of APP and HSPB1 by racial group across six datasets.

		HSPB1[a]		APP[a]	
		NHW	**AA/Black**	**NHW**	**AA/Black**
AUCs	ROSMAP	0.68	**1.00**	**0.91**	0.50
	MSBB-Full	0.81	**0.86**	**0.92**	0.84
	MSBB-Bai	0.88	**1.00**	1.00	**1.00**
	Pitt ADRC-Hippocampus	0.84	**0.93**	0.88	**0.93**
	Pitt ADRC-IPL	0.76	**1.00**	**1.00**	0.85
	Pitt ADRC-GP	0.32	**0.75**	0.52	**0.88**
FCs	ROSMAP	1.20	**1.23**	**1.81**	1.08
	MSBB-Full	1.46	**1.52**	**3.40**	2.24
	MSBB-Bai	1.4	**1.70**	**6.93**	6.35
	Pitt ADRC-Hippocampus	1.45	**1.75**	**6.03**	2.58
	Pitt ADRC-IPL	1.12	**1.30**	**7.80**	4.40
	Pitt ADRC-GP	0.84	**1.29**	1.12	**1.41**

[a]Bold indicates the higher AUC or fold change value between the two racial groups.

Abbreviations: APP, amyloid-β precursor protein; HSPB1, heat shock protein β-1; AUC, area under the curve; NHW, non-Hispanic White; AA, African American; ROSMAP, Religious Orders Study and Rush Memory and Aging Project; MSBB, Mount Sinai Brain Bank; Pitt ADRC, University of Pittsburgh Alzheimer Disease Research Center.

Table 4.

Classification and AUC metrics for APP, HSPB1, and age features.

Datasets	% Non-Hispanic White	APP	APP + HSPB1	APP + Age	All 3
MSBB-Full					
AUC	76.8%	0.89	0.93	0.90	0.93
NHW % error		15	13	13	14
AA % error		30	22	30	22
H % error		24	6	18	6
ROSMAP					
AUC	98.4%	0.89	0.91	0.92	0.93
NHW % error		13	11	12	12
AA % error[a]		33	33	33	33
Pitt ADRC-Hippocampus					
AUC	55.6%	0.76	0.89	0.90	0.91
NHW # error		2	3	3	3
AA # error		1	1	1	0
Pitt ADRC-IPL					
AUC	52.6%	0.89	0.98	0.89	0.96
NHW # error		0	2	0	1
AA # error		1	1	1	1

[a]33% of the African American/Black participants is equal to one sample. The AUCs from classification with APP alone and APP + HSPB1 were compared using DeLong's test, resulting in the following p-values: MSBB-Full, p = 0.01; ROSMAP, p = 0.09; Pitt ADRC-hippocampus, p = 0.20; Pitt ADRC-IPL, p = 0.39.

Abbreviations: AA, African American/Black; APP, amyloid-β precursor protein; H, Hispanic; HSPB1, heat shock protein β-1; MSBB, Mount Sinai Brain Bank; NHW, non-Hispanic White; ROSMAP, Religious Orders Study and Rush Memory and Aging Project; Pitt ADRC, University of Pittsburgh Alzheimer Disease Research Center; IPL, inferior parietal lobule.